Probing the restorative potential of dates (*Phoenix dactylifera*) against atherogenic diet induced cardiac and hepatic stress

by

Kanza Aziz Awan

Reg No. 2007-ag-1134
M.Sc. (Hons.) Food Technology (UAF)

A dissertation submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
IN
FOOD TECHNOLOGY

NATIONAL INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY
FACULTY OF FOOD, NUTRITION AND HOME SCIENCES
UNIVERSITY OF AGRICULTURE
FAISALABAD
2017
DECLARATION

I hereby declare that the work presented in this thesis “Probing the restorative potential of dates (*Phoenix dactylifera*) against atherogenic diet induced cardiac and hepatic stress” is my own effort except where other acknowledged and that the thesis is my own composition. No part of the thesis has previously been presented for any other degree. University may take action if information is incorrect at any stage.

Kanza Aziz Awan
2007-ag-1134
To
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ACKNOWLEDGEMENT

I surrender myself before the Almighty Allah, who is the entire source of all knowledge and wisdom endowed to mankind. Trembling lips and wet eyes praise for the Holy Prophet Muhammad (PBUH) for enlightening our conscience with the essence of faith in Allah, converging all His kindness and Mercy upon him.

I deem it a great honor and privilege to record my gratitude to my respectable supervisor Prof. Dr. Masood Sadiq Butt, Dean, Faculty of Food, Nutrition and Home Sciences for his dynamic supervision, constructive guidance, encouraging and cooperative behavior and indefatigable support during the entire degree program. I am also indebted to the sincere contributions of my committee members, Dr. Mian Kamran Sharif, Assistant Professor, National Institute of Food Science and Technology, University of Agriculture Faisalabad and Dr. Fatma Hussain, Lecturer, Department of Biochemistry, Faculty of Sciences, University of Agriculture, Faisalabad. I would also like to thank all my teachers for their valuable suggestions and guidance.

My heartfelt thanks to my friends, colleagues and juniors for their motivation, endorsing support and encouragements throughout my studies. Last but not the least, I wish to express my feelings of obligations towards my affectionate father Dr. Javaid Aziz Awan and my dearest mother for their unconditional love, support and meticulous guidance throughout the life and for making me what I am. I am also indebted to my Aunt and all other family members who encouraged, inspired and supported me throughout my life.

Kanza Aziz Awan
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ABBREVIATIONS

ABTS 2, 2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid
AI Atherogenic index
AIP Atherogenic Index of plasma
ALP Alkaline Phosphatase
ALT Alanine Aminotransferase
AST Aspartate Aminotransferase
CAT Catalase
CK Creatine kinase
CK-MB Creatine kinase-MB
CRI (I) and (II) Castelli’s risk index I and II
D₀ Control (Traditional Halwa)
D₁ Dhaki Date Based Halwa
D₁a Dhaki Date Extract Based Halwa
D₂ Aseel Date Based Halwa
D₂a Aseel Date Extract Based Halwa
D₃ Zahidi Date Based Halwa
D₃a Zahidi Date Extract Based Halwa
DPPH 1, 1-diphenyl-2-picrylhydrazyl
EGCG Epigallocatechin gallate
FER Feed Efficiency Ratio
FRAP Ferric reducing antioxidant power
G₁ Normal diet
G₂ Date fruit + normal diet
G₃ Date extract + normal diet
G₄ Atherogenic diet
G₅ Date fruit + atherogenic diet
G₆ Date extract + atherogenic diet
GAE Gallic Acid Equivalent
Hb Hemoglobin
HDL High Density Lipoproteins
LDH Lactate dehydrogenase
LDL Low Density Lipoproteins
MCH Mean Corpuscular Hemoglobin
MCHC Mean Corpuscular Hemoglobin Concentration
MCV Mean Corpuscular Volume
MDA Malondialdehyde
NFE Nitrogen Free Extract
nHDL Non-High Density Lipoprotein
QE Quercetin Equivalent
RBC Red Blood Cells
SOD Superoxide Dismutase
TBARS Thiobarbituric Acid Reactive Substances
TC Total Cholesterol
TFC Total Flavonoid Content
TG Triglycerides
TPC Total Phenolic Content
VLDL Very Low Density Lipoprotein
WBC White Blood Cells
γ-GT γ-glutamyl transferase
ABSTRACT

In the recent years, there has been a paradigm shift towards diet’s elementary role as an energy and body building source to more elusive action of food bioactives on human health and well-being. In this context, the present research was designed to explore the functional worth of three locally available date varieties, namely Dhaki, Aseel and Zahidi against atherogenic diet induced oxidative stress mediated dysfunctions. Amongst the varieties, Zahidi exhibited better nutritional profile followed by Dhaki and Aseel. The Zahidi dates proved to be a good source of potassium (870.83±32.39 mg/100g), calcium (96.86±3.18 mg/100g) and iron (3.23±0.11 mg/100g) along with other micronutrients. Moreover, vitamin C content was also higher in Zahidi (63.38±0.95 mg/100g) as compared to others, while maximum β-carotene content *i.e.* 0.54±0.01 mg/100g was found in the Dhaki cultivar. The HPLC determination of sugars also revealed highest reducing and non-reducing sugars in Zahidi dates. The chromatographic phytochemical analyses demonstrated maximum gallic and caffeic acids, kaempferol and quercetin in Zahidi while, myricetin was only found in Dhai. Nevertheless, chlorogenic and vanillic acids were prominent in Aseel date variety. Antioxidant potential reflected by DPPH, TEAC and H$_2$O$_2$ scavenging capacity assays indicated better performance of Zahidi extract, whereas Dhaki extract exhibited higher FRAP and reducing power capacity. The selected varieties along with their extracts were used in the development of date halwa that proved to be an energy rich nourishing product with good physicochemical and sensorial attributes having shelf stability of two weeks without any preservative. Considering the phytochemical screening tests and hedonic response, Zahidi date fruit and extract were selected for the bioefficacy assessment. Purposely, bioevaluation trials were conducted on Sprague Dawley rats for a period of twelve weeks comprising of six groups. The rats were divided into two broad categories; one fed on normal diet, whereas the others were administered atherogenic diet to induce oxidative stress. Each module was then further divided on the basis of date fruit and date extract consumption. The formulated groups were G$_1$ (Normal diet), G$_2$ (Date fruit + normal diet), G$_3$ (Date extract + normal diet), G$_4$ (Atherogenic diet), G$_5$ (Date fruit + atherogenic diet) and G$_6$ (Date extract + atherogenic diet). The findings showed that date based dietary regimen has proven effectual in mitigating cardiac, hepatic and renal oxidative stress. Date extract effectively ameliorated lipid biomarkers by reducing cholesterol, triglycerides and LDL by 18.55, 14.51 and 25.98%, respectively and elevating HDL by 10.22% in atherogenic rats. Date extract supplementation alleviated the atherogenic diet induced cardiac stress by lowering serum AST (19%), CK (19.38%), CK-MB (18.02%) and LDH (22.51%), besides improving myocardial endogenous enzyme status and histoarchitecture. It also modulated hepatic biomarkers by reducing the serum ALT (21.45%), ALP (14.77%), γ-GT (18.88%) and total bilirubin (10.33%) with concomitant decrease in lipid peroxidation in hepatic parenchyma. Likewise, date enriched diet improved renal health in oxidative stress induced rats. Furthermore, the hematological analyses were within normal range with non-significant effect of diets on most parameters indicating safety of use. Conclusively, date fruit encompasses beneficial nutritional matrix that need to be promoted to develop designer foods against various lifestyle related disorders with special reference to cardiac, hepatic and renal stress related ailments.
CHAPTER 1

INTRODUCTION

The introduction of diet based therapeutic interventions around the globe are aimed to utilize food and its constituents as remedial agents against prevailing metabolic ailments. The role of preventive approaches in health maintenance resulted in the development of novel health care practices that are supported by strong scientific evidences. This is leading to a paradigm shift from the intervention-oriented and technology-driven outlooks of the previous era to more advanced prophylactic and molecular based tactics (Joseph et al., 2016). The diet-health linkages and their economic and social implications are gaining wide public acceptance globally. The knowledge about the health-benefiting role of food and its components has grown immensely over the past few decades. This has resulted in increased consumer desire and demand for the healthy diets. Hence, natural foods are being used that exert affirmative effects on the human health. It is envisaged that the exploration of nature-based foodstuffs will lead to reduced dependence on chemical-based drugs. The bounties of nature’s pharmacy are often described as nutraceutical and functional ingredients. The term nutraceutical was coined by the portmanteau of pharmaceutical and nutrition i.e. employing food components to mitigate the prevailing lifestyle related disorders (Pathak, 2009). The functional foods, alongside provide basic nutrition, contain an optimal mix of biologically active constituents often termed as nutraceutics. These are involved in improving and protecting the physiologic functionality (Goldberg, 2012; Lu and Yen, 2015).

Scientific evidences support the salubrious role of fruits and vegetables in lowering oxidative stress related malfunctions, arising due to imbalance among the reactive oxygen species and natural antioxidant defense mechanisms in the cells and tissues (Halliwell, 2005). Nowadays, consumers have started subscribing to dietary regimens that prevent the onset and progression of various diseases. In this context, several fruits and vegetables have been studied that possess affirmative health boosting properties. Amongst, dates are being consumed since the primeval times. Date palm and date fruits are valued across the globe due to their significance in the three major religions of the world, Judaism, Christianity and Islam. In Jewish scriptures, dates are among the seven holy foods. Numerous references in the Holy Bible testify the manifold virtues of dates and date palm. However, Islam has given a very high priority to the dates that are regarded as a blessing of the paradise (Holy
Quran, 55:68). Health benefits of dates and their nutritional significance have been mentioned in the Holy Quran in several Ayats (Quran 19:23-26). Prophet Muhammad also recommended the consumption of dates owing to its therapeutic properties (Ali et al., 2012; Azarpour et al., 2014; Rahmani et al., 2014). Consumption of dates varies during the year and reaches its peak in the Holy month of Ramadan (Islamic fasting month) when it is used to break the fast. Socioeconomic values also contribute to the consumption pattern depending on the food choices, habits, continued urban drifts as well as the availability of other fruits (Ismail et al., 2006).

Date palm (Phoenix dactylifera) belongs to the family Arecaceae and grows in the hot arid regions of the world. The date fruit is savored for its sweet taste and fleshy mouth feel. About 100 million date palms are present around the globe in nearly 34 countries. The major date producing countries are Egypt, Saudi Arabia, Iran, UAE, Pakistan, Libya, Iraq, Oman and Tunisia (Al-Shahib and Marshall, 2003; Zaid, 2006). In Pakistan, several varieties are being produced particularly in southern Punjab, Sindh and Baluchistan regions, nonetheless, Dhaki, Aseel and Zahidi are the prominent ones (Khan et al., 2008).

Dates are categorized on the basis of shape, moisture content, sugar level and sensory properties. More than 600 varieties of date fruit have been identified and are consumed globally (Baliga et al., 2011). Date fruit is valued as an important subsistence crop in the desert areas throughout the world (Aleid, 2012). Date palm fruits once in a year and passes through five different developmental stages. The sweetness, texture and polyphenolic profile varies during all the stages. In the first stage called the ‘Hababouk’, the fruit is pea-sized and immature. This develops into ‘Kimri’ or green stage in which the fruit attains its full length. Here, the green colored fruit forms the characteristic oblong shape, tastes bitter and is unsuitable for consumption. This is followed by ‘Khalal’ or color stage i.e. characterized by the change in color, depending on the variety. The fruit attains maximum size and weight at the end of this phase with rapid rise in sugar and decline in the water contents. In the next stage, ‘Rutab’, the fruit becomes soft in texture and often changes to brown or black in color. The fruit weight decreases due to loss in moisture content. At this stage, more sucrose is converted to simple sugars. Finally, in the last stage of development, the ‘Tamr’ or the full ripe phase, the flesh shrinks, dates appear dehydrated and wrinkled due to loss of moisture. The flesh also darkens with time. Dates are mostly harvested in ‘Rutab’ or ‘Tamr’ stages depending upon the variety, end-use and consumer demand (Fadel et al., 2006; Baliga et al., 2011).
Dates are valued for the presence of readily available sugars particularly fructose, glucose and sucrose that constitute almost 70% of the edible fruit. These sugars, after consumption, are immediately utilized as instant source of energy. Dates are also considered as a rich source of dietary fiber. However, proteins, lipids and ash are present in small amounts. Nevertheless, 23 amino acids are present in the date fruit with high contents of aspartic and glutamic acids, leucine, lysine, serine and alanine (Borchani et al., 2010; Tang et al., 2013). Minerals like calcium, potassium, magnesium, phosphorous, manganese, sodium, copper, zinc and selenium are also present. Amongst these minerals, potassium content is highest up to 0.9% in the fruit (Khan et al., 2008; Tang et al., 2013). Vitamins such as A and C, thiamine, riboflavin, niacin and folic acid are also found in the date fruit in reasonable amounts (Al-Shahib and Marshall, 2003; Chaira et al., 2007).

Numerous epidemiological investigations have suggested an inverse association between the diseases of affluence, such as cardiovascular ailments, hepatic disorders, hypercholesterolemia, diabetes and oncogenesis and the consumption of fruits and vegetables. These commodities contain a myriad of phytoceutics that pose positive effects on the human health. Various phytochemical moieties are present in the date fruit, which are responsible for their antioxidant and disease ameliorating characteristics. The fruit pulp contains phenolics, flavonoids, carotenoids, sterols, anthocyanins and procyanidins (Al-Farsi et al., 2005a). Dates contain carotenoids and such phenolics as cinnamic acids, sinapic, ferulic, vanillic, syringic, caffeic, chlorogenic, coumaric and protocatechuic acids and their derivatives. Dactilyferic acid; a derivative of hydroxycinnamic acid, has also been identified (Mansouri et al., 2005; Borochov-Neori et al., 2013; Habib et al., 2014). Among the flavonoid glycosides quercetin, luteolin, methyl quercetin and methyl luteolin are present. Studies also reveal the presence of catechins, epicatechins and anthocyanins (Al-Farsi et al., 2005a; Rock et al., 2009; Habib et al., 2014). The presence of these compounds and quantity depends on the fruit variety and type, maturation stage and the prevailing environmental conditions (Baliga et al., 2011).

The antioxidant status and polyphenolic composition is directly dependent on the extraction method used. Numerous extraction technologies are in vogue depending upon the raw material and certain physicochemical factors. The economic feasibility and viability of the extraction process is highly dependent on the cost as well as the safety of the process (Singh et al., 2011b). Amongst all the methods, conventional solvent extraction is one of the favored partitioning technique owing to its speed, simplicity and wide scope (Kislik, 2011).
The phenolic content recovery is influenced by solvent polarity and component solubility (Alothman et al., 2009). The bioactives from date fruits are usually extracted using water due to their high affinity in the aqueous phase. However, due to high polarity of methanol it is being widely used to obtain maximum amount of polyphenols, but the safety concerns restrict the use of methanolic extracts in food products, thus the aqueous extract is preferred for food applications (Vayalil, 2002; Al-Farsi et al., 2008; Kchaou et al., 2013).

Dates are consumed as fresh, dried or semi-dried. Moreover, they are also being processed into several products owing to their better hedonic response and energy dense nature. In the processed form, they are mainly transformed into syrups, pastes, jams and jellies. Besides, these are also used in various confectionary products such as tarts, bars, biscuits and pies (Awan and Sohail, 1999). Date fruit is also employed in designing new products along with other ingredients such as chocolate, nuts, honey, cereal flours depending upon the product nature (Al-Rawahi et al., 2006; Besbes et al., 2009; Masmoudi et al., 2010).

Oxidative stress is triggered by the production of reactive oxygen and nitrogen species that performs an important role in the etiology of numerous disorders like cardiac ailments, atherosclerosis and hepatotoxicity (Sankhari et al., 2012). Globally, cardiovascular disparities have become the primary cause of morbidity and mortality. According to the WHO estimates, by 2030, cardiovascular ailments would be responsible for 24.2 million deaths, world widely (Miltonprabu and Thangapandiyan, 2015). These disorders do not arise spontaneously, nevertheless, there are several other pathological conditions triggering the onset of cardiac stress. Amongst, major driving force is the oxidative stress that adversely affects the physiologic system. Liver plays vital part in the maintenance and regulation of homeostasis in the physiological system. It is primarily involved in all biochemical pathways of growth, reproduction, energy provision, disease protection and xenobiotic detoxification. Hence, maintenance of healthy liver is important for overall health and wellness (Pandit et al., 2012). Raised lipoprotein level, lifestyle and several environmental factors result in cellular damage via reactive oxygen species-mediated hepatotoxicity (El Arem et al., 2014a). Phytocutics present in plant foods and are responsible for their defensive roles against several physiological threats (Quiñones et al., 2013). In this milieu, date fruits having rich polyphenolic profile along with distinct mineral elements and vitamins, possess cardio- and hepatoprotective potentials. Anthocyanins, catechins and carotenoids act as scavengers of active oxygen species in the biological system. Evidence based insights have revealed inverse association between catechin intake
and ischemic heart diseases. Moreover, dates contain ample amounts of dietary fiber and potassium, both of which are heart and liver friendly, imparting positive effects (Devika and Prince, 2008). Similarly, date fruit extract inhibits lipid peroxidation, scavenges superoxide and hydroxyl radicals, thereby reducing the oxidative stress (Mukherjee et al., 2001). LDL oxidation plays a key role in the initiation of atherosclerotic lesions. Flavonol fractions from the date fruit effectively control LDL oxidation as compared to the phenolic acid fractions. Similarly, these fractions also stimulate the cholesterol removal from the macrophages, thus demonstrating strong anti-atherogenic potentialities (Borochov-Neori et al., 2015). The methanolic, ethyl acetate and water extracts of Ajwa dates significantly inhibit lipid peroxidation and cyclooxygenase enzyme activities i.e. COX-1 and COX-2 (Zhang et al., 2013). The prophylactic role of date fruit extracts against dimethoate-induced liver damage reveals the ability of its aqueous extract to inhibit the lipid peroxidation and also improves the glutathione peroxidase, superoxide dismutase and catalase levels. Positive histopathological changes are also recorded (Saafi et al., 2011). The date fruit extracts attenuate chemically induced hepatic damage by lowering the liver TBARS (thiobarbituric acid reactive substances) content while restoring the activity of hepatic enzymes and DNA fragmentation (El Arem et al., 2014a).

Considering the above-mentioned facts, the present research work was designed to characterize three locally grown date varieties i.e. Dhaki, Aseel and Zahidi with special reference to their phenolic profile and antioxidant activity. The quantification of bioactives present was also carried out using HPLC. Alongside, the development of a date based product (halwa) using all the cultivars and comparing them with the traditionally consumed halwa was another feature of core interest. Besides, restorative potential assessment of date fruit against cardiac and hepatic stress through bioefficacy trials is the limelight of this study. The objectives of this research are:

- Compositional profiling and phytochemical characterization of three locally grown dates; Dhaki, Aseel and Zahidi
- Designer date based halwa evaluation via physicochemical and sensory indicators
- Bioassessment of selected date variety to attenuate diet induced cardiac and hepatic oxidative stress using Sprague Dawley rats
CHAPTER 2

REVIEW OF LITERATURE

Since antiquity, plants having significant therapeutic potential, have played a crucial role in the global health and fathered the evolution of contemporary medicine (Ramzan and Li, 2015). Thus, the current dietary interventions are focusing on the consumption of natural foods containing vista of health enhancing bioactive ingredients for disease prevention and health management. In this milieu, date fruit contains an optimal blend of nutrients along with an array of nutraceutical moieties. The fruit is consumed as such and its addition to dishes makes them more appealing and nourishing. It is an instant source of energy, thus finds applications in children meals. Being rich in nutraceutics, the fruit and its extract can be employed to modulate certain physiological threats. Several lines of evidences suggest its prophylactic role in cardiac, hepatic and nephrotic malfunctions. This research was designed to probe the restorative ability of dates against oxidative stress mediated diseases. The literature relating to various facets of the present research has been reviewed under the following titles:

2.1. Nutrition and health
2.2. Date Fruit-an overview
2.3. Compositional profiling of date fruit
2.4. Date preservation and novel product formulations
   2.4.1. Date based liquid products
   2.4.2. Date based confections
2.5. Phytochemistry of date fruit
   2.5.1. Extraction of date fruit phytoceutics
   2.5.2. Phytochemical fingerprinting of date fruit
   2.5.3. In vitro antioxidant properties
2.6. Restorative potential of date fruit
   2.6.1. Cardioprotective aspects of date fruit
   2.6.2. Hepatoprotective ability of date fruit
   2.6.3. Renoprotective potential of date fruit
2.1. Nutrition and health

Drastic socio-economic demographic transitional developments accompanied by increased urbanization, globalization and Westernization have transformed the living patterns and dietary practices of Asian populaces over the past few decades (Pingali, 2007; Brandtzaeg et al., 2009). Various epidemiological investigations reveal increasing incidence of chronic diseases such as hyperglycemia, obesity, hyperlipidemia, hypertension, cardiovascular disorders, stroke, osteoporosis and certain malignancies (Bingham and Riboli, 2004). These are attributed to rapid adaptations in lifestyles especially regarding food consumption patterns (Hu, 2002). The dietary diversification from a traditional Asian diet comprising of whole cereals and low fat plant based products to a more contemporary western diet, having more processed and refined products and increased consumption of hyper caloric fat and sugar based snacks and confections, respectively are becoming the leading cause of numerous physiological threats (Pingali, 2007; Reardon et al., 2014).

Nutrition plays an imperative role in the primary and secondary prophylaxis of non-communicable diseases (NCDs). These ailments are generally of long term nature characterized by slow progression. More often, these NCDs are termed as degenerative diseases as they affect the structure and function of tissue gradually leading to deterioration. The reasons lying behind are mostly lifestyle preferences such as deskbound and robotic living, lack of exercise and primarily poor eating and drinking habits. Stress, anxiety along with under nutrition over a long period results in toxic overload in mitochondrial decay or aging. As a consequence several other degenerative non-communicable disparities such as brain dysfunction, immune system decline, cataracts, cardiac complications including CVDs specially stroke and atherosclerosis that are the root cause of disability and death take place (Olaiya et al., 2016). In this vista, fruits and vegetable consumption is an essential recommendation to prevent the incidence and prevalence of NCDs. Fruit and vegetable consumption is becoming an integral part of dietary interventions. The World Health Organization (WHO) has recommended a daily intake of 400-500 g fruits and vegetables in context of reducing the risk of morbidity and mortality resulting from cardiovascular and oncogenic events (WHO, 2003; Biesalski et al., 2009; Pysz et al., 2016). Scientific evidences also suggest a daily intake of 3-5 servings of fruits and vegetables that provides the body with numerous non-nutrient bioactive components that are helpful in mitigating the threats of various non-communicable diseases (Slavin and Lloyd, 2012). Moreover, the American Dietary Guidelines 2010 also endorse the consumption of food...
plate having one half portion from fruits and vegetables (USDHHS, 2010). Fruits are an essential part of routine diet. Besides, they are also consumed in religious and traditional practices in different parts of the globe. Numerous studies also indicate strong potential of fruits in disease prevention as compared to the vegetables owing to the presence of several essential vitamins, minerals and phytoceutical compounds (Habauzit and Morand, 2012; Chang et al., 2016).

The phytoceutics employed in therapeutic interventions are termed as phytotherapies and are an integral part of herbal pharmacology. However, no clear border exists between food and phytotherapies since most foods comprise of several phytochemicals. In the western world, the emphasis and application of natural phytotherapies is growing at a phenomenal rate owing to their harmless nature, posing no adverse effects. Phytopharmacology is the study of the function and mode of action of phytoremedial agents in the physiologic systems and the pharmacokinetics of such compounds with scientific principles to comprehend the underlying nature of their possible clinical applications. The biologically active components can act on multiple pharmacologic targets. The research techniques that authenticate their bio efficacious nature involve in vitro studies at the tissue or cellular level or to more complex in vivo animal studies to assess their preclinical and pharmacokinetic properties (Ramzan and Li, 2015).

Nevertheless, these therapeutic plants have been employed in the Arab-Islamic medicine since time immemorial. Islamic teachings also stressed on the consumption of God gifted natural fruits, vegetables and herbs for the prevention as well mitigation of diseases. Prophet Muhammad (PBUH) revealed the benefits of natural foods and their therapeutic applications in numerous ahadith more than fourteen hundred years ago. Various religious intellectuals complied them in a book and then titled as “Tib-e-Nabvi” (the Prophet’s Cure/medicine). These concepts are the backbone of today’s functional food approach. In His saying, He said that Allah has sent both disease and its cure and that there is no disease created by Allah without cure except aging. These ahadith (sayings of the Prophet) encouraged the early Muslims to seek remedies from food, hence initiated the Arab-Islamic Medicine. Muslims of that era utilized numerous animal and plant products mentioned in the Quran and ahadith such as dates, olives, honey, black cumin, camel milk, etc. that are mentioned in detail in the Tib-e-Nabvi. Then, these remedies spread to other regions of the world and founded the Greco-Arab and Islamic medicine that laid the foundations of modern medicine. Dates are mentioned at about 20 places in the Holy Quran. The
importance lies in the fact that the Prophet advised to eat dates to break the fast (Ramzan and Li, 2015).

2.2. Date fruit – an overview

Date fruit has been categorized as an emerging healthful food on account of its diverse functionality and nutritious nature (Sirisena et al., 2015). Date palm is usually called ‘the tree of life’ and ‘the King of Oasis’, botanically known as Phoenix Dactylifera L. and belongs to the Arecaceae family. The genus contains 12 species amongst which dactylifera is of prime importance owing to its commercial value and food applications. Date palm is dioecious plant, having separate male and female cultivars (Zaid and Arias-Jimenez 2002). It grows in a wide geographical range but certain conditions are mandatory for proper fruit bearing (Burt, 2005). Dates are considered as a subsistence crop and imperative nutritional source especially in arid regions of the globe, where only few plants can grow due to climatic extremities. The global date fruit production accounted to almost 7 million in 2012 with an overall market value more than 1 billion USD. Seventy five percent total date production is from the top six countries including Pakistan. Pakistan is the sixth largest producer of date fruit with production of 600,000 tons annually as reported in 2012 (FAO, 2014). Date fruit is an essential component of the Muslim diet owing to religious significance and is mentioned at numerous places in the Holy Quran. During the month of Ramzan, date fruits are customarily consumed to break the day long fast (Baliga et al., 2011).

The monocotyledon date palms are cultivated all over the globe, especially in the Southern Europe, Middle East, North Africa, in some parts of Central and North America and the Indo-Pak region (Al-Shahib and Marshall, 2003). Date fruit is oval shaped having single stone grooved down one side. The fruit varies in color, texture and sensory properties depending upon the cultivars. More than 600 varieties of dates have been reported to date that have been developed by years of seedling selection and merely those possessing desirable traits have been propagated. The varieties are greatly influenced by the environmental conditions, geographical location and maturation process. Depending upon the sugars and moisture content, dates are categorized as dry with 8-10% moisture content, semi-dry having 13-15% and soft 18 to 24% moisture content (Biglari et al., 2008). In dry dates, 20-40% dry matter is invert sugars and 40% is sucrose, whilst in semi dry 40% of dry matter is sucrose and 40% is invert sugars. However, in case of soft dates invert sugars are up to 80% (Bender, 2009). Often cultivars possessing similar morphological
characteristics possess same varietal name with regional differentiation as Aseel and Aseel Sindh. The main varieties cultivated around the globe are Ajwa, Al-Barakah, Barhe, Aabel, Barakwi, Deglet Noor, Fard, Hallawi, Khlas, Khadrawy, Rotab, Saidi and Zahidi (Fadel et al., 2006; Chaira et al., 2009; Baliga et al., 2011).

Pakistan is an ideal date growing region due to its soil quality and climatic conditions. Dates are commercially grown in Sukh and Khairpur District in Sindh and Makran, Kech, Turbat, Punjgur and Kalat in Baluchistan. In the Khyber Pakhtunkhwa dates are being cultivated in the Bannu and Dera Ismail Khan regions. While, Multan, Dera Ghazi Khan, Muzaffarghar and Jhang are the prominent date growing areas in the Southern Punjab (Abul-Soad et al., 2015). However, Sindh and Baluchistan are the main regions contributing to about 90% of total date production in the country, followed by Southern Punjab. The important cultivars grown in Pakistan are Dhaki, Zahidi, Aseel, Aseel Sindh, Hillawi, Beghum Jhangi, Karblain, Karbalain Sindh, Rabbai and Dora (Nadeem et al., 2011). The reported date production is about 725,000 metric tons at an area of 97,300 hectares. Dates are the third commercial fruit crop after mango and citrus according to Pakistan Horticulture Development and Export Board (Jatoi et al., 2009; Fatima et al., 2016).

Date fruits are matured by passing through distinct developmental stages involving physical and chemical transformations to attain desired maturity stage. All the stages play an imperative role in date marketing. The sweetness, color and texture are indicators of fruit maturity and ripeness (Al-Farsi and Lee, 2011). The five date developmental stages are named in Arabic as Hababouk, Kimri, Khalal, Rutab and Tamr. The ‘Hababouk’ stage continues for 4-5 weeks, these dates are usually immature, pea sized and covered by a calyx weighing about a gram. This stage is followed by ‘Kimri’ and the longest developmental stage that lasts for about nine to fourteen weeks. In this stage, the fruit changes from small berry to oblong shape, bitter and unsuitable for eating due to its hard texture, low sugar content (50%) with high moisture content and acidity. In the next stage called ‘Khalal’, the fruit weight and moisture content decrease with an increase sugar content increases and there is a visible transition in the color of the fruit from greenish to yellowish or reddish depending upon the variety. This phase normally extends for about six weeks. By the end of this stage, the fruit attains maximum size and weight and is physically mature and hard in texture. The moisture content decreases with an increase in sugar content during this stage. This is followed by soft ripe ‘Rutab’ stage that lasts between two to four weeks. The
fruit texture becomes soft and the apex starts to ripen. The astringency is lost gradually and color darkens. The moisture content decreases resulting in weight loss, while the sugar content and rate of sugar conversion to simpler forms increases. Tamr stage is the final full ripe stage in which the dates are firm in texture having dark color and less moisture content as compared to the preceding stages. At this point, dates contain higher sugars and proteins. Moreover, fruit weight, moisture and lipids also reduce within this stage. In most fruit cultivars, the outer skin sticks to the inner flesh and wrinkles appear due to inner flesh shrinkage (Baliga et al., 2011; Sahar et al., 2013). Mostly, date fruits are consumed at the ‘Rutab’ semi ripe stage and/or ‘Tamr fully ripe stage’ with minute or merely no processing (Chandrasekaran and Bahkali, 2013).

2.3. Compositional profiling of date fruit

Date fruit is a berry comprising of seed and fleshy pericarp i.e. about 85 to 90% of total fruit weight. It is among the rare plants of which all parts such as flesh, pit, leaf, etc. can benefit the food chain if processed suitably. The water extracted from the spathe of date palm known as “Tarooneh” is widely employed in Persian traditional medication. It contains numerous volatile compounds and essential oil, and is also used as a tranquilizer (Pourdarbani et al., 2012; Hamedi et al., 2013; Mohamadi et al., 2014). Similarly, the date pit also possesses several edible benefits; the roasted pits are often used as an alternative to the caffeine free coffee. The seed oil contains tocopherols, sterols and essential fatty acids that can be employed as a source of edible oil. The investigations based on its antioxidant properties are also opening new horizons for researchers to seek evidences regarding the disease preventing ability of date fruit. Moreover, being a promising source of dietary fiber, fatty acids and minerals it is also extensively used in animal feeds (Alsaif et al., 2007; Nehdi et al., 2010; Al-Farsi and Lee, 2011; Abdul Afiq et al., 2013). Likewise, other parts of the date palm such as leaves and branches are used in artifact industries and paper mills. Hence, the mechanizational and post-harvest operations regarding date fruit require a sound knowledge of its physical characteristics. The sorting and grading is also based on the shape and size of the fruit. In the same way, the packaging and handling also demands proper know-how of the fruit dimensions.

The physicochemical traits of the date fruit are entirely dependent on the type of cultivar, agronomic practices and ripening stage. These characteristics vary from variety to variety and even the variations exist amongst the fruits of the same variety. The date fruit weight of seven Saudi cultivars ranges between 4.99 to 7.95 g (Al Juhaimi et al., 2014). Similarly,
maximum fruit weight \textit{i.e.} 12.78 g is observed in Sudanese date variety (Black Gau) with 5.1 cm length and 1.82 diameter \textit{i.e.} maximum amongst all the selected varieties. However, the highest pit percentage (15\%) is recorded in Barakawi weighing about 7.90 g and the least diameter 1.38 cm is also reported for Barakawi (Sulieman, 2012).

Chemically, date fruit contains carbohydrates and dietary fiber as main constituents, whereas proteins and fats are also present, it encompasses certain vitamins and minerals required for optimal physiologic functioning. Like the physical traits, the fruit chemistry also differs according to the cultivar, growing region, ripening stage, environmental conditions and postharvest practices (Manickavasagan \textit{et al.}, 2012; Tang \textit{et al.}, 2013). Usually moisture content ranges from 12-40\% depending upon the type of date variety. The moisture content signifies the estimated shelf stability of the fruit as well as its keeping and eating quality. The textural parameters such as chewiness, hardness and resilience also rise exponentially with the decreasing water content. Dates having more moisture are often dried and dehydrated to increase their shelf stability (Rahman and Al-Farsi, 2005). As the composition varies within the varieties and substantial regional variations are also present. Table 2.1 explains the region-wise compositional profile of date fruit.

Amongst the sugars, glucose, fructose and sucrose are the predominant. However, their content also depends upon the variety and ripening stage. Evidences suggest lower quantity of glucose and fructose at ‘Khalal’ stage and higher at the ‘Tamr’ stage. In eight Pakistani date palm cultivars, the glucose and fructose content varies from 25.7-31.66\% and 22.48-30.58\% respectively at the ‘Tamr’ stage (Haider \textit{et al.}, 2014). Though, sugar content is higher in dates, yet the glycemic index (GI) is lower than apples, apricots and bananas (Jenkins \textit{et al.}, 2002; Miller \textit{et al.}, 2003). Date fruit cultivars are low in lipid contents containing a relatively high percentage of unsaturated fatty acids. About eighty volatile components are identified in the studied Tunisian varieties, amongst, 34 components were new and specific for Tunisian date fruits (Amira \textit{et al.}, 2011). About 23 amino acids have been reported in the date fruit that vary with maturation stage. High content of aspartic acid, glutamic acid, leucine, lysine, serine and alanine are found at the Kimri stage, whilst aspartic acid, glutamic acid, leucine, lysine, proline and glycine are most abundant during the ripening stages (Ishurd \textit{et al.}, 2004).
Table 2.1. Region-wise compositional profiling of date fruit

<table>
<thead>
<tr>
<th>Region</th>
<th>Moisture (%)</th>
<th>Carbohydrates (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Fiber (%)</th>
<th>Ash (%)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Sudan</td>
<td>8.78-10.68%</td>
<td>78.72-80.41%</td>
<td>3.69-4.09</td>
<td>1.71-2.00</td>
<td>2.37-3.14</td>
<td>1.96-2.50</td>
<td>(Mohamed et al., 2014)</td>
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<td></td>
<td>12.6-18.5</td>
<td>77.13-83.41</td>
<td>1.47-1.68</td>
<td>0.52-1.41</td>
<td>6.26-8.0</td>
<td>1.49-1.79</td>
<td>(Al-Farsi et al., 2005b)</td>
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<td></td>
<td>18.77-23.71</td>
<td>-</td>
<td>1.28-1.89</td>
<td>1.24-2.37</td>
<td>1.66-2.38</td>
<td>1.12-1.55</td>
<td>(Ali et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>15.00-21.00</td>
<td>74.5-82.4</td>
<td>1.8-3.8</td>
<td>0.1-0.7</td>
<td>1.0-2.5</td>
<td>1.0-2.0</td>
<td>Al-Harrasi et al., (2014)</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>1.97-4.82</td>
<td>-</td>
<td>1.51-2.41</td>
<td>-</td>
<td>1.91-3.90</td>
<td>-</td>
<td>(Al Juhaimi et al., 2014)</td>
</tr>
<tr>
<td>UAE</td>
<td>13.34-20.10</td>
<td>67.33-75.30</td>
<td>1.89-2.73</td>
<td>0.12-0.22</td>
<td>5.52-9.11</td>
<td>1.45-1.89</td>
<td>(Habib and Ibrahim, 2011)</td>
</tr>
<tr>
<td>Tunisia</td>
<td>-</td>
<td>79.93-88.02</td>
<td>0.66-2.85</td>
<td>0.06-0.57</td>
<td>8.09-20.25</td>
<td>1.73-2.59</td>
<td>(Borchani et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>79.1</td>
<td>2.10</td>
<td>-</td>
<td>14.4</td>
<td>2.50</td>
<td>(Elleuch et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>14.30-17.45</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.06-7.87</td>
<td>(Khan et al., 2008)</td>
</tr>
<tr>
<td>Pakistan</td>
<td>9.90-14.81</td>
<td>-</td>
<td>2.1-2.7</td>
<td>0.2-0.4</td>
<td>-</td>
<td>1.4-1.9</td>
<td>(Anjum et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>6.80-31.90</td>
<td>-</td>
<td>1.9-3.24</td>
<td>0.1-0.46</td>
<td>-</td>
<td>2.00-3.46</td>
<td>(Hasnaoui et al., 2011)</td>
</tr>
<tr>
<td>Morocco</td>
<td>13.80</td>
<td>73.00</td>
<td>3.00</td>
<td>2.90</td>
<td>5.20</td>
<td>2.13</td>
<td>(El-Sohaimy and Hafez, 2010)</td>
</tr>
</tbody>
</table>
Another nutritious quality of dates is being very low in fat, an ideal diet for patients suffering from dyslipidemia, atherosclerosis and other cardiovascular disorders. Amongst the fatty acids, lauric, myristic, oleic and palmitic acid are dominant in the date fruit (Habib et al., 2013; Tang et al., 2013). Dates are a promising source of soluble and insoluble dietary fiber ranging from 6.4 to 11.5% in different cultivars (Al-Shahib and Marshall, 2003). The high fiber content of dates makes them easier to digest alongside regulating the bowel movements and aid constipation thus, can be used as a natural laxative. The fruit can be utilized in the development of energy dense bars due to the presence of sugars providing the body an instant source of energy (El-Sohaimy and Hafez, 2010). Evidences suggest that the total fiber concentration decreases during the ripening as the fruit becomes soft at the final stage. It is presumed that enzymes gradually breakdown the polysaccharides during the ripening process thus increasing the fruit softness thereby decreasing the fiber content (Mrabet et al., 2012). Mineral composition of seven commercially available date varieties in Pakistan, namely, Basra, Aseel, Begun, Mazoe, Zahidi, Rabee and Janshoor was elucidated. The varieties proved to be a rich source of potassium about 403-632 mg/100 g and calcium 27.41-81.47 mg/100 g. Micronutrients such as iron, zinc, nickel and copper are also present ranging from 1.16-2.19 mg per 100 g, 0.55-1.17 mg per 100 g, 0.45-0.98 mg per 100 g and 0.10-0.20 mg per 100 g respectively. Moreover, gradual increase in potassium and copper content is also found with maturation (Khan et al., 2008). Date fruit also possess certain aromatic components responsible for the characteristic fresh date aroma. Amira et al. (2011) detected 80 volatile constituents that account for 90.70 to 99.60% of total aroma comprising of 20 esters, 19 alcohols, 13 aldehydes, 12 hydrocarbons, 10 terpenes, 6 ketones and 1 lactone. However, these compounds differ during the maturation stages, decrease from Besser to Tamr stages, and are area specific as well.

Significant quantity of vitamin C ranging from 97.18 mg/100g to 145.31 mg/100g was reported in another scientific work suggesting date fruit as a good source of vitamin C (Al Juhaimi et al., 2014). Vitamin C is considered as an antioxidant that substantially decreases the adverse effects of the reactive oxygen and nitrogen species. It is also essential for the carnitine, collagen and neurotransmitters biosynthesis (Naidu, 2003). Ascorbic acid enhances iron absorption needed for buoyant health, endurance and vitality. It improves wound healing and prevents against stress (Bakhru, 2005). Besides, date fruits contain a variety of B group vitamins predominantly, thiamine (B1), riboflavine (B2), nicotinic acid (B3), pantothenic acid (B5), pyridoxine (B6), folate (B9) and fat soluble vitamins K and A.
Nevertheless, the content of these vitamins is low, still some vitamins $B_3$, $B_5$, $B_6$ and $B_9$ are higher in concentration as compared to oranges, apples and berries (Siddiq and Greiby, 2013). These vitamins are essential for maintenance of a healthy body performing and aiding in numerous metabolic functions. Perform significant role in maintain blood glucose levels and metabolize carbohydrates and fatty acids. They also aid in the hemoglobin, red and white blood cell synthesis process. Dates also contain considerable quantity of minerals such as magnesium, calcium and phosphorous that are essential for healthy bone development and energy metabolism. Phosphorous also helps in proper muscle functioning. Considerable quantity of iron is present in the fruit i.e. essential for the production of red blood cells that carry the nutrients to the cells throughout the body. Dates are rich source of potassium required for healthy functioning of nervous system. Potassium is also important for proper functioning of heart and maintains a normal heartbeat. It is also helpful in reducing the blood pressure and blood detoxification through kidneys (El-Sohaimy and Hafez, 2010). Dates contain 2.5 times more potassium than bananas (Siddiq and Greiby, 2013). Selenium is also present in the date fruit and helps in cell growth and repair, maintaining youthful elasticity in tissues. Studies also suggest the cancer preventive role of selenium (Bakhru, 2005; El-Sohaimy and Hafez, 2010).

Consumption of 100 g dates can provide more than fifteen percent of the daily RDA to adequate intakes of magnesium, potassium, selenium and copper (Al-Farsi and Lee, 2008).

2.4. Date preservation and novel product formulations

The remarkable nutritional profile of the date fruit makes it highly suitable food that can be eaten fresh or can be utilized in the development of value added products. The massive production of the fruit in the country also requires methods for value addition and preservation to avoid wastage and spoilage losses. Food preservation is an ideal contribution to food security and food safety in societies with limited access to external food sources. However, it is also an imperative way to produce novel value added products. The raw materials are quite often preserved by heating, drying, pickling, salting, smoking, curing and/or fermentation. Sustainable elimination of pathogenic microbes, sporulation and growth of zoonotic and foodborne microorganisms is essential to enhance food safety and ensure food security by reducing the post-harvest losses and food waste (Jans et al., 2016). Other than the quantitative losses, certain qualitative losses also exist that are difficult to assess. The losses majorly focus on the reduction of product acceptability by the consumers. May be a consumer finds a raw fruit unacceptable due to taste preferences,
however, the same can be acceptable in processed form. Thus, higher variety of products as per the consumer demand are required to reduce both the qualitative as well as quantitative losses. Hence, strategic approach involving the improvement in handling systems and the infrastructure and encouraging vertical integration among the producers and marketers is required (Kader, 2004). Besides, in this competitive world, food companies always seek new products for profitability and survival. The development of newfangled products or transforming the old into new ones is essential for continued growth and development of a food company (Fuller, 2016). In this context, the processing of date fruit is no exception and is required both for preservation and product development perspectives. It can also devise new ways and date based valued products for the industries.

Dates are relished in fresh form by majority of the consumers, nevertheless, new date based products are also seen on grocery shelves. Dates having varied amount of moisture and sugar content demands certain preservation conditions for a longer shelf life. The fruit is prone to deterioration through various processes such as fermentation, darkening due to browning, molding and rancidity. Deterioration is triggered with increased moisture content; thus, the preservation techniques are often aimed at reducing the free water. Since, dry and semi dry dates have more shelf life as compared to soft ones. High temperature and high moisture contents result in enzymatic as well as non-enzymatic browning. To preserve the raw dates, cold storage, drying and fumigation techniques are applied. Irradiation is also practiced in certain areas but is limited. Dates are also pasteurized at 66 ºC for about 30 minutes to ensure microbial inactivation. The dates are also coated with edible oil to smoothen the surface and increase the glossiness. Moreover, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are also used for smooth and glittering surface. The surface stickiness of dates is removed by dipping the fruit into starch or methylcellulose based solution. Cold storage conditions are recommended for longer shelf stability (Barreveld, 1993).

The processed date based products are also gaining importance globally. The simplest processing involves fruit grading and sorting, followed by washing and drying and then packing. Grading of dates is based on the quality profile of the fruit that encompass four attributes (a) physical traits such as uniformity in size, color, shape, texture, taste and flesh/pit ratio (b) chemical composition particularly moisture, sugar and fiber content (c) any physical defects, discoloration, blemishes, broken skin, deformity etc. (d) foreign matter, insect or mold infestation etc. The A grade dates are the table dates that are primarily
used for eating purposes. Though, the B, C and D grade dates are employed for value addition purposes. In Pakistan, dates are classified into extra class, select-A, select-B, good average quality, fair average quality and industrial grade (Siddiq and Greiby, 2013).

Adding value to the raw dates may involve simple removal of the cap, pitting and packaging to more advanced date based value added products. In some countries, the pit is removed and almonds are added; chocolate or sesame seeds coated dates are also popular among the consumers. These dates are packed in an appealing way and are also used for gifting purposes, especially among Muslim community as a souvenir upon arrival from the Holy places (Kamal-Eldin et al., 2012). The other two grades of dates are mostly used for the preparation of pulp, dices, syrups and other such products. The optimal balance of nutrients in date fruit makes it suitable for incorporating into confections and breakfast cereals. Higher sugar content in the pastes is sometimes used as natural sweeteners, binding and filling agents in baked confectionaries. Date fruit based halwa is also prepared in the subcontinent especially in winters. Moreover, fruit bars are prepared as instant source of energy using dates as major ingredient. Date milkshake is relished during the Muslim fasting month. Several thermal and non-thermal processes are in vogue for preserving the fruit quality and easy acceptability of the consumers (Ashraf and Hamidi-Esfahani, 2011; Aleid, 2014; Homayouni et al., 2015). The products that are developed using the date fruit can be divided into two categories viz. liquid products including juices and syrups and solid date based confections containing diced dates, date paste, minced dates etc. (Aleid, 2014). Some products are discussed below:

2.4.1. Date based liquid products

The low quality or the industrial grade dates are used for the production of date concentrates and syrups. Fruit being rich in sugars, contributes to the physical appearance and hygroscopy of the syrups. These syrups can be used as sugar replacers in foods where color is not the critical factor. For the preparation of syrup, dates are mixed with water in a suitable ratio at about 50-60 °C for 60 minutes or more. Sonication can also be applied for heating under optimal conditions for better extraction and microbial inactivation (Entezari et al., 2004). Date juice is developed in Gujrat-India using immature fruits of less commercial value to prevent spoilage due to monsoon rains. Crushed dates are treated enzymatically using pectinase @ 0.1% for 2 hours. The juice obtained contains 16.1% reducing sugars and 18.3% total sugars. The juice is then pasteurized, cooled and clarified. Brix is maintained at 76° by concentrating the juice (Kulkarni et al., 2010). Earlier, Al-
Farsi (2003) worked on the utilization of low quality dates to produce a high quality date syrup using a modified clarification procedure. Five types of clarification processes were employed out of which the filtration and activated carbon filtration were found to be the successful methods. Highest syrup purity *i.e.* 97.7% was reported in the filtered treatment along with ash and color reduction. However, the activated carbon filtration results in decreased color and total ash with 92.2% purity. Liming increases the ash and color matter, thus, seems to be an unsuitable method. Nevertheless, lower phenolic content was reported in date syrups as compared to fruit and flesh (Al-Farsi *et al.*, 2007). Date fruits are also used to produce high fructose date syrup that is preferred owing to its sweeter taste and different absorption mechanism as compared to glucose. The chromatographic separation of fructose is an accessible approach that depends on several parameters as the characteristics of the mixture and resin and type of separation *i.e.* ion exchange or ligand (Mostafazadeh *et al.*, 2011).

Dates are readily fermented under optimum conditions due to the presence of sugars. The fermentation process is used for the development of date wine and vinegar since primeval times. Different extraction methods are employed to extract juice from dates ranging from simple hot water extraction to detailed enzyme extraction technologies. It was found that the wine fermented from heating dates with water (1:5) for 5 hours appears brown is color, having mellow date aroma but slightly bitter taste. However, pectinase extracted date juice (50 °C for 4 hours and 0.2% pectinase) fermented wine has the same color and aroma is that of date fruit with better hedonic response then the hot water extracted one (Peng-bao *et al.*, 2011). In a previous study it was reported that less extraction and fermentation time is achieved through microwave assisted extraction with higher ethyl acetate content and special aroma as compared to the hot water and pectinase extraction methods (Zheng and Lin, 2006). The effect of heating date syrup was studied by Abbes *et al.* (2013). The authors stated that heating the syrup at temperature up to 100 °C could improve the type of antioxidants subsequently increasing the antioxidant activity. Heating the syrup leads to the formation of Maillard reaction and caramelization products such as 5-Hydroxymethyl-2-furfuraldehyde that possess significant antioxidant ability. Thus, date syrup can also be used for value addition in food industry for products to enrich them with natural and health promoting antioxidants.
2.4.2 Date based confections

Development of date paste is an ideal and convenient way to preserve the fruit goodness that offers flavorful choices of paste addition into products. The developed paste is suitable for use in bakery and confectionary products (Barreveld, 1993). Moreover, the paste also gains importance for the food industry since it is easy to handle and also reduces the transportation and storage costs (Hui, 2006). The paste is produced by steaming the washed, pitted dates for 3 minutes at 69 KPa or soaking in hot water (95 ºC) for 5 to 15 seconds. Afterwards, the dates are minced to form date paste. Ascorbic acid and citric acid are added to prevent color change and to maintain the desirable pH of the end product (Shi et al., 2005). The physicochemical properties including total solids, sugar profile, water activity, color, texture, glass transition and melting point of three commercial date cultivars based date paste from UAE were investigated. No considerable differences were found regarding the TSS, sugars, water activity and glass transition temperature. The glass transition temperature indicates the water mobility in the foods and an important tool to assess shelf life that ranges from 30.67 and 33.39 ºC. However, texture profile and melting point vary among the cultivars. The rheology of paste reflects the viscoelastic nature of all pastes (Ahmed and Ramaswamy, 2006). Date paste is also prepared using hot water in 1:1 ratio for 3 minutes. After manual removal of seeds, the date flesh mixture is crushed to a homogenous paste. The blanching water is filtered from the paste and the product is pasteurized at 80 ºC. The analyses of the paste reveals the presence of dietary fiber, antioxidants and nutraceuticals. The authors conclude that the presence of sugars, particularly reducing sugars, and neutral pH of the paste makes it suitable to be used in bakery, confectionary and dairy industry (Trigueros and Sendra, 2014). In another study, second grade Deglet Nour dates were used for paste development and its subsequent use in the manufacturing of date jelly. Fully ripened dates (tamr stage) were washed and dried at 45 ºC for 12 hours. Afterwards, the pulp was milled to obtain paste which was then further used (Masmoudi et al., 2010).

Date paste is an ideal ingredient whose addition can make the product more nutritious and appealing. Date paste is incorporated in bread formulations about 4-8% that results in considerable improvements in the rheological properties of the date-based bread. It delays gelatinization and improves gas production and retention capacity. Moreover, date paste addition improves the overall texture and crumb and crust characteristics. It also prolongs the shelf stability and delays the staling process (Yousif et al., 1991). In a more recent
study, date pit fortified pan bread was prepared using 5-10% levels. The addition increases the water absorption, dough development time, dough stability and softness of the product (Halaby, 2014).

Date paste supplementation in cookies results in 20% higher spread ratio, while sugar crystallization is prevented during the cool off period. Date pulp with 65° and 73° Brix is also used for the development of date jam and jelly, respectively (Shi et al., 2005). Similar to the peanut butter, date butter has also been developed using fully ripe dates with high sugar content (Hui, 2006). Nutritional quality of biscuits supplemented with date fruits and wheat bran was studied by El-Sharnouby (2012). Wheat flour was partially replaced with wheat bran and date fruit powder (1:1) @ 10, 20, 30 and 40% levels. Results indicated increased water absorption with decrease in mixing tolerance index and dough stability that might be attributed to the addition of wheat bran. However, the mineral contents increased by increasing the powder levels while the spread of biscuits decreased from 55.66 to 52.82 mm by the addition of wheat bran and date fruit mixture. The sensory qualities of biscuits proved to be acceptable to the consumer.

Likewise, Elleuch et al. (2014) reported that the addition of date fiber concentrate and sesame seed coats significantly improve the nutritional and sensory quality of date halwa. Chopped dates, paste and syrup was added to traditional Indian dish called ‘idli’ to replace sugar. The results show higher vitamin C and polyphenolic content in the date containing ‘idli’ as compared to the traditional one (sugar containing). Date based treatments attain better sensory scores or at least equal with the control. Consequently, the dates can also be added with the aim of reducing the sweet white poison, sugar (Manickavasagan et al., 2013).

Date candies and bars are among the popular date based products prepared using paste along with nuts and often coated with chocolate having good sensory profile. For bars, skim milk powder and oat flakes are used for high energy products. Ice creams, puddings, jams and jellies have been developed using date fruit (Awan and Sohail, 1999; Besbes et al., 2009). In this arena, Nadeem et al. (2012) designed a nourishing date bar for children especially the school going ones to fulfil their nutritional requirements. Whey protein was added as a protein source. Date fruit containing functional yogurt was also prepared previously (Gad et al., 2010). However, still commercial scale processing and technological applications of the date fruits are limited and new possibilities need to be explored. The processing is not on the scale similar to other tropical fruits especially in Pakistan.
2.5. Phytochemistry of date fruit

According to Quiñones et al. (2013) phytoceutical moieties are secondary metabolites that are present in varied amounts and types in the plant foods and are recognized for their disease ameliorative roles. Evidence based insights reveal the salubrious properties of these phytoceuticals against several against several physiological threats like cardiovascular ailments, hepatotoxicity, renal-toxicity, hypertension, dyslipidemia and oncogenesis. The potential of these biomolecules to modulate the enzyme activity and interfere in various cellular pathways permits them to participate in numerous metabolic redox reactions. Antioxidants are the chemical entities present naturally in the biological systems that can delay, inhibit or prevent the substrate oxidation by their free radical scavenging mechanisms. However, in diseased conditions, the defensive mechanisms weaken leading to increased oxidative load on the physiological system. In such circumstances, external supply of antioxidants is necessary to counter veil the deleterious effects. Most disparities are a result of suboptimal living. The risk factors accountable for increased free radical generation and disease progression are primarily ascribed to poor lifestyle habits; deskbound and mechanized living, smoking, malnutritional practices, focusing particularly on the elevated intake of refined and empty caloric foods. Excessive cholesterol consumption is an additional modifiable risk factor that is concomitant with oxidative stress mediated complications in the body organs particularly affecting heart, liver and kidney (Santilli et al., 2015).

The researchers, medical practitioners and health care professionals are focusing on the active role of food in increasing the healthy life expectancy by modulating various physiological functions and preventing chronic maladies. Several epidemiological and clinical studies advocate an inverse association between diseases of affluence, such as cardiovascular ailments, hepatic disorders, hypercholesterolemia, diabetes and oncogenesis, and the consumption of fruits and vegetables. These commodities encompasses a myriad of phytoceuticals that pose positive effects on the human health and well-being. Various phytochemical moieties are abundant in the date fruit that are responsible for its antioxidant and disease ameliorating characteristics. Dates contain carotenoids, phenolics such as cinnamic acids, sinnapic, ferulic, vanillic, syringic, caffeic, chlorogenic, coumaric and protocatechuic acids and their derivatives. Dactilyferic acid, a derivative of hydroxycianmic acid, has also been identified (Mansouri et al., 2005; Borochov-Neori et al., 2013; Habib et al., 2014). Among the flavonoid glycosides
quercetin, luteolin, methyl quercetin and methyl luteolin are present. Studies also reveal the presence of catechins, epicatechins and anthocyanins (Al-Farsi et al., 2005a; Rock et al., 2009; Habib et al., 2014). Nevertheless, their extraction and quantification is of considerable importance, the content and nature of the phytochemicals and their subsequent antioxidant activity depends on the type of technique used.

2.5.1. Extraction of date fruit phytoceutics

Natural bioactives include a broad spectrum of structures and functionalities providing a tremendous molecular pool for the production of nutraceuticals, designer foods and natural food additives. These compounds vary in concentration in different commodities: often very low levels are present that require extensive harvesting to obtain sufficient quantities. In such cases, the structural complexity and diversity makes the chemical synthesis unprofitable. The intrinsic hurdles in quantifying and isolating these beneficial components have led to the development of cutting-edge extraction technologies. The commonly used extraction methods include the conventional solid-liquid or liquid-liquid extraction, while the advanced methods include supercritical and subcritical fluid extractions, pressurized liquid extraction, ultrasound- and microwave-assisted extraction techniques. The commercial application of these technologies can lead to the development of designer and personalized diets to cope with the increasing dilemma of physiological threats (Gil-Chávez et al., 2013).

The extraction procedure involves sequential and systematical release of the active components from their respective forms (Ignat et al., 2011). The extraction of phenolic compounds from a wide range of test samples is dependent on the nature of sample matrix and molecular structure of phenolic compounds present, number of hydroxyl and aromatic groups and polarity. For optimal extraction, the samples are minced or ground to small particle size for better efficient extraction. High fat samples also require prior defatting to avoid any hindrance during extraction. The yield of phenolics is influenced by such factors as extraction time and temperature, solvent type, solvent-to-sample ratio as well as the number of repeated extractions (Khoddami et al., 2013). Extraction efficiency is considered as function of the aforementioned process conditions.

Usually, solvent extraction methods are mostly employed for date extract preparation. The most frequently used solvents for date extraction are water, methanol, ethanol and acetone. Dates are mixed with specified ratio of solvent and are homogenized for few hours using
orbital shaker, sometimes overnight stay is also preferred for maximum extraction. The obtained extracts are then filtered or centrifuged followed by rotary evaporation to obtain the crude extract (Al-Qarawi et al., 2004; Al-Farsi et al., 2005a; Biglari et al., 2008; Anjum et al., 2012; Amira et al., 2012; Benmeddour et al., 2013; Al-Najada and Mohamed, 2014; Al-Asmari et al., 2017).

Herch et al. (2014) compared three extraction methods viz soxhlet, hexane and modified Bligh-Dyer methods for the extraction of date flesh and date seed oil. Maximum extraction yield is obtained through soxhlet extraction technique i.e. 7.11% followed by the modified Bligh-dryer method (5.70%) and hexane extraction technique (5.12%). Extraction time, temperature and solvent type have greater influence on the yield and efficiency of extracts. Kchaou et al. (2013) studied the effect of solvent system on the phenolic recovery from date extracts. The polarity of extraction solvent and compound solubility are amongst the crucial factors for maximum recovery. They used different solvents such as aqueous acetone, aqueous methanol, ethanol, acidified methanol (0.1% formic acid) and water. Amongst these, 70% acetone extract is considered as the most efficient solvent for maximum phenolic recovery, whilst lowest recovery of phenolics is obtained in the ethanolic extracts. Whereas, in case of antioxidant potential maximum activity is recorded in the methanolic extracts, while minimum in ethanolic ones. The methanolic extracts show maximum antioxidant activity in Pakistani date cultivars, while hexane proves to be less efficient (Anjum et al., 2012). However, Saleh (2011) stated that water extract contains considerably higher phenolic moieties particularly hydrophilic phenolics (catechin, rutin and caffeic acid) as compared to the alcoholic extracts. Previously, Biglari et al. (2008) declared methanol-water (4:1 v/v) as the most efficient solvent for highest yield of phytocutetics (phenolics and flavonoids). Earlier, Al-Farsi et al. (2005a) compared seven extraction solvents. The authors conclude that mostly the antioxidants present in date are hydrophilic in nature, therefore, aqueous methanol (50:50 v/v) yields highest recovery of polyphenolic constituents.

Supercritical fluid extraction (SFE) is a novel technique that is considered as an environmentally friendly alternative to the conventional solvent extraction. It is gaining global popularity owing to its high selectivity and diffusivity. Carbon dioxide is the most commonly used supercritical fluid with critical temperature 31ºC and pressure 73 atm. It is preferred because of its inexpensive, non-flammable, inert and nontoxic nature. This technology is not used for date fruit extraction mainly due to high sugar content of the fruit.
making it stickier. However, the oil extraction from date seeds can be carried out using supercritical fluid extraction technique. In this context, Aris et al. (2013) extracted date seed oil via SFE. The highest oil yield was obtained at 41.4 Mpa, 70 ºC and 24 mL/minute CO₂ flow rate for 40 minutes. The GC-MS component analysis of the obtained oil revealed the presence of lauric, myristic, elaidic, palmitic, capric and caprylic acids. Earlier, Liu et al. (2012) used SFE for the first time for the extraction of phenolics from date pits. The extraction was carried out at 350 bar pressure, 50 ºC for 2 hours with two repeated extractions. The phenolic content of the obtained extract was 441.57 mg GAE/100g F.W. Several individual phenolic acids such as rutin, chlorogenic acid, ellagic acid and quercetin were also detected in the date seed supercritical extract.

The emergence of nutraceuticals as a disease therapy has resulted in more focused extraction for specific moieties. This demand for a highly selective and sensitive polyphenols determination method. A number of spectroscopic methods have been developed based on the presence of structural groups on the phenolic compounds. Most of these are employed for overall quantification and few rely on ascertaining the individual components on the basis of their extinction coefficients such as anthocyanins, betalains, etc. However, screening and quantification of individual polyphenols require methods that are more advanced. In this regard, HPLC is the preferred, dominating and widely used technique. The extraction for HPLC quantification for polyphenol determination also varies with respect to the type of moieties to be analyzed and system used. For instance, the simple polyphenols including the monomeric catechins, hydroxycinnamic acid, flavonoids and procyanidins are extracted from the dates using acidified methanol. Acidification is usually carried out by the addition of acetic acid or formic acid up to 1%. For better results depolymerization of the procyanidin structures is carried out in the presence of phloroglucinol that converts the procyanidin oligomers to monomeric units (Hammouda et al., 2014). The most commonly employed HPLC system uses a reverse phase C₁₈ column, a UV-VIS diode array detector and solvent system. Diode array detectors and mass spectroscopy are also used for polyphenol detection (Ignat et al., 2011).

2.5.2. Phytochemical fingerprinting of date fruit

Chemical fingerprints are obtained by chromatographic techniques as HPLC (high performance liquid chromatography) or HPTLC (high performance thin layer chromatography), spectroscopic or hyphenated techniques, for identification and quality evaluation of medicinal plants and phytomedicines (Rawal et al., 2010; Steinmann and
Ganzera, 2011). The phytochemical fingerprinting analysis is a comprehensive approach to provide the characteristic phytoceutic profile of the analyzed material (Ramzan and Li, 2015). Apart from these highly specific methods certain non-specific methods like determination of total phenolics and flavonoids, carotenoid content and total anthocyanins by pH differential methods are also widely used for overall content quantification (Chang et al., 2016). Nevertheless, the non-specific methods are generally used for getting an overall picture regarding the antioxidant capacity of the studied commodity. It is anticipated that greater the phenolic content more would be the antioxidant activity and vice versa.

Previously, Farag et al. (2014) elucidated the metabolite profiles of twenty one Egyptian date varieties. The authors reported that the total polyphenols in the selected varieties range from 233-1897 mg/100g D.W. The UPLC/PDA/ESI–qTOF- MS quantification reveal the presence of glycosides of apigenin and luteolin and conjugates of quercetin. Amongst the phenolics, caffeoyl shikimic acid (hydroxycinnamic acid conjugate) was present in higher amount than others. Aqueous date fruit extract (Egyptian dates – Tamr stage) was compared to ethanolic extract. The results reveal that the aqueous fruit extract contained 14.80 mg GAE/g total phenolics as compared to the ethanolic extract 10.31 mg GAE/g. The HPLC quantification reveals the presence of aesculetin and tannic acid in higher amounts as compared to gallic, itaconic and ferulic acids (El Sohaimy et al., 2015). However, lower total phenolic content i.e. 76.74 to 122.20 mg GAE/100g, is reported in four commercial Saudi date cultivars (Al-Asmari et al., 2017).

Polyphenolic moieties in date fruit were characterized using different approaches involving the direct separation of procyanidin oligomers employing HPLC in methanolic extracts or date fruit powder and by using the acidic cleavage method (phloroglucinolysis) prior to the solvent extraction enabling the extraction of both extractable and non-extractable procyanidins (Kennedy and Jones, 2001). On the basis of acid depolymerization method, Hammouda et al. (2014) classified phenolics present in date fruit in five distinct classes; hydroxycinnamic acid and its derivative, flavonoids, flavones, catechin monomers and procyanidin oligomers. They found that about 82% of the total polyphenolic entities in the date fruit are present in the flesh and peel portion. The procyanidins based on the (-) epicatechins structure were considered to be the most concentrated phytoceutics in Tunisian dates at Tamr stage accounting for about 95% of the phenolics with an average content of about 14 g/kg in the edible portions. Moreover, Eid et al. (2013) studied the polyphenolic content of three Saudi varieties namely Barni, Khalas and Ajwa. All the cultivars contained
hydroxybenzoic, hydroxycinnamic, gallic, vanillic, protocatechuic, isovanillic, chlorogenic, syringic, caffeic, ferulic, p-coumaric, sinapic and isoferulic acids. Among the flavonols, catechins were detected. The flavonoid glycosides such as aglycones quercetin, myricetin, kaempferol, naringenin, apigenin and luteolin were also identified. In the Ajwa variety, significant anthocyanidins are present unlike the other two varieties and also contains highest polyphenols. One of their peers, Benmeddour et al. (2013) identified and quantified four phenolic acids namely gallic, ferulic, coumaric and caffeic acids along with five flavonoids i.e. quercetin, isoquercetrin, quercetrin, luteolin and rutin in ten Algerian date fruit cultivars. In another study, sixteen phenolic compounds were characterized in the methanolic extracts of four Tunisian date cultivars during ripening. The most predominant were the hydroxycinnamic acids (16.75-19.40 mg/100 g F.W.) followed by ferulic acid (3.48-5.96 mg/100 g F.W.) and caffeic acid (3.04-5.75 mg/100 g F.W.). Syringic acid (1.33-4.95 mg/100 g F.W.) and protocatechuic acid (3.24-4.41 mg/100 g F.W.) at ‘Besser’ and ‘Tamr’ stages were also quantified. However, syringic acid is not detected in Khalat Dhabhi variety. Moreover, lower content, i.e. 0.70-2.364 mg/100 g F.W., of gallic acid was reported. Amongst the flavonoids, catechin group is the dominant one with content ranging from 3.34-3.84 mg/100 g F.W. (Amira et al., 2012). Similarly, Saleh (2011) determined hydrophilic antioxidants mainly catechin, rutin and caffeic acid in three Saudi date varieties. The content range between 5.00-7.30, 3.60-8.10 and 5.40-7.40 mg/kg for catechin, rutin and caffeic acid respectively. Earlier, Al-Farsi et al. (2005a) compared the antioxidant activity and phenolic profile of three Omani date varieties. The results revealed the presence of free and bound phenolic acids ranging between 2.61-12.27 mg/100g and 6.84-30.25 mg/100 g respectively. Amongst the free phenolic acids protocatechuic, ferulic, syringic and vanillic acids are quantified. Nevertheless, bound phenolics namely gallic, p-hydroxybenzoic, caffeic, p-coumaric and o-coumaric acids are also present in the Omani dates.

2.5.3. In vitro antioxidant properties

The chemical diversity of antioxidants makes them difficult to isolate or quantify in the biological matrix. Numerous tests are used for the determination of antioxidant activity, nonetheless, every method has certain limitations as total antioxidant activity cannot be assessed directly by the mechanism that involves measuring the effect of antioxidants in controlling the rate of oxidation in complex multiphase systems. Therefore, no single method can give whole picture so, several test models are used to explain the antioxidant
activity of a particular extract (Antolovich et al., 2002; Roginsky and Lissi, 2005). The commonly adopted techniques are the free radical quenching ones such as DPPH method, FRAP assay, ABTS decolorization methods, hydrogen peroxide scavenging activity, reducing power assay, nitric oxide scavenging capacity, oxygen radical absorbance capacity and many others. Amongst these, DPPH is the widely used procedure owing to its simple and inexpensive nature. It is a quick method involving only few steps and reagents. Whereas, the ABTS assay is applicable to both lipophilic and hydrophilic antioxidants, therefore considered as a reliable method (Alam et al., 2013). These radical scavenging analyses are based on either the transfer of hydrogen atom or single electron mechanisms (Apak et al., 2013; Shahidi and Ambigaipalan, 2015). Several lines of evidences suggest a positive association between the antioxidant potential and the content of compounds possessing the antioxidant capacity primarily the flavonoids and polyphenolic constituents (Mansouri et al., 2005; Amorós et al., 2009; Amira et al., 2011; Awad et al., 2011; Amira et al., 2012). Therefore, non-specific determination of phenolic and flavonoid content often relates to the antioxidant potential of the studied samples.

The phenolic content in dates varies with the ripening stage. In an investigation, methanolic extracts of six Mauritian date cultivars were studied at two edible stages i.e. ‘Khalal’ and ‘Tamr’. It was found that more phenolic content i.e. 728.5 mg GAE/100g DM is present at ‘Khalal’ stage as compared to ‘Tamr’ (558.9 mg GAE/100g). Similarly, the flavonoid content is also higher in the ‘Khalal’ stage (119.6 mg QE/100g DM) as compared to Tamr (67.3 mg QE/100g DM). Significant positive correlation is witnessed between the polyphenolic content and antioxidant activity as highest TEAC capacity is reported at Khalal stage (Lemine et al., 2014). Likewise, significant differences have been recorded in the phenolic content in three Tunisian varieties that range from 240.38 mg GAE/100g-505.49 mg GAE/100g date extract. The radical quenching ability of the extracts demonstrated by DPPH scavenging ability also shows varietal variations. The highest reported scavenging capacity is in Allig variety i.e.58.77% (Kchaou et al., 2014). The antioxidant potential of three Pakistani date cultivars namely, Dhaki, Dora and Karbaline were investigated by Anjum et al. (2012). Amongst the varieties, methanolic extract of Karbaline shows highest DPPH activity (90.96%). Al-Najada and Mohamed (2014) studied the changes in the antioxidant activity during storage in some Saudi date varieties and reported a slight increase in phenolic and flavonoid content at 4 ºC for 6 to 12 months storage. However, the DPPH and FRAP activity decreases up to threefold after 12 months.
storage study. The authors deduced that these changes in the antioxidant potential could be ascribed to the appearance of new phenolics with lower antioxidant potential.

Al-Jasass et al. (2015) studied six commercial date cultivars in United States for their polyphenolic contents and the antioxidant capacity. Significant variations were recorded amongst the studied parameters; the total phenolic content varied between 33 to 125 mg GAE/100g DM, while ABTS activity was found between 341-1300 μmol TE/g dry weight with highest in Deglet Nour variety and lowest in Khudri dates. Similar results are reported for DPPH, ORAC and FRAP ranging from 3.27-3.54, 189-243 and 3.29-5.22 μmol TE/g dry basis, respectively. Considerable antioxidant activity in date fruit syrups is reported using TBARS assay, DPPH method and H2O2 scavenging ability (Al-Mamary et al., 2014). The TPC of 10 Algerian date palm cultivars range from 167-709 mg GAE/100g F.W. Highest phenolic content is reported for Ghazi variety and lowest in the most commercially used *i.e.* Deglet Nour. Similar trend is observed regarding the flavonoid content that ranges from 11.52-225.77 mg QE /100 g F.W. The reducing power of the extracts varies from 272 to 1175 mg GAE/100g, while the DPPH radical activity is in the range of 32.4% to 86% in Thouri and Ghazi, respectively. The hydrogen peroxide scavenging capacity in the extracts ranges from 14.9 to 97.5%. The ferrous ion chelating activity also differs significantly with up to 95.7% in Ghazi and 48.9% in Deglet Nour (Benmeddour et al., 2013). Likewise, Singh et al. (2012) compared the polyphenolics and antioxidant activities of Omani date cultivars. The total phenolic and flavonoid content ranges from 194-234 mg GAE/100g and 25-34 mg CE/100g at the ‘Tamr’ stage, correspondingly. The ABTS activity in the selected varieties varies from 84-92%, while 70-73% DPPH activity is reported. Contrarily, very low phenolic content is reported in Algerian date varieties *i.e.* 2.49±0.01 to 8.36±0.60 mg GAE/100g F.W. However, high correlation ($R^2=0.975$) was obtained for the phenolic content and antioxidant activity (Mansouri et al., 2005). Water extract of Ajwa date extract exhibits significant phenolic content 455.88 mg/100g as compared to the alcoholic extract 245.66 mg/100g (Saleh, 2011).

Dose dependent inhibition of hydroxyl and superoxide radicals results by the use of aqueous date fruit extract. It was observed that 0.8 mg/mL extract is necessary to quench 50% of the superoxide radicals in the riboflavin photoreduction assay. However, 2.2 mg/mL extract scavenges half of the hydroxyl radicals in the deoxyribose degradation method. Similarly, the extract significantly inhibited the lipid peroxidation and protein oxidation in a dose dependent method. Two mg/mL date extract completely hinders the
TBARS formation. Date extract also proves to be potent in the dose-dependent inhibition of benzo (a) pyrene-induced mutagenicity on the salmonella strains. Hence, the findings indicate that antioxidant and antimutagenic potential in date fruit implicates the presence of chemical entities with potent free-radical-quenching abilities (Vayalil, 2002). The antioxidant activity assessment of sixteen Bahraini cultivars reveals 1.2 mmol/100 g average FRAP values on fresh weight basis in fully ripe dates (Allaith, 2008). Amongst the 28 studied fruits, dates stand second highest in terms of antioxidant activity after hawthorn fruit determined by the FRAP assay (Guo et al., 2003).

2.6. Restorative potential of date fruit

Recent years have witnessed great deal of attention towards the free radical chemistry and their role in disease pathogenesis leading to a medical revolution promising new era of disease and health management. Oxygen, an indispensable life element, has deleterious effects on human body under certain conditions (Aruoma, 2003). Free radicals are the reactive oxygen and nitrogen species that are generated by the human body due to several endogenous and exogenous factors. These are molecular species containing an unpaired electron in the atomic orbital capable of existing independently usually being unstable and highly reactive. Due to the ability to accept or donate electrons, they exist as reductants or oxidants. Amongst, the oxygen-containing free radicals are singlet oxygen, hydrogen peroxide, hypochlorite, superoxide anion, nitric oxide, peroxynitrite and hydroxyl radicals that perform key functions in the etiology of numerous chronic physiologic dysfunctions. These free radicals attack macromolecules leading to homeostatic disruption and cellular damage (Young and Woodside, 2001).

For the optimal physiologic functionality, balance between the free radical species and antioxidants is necessary. If there is an excess of free radicals, the body moves to a state of oxidative stress altering the proteins, lipids, nucleic acids, thus triggering a number of disorders (Lobo et al., 2010). Oxidative stress is amongst the main causative factor that associate hyperlipidemia with atherogenesis and myocardial infarction along with disturbing the liver marker enzymes (Lee et al., 2007). Reactive oxygen species play an imperative role in the disease pathogenesis in several physiological processes. Increasing scientific evidences declare abnormal production of free radicals as determinants of cellular stress and changes in several molecular pathways that underpins the pathogenesis of various disorders such as cardiovascular ailments, hepatotoxicity, nephrotoxicity, neurological disorders and oncogenesis (Santilli et al., 2015). Likewise, the oxidative stress
is also proposed to be the key factor in the etiology of type 2 diabetes mellitus. Hyperglycemia coupled with increased free fatty acid content results in increased production of mitochondrial ROS, ultimately augmenting the oxidative stress conditions. This damages a variety of cellular macromolecules including lipids, proteins and nucleic acids in case of inappropriate compensatory antioxidant response (Evans et al., 2005; Liu et al., 2015).

The multiple risk factor syndrome, often known as metabolic syndrome encompasses several medical complications including atherosclerosis, dyslipidemia, high blood glucose and hypertension in the same individual. Such disorders are increasing manifold especially in the industrialized countries. The core causative factor behind such complications is obesity. High fat diet or fat accumulation leads to escalated oxidative stress resulting in obesity-associated metabolic disorders (Furukawa et al., 2004).

Considerable antioxidant activity along with a myriad of polyphenolic biomolecules, explains significantly, the disease preventive properties of date fruits. These prophylactic properties are being used in the folklore medicine systems since antiquity. An ethnomedical survey in the southeastern Moroccan region revealed that dates are customarily used as a remedy for diabetes and hypertension. In the Indian Ayurveda, the fruit pulp is considered as a laxative, diuretic, antitussive, expectorant, demulcent and restorative (Khare, 2007; Tahraoui et al., 2007). The date fruits also possess certain anti-aging properties, it strengthens the body and prevents the formation of wrinkles giving skin a healthy radiant look (Bauza et al., 2001). The date consumption is also considered beneficial for the pregnant and lactating mothers. Infants having teething problems are also fed on date fruits as believed to harden the gums (Baliga et al., 2011; Al-Kuran et al., 2011). Date fruit extracts possess ameliorative potential against gastric ulceration and also mitigate increased histamine and gastrin contents in ethanol-induced ulceration (Al-Qarawi et al., 2005). The methanolic date extracts exhibit considerable neuroprotection against free radical mediated oxidative stress, spatial learning, neuronal damage and memory impairment (Pujari et al., 2014). Date fruit extract also ameliorates diabetic deterioration and improves the pathological parameters of diabetic nephropathy in rats (Zangiabadi et al., 2011). The polyphenols in date fruit particularly caffeic, chlorogenic, pelargonin and ferulic acids significantly stimulate the IFN-γ mRNA expression in the mouse Peyer’s patch cell cultures indicating the ability of this fruit to stimulate the cellular immune system in mice (Karasawa et al., 2011). Several studies validate the antibacterial, immunomodulatory, anti-inflammatory, hepatoprotective and nephroprotective properties of date fruits (Ali et al., 2012; Tang et
The increasing ailments in the societies are creating an urge for exploration of natural ingredients for their preventive properties. Most of these life-threatening ailments involve imbalance between the reactive oxygen species creating a stressed condition in body organs resulting in disease progression. In this regard, the phytochemicals in the date fruit are scientifically proved effective in modulating the oxidative stress mediated dysfunctions. The protective role of dates against oxidative stress conditions in heart, liver and kidney have been vigorously reviewed. The data have been divided into three parts depending upon the available literature. The overall effect of date fruit and influence of its main bioactives is described to draw a more conclusive approach regarding the molecular targets of date fruit. The conditions that results in stress and the underlying pathology have been discussed in the next sections.

2.6.1. Cardioprotective aspects of date fruit

Cardiovascular disorders are among the major causes of morbidity leading to mortality. There are certain risk factors responsible for developing these complications. They can either be non-modifiable such as gender, age, inherited once or can be modifiable factors including unhealthy diet, excessive alcoholism and deskbound living. Therefore, a behavioral and lifestyle improvement can reduce the peril of prevailing circulatory disorders (Guo et al., 2016). Scientific investigations strongly link oxidative stress with accelerated atherosclerosis leading to the development of CVDs. The origin begins with the damage of vascular endothelium due to increased rate of metabolic stress conditions like hypercholesterolemia. It is now believed that ROS plays key role in the development or initiation of atherosclerosis. The identification, targeting and reduction of lifestyle factors is by far the greatest potential for lowering cardiac complications along with allied ailments (Deepa and Varalakshmi, 2003; Elahi et al., 2009). Numerous scientific evidences have laid stress on the inverse association between consumption of antioxidant rich diet to mitigate lipidemic oxidative stress induced atherosclerosis (Zhong et al., 2015). In this regard, date fruit contains certain bioactives responsible for its restorative potential on the cardiac functionality.

The hypolipidemic effect of date fruit extract on high fat diet induced rats reveals that its oral administration alters the disturbed lipid profile to normal level indicating hypolipidemic effect of dates (Vembu et al., 2012). The anti-inflammatory and antioxidant analyses of the Ajwa dates was confirmed through lipid peroxidation, MTT and COX-1 and COX-2 assays. The aqueous extract of Ajwa dates are potent in the MTT analysis @
250 μg/mL and inhibits the lipid peroxidation by 91%. Whilst, 32% and 45% inhibition is recorded for COX-1 and COX-2 activities, respectively. These cyclooxygenase enzymes convert arachidonic acid to the prostaglandins, thereby initiating the inflammatory process in the physiological system (Zhang et al., 2013).

Rock et al. (2009) deliberated the influence of Medjool and Hallawi date consumption on glycemic and lipidemic profiles and also analyzed the oxidative stress markers in healthy subjects. Dates @ 100 g/day were administered on 10 healthy subject for a period of 4 weeks. It was found that date consumption has no significant effect on the body mass index and cholesterol levels. Dates, being sweet in nature are not considered suitable for the glycemic profile. However, no increment was observed on the blood glucose level. The serum triacylglycerol levels significantly reduce up to 15% by the consumption of Medjool dates and 8% by Hallawi date consumption. Considerable reduction i.e. 33% is recorded in the basal serum oxidative stress and lipid peroxidation also decreased by 12%. The authors conclude that the dates can be considered as an anti-atherogenic agent. Likewise, the effect of date supplementation on lipid profile was studied on hypercholesterolemic hamsters by Alsaif et al. (2007). Decrease in body weight in normal as well as hypercholesterolemic hamsters is observed with 13-week date supplementation from 38.78±2.26 g to 35.24±2.16 g in normal and 48.75±3.04 to 39.13±2.32 g in hypercholesterolemic groups. The serum lipid biomarkers also reduce by the administration of date fruit. The cholesterol level decreased from 109.70±11.63 to 97.30±14.08 mg/dL in the normal group, while in cholesterolemic group the serum cholesterol level rises to 309.90±20.15 mg/dL due to high cholesterol diet that reduces through date fruit containing diet (240.80±22.25 mg/dL).

Similarly, the triglyceride and LDL levels decrease drastically in normal group from 95.90±10.26 to 68.40±10.49 mg/dL and 16.90±4.45 to 10.60±1.79 mg/dL respectively. Significant reduction is observed in hypercholesterolemic group, the triglyceride level in the high cholesterol diet group decreased from 210.90±3.80 mg/dL that to 142.90±22.78 mg/dL by date fruit containing diet. The LDL decreases from 61.50±9.47 to 27.40±4.77 mg/dL in the hypercholesterololemic group fed on date fruit based diet. Substantial increase in the HDL level is also observed. Furthermore, Rosenblat et al. (2015) elucidated the combined effect of date and pomegranate polyphenols on E° mice. Two date varieties namely Amari and Hallawi were studied. The combination of date fruit and seed extract along with pomegranate extract administered for 3 weeks significantly lowered serum
triglyceride and cholesterol levels, while serum paraoxonase (PON1) activity increases that is preventive against lipid oxidation and prevents accumulation of lipoperoxides in LDL.

Several prospective cohort studies indicate inverse associations between polyphenol or flavonoid enriched foods and CVDs. Protective effects of such foods on intermediate markers of cardiac disorder has been indicated in various intervention studies. Recently, the cardioprotective, anti-inflammatory and lipid lowering potential was studied by Al-Yahya et al. (2016). The authors reported that lyophilized Ajwa date extract attenuates cytotoxicity and enhances the H9C2 proliferation by 40%. Moreover, the oral treatment of the extract @ 250 and 500 mg/kg B.W. is effectual in restoring the endogenous enzyme activities (SOD, CAT and NO) and decreasing the lipid peroxidation levels. Likewise, it also down regulated the proinflammatory cytokine expression and the anti-apoptotic protein. Histoarchitectural studies indicated the prophylactic effect of Ajwa date extract by the reduction of necrosis, inflammatory cells, infiltration and edema. The lipidemic and cardiac stress biomarkers are also significantly ameliorated. Thus, suggesting the therapeutic role of date fruit against cardiac dysfunctions with special reference to disrupted lipoprotein metabolism.

The polyphenolic moieties in date fruit are potent anti-atherogenic agents which was investigated by Borochov-Neori et al. (2015) on two date cultivars (Amari and Hallawi). The studied date extracts exhibits varied radical scavenging properties and also differ in their ability to inhibit the LDL oxidation. The flavonol fractions perform better in inhibiting the LDL oxidation as compared to the phenolic fractions. The flavonol fraction also stimulate the removal of cholesterol from the macrophages that play a key role in the lipid metabolism. Excessive uptake of native as well as modified lipoprotein lead to the formation of foam cells which accumulate and develop into fatty streaks i.e. the principal feature of developing the atherosclerotic lesion. In this regard, EGCG, a potent flavonol, activates eNOS and SIRT1 regulated phosphorylation of AMPK, thus combating the effect of atherogenic diet (Zhong et al., 2015). SIRT1 is a signaling molecule that is present in the endothelium and improves its function. It binds directly to the eNOS and targets it for deacetylation, hence stimulating the production of nitric oxide (NO) resulting in vascular relaxation exerting athero-protective effects. Whilst, the homeostasis is controlled primarily by the AMPK sensor and helps in endothelial dilation and relaxation. This sensor is also involved in managing the lipid metabolism by switching the oxidative pathways for fatty acids and constraining the lipid biosynthesis (Steinberg and Kemp, 2009; Stein and
Matter, 2011). Dower et al. (2015) also suggested the cardioprotective effects of epicatechin through a double blind, placebo controlled, randomized cross-over trial. Epicatechin reduces the circulatory inflammatory markers and possess anti-inflammatory effects. It was found that it decreases the atherosclerotic lesion area by 27% in rats fed on an atherogenic diet and impair the lesion progression as well (Morrison et al., 2014). Catechins have been shown to prevent the low-density lipoprotein from the oxidative damage. They also interfere with the atherosclerotic process and lowers thrombosis (Zhu et al., 1999; Kris-Etherton and Keen, 2002).

Quercetin is also present in date fruit in considerable amounts (Farag et al., 2014) and evidence based insights suggest its cardioprotective role. In this regard, the effect of quercetin treatment was investigated on 4 week and 12-week-old rats. A dose of 20 mg/kg/day was administered for 4 weeks; the results revealed that quercetin post ischemic recovery of left ventricular developed pressure as well as the contraction and relaxation markers were also recovered in young rats. However, non-significant effect is recorded in case of adult rats (Bartekova et al., 2016). It is also suggested that quercetin triggers the cardio prophylaxis against oxidative stress induced cell death, however, prolonged exposures may lead to cardiotoxicity as well (Daubney et al., 2015). Besides, rutin also provides significant defense against diabetes associated oxidative stress, improves ECG parameters and also prevents degenerative changes in cardiac tissue (Saklani et al., 2016).

The efficacious nature of several flavonoids has been identified and studied. Kaempferol, a phytoestrogen, is found in various cultivars of date fruit (Eid et al., 2013). Kaempferol possess certain anti-apoptotic and anti-inflammatory properties. Inverse association have been found between kaempferol consumption and cardiac ailments (Lin et al., 2007; Kim et al., 2010; Bhouri et al., 2011; Jang et al., 2011). In this regard, Xiao et al. (2012) investigated the protective effect of kaempferol against doxorubicin induced cardiotoxicity. The kaempferol treatment @ 1-20 mg/kg before doxorubicin injection results in a dose dependent restoration of heart and bodyweights. Moreover, it also ameliorates the catalase and superoxide dismutase levels in tissue and lactate dehydrogenase activity in serum, therefore, indicating its effectiveness against cardiac stress. Zhou et al. (2015) studied the restorative potential of kaempferol in alleviating myocardial ischemia/reperfusion injury in rat heart. Significant recovery in cardiac functionality is observed in the kaempferol treated rats. It also weakens the apoptosis induced by the injury.
The phenolic acid p-coumaric acid is a precursor of other phenolic compounds, besides possessing the ability to attenuate oxidative stress in doxorubicin induces myocardial injury. p-coumaric acid administration at 100 mg/kg for five days in stressed animals results in 39.65% and 32.89% amelioration in CPK and LDH enzyme activities, respectively. Moreover, the reduced superoxide dismutase and catalase activities in stress induced rats also increase by 41.13 and 15.44%, correspondingly through p-coumaric acid treatment (Abdel-Wahab et al., 2003).

The cardioprotective potential of date fruit may also be accredited to the presence of gallic acid (Amira et al., 2012), a metabolite of propyl gallate. The evidences suggest that the principal determining factor of its antioxidative properties is attributed to the binding ability of gallate compounds to the lipid membrane (Shahrzad et al., 2001). In a study conducted by Priscilla and Prince (2009), 7.5 and 15 mg/kg gallic acid was administered in ISO treated rats. After 10-day study, it was observed that gallic acid @ 15 mg/kg dose rate decreases the increased levels of lactate dehydrogenase (LDH), creatine kinase (CK) and creatine kinase-MB (CK-MB). The CK level reduces from 280.98±27.18 to 180.37±15.76 IU/L in the ISO-induced rats. CK-MB also decreases from 195.31±15.37 to 105.73±9.07 IU/L in the same group. Slight reduction is also observed in the normal groups. The AST, ALT and LDH levels in serum also decreases from 55.25±5.39-41.29±3.88, 42.54±3.57-30.91±2.76 and 152.51±13.43-88.84±8.61 IU/L respectively in the ISO-induced group. The ISO-induced rats exhibit a substantial decrease in the heart enzymatic antioxidants, SOD, CAT, GST. The histopathological examination of ISO-induced cardiac tissue shows infarction with inflammatory cells and splitting of the heart muscle fibers. However, gallic acid pretreatment results in mild hyalinization. The effect of gallic acid on dyslipidemia, obesity and oxidative stress was also studied by Hsu and Yen (2007). Obesity was induced through the administration of high fat diet. Two levels of gallic acid, 0.1% and 0.2%, were evaluated in normal and high fat diet fed rats. Gallic acid treatment results in significant decrease in body weight of high fat diet fed rats. Substantial reduction is also observed in the serum triacylglycerol and LDL levels in gallic acid fed obese rats and ameliorates the hepatic steatosis conditions. Moreover, the combination of gallic acid along with cyclosporine A (immunosuppressant drug) improves the cardiac marker enzymes, mitochondrial preservation and cell membrane integrity against ischemia/reperfusion oxidative stress (Badavi et al., 2014). Hence, gallic acid can possibly play a key role in lipid metabolism and oxidative stress reduction.
Apart from these aforementioned polyphenolic moieties, dietary fiber, vitamins and minerals in the date fruits may also contribute to the cardioprotective potential of the date fruit. However, more detailed investigations are required for better understanding regarding the role of date fruit in mitigating cardiac stress.

2.6.2. Hepatoprotective ability of date fruit

Liver plays an imperative role in the xenobiotic metabolism. The drugs and other chemicals need to be transported to the hepatic system to be metabolized. These entities are often bio transformed under various phase-I & II detoxification mechanisms into water soluble moieties. These are excreted out of the liver into either the bile or the blood to be eliminated via urine. Liver disease pathogenesis is triggered by the accumulation of triglycerides in the hepatocytes in a condition known as steatosis. Moreover, oxidative stress arising due to obesity as well as genetic factors influences the susceptibility of non-alcoholic fatty liver disease. The stress conditions disrupt the mitochondrial functioning and the nitric oxide signaling, thereby, worsening the steatosis and initiating the progression of steatohepatitis and fibrosis. Numerous antioxidants have been proposed to have beneficial effects towards disease mitigation and prevention. These bioactive moieties reverse the pathological conditions presumably through their ability to mitigate the oxidative stress (Mantena et al., 2008).

The hepatoprotective effect of aqueous date extracts were evaluated by Bastway et al. (2008) on thioacetamide induced hepatotoxicity in rats. The results show that aqueous date extract significantly reduces the plasma bilirubin concentration and hepatic enzymes elevated by thioacetamide. Serum alkaline phosphatase (ALP) activity was 60.04±2.35 IU/L in normal rats, which increased to 125.38±5.55 IU/L in hepatotoxic rats. The date fruit extract treatment @ 4 mL/kg body weight results in considerable decrease in ALP, up to 88.03±2.45 IU/L, in the hepatotoxic group. Similarly, the alanine amino transferase (ALT) activity reduces from 78.38±1.61 to 48.39±2.08 IU/L and aspartate amino transferase (AST) activity from 117.73±5.06 to 62.50±4.04 IU/L.

The date fruit extracts attenuate chemically induced liver stress by lowering the hepatic TBARS (thiobarbituric acid reactive substances) levels and restoring the activity of hepatic enzymes along with reducing the hepatic DNA fragmentation (El Arem et al., 2014a). Aqueous date extract (4 mL/kg) was administered on male Wistar rats fed on dichloroacetic acid (0.5 and 2 g/L) for 2 months. The results show restorative potential of fruit extract on
serum biochemical parameters. For instance, the recorded AST activity in the toxic group fed on dichloroacetic acid 0.5 g/L and 2 g/L was 124.50±2.50 and 157.72±1.74 IU/L respectively. The AST indices decreases upon the administration of aqueous date extracts to 115.00±1.56 and 132.75±1.16 IU/L respectively. Similarly, the ALT level decreases from 60.81±0.70 to 57.31±0.93 IU/L in 0.5 g/L dichloroacetic acid induced toxic group and from 68.75±0.42 to 62.75±0.62 IU/L in the other group. Maximum decrease in LDH level and γ GT is observed in the group fed on date extract with higher dose of dichloroacetic acid as 734.62±2.95 to 686.01±3.97 IU/L and 56.67±0.59 to 39.31±0.39 IU/L, respectively. In case of lipid peroxidation, dose dependent increase was observed through DCA treatment. However, aqueous date extract treatment ameliorates the lipid peroxidation as well as raises the levels of hepatic antioxidant enzymes (SOD, CAT and GPx). The hepatic histoarchitecture shows marked alterations in the hepatotoxic groups, while normal structure is observed in the normal groups. Aqueous date extract treatment improves the altered hepatic histology in the hepatotoxic groups. The absence of necrotic cells and vacuolization clearly indicates the hepatoprotective role of date fruit. The prophylactic potential of date fruit extracts against dimethoate induced liver damage reveals the ability of aqueous extract to inhibit the lipid peroxidation and improves the superoxide dismutase, glutathione peroxidase and catalase activities. The histopathological examinations reveal severe changes involving mononuclear cell infiltration, enlargement of hepatic sinusoids, congestion and hepatocellular damage in the dimethoate-intoxicated group. Contrarily, the date fruit pretreatment in the dimethoate induced hepatotoxic group results in improvement in overall liver morphology, however, mild degree of inflammation is observed but the absence of vacuolization and necrotic cells shows the ameliorative potential of date fruit extract on the hepatic tissues (Saafi et al., 2011).

Ajwa date varieties grown in Madinah-Saudi Arabia are considered the most luxurious dates. The hepatoprotective effect of Ajwa dates was analyzed by Sheikh et al. (2014) on male Wistar rats. Purposely, 1 g/kg/ body weight date extract was administered to rats that was supposed to be equal to 7 dates/person/day. The effect of date extract was studied on CCl₄ induced hepatotoxic rats for four weeks and twelve weeks. The findings reveal significant reduction in serum AST and ALT levels in date fruit fed groups as compared to the control hepatotoxic groups. However, more profound decrease is observed in the 12th week. The date fruit extract supplementation results in decrease in serum AST levels from 235.6±22.0 to 84.4±6.5 IU/L and serum ALT from 166.4±7.3 to 56.7±7.1 IU/L. Significant
improvement in the histological architecture is also reported. At the 12th week, the histological alterations such as tissue congestion, necrosis, dilation of veins and nuclear pyknosis due to CCL4 were reduced immensely by the administration of aqueous Ajwa extract. One of their peers, Ramadhas et al. (2014) studied the effect of date fruit on lambda cyhalothrin (LTC) induced biochemical and histological alterations in male Wistar rats. LTC is commonly used as an insecticide but is also known to cause several pathological conditions leading to genotoxicity, cardiotoxicity and hepatotoxicity. LTC results in lipid peroxidation in the experimental groups; however, date extract treatment considerably reduces the TBARS levels. The activities of the endogenous antioxidants restores in the date fruit fed LTC induced rats as compared to control. LTC induced rats show adverse effects on the activities of CAT, SOD, GPx, GST and GR. Along with marked increase in the liver marker enzymes (AST, ALT, ALP and LDH). However, the markers substantially decreased in the date extract treated group viz AST, ALT, ALP and LDH levels in the LTC induced group were 246±5.09, 93±9.11, 6.27±0.49 and 9.73±0.75 IU/L respectively that decreased in the date fruit treated group as 166±15.10, 54±4.80, 3.98±0.27, 7.45±0.51U/L, respectively. LTC exposure lead to adverse effects on the hematological traits (RBCs, WBCs, Hb, Hct, MCV and MCH) which were also normalized in the date fruit fed groups thus, highlighting its efficaciousness against such pathophysiological conditions. Date fruit extract is reported to ameliorate CCl4 induced liver damage in New Zealand rabbits (El-Gazzar et al., 2009).

Al-Qarawi et al. (2004) also demonstrated the disease modulatory effect of dates on CCl4 induced liver cirrhosis. Pre-and post-treatment with date fruit extract results in significant reduction in AST, ALT and ALP values. In the post treatment, the fruit extract was fed for 14 days and CCl4 was injected intraperitoneally at 1, 2, 3 day of the treatment period. In case of pretreatment, the CCl4 was injected at the 14th, 15th and 16th day. Maximum reduction in AST and ALT is observed in post treatment date flesh fed rats from 282.6±2.96 to 124.4±4.27 U/L and from 85.6±1.99 to 43.68±0.64 IU/L, respectively accounting for about 55.98 and 48.97% decrease, correspondingly. The authors suggest that up to 80% protection against CCl4 poisoning can be achieved through daily consumption of aqueous date fruit extract.

Catechin and quercetin present in date fruit are also known to encompass several hepatoprotective properties. In a study conducted by Uzun and Kalender (2013), the restorative role of these two compounds was studied against chlorpyrifos induced
hepatotoxicity in rats. After the four-week trial, 76% increase in lipid peroxidation levels was recorded in the hepatotoxic group. However, 31 and 33% reduction in the lipid peroxidation was observed with catechin and quercetin treated groups, correspondingly. Statistically significant reduction is recorded in ALP, AST, ALT and LDH activities by the administration of quercetin (14, 17, 19 and 12%, respectively) and catechin (15, 28, 18 and 14% respectively) in the hepatotoxic groups. The effect of epigallocatechin gallate against atherogenic diet induced hepatic oxidative injury was evaluated by Ramesh et al. (2009). Atherogenic diet significantly lowers the hepatic antioxidant enzyme activities that are restored in the green tea catechins fed rats. Higher serum AST, ALT, ALP and LDH activities are reported in atherogenic diet fed rats. The hepatoprotective properties of the studied green tea catechins are mainly attributed to epigallocatechin gallate - the major green tea catechin that is also present in date fruits.

### 2.6.3 Renoprotective potential of date fruit

Kidney is an imperative organ of the urinary system that is involved primarily in blood purification and waste removal, maintenance of electrolyte balance, fluid homeostasis and blood pressure. About 180 L of blood is filtered daily by the renal system (Awan, 2011). However, kidneys are vulnerable to injury because of toxic substance exposure leading to nephrotoxicity. Kidney disease is amongst the leading cause of morbidity and mortality in the developing world. It is estimated that kidney dysfunction is the 9th leading cause of death, globally (Janakiraman and Jeyaprakash, 2016). The development of renal complications are often based on excess nutrient consumption that results in oxidation and generation of reactive species. The disturbance in the balance of pro-oxidants and antioxidants leads to lipooxidation and glycation, generating highly reactive compounds as malondialdehyde, acrolein, 4-hydroxy-nonenal and glyoxals (Aldini et al., 2011). These substances exhibit high affinity with the proteins and results in the formation of advanced glycation end products that are chiefly responsible for damaging the renal tissue (Pierine et al., 2015).

The protective role of date fruit against renal damage was elucidated by El Arem et al. (2014c). Date fruit extract @ 4 mL/kg B.W. administration on trichloroacetic acid intoxicated rats for 2 months proved effective in restoring the endogenous enzymes and mitigating lipid peroxidation. The date extract also improved the renal histology indicating its protective role towards renal damage. In another study, it was explicated that feeding Ajwa date extract @ 300 mg/kg/day to oxidative stress induced rabbits results in 19.78 and
14.54% reduction in serum creatinine and urea levels, respectively in a 14 day experimental period (Ragab et al., 2013). Berne date extract @ 300 mg/kg for 14 days before and 7 days after the administration of cisplatin in rats proved to be protective in ameliorating the nephrotoxicity due to its antioxidant, anti-apoptotic and anti-inflammatory potentials. The levels of TNF-α, urea and creatinine reduces up to 57, 73 and 72% by date extract treatment in the nephrotoxic rats, it also mitigates the histological alterations induced by cisplatin (El-Sayed et al., 2015).

Recently, El-Mousalamy et al. (2016) elucidated the effect of aqueous and methanolic extract of date fruit on diabetic nephropathy in rodent experimental modeling. The results demonstrates renoprotective effects of date extracts by improving the lipid and glucose homeostasis and antioxidant properties. The body weight increases in the diabetic control to 248.34 ± 15.9 g that decrease by aqueous and methanolic date extract to 194.60 ± 27.2 and 196.29 ± 10.5 g, respectively. The fasting blood glucose level in diabetic group was 464.71 ± 43.96 mg/dL that reduces to 125.00 ± 13.6 and 154.71 ± 17.4 mg/dL in the aqueous and methanolic extract administered groups, correspondingly. The researchers also observed significant decrease in the elevated lipidemic biomarkers as cholesterol, triglycerides and LDL with increase in HDL. Serum creatinine reduces from 2.92 ± 0.23 to 1.44 ± 0.097 mg/dL due to aqueous date extract treatment. Likewise, the urea level was 71.57 ± 2.14 mg/dL in the diabetic control that reduces to 34.14 ± 3.71 mg/dL in the aqueous date extract treated rats. Their study suggested better ameliorative potential of aqueous date fruit extract as compared to the methanolic one.

Vitamin C is abundant in date fruit, numerous studies indicate the mitigating potential of date fruits against renal dysfunctions (Das and Buchner, 2007; Karabulut-Bulan et al., 2008). Vitamin C pretreatment @ 16.6 mg/kg for seven days in nickel induced oxidative stressed mice results in significant reduction in serum urea, uric acid & creatinine and decreases the nickel accumulation in the nephrotic tissue (Kadi and Dahdouh, 2016). Date fruit also contains quercetin in substantial quantities (Tang et al., 2013). Several studies validate the nephroprotective effect of quercetin. It is believed that quercitin possess certain vasodilator properties that contributes in increasing the renal blood flow (Behling et al., 2006). The protective role of quercetin against fluoride induced nephrotic damage was studied by Nabavi et al. (2012). Quercetin @ 10 mg/kg and 20 mg/kg was administered to rats along with simultaneous provision of sodium fluoride (600 ppm) containing drinking water for 7 days. The glomerular damage markers (urea and creatinine) increases
significantly due to fluoride toxicity with downregulations in the antioxidant defense. Besides, quercetin treatment results in dose dependent amelioration of renal biomarkers in serum as well as tissues comparable to the positive control (vitamin C).

Conclusively, date fruit and its phytoceutic entities hold significant functional and nutraceutical worth to address metabolic disorders. Date fruit extract has been studied extensively for its hepatoprotective aspects. However, the study regarding the impact of whole fruit on the hepatic parameters was scarce hence need to be investigated. Thus, scientific studies were planned to analyze the effect of whole fruit instead of extract. Likewise, extensive research with special reference to cardioprotective abilities of dates was necessitated owing to myriad of heart beneficial agents. Moreover, in the literature review, it was found that varieties might differ based on phytoceutics composition and content, therefore this reason was considered while designing the research plan.

Based on aforementioned facts, it is hypothesized that date fruit, containing a vista of bioactives, is beneficial for hepatic and cardiac disorders. Therefore, present research is planed to validate the stated hypothesis.
CHAPTER 3

MATERIALS AND METHODS

The current investigation was carried out in the Functional and Nutraceutical Food Research Section, National Institute of Food Science and Technology, University of Agriculture, Faisalabad. Accordingly, three locally available date varieties namely Dhaki, Aseel and Zahidi were characterized for their nutritional and phytochemical contents. Date based halwa was developed using the varieties and extracts. Finally, the best selected variety and its extract was employed for the bioefficacy assessment. The materials and protocols followed are elaborated herein.

3.1. Materials

Three varieties of date fruit grown in Pakistan were selected namely, Dhaki, Aseel and Zahidi. Dhaki is considered to be the best table date variety and Aseel and Zahidi are amongst the most commonly consumed cultivars. Dhaki dates were obtained from the Date Research Institute D.I. Khan. Aseel and Zahidi were purchased from the Faisalabad local market. The sample selection was random and based on certain quality characteristics (like color uniformity, size, shape and being free from abrasion). The samples were graded and washed before refrigerating at 4°C for further analysis and potential applications. Standards and reagents (analytical and HPLC grade) were procured from Merck, Darmstadt and Sigma-Aldrich Tokyo. Male Sprague Dawley rats were housed in the Animal Room of NIFSAT. The biological efficacy assessment trials were carried out using the diagnostic kits procured from Sigma-Aldrich, Bioassay Germany and Cayman Chemicals Europe, Estonia.

3.2. Sample preparation

The date fruit samples were cleaned and pitted. The fruit pulp was minced using an electric mincer and the samples were kept in polyethylene bags at 4°C for further analyses.

3.3. Compositional profiling of date fruit

In the first part of the study, the selected varieties were analyzed for their chemical composition.
3.3.1. Chemical characterization of date fruit

The date fruit varieties were analyzed for various compositional traits including proximate assay, mineral, vitamin C, β-carotene and sugar profiles.

3.3.1.1. Moisture content

Date pulp was analyzed for moisture content determination following AOAC (2006) Method No. 934-01. Purposely, 10 g of the date sample was dried in the hot air oven (Model: DO-1-30-02, PCSIR, Pakistan) at a temperature of 105±5 °C until constant weight.

\[
\text{Moisture (\%)} = \frac{\text{Fresh sample weight (g)} - \text{Dried sample weight (g)}}{\text{Fresh sample weight (g)}} \times 100
\]

3.3.1.2. Crude protein content

Kjeltech apparatus (Model: D-40599, Behr Labor Technik, Germany) was employed to measure the percent nitrogen in date samples according to the AOAC (2006) Method No. 984-13. In this regard, 500 mg of each date sample was digested with concentrated H₂SO₄ by using the digestion mixture (K₂SO₄:FeSO₄:CuSO₄:: 100:5:10) until the color was light greenish (three to four hours). The digested sample was then added to 250 mL volumetric flask and volume was made up to the mark using distilled water. Afterwards, 10 mL of 40% sodium hydroxide and 10 mL digested sample were taken in the distillation apparatus, where the liberated ammonia was added to the beaker containing 4 percent solution of boric acid using methyl red indicator. This lead to the formation of ammonium borate that was employed for the determination of nitrogen in the sample. The nitrogen percentage in the date samples was assessed by titrating distillate against 0.1 N solution of H₂SO₄ till the appearance of light golden color. The content of crude protein was determined by multiplying the nitrogen percentage with the factor 5.80.

\[
\text{Nitrogen (\%)} = \frac{\text{Volume of 0.1 N sulphuric acid used} \times 0.0014 \times 250}{\text{Sample weight} \times \text{Aliquot volume}} \times 100
\]

\[
\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 5.80
\]

3.3.1.3. Crude fat

The crude fat in the date fruit samples was ascertained according to the Method No. 920-39 of AOAC (2006). For the purpose, 2 g moisture free sample was weighed and taken in a thimble. Then, 50 mL n-hexane as a solvent was added into the flask that was attached to
the soxhlet (Model: H-2 1045 Extraction Unit, Hoganas, Sweden). Fat content in the date sample was extracted for 2-3 hours in the soxhlet by regulating the flow rate to 3-4 drops per second of n-hexane. After six to seven siphon back, the thimble was removed and dried in an hot air oven at constant temperature (105 ºC) for constant time (1 hour) and then weighed using electric balance.

\[
\text{Crude fat (\%) = } \frac{\text{Weight of hexane extract (g)}}{\text{Weight of sample}} \times 100
\]

3.3.1.4. Crude fiber

The content of crude fiber in date samples was measured by using the Method No. 978-10 as per the guidelines of AOAC (2006). Purposely, 2 g fat free date sample was digested using 1.25% of boiling H\textsubscript{2}SO\textsubscript{4} (200 mL) for 30 minutes in the Fibertech apparatus (Labconco Corporation, Kansas, USA). Sulphuric acid was drained out and the digested sample was then filtered and washed thrice using boiling distilled water to ensure acid free sample. Afterwards, it was again digested for 30 minutes in 200 mL boiling sodium hydroxide (1.25%). The sodium hydroxide was then drained out and the digested sample was again filtered and washed three times with boiling distilled water to make free from alkali residues. The residue obtained was then dried at predefined temperature (130ºC) and time (2 hours) and weighed (W\textsubscript{1}). The oven dried sample was ignited in the muffle furnace (MF-1/02, PCSIR, Pakistan) at temperature of 550-650°C till white ash was obtained and reweighed (W\textsubscript{2}). The percent crude fiber in the date fruit samples was obtained according to the expression given below:-

\[
\text{Crude fiber (\%) = } \frac{W_1 - W_2}{\text{Sample weight (g)}} \times 100
\]

3.3.1.5. Ash content

Total ash content was assessed by incineration of the sample by following the procedure as mentioned in AOAC (2006) Method No. 942-05. Accordingly, 5 g sample was taken in a crucible and directly charred on flame until fumeless. Afterwards, the sample was ignited in the muffle furnace (MF-1/02, PCSIR, Pakistan) at temperature of 550-600 ºC for 5-6 hours till greyish white residues. The ash percentage in each date sample was computed using the following mathematical expression:

\[
\text{Ash (\%) = } \frac{\text{Weight of Ash (g)}}{\text{Weight of sample (g)}} \times 100
\]
3.3.1.6. **Nitrogen free extract (NFE)**

The NFE in date samples was calculated according to the following formula:

\[
NFE = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ crude fat} + \% \text{ crude fiber} + \% \text{ crude protein})
\]

3.3.1.7. **Total carbohydrate content**

The carbohydrate content in date samples was also calculated using the following mathematical expression:

\[
\text{Total Carbs (\%)} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ crude fat} + \% \text{ crude protein})
\]

3.3.1.8 **Mineral profile**

Date fruit samples were analyzed for their mineral contents following AOAC (2006) method. Purposely, 3 g date sample was ignited and ashed at 550°C in the muffle furnace. The obtained ash was then dissolved in 5 mL of fuming nitric acid and shifted to 50 mL volumetric flask using double distilled deionized water. For the preparation of standard solutions of each element, 250 µL was taken from the standard stock solution (1000 mg/L) in a 25 mL volumetric flask and made up to the mark with 0.5 N HCl solution hence prepared the intermediate standard solution (10 mg/L). Working control solutions suitable to the concentration of each element in the sample solution were prepared. The date samples and controls were ascertained using Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge) for potassium and sodium as per method no 956.01 (AOAC 2006). While, calcium, copper, cobalt, magnesium, iron, manganese and zinc in the resultant samples were determined by using Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) following the conditions described in AOAC (2006) Method No. 975.03 (b) and 991.11. Commercially available stock solution (Applichem®) in the form of an aqueous solution (1000 ppm) was used for the preparation of calibrated standards. While, the working standards were prepared using highly purified de-ionized water. All the glass apparatus employed throughout the analytical process were immersed in 8 N HNO₃ solution overnight and washed several times with de-ionized water before using.
3.3.1.9. Vitamin C content

The content of vitamin C in the date fruit cultivars was measured using High Performance Liquid Chromatography (HPLC) employing the method of Van de Velde et al. (2012). For the extraction, 5 g homogenized sample was added to 25 mL extracting solution (HPLC grade water). The mixture was homogenized for 20 minutes at 280 rpm using orbital shaker and allowed to rest for 2 hours at room temperature. Afterwards, it was centrifuged using centrifugal machine (M-3k30, Sigma, Germany) at 5000 rpm for 10 minutes at 4 °C. The collected supernatant was filtered via 0.45-μm Millipore membrane. The residue was then dissolved in mobile phase (70% methanol) and filtered through 0.25 μm membrane filters; 20 μL was injected for HPLC analysis @ 1 mL/minute flow rate. Analysis was performed using Shimadzu LC-20AC (Shimadzu, Kyoto, Japan) pumps with UV-Visible detector while chromatographic separations were performed on a Shim-Pack CLC-ODS (C-18), 25cm x 4.6nn, 5μm column. The column was set at 20 °C. The absorbance was read at 280 nm.

3.3.1.10. Quantification of β-carotene

β-carotene content in the date samples was determined using HPLC in gradient mode following the procedure of Siriamornpun et al. (2012). Purposely, 2 g of minced fresh date samples were placed in a light protected vessel using aluminum foil. The sample mixture was then mixed with the extraction solvent 100 mL (hexane: ethanol: 75:25 v/v). The mixture was magnetically stirred for 30 minutes and then 15 mL of HPLC grade water was added to it. The upper layer was placed in a round-bottom flask and an aliquot of 10 mL date extract was evaporated till dried. The residue was dissolved in methanol/acetonitrile (1:1 v/v) and diluted with methanol/acetonitrile (50:50) to final volume of 4 mL. The resultant solution was then filtered through 0.45 μm membrane filters and 20 μL were injected for HPLC analysis performed using Shimadzu LC-20AC (Shimadzu, Kyoto, Japan) pumps, SPD-M20A with diode array detector and the chromatographic separations were done on a Shim-Pack CLC-ODS (C-18), 25cm x 4.6nn, and 5μm column. For the separation, the mobile phase comprised of acetonitrile (solvent A)/water 10:90 (v/v) at a flow rate of 0.8 mL/minute. The column temperature was set at 30 °C and the absorbance was read at 450 nm.
3.3.1.1. Sugar content determination

The sugar content in the selected date fruit samples were determined according to the protocol described by Chaira et al. (2009). The fresh date flesh weighing 5 g was refluxed with 25 mL HPLC grade water. The mixture was homogenized for 5 minutes at 280 rpm using orbital shaker and allowed to rest for 2 hours. Afterwards, it was centrifuged using at 5000 rpm for 10 minutes at 4°C. The supernatant was filtered via 0.45 µm membrane filter. HPLC was used to quantify the reducing and non-reducing sugars in the date samples. The separation was conducted at 20°C room temperature on Lichrospher® 100 NH₂ Purospher®STAR NH₂ 5 µm column. The mobile phase comprised of acetonitrile and ultrapure water (80/20 v/v). The liquid chromatographic system was connected to refractive index detector 10 A. 0.8 mL/minute flow rate and 20 mL injection volume was set throughout the test. The integrator was calibrated using the external standards consisting of 2% fructose, 2% glucose and 1% sucrose solutions. The total reducing sugars were calculated by adding the obtained glucose and fructose contents.

3.4. Phytochemical and antioxidant activity assessment of date fruit

Date fruit extracts were prepared and analyzed for their phytochemical constituents and antioxidant activities as described below:

3.4.1. Extraction protocol

Bioactive moieties from the three selected date cultivars were extracted using aqueous methanol through the defined protocol (Al-Farsi et al., 2005a; Kchaou et al., 2014). Purposely, 100 g date sample was subjected to solvent extraction by the addition of 200 mL extraction solvent comprising of methanol, containing 0.1% acetic acid/water (80:20). The samples were kept overnight at 4°C. Afterwards, the samples were subjected to orbital shaking at 280 rpm at 20°C for 4 hours. The extracts were filtered and the solvent was evaporated at 40°C using rotary evaporator. The obtained extracts were stored in bottles at -40 °C prior to use. The prepared extracts were further employed to determine the polyphenolic content and antioxidant activity.
3.4.2. Phytochemistry

3.4.2.1. HPLC quantification of bioactives

Bioactive moieties mainly phenolic acids, flavonols and flavan 3-ols were quantified using HPLC. The procedures followed are described as under:

3.4.2.1.1. Sample preparation for phenolic acids and flavonols

Acidified methanol (25 mL) containing 1% (v/v) HCl was added to 5 g of minced date samples and the mixture was stirred thoroughly at 60°C under reflux for 2 hours to obtain aglycons of flavonols glycosides. The obtained extract was then cooled to room temperature (20°C) and centrifuged at 5000 rpm for 10 minutes. Upper layer was sonicated for 5 minutes in order to remove air. The final extract was then filtered through 0.45 Millipore filter before injecting to the HPLC system.

3.4.2.1.2. Phenolic acid determination

Quantification of phenolics in date flesh was carried out by following the protocol of Shabir et al. (2011). For the purpose, a Varian HPLC system containing ODS2 C_{18} reversed phase column (25 cm x 4.6 mm, 5.0 μm particle size) with UV/Vis detector was used for the analysis. The HPLC quantification was performed using acidified acetonitrile (99.5%) as mobile phase in the isocratic mode with a constant flow rate of 1 mL/minute and detection at 280 nm with sample injection volume of 20 μL. Polyphenolic moieties in each date fruit sample were identified by matching their relative retention times with the standard chromatogram. The concentration of every individual compound was calculated on the basis of peak area measurement and the results were then converted to mg phenolics/100g fresh weight basis.

3.4.2.1.3. Flavonoid quantification

Flavonols such as kaempferol, quercetin and myricetin in date samples were analyzed through HPLC system according to the procedures of Sultana and Anwar (2008). An HPLC (model LC-10A, Shimadzu, Kyoto, Japan) and data acquisition class LC-10 software was employed. The system was equipped with two LC-10 AS pumps, Rheodyne injector, SCL-10A system control unit, SPD-10A UV-vis detector and CTO-10A column oven. Twenty μL volume of the filtered date extract sample was injected into analytical Supelco (Supelco Inc., Supelco Park, PA, USA) ODS reverse phase (C_{18}) column. Two solvent systems A: containing 3% trifluoroacetic acid and B: containing acetonitrile and methanol (80:20 v/v)

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were used. The chromatographic separation was carried out by the isocratic elution of mobile phase *i.e.* mixture of solvent A and B (50:50 v/v). This was vacuum filtered through 0.45 µm membrane prior to injecting at a flow rate of 1.0 mL/minute at 30°C. Detection was conducted at a wavelength of 360 nm. Identification of flavonols (kaempferol, quercetin, myricetin) was performed by comparing their retention times with those of authentic standards. Standard calibration curves were used for the quantitative determination.

Flavanols particularly catechin and epigallocatechin gallate contents were quantified as per the procedure described by Longo *et al.* (2008). Date fruit extracts were prepared using HPLC grade acetone as the extracting medium. Purposely, 10 g sample from each variety were subjected to solvent extraction by the addition 30 mL extraction solvent comprising of aqueous acetone, containing 0.1% acetic acid/water (80:20). The samples were kept overnight at 4°C. Afterwards, the resultant samples were subjected to orbital shaking at 280 rpm, 20°C for 4 hours. The extracts were filtered and the solvent was evaporated at 40°C using rotary evaporator. The obtained extracts were then filtered with 0.45 µm membrane filter. The analytical determination was performed using reverse-phased HPLC system in isocratic mode. The separation was carried out on a 25 cm x 4.6 mm, 5.0 µm particle size ODS2 C-18 reversed phase column. The samples were eluted at room temperature using mobile phase consisting of water: methanol: acetic acid (70:30:0.5) at a flow rate 1.0 mL/minute. All extracts were prepared in triplicates and detection was carried out. The UV detection was carried out at 280 nm.

### 3.4.3. *In vitro* antioxidant activity

#### 3.4.3.1. Total phenolics

The total phenolic content in each date extract sample was ascertained by following the protocol described by Lemine *et al.* (2014) employing Folin-Ciocalteu reagent based method and using gallic acid as a standard. Purposely, aliquots of 0.2 mL of each sample were evaluated. Folin-Ciocalteu phenol reagent (0.2 M/L) 0.5 mL was added to each test tube and they were then kept at room temperature for 5 minutes. Afterwards, 0.4 mL of 7.5% sodium carbonate (Na₂CO₃) was added and mixed thoroughly. The samples were then incubated for 60 minutes at 25°C. The absorbance at 750 nm was taken using a microplate reader (ELx-800 BioTek, USA). Results were articulated as milligram gallic acid
equivalents (GAE)/100g. The standard curve was prepared using gallic acid at different concentrations.

### 3.4.3.2. Flavonoids

The flavonoid content was measured in the samples by following the procedure of Amira et al. (2012). Accordingly, 250 µL of each extract or the standard solution was mixed with 1.25 mL of double distilled H₂O and 75 µL of 5% NaNO₂ solution. After 6 minutes, 150 µL of AlCl₃. H₂O solution was added. After next 5 minutes, 0.5 mL of 1 M sodium hydroxide solution was added and then the total volume was made up to 2.5 mL with distilled water. The absorbance against blank was determined at 510 nm using microplate reader. The standard curve was prepared using quercetin and the results were expressed as milligram quercetin equivalents (QE)/100g.

### 3.4.3.3. DPPH (1,1-diphenyl-2-picrylhydrazyl) antiradical ability

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was measured by the procedure of Al-Najada and Mohamed (2014). For the purpose, 0.1 mL methanolic date extract was added to 0.9 mL freshly prepared DPPH solution (0.1 mM). For the control sample equal amount of methanol was used for comparison. After incubating the samples for 30 minutes in dark at room temperature, the optical density (OD) was measured at 517 nm using a microplate reader. Scavenging activity (%) was calculated using the following formula:

$$\text{DPPH antiradical activity} \% = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

### 3.4.3.4. Ferric reducing antioxidant power (FRAP)

The FRAP analysis was also performed according to the method of Al-Najada and Mohamed (2014). The principle of this technique is based on the reduction of ferric-tripyridyltriazine complex in the presence of antioxidants to its ferrous colored form. Concisely, the freshly prepared FRAP reagent containing 2.5 mL of 10 mM/L TPTZ (2,4,6-tripyridy-s-triazine) solution in 40 mM/L HCl + 2.5 mL of 20 mM/L FeCl₃ and 25 mL of 0.3 M/L acetate buffer at pH 3.6 and was warmed at 37°C. Afterwards, 40 µL of the supernatant was mixed with 0.2 mL distilled water and 1.8 mL FRAP reagent. The absorbance of the reaction mixture was measured spectrophotometrically at 593 nm after incubating at 37°C for 10 minutes. Aqueous standard solutions of FeSO₄.7H₂O (20-100
µM) were employed for the calibration curve and the results were expressed as the FRAP value µM Fe^{2+}/g.

3.4.3.5. Trolox equivalent antioxidant capacity assay (TEAC)

The TEAC assay based on the ABTS radical scavenging activity of date flesh was determined according to the method described by Biglari et al. (2008). The ABTS radical cation (ABTS⁺) solution was prepared through the reaction of 2.45 mM potassium persulphate and 7 mM ABTS, after incubation at 23°C in dark for 16 hours. The ABTS⁺ solution was diluted with 80% ethanol to obtain an absorbance of 0.700±0.005 at 734 nm. 3.9 mL ABTS⁺ solution with the absorbance of 0.700±0.005 was added to 0.1 mL of the test samples and mixed vigorously. The reaction mixture was allowed to rest at 23°C for 6 minutes and the absorbance at 734 nm was instantly noted. Trolox standard solution at different concentrations ranging from 0 to 15 mL in 80% ethanol was used to obtain the standard curves. The sample absorbance was compared to that of the Trolox and the results were expressed in terms of Trolox equivalents.

3.4.3.6. Hydrogen peroxide scavenging capacity

The scavenging activity of date extracts on hydrogen peroxide was measured by using the method of Atmani et al. (2009). Accordingly, test tubes were prepared with 2.0 mL of date fruit extracts and solution of H₂O₂ (1.2 mL, 40 mM) in phosphate buffer having pH 7.4. A blank solution without H₂O₂ was prepared in the same way. After incubation of the reaction mixture for 10 minutes, the absorbance at 230 nm was recorded. The scavenging activity was calculated using the following formula:

\[
\text{Hydrogen peroxide scavenging activity } \% = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100
\]

3.4.3.7. Reducing power

Reducing power of the extracts was determined by following the protocol of Kchaou et al. (2014). Accordingly, 1 mL date extract was mixed with 2.5 mL potassium ferricyanide [K₃Fe(CN)₆] (1%) and 2.5 mL of phosphate buffer (0.2 M/L, pH 6.6). The resultant mixture was then incubated for 20 minutes at 50°C. Afterwards, 2.5 mL of 10% trichloroacetic acid was mixed with 0.5 mL of 0.1% ferric chloride (FeCl₃) and 2.5 mL distilled water. The absorbance was measured @ 700 nm against blank. Increased reaction mixture absorbance specifies improved reducing power capacity of the date sample.
3.5. Product development

For the product development phase, six treatments of date enriched traditional halwa were prepared (Table 3.1). For halwa preparation, date varieties and respective extracts were used to determine their impact on product characteristics. All the treatments had the same recipe except the difference of varieties and their extracts. A control treatment comprising of traditional halwa with no dates was used for comparison purpose. High energy and nutritious date based halwa was prepared using clarified butter, gram flour, semolina, date paste/date extract, sweetened condensed milk, almonds and pistachio. All the ingredients were carefully selected. Initially, clarified butter along with few cardamoms were heated in a wok followed by the addition of semolina flour. The flour was heated until it became light brown in color. Afterwards, roasted gram flour was added and mixed continuously to avoid the formation of lumps. After heating for three more minutes with constant stirring, date fruit/extract was added and mixed thoroughly. Then, milk was added and cooked until the final texture. Nuts were sprinkled and the treatments were kept under refrigeration conditions throughout the storage for physicochemical analyses and sensory response assessment (Appendix I).

Date paste preparation

The date flesh was minced using a meat mincer. The paste was kept overnight in 100 mL water to get smooth textured halwa. Prior to addition in halwa, the date water mixture was also blended for uniform particle size. 350 g date paste was added to each date fruit containing treatment.

Date extract preparation

Date extract was prepared by dissolving date paste into water (1:3 w/v). The obtained mixture was kept at 4 °C for 48 hours. Afterwards, it was shaken at 400 rpm for 45 minutes, followed by centrifugation at 5000 rpm at 4 °C for 45 minutes. Supernatant was collected and used in the product development phase as well as in the bioefficacy trials (El Arem et al., 2014a).
Table 3.1. Treatments used in the product development

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>D&lt;sub&gt;0&lt;/sub&gt;</td>
<td>Control (Traditional Halwa)</td>
</tr>
<tr>
<td>D&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Dhaki Date Based Halwa</td>
</tr>
<tr>
<td>D&lt;sub&gt;1a&lt;/sub&gt;</td>
<td>Dhaki Date Extract Based Halwa</td>
</tr>
<tr>
<td>D&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Aseel Date Based Halwa</td>
</tr>
<tr>
<td>D&lt;sub&gt;2a&lt;/sub&gt;</td>
<td>Aseel Date Extract Based Halwa</td>
</tr>
<tr>
<td>D&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Zahidi Date Based Halwa</td>
</tr>
<tr>
<td>D&lt;sub&gt;3a&lt;/sub&gt;</td>
<td>Zahidi Date Extract Based Halwa</td>
</tr>
</tbody>
</table>

3.5.1. Compositional profile and calorie count

The developed samples were analyzed for their moisture, crude protein, crude fat, ash and total carbohydrate content according to the methods of AOAC (2006) as described earlier (Sec. 3.3.2). However, the energy provided by each sample was calculated using the mathematical expression described by Hunt et al. (1987) as follows:

\[
\text{Total energy (kcal)} = (\text{Protein (g)} \times 4) + (\text{fat (g)} \times 9) + (\text{total carbohydrates (g)} \times 1.1 \times 3.75)
\]

3.5.2. Storage studies

The prototypes were stored for two weeks at 4 °C. Following analyses were conducted during the defined storage intervals (0, 7, and 14 days):

3.5.2.1. Physical attributes

3.5.2.1.1. Color

The developed halwa prototypes surface color, L* = lightness, a* = -a greenness; +a redness, and b* = -b blueness; +b yellowness were determined using Color Meter (CIELAB SPACE, Color Tech-PCM, USA). The samples were placed in a transparent Petri dish and positioned directly on the light path to measure the color parameter values of L*, a* and b*. The obtained data were used to compute chroma and hue angle as per the method described by Elleuch et al. (2014).

\[
\text{Chroma} = \sqrt{a^* + b^*} \\
\text{Hue angle} = \tan^{-1}(b^*/a^*)
\]
3.5.2.1.2. Texture profile

The triple beam snap (three-point break) method of Texture Analyzer (TA.XT plus, Stable Microsystems, UK) was used for assessing the texture of the halwa samples. A crosshead speed of 2 mm/second with a load cell of 50 kg was used. The force required to break the individual product was recorded and average values were calculated following the method described by Elleuch et al. (2014).

3.5.2.1.3. Water activity

Water activity in the date based halwa samples was measured using a hand held water activity meter (Rotronic aw-Dio) and the analysis was performed at 34°C.

3.5.2.2 Antioxidant profiling

The total phenolic and flavonoid contents of the developed products were measured following the procedures of Lemine et al. (2014) and Amira et al. (2012), respectively. Likewise, antioxidant activity of halwa samples was determined using TEAC, DPPH and FRAP assays as described earlier (Biglari et al., 2008; Al-Najada and Mohamed, 2014).

3.5.3 Sensory evaluation

Sensory evaluation of date halwa was carried out according to the method described by Lawless and Heymann (2010). The samples were presented in a perfectly homogeneous way i.e. identical conditions of preparation and presentation under soft white lighting conditions. They were evaluated for color, appearance, taste, texture and aroma (Appendix II). The mean value of these sensory properties indicated the overall acceptability of the samples. Bottled water along with unsalted crackers were served in between the sample to neutralize the palate. The samples were assessed based on a nine-point hedonic scale system, where one represents “disliked extremely” and 9 represents ‘liked extremely”. Sensory evaluation was done by a panel of judges (n=15) subjects from students and staff members of the National Institute of Food Science and Technology, University of Agriculture, Faisalabad. Date halwa samples were compared with the most consumed traditional halwa in this region, in order to predict the acceptance of the date halwa by the consumers.

3.6. Selection of best treatment

Based on in vitro analyses and hedonic response, the best date variety and extract were screened for the in vivo efficacy studies.
3.7. Bioefficacy studies

To explore the functional worth of dates against diet related disorders with special reference to cardiovascular complications and hepatic disorders, a model feeding trial was carried out. Purposely, 60 male Sprague Dawley rats (four weeks old) were placed in the animal room of National Institute of Food Science and Technology, University of Agriculture, Faisalabad. The rodents were housed individually in stainless steel cages in an air-conditioned room at 23±2°C, 55-60% relative humidity and 12-hour light-dark cycle. The animals were acclimatized by feeding basal diet for one week. They were fed on specific diets and tap water *ad libitum* throughout the experimental period.

**Experimental design**

Rats were randomly divided into six groups (n=10). G₁ served as control comprising of rats fed on normal diet, whereas in G₂ and G₃ groups, rats were fed on normal diet in conjunction with functional date fruit and date extract, respectively. However, in G₄, rats were given atherogenic diet comprising of normal rat chow along with cholesterol (1.5%), cholic acid (0.5%) and thiouracil (0.1%) to induce cardiac and hepatic stress while, in G₅ atherogenic rats were administered on date fruit based diet whilst, the rats in G₆ were fed on date extract along with simultaneous provision of atherogenic diet (Table 3.2). The dietary supplementation of date fruit @ 20% was used in case of date fruit fed rats while equivalent amount of extract was added to the diet of the date extract fed rats. The composition of diet is described in Appendix III. After twelve weeks trial, the overnight fasted rats were sacrificed and sera were collected for biomarkers assessment including lipidemic profile, antioxidant status as well as cardiac, hepatic and nephrotic stress indicators. The major organs including liver, kidney and heart were also collected and analyzed for stress indicators and their histoarchitecture was also studied.
3.7.1. Growth performance parameters

Net feed and drink intakes of rats were measured on daily basis during the entire study period. Growth performance of rats was assessed by measuring the change in body weight of experimental groups on weekly basis throughout the study period. Relative heart, liver and kidney weight were recorded immediately after the animals were killed. Likewise, feed efficiency ratio was calculated using following mathematical expression (Slemmer et al., 2012):

\[
\text{FER (\%)} = \frac{\text{Body weight gain (g)}}{\text{Feed intake (g)}} \times 100
\]

3.7.2. Serum lipidemic profile

Serum triglycerides (TG) and total cholesterol (TC) were determined using the commercially available Fluitest TG (Triglyceride GPO-PAP) and Fluitest Chol (Cholesterin CHOD-PAP) kits (Biocon, Vöhl-Marienhagen, Germany), respectively. However, high-density lipoproteins (HDL) were ascertained by HDL precipitant method using commercially available Ecoline kits (Merck, Germany). The analysis was performed on Semi Automated Clinical Chemistry Analyzer (Microlab 300, Merck, Netherlands). Moreover, Friedewald formula as described by Friedewald et al. (1972) was used for calculating low density lipoproteins (LDL), very low density lipoprotein (VLDL) and non-high density lipoprotein (nHDL):

\[
\text{LDL} = \text{Total cholesterol} - \text{HDL} - \text{VLDL}
\]
VLDL = \frac{\text{Triglycerides}}{5} \\
\text{nHDL} = \text{Total cholesterol} - \text{HDL}

### 3.7.2.1 Atherogenic ratios

Lipoprotein risk ratios including atherogenic index of plasma (AIP), atherogenic coefficient (AC), Castelli risk index (CRI) I and II were calculated using mathematical expressions (Jamil and Siddiq, 2012; Hassan et al., 2015).

\[
\text{AIP} = \frac{\log \text{TG}}{\text{HDL}} \\
\text{AC} = \frac{\text{TC} - \text{HDL}}{\text{HDL}} \\
\text{CRI(I)} = \frac{\text{TC}}{\text{HDL}} \\
\text{CRI(II)} = \frac{\text{LDL}}{\text{HDL}}
\]

### 3.7.3. Biomarkers of cardiac, hepatic and renal damage in serum

#### 3.7.3.1. Cardiac stress analyses

Aspartate transaminase (AST), creatine kinase (CK), creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) in rat serum were measured using commercial kits according to Miltonprabu and Thangapandiyan (2015). The AST level was determined by following the IFCC method (EC 2.6.1.1), while for the analysis of creatine kinase level in serum, Creatine Kinase Activity Assay kit (Colorimetric) (CK NAC Innoline, Merck) was used. Whereas, CK-MB was measured using Commercial kit method (Breurer and Breurer Diagnostic, Germany). Likewise, the LDH DGKC Kit (Breurer and Breuer Diagnostic, Germany) was used for the LDH assay.

#### 3.7.3.2. Hepatic stress indicators

Alanine transaminase (ALT) and alkaline phosphatase (ALP), gamma-glutamyl transferase (γ-GT) and total bilirubin (direct and indirect) were determined spectrophotometrically using manual commercial reagent kits (El Arem et al., 2014a). The AST/ALT ratio was also calculated. Optimized UV-test (Mod. IFCC method) through commercial kit was used for the ALT analysis. Alkaline phosphatase was analyzed via a kinetic photometric kit method (DGKC-Breuer and Breuer Diagnostic Germany). Commercial Kit (Gamma GT SL, Merck) was used for the analysis of γ-GT. The direct and total bilirubin was determined
using Jenrassik-Gróf method using commercial kit (Ecoline, Merck Germany). The indirect bilirubin is computed from the difference between the total and direct bilirubin.

**3.7.3.3. Renal stress markers**

The serum samples were analyzed for urea by Urease-GLDH: enzymatic UV test and creatinine according to Jaffé method employing commercial kits as described by El Arem *et al.* (2014b) to assess proper renal functionality. Serum glucose level was also measured to analyze the effect of date fruit containing diets by the enzymatic photometric test method GOD-PAP (Breuer and Breuer Diagnostic Kit, Germany).

**3.7.4. Serum and tissue antioxidant status and lipid peroxidation**

The collected serum was analyzed for superoxide dismutase, catalase and lipid peroxidation. However, the tissues mainly cardiac, hepatic and renal from all groups were excised, washed with ice, weighed, homogenized (10% w/v) in chilled phosphate buffer (50 mM, 300 mM NaCl, pH = 7.4) and centrifuged at 5000 rpm for 20 minutes in a high-speed cooling centrifuge (4 °C). The supernatant fractions were aliquoted, transferred in Eppendorf tubes and stored at -40°C for further assays. The clear supernatant was employed for assaying superoxide dismutase, catalase and lipid peroxidation.

**3.7.4.1. Superoxide dismutase activity**

SOD activity was assayed spectrophotometrically as described by Zargar *et al.* (2015). The enzyme activity was determined by mixing 250 µL phosphate buffer (50 mM, pH 7.8), containing 0.3 mM EDTA, 100 µL L-methionine, 100 µL trition X, 50 µL nitroblue tetrazolium and 400 µL distilled water. Then, 50 µL of the supernatant was added to the reaction mixture followed by the addition of 50 µL riboflavin. Later, the tubes were illuminated under UV light for 20 minutes. A control tube, in which the sample was replaced by a buffer, was also analyzed and the absorbance was noted at 560 nm. 1 unit of SOD represents the amount of enzymes that are required to inhibit the rate of NBT oxidation by 50% at 25°C. The SOD activity was calculated in Unit/mL for serum and Unit/g for tissue.

**3.7.4.2. Catalase**

The catalase activity in the rat tissues was measured according to the method described by Bulucu *et al.* (2008). The principle is based on the rate constant determination or the H₂O₂ decomposition rate at 240 nm. Purposely, 20 µL of the supernatant was added to a cuvette
containing 780 μL of phosphate buffer (pH 7.5). The reaction was initiated by the addition of 100 μL freshly prepared 500 mM hydrogen peroxide solution. The H₂O₂ disappearance was monitored kinetically at 240 nm for 1 minute at 25 °C. Enzyme content was calculated using an extinction coefficient of 0.0436 mM⁻¹cm⁻¹. One unit of activity is equal to 1 μmol of H₂O₂ destroyed/minute. The activity was expressed in Units/mL serum and Unit/g tissue for serum and tissue samples, respectively.

3.7.4.3. Lipid peroxidation (MDA)
Lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) in serum and tissue specimens using an established protocol (Li et al., 2000). For each sample, 0.5 mL serum/tissue homogenate was transferred into 3.0 mL of 20% TCA solution containing 0.5% TBA. After vortexing, each sample mixture was incubated in a 60°C water bath for 30 minutes, cooled in ice water bath and allowed to rest for 30 minutes. After cooling, each sample mixture was centrifuged at 10°C at 3000 rpm for 15 minutes. The absorbance of the upper organic layer of the centrifuged solution was measured at a wavelength of 532 nm and 600 nm with a microplate reader. The final unit of TBARS value was calculated and expressed as nM/mL in case of serum and nM/g in case of tissue in form of MDA (malondialdehyde) content.

3.7.5. Histopathology of cardiac, hepatic and renal tissues
Histopathological examination of heart, liver and kidney tissues was performed according to the protocols of Ragab et al. (2013) and Miltonprabu and Thangapandiyan (2015). The tissues (cardiac, hepatic and nephrotic) obtained from all experiment groups were fixed for 48 hours in ten percent buffered neutral formalin solution and dehydrated by passing successfully in different mixtures of ethyl alcohol, water, cleaned in xylene and embedded in the paraffin wax. Tissue sectioning (5-6 µm thick) were prepared by employing a rotary microtone and stained with hematoxylin and eosin dye that was mounted in a neutral deparaffined xylene medium. Then, the tissues were observed under light microscope and the photomicrographs were taken. Digital images were produced using a light microscope (MCX 100, Micros Austria).

3.7.6. Hematological analyses
Erythrocyte indices including hemoglobin (Hb), hematocrits (HCT), total red blood cells (RBC), mean corpuscle volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were assessed. Whilst, leukocyte indices
i.e. neutrophils, lymphocytes, monocytes, eosinophils, and basophils and thrombocytes were also analyzed. Moreover, the clotting profile was indicated through the prothrombin time using Soluplastin kit (Wiener Lab, Rosario, Argentina). To conduct the above analyses, Medonic M series (Boule Diagnostics Int AB, Stockholm, Sweden) hematology analyzer was used. Besides, the inflammatory marker i.e. erythrocyte sedimentation rate (ESR) was determined by Westergren’s method using an automated ESR system (Urasoko et al., 2009; Uzun and Kalender, 2013).

3.8. Statistical modeling

The data obtained for each parameter were subjected to statistical analysis to determine the level of significance. Completely randomized design (CRD) was applied using Statistical Package (Statistix 8.1). The levels of significance (P<0.05 and P<0.01) were determined by employing 2-factor factorial CRD in case of product storage study. Simple CRD was applied to the bioefficacy assessment data following the principles outlined by Mason et al. (2003). Significant ranges were further compared by post-hoc Tukey’s HSD test.
CHAPTER 4

RESULTS AND DISCUSSION

Diet and its imperative health effects have become a leading debate for the past few decades. The growing interest in the chemotherapeutic, disease prophylactic and preservative role of the phytoceutics in food and biological systems has created an urge among the food and health care professionals to utilize the nature blessed ingredients for better health and well-being. Purposely, the present research was tailored to elucidate the restorative potential of date fruit against oxidative stress mediated diseases with special reference to cardiac and hepatic stress. This study was divided into four modules; in the first phase locally available date varieties were ascertained for their compositional characteristics. Further, the bioactives were quantified using HPLC and the antioxidant activity was assessed. In the third module, the varieties and their respective extracts were then utilized in the development of date-based halwa. The physicochemical behavior, storage stability and hedonic response of the developed product was also determined. Finally, in the last part of the study, the bioefficacy assessment of the date fruit and extract was carried out on atherogenic diet induced oxidative stressed rats. Consequently, the obtained data were inferred statistically to determine the level of significance. The study was divided into following categories and subcategories:

Section 1: Compositional characterization
Section 2: Phytochemistry
Section 3: Date based functional product development
Section 4: Bioefficacy studies
   Part 1: Serum lipidemic and oxidative stress biomarkers
   Part 2: Cardiac stress biomarkers
   Part 3: Hepatic stress biomarkers
   Part 4: Renal stress biomarkers
   Part 5: Hematological aspects
SECTION 1: COMPOSITIONAL CHARACTERIZATION
4.1. Compositional profiling

The compositional traits of date fruit are imperative in determining its degree of maturation and predicted shelf life. These traits are related to its nutritional significance and possible health benefits. The first module of the study comprised of the detailed compositional profiling of the selected date varieties. The results regarding the composition are discussed below:

4.1.1. Proximate composition

Dates were analyzed for various quality attributes. The statistical analysis depicted significant variations in proximate composition of Dhaki, Aseel and Zahidi. It is evident from Table 4.1 that Aseel contains maximum moisture (17.71±0.67%) trailed by Dhaki (15.54±0.54%) and Zahidi (10.50±0.39%) cultivars. The protein content is more in Zahidi as 4.60±0.18% followed by Dhaki and Aseel as 4.22±0.15 and 3.72±0.14%, respectively. Dhaki, Aseel and Zahidi dates contain 1.28±0.04, 2.61±0.08 and 2.08±0.07% crude fat, respectively. Whereas, the fiber content was highest in the Zahidi cultivar (7.19±0.27%) followed by Dhaki (6.47±0.23%) and Aseel (4.84±0.17%). The ash content was recorded as 1.54±0.06, 0.94±0.03 and 1.91±0.07% in Dhaki, Aseel and Zahidi, correspondingly. The maximum NFE was found in Zahidi dates (73.72±2.65%), while minimum in the Aseel dates (70.19±2.11%).

The results are in general agreement with those reported previously (Al-Shahib and Marshall, 2003; Al-Farsi et al., 2005b; Bouhlali et al., 2015). The findings of the current investigations are also in line with the work of Mohamed et al. (2014) on five Sudanese date varieties in which the moisture content ranged from 8.78-10.68 g/100g and ash 1.96-2.50 g/100g. The varieties contained 78.73-80.41 g/100g carbohydrates, 2.37-3.14 g/100g fiber, 3.69 to 4.09 g/100g protein and 1.71-2.06 g/100g fat. Results suggest significant variations in the compositional traits like moisture, ash, carbohydrates and fat among the varieties. However, all the cultivars contained almost same amount of protein. El-Sohaimy and Hafez (2010) elucidated the biochemical profile of Egyptian dates and found that those cultivars contain moisture (13.80%), protein (3.00%), fat (2.90%), crude fiber (5.20%) and ash (2.13%). The overall chemical properties also relate to the work of Borchani et al. (2010) who studied the biochemical composition of 11 Tunisian date cultivars and concluded that Tunisian dates contain 9.43 to 23.34% moisture, 0.46 to 2.85% protein, 0.06 to 0.57% fat, 1.58 to 2.59% ash and 8.09 to 20.25% total dietary fiber. Similar compositional traits were reported by Ali et al. (2009) for Omani date varieties as moisture
18.77-23.71 g/100g, crude protein 1.28-1.89 g/100g, ash 1.12-1.55 g/100g, crude fat 1.14-2.37 g/100g and NFE 68.53-75.37 g/100g.

The health promoting potential of dates can also be attributed to the healthy components in the matrix, it contains less amount of fat along with more fiber and the higher ash content that also signifies the presence of more minerals. Moreover, the protein content is not very high but the scientific investigations declare that dates contain suitable amounts of several essential amino acids that include glycine, valine, histidine, aspartic acid, proline, arginine and leucine (El-Sohaimy and Hafez, 2010). Bouhlali et al. (2015) stated that 100 g dates can provide 9.94 to 18.86% of an adult daily energy requirements. In the present study, the compositional traits varied significantly with respect to varieties. However, the cultivar having the lowest moisture content i.e. Zahidi contained higher amounts of protein, fiber, ash and NFE, while Dhaki also possessed good composition after Zahidi. Besides, the Aseel variety contented highest moisture and fat content among the three cultivars. The high fat content could be due to the fact that the Aseel dates are coated with a very thin layer of oil to improve their shelf stability and luster before reaching to the market. These results and previous findings clearly shows that considerable variations exist among the varieties of same region as well as different regions, these differences can be related to the geographical conditions of a particular area and genetic variations among the cultivars.

4.1.2. Mineral profile

Micronutrients are involved in all cellular and metabolic processes. They play significant role in the growth and development. These entities are required in small amounts but their absence may lead to severe complications. It is evident from Table 4.2 pertaining the F statistic values that treatments exhibited substantial variations regarding the mineral profile of selected cultivars. Mineral content determination indicated that potassium is dominant in all the selected varieties. Highest potassium content was found in Zahidi dates (870.83±32.39 mg/100g), followed by Dhaki (640.23±24.97 mg/100g) and Aseel (577.30±17.90). Calcium content was also higher in Zahidi as 96.86±3.18 mg/100g trailed by Aseel (58.42±2.04 mg/100g) and Dhaki (37.19±1.30 mg/100g). Similar trend was observed in case of sodium, as highest content of 8.49±0.31 mg/100 g was found in Zahidi and lowest in Dhaki (3.27±0.10 mg/100 g) on fresh weight basis.
Table 4.1. Proximate composition of selected date cultivars

<table>
<thead>
<tr>
<th>Compositional analyses</th>
<th>Dhaki</th>
<th>Aseel</th>
<th>Zahidi</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>15.54±0.54b</td>
<td>17.71±0.67a</td>
<td>10.50±0.39c</td>
<td>108.82**</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>4.22±0.15ab</td>
<td>3.72±0.14b</td>
<td>4.60±0.18a</td>
<td>14.10*</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>1.28±0.04c</td>
<td>2.61±0.08a</td>
<td>2.08±0.07b</td>
<td>215.76**</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>6.47±0.23a</td>
<td>4.84±0.17b</td>
<td>7.19±0.27a</td>
<td>49.38**</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.54±0.06b</td>
<td>0.94±0.03c</td>
<td>1.91±0.07a</td>
<td>177.97**</td>
</tr>
<tr>
<td>Nitrogen free extract (%)</td>
<td>70.95±2.36</td>
<td>70.19±2.11</td>
<td>73.72±2.65</td>
<td>1.83NS</td>
</tr>
</tbody>
</table>

Values represent mean±SD; One-way ANOVA followed by Tukey’s HSD multiple comparison tests
* = Significant
**= Highly significant
NS = non-significant

Table 4.2. Mineral content distribution in selected date cultivars

<table>
<thead>
<tr>
<th>Minerals (mg/100g F.W.)</th>
<th>Dhaki</th>
<th>Aseel</th>
<th>Zahidi</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>640.23±24.97b</td>
<td>577.30±17.90c</td>
<td>870.83±32.39a</td>
<td>132.98**</td>
</tr>
<tr>
<td>Calcium</td>
<td>37.19±1.30c</td>
<td>58.42±2.04b</td>
<td>96.86±3.18a</td>
<td>512.11**</td>
</tr>
<tr>
<td>Sodium</td>
<td>3.27±0.10c</td>
<td>6.37±0.27b</td>
<td>8.49±0.31a</td>
<td>335.05**</td>
</tr>
<tr>
<td>Iron</td>
<td>1.98±0.07b</td>
<td>1.58±0.04c</td>
<td>3.23±0.11a</td>
<td>341.43**</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.33±0.01c</td>
<td>0.82±0.03a</td>
<td>0.52±0.01b</td>
<td>587.61**</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.41±0.02b</td>
<td>0.41±0.02b</td>
<td>0.52±0.02a</td>
<td>53.42*</td>
</tr>
<tr>
<td>Copper</td>
<td>0.25±0.01c</td>
<td>0.34±0.01b</td>
<td>0.65±0.02a</td>
<td>178.47**</td>
</tr>
<tr>
<td>Cobalt</td>
<td>0.50±0.02a</td>
<td>0.48±0.02a</td>
<td>0.39±0.01b</td>
<td>12.33*</td>
</tr>
</tbody>
</table>

Values represent mean±SD; One-way ANOVA followed by Tukey’s HSD multiple comparison tests
* = Significant
**= Highly significant
However, Aseel contained 6.37±0.27 mg/100g sodium. Iron was also higher in Zahidi variety as compared to the other two cultivars. The recorded iron contents for Dhaki, Aseel and Zahidi were 1.98±0.07, 1.58±0.04 and 3.23±0.11 mg/100g, respectively.

Micro mineral profile showed maximum zinc content in Aseel as 0.82±0.03 mg/100g followed by Zahidi 0.52±0.01 mg/100g and lowest in Dhaki 0.33±0.01 mg/100g. The manganese content was higher in Zahidi (0.52±0.02 mg/100g), while Dhaki and Aseel contained similar amounts as 0.41±0.02 and 0.41±0.02 mg/100g, respectively. Likewise, copper content was more in Zahidi as 0.65±0.02 mg/100g trailed by Aseel 0.34±0.01 and Dhaki 0.25±0.01 mg/100g. Maximum cobalt content was found in Dhaki variety (0.50±0.02 mg/100g), followed by Aseel (0.48±0.02 mg/100g) and lowest in Zahidi (0.39±0.01 mg/100g).

Mineral content in a particular food also depends on the type of soil. Similar results were obtained by Mohamed et al. (2014) for six Sudanese date varieties. Their results reveals the presence of calcium (222.20-293.04 mg/100g), magnesium (66.30-120.88 mg/100g), sodium (55.56-139.11 mg/100g), potassium (691.67-1088.40 mg/100g) and phosphorous (150.19-232.04 mg/100g). Among the micro minerals, iron (4.06-7.06 mg/100g), copper (0.71-1.86 mg/100g), zinc (0.66-1.00 mg/100g) and manganese (0.54-0.78 mg/100g) were present. The broader ranges covers almost all characterized minerals in the present study, however, low sodium content was found the Pakistani varieties as compared to the Sudanese. The potassium and calcium content in the Egyptian date cultivars was similar to the Aseel cultivar i.e. 521 and 65 mg/100g, respectively as reported by El-Sohaimy and Hafez (2010). In 11 explored Tunisian date cultivars, the potassium content ranged from 404.19 to 774.71 mg/100g that is slightly lower from the potassium content in the studied Zahidi dates, while the other two varieties fits well in the given range. Likewise, those cultivars also contain lower calcium content than Zahidi with maximum value of 36.52 mg/100g. The sodium and iron content in Tunisian dates range from 5.27 to 25.14 and 1.06 to 2.30 mg/100g, respectively (Borchani et al., 2010) that are somewhat similar to the studied dates. The results are also in corroboration with the findings of Bouhlali et al. (2015) who studied the mineral composition of eight date varieties from Morocco having higher potassium, calcium and magnesium content as compared to the other minerals. The variations in the mineral content as explained earlier might be a reason of varietal, environmental, genetic and phenotypic dissimilarities amongst the cultivars.
The results clearly show that dates are good source of minerals especially potassium, calcium, iron and zinc. Studies also suggest the presence of selenium in date fruit that can also responsible for its beneficial activities (Baliga et al., 2011). Scientific evidences suggest that dietary intake of copper and manganese plays an imperative role in modulating the blood pressure and ameliorating the lipid profile (Kim and Choi, 2013). These minerals along with iron and zinc are integral to many metabolic pathways. Specially zinc, iron and manganese are amongst the highly utilized metals by the enzymes in the biological catalysis (Andreini et al., 2008). Besides, Aaron and Sanders (2013) deduced that dietary potassium attenuates endothelial dysfunction, hypertension and kidney disease progression as a result of excessive salt intake. Recent meta-analysis and clinical trials are also focusing on sodium reduction and potassium supplementation for lowering the incidence of hypertension and morbidity from the cardiovascular events (Whelton and He, 2014). Higher potassium intake is associated with a 24% decreased risk of stroke according to a meta-analyses (Aburto et al., 2013). Hence, high potassium content in date fruit coupled with low sodium content may serve as an approach towards the control of hypertension and related ailments. Among the analyzed cultivars, Zahidi cultivar contains better potassium, calcium, copper, iron and manganese content as compared to Dhaki and Aseel. Incorporating Zahidi dates in the daily diet could be a preventive measure against various physiological threats.

4.1.3. \( \beta \)-Carotene

\( \beta \)-carotene is the most potent precursor of vitamin A having significant antioxidant activity (Gul et al., 2015). \( \beta \)-carotene was quantified using High Performance Liquid Chromatography (HPLC). The F statistic relating to \( \beta \)-Carotene content explicates momentous varietal variations (Table 4.3). The results depict that \( \beta \)-carotene content was higher in Dhaki and Zahidi as 0.54±0.01 and 0.47±0.01 mg/100g, respectively and lower in Aseel (0.32±0.01 mg/100g).

Carotenoids are the fat soluble pigments that impart color to the plants apart from being an important source of vitamin A. They also act as antioxidants, preventing the cells from free radical damage. Humans lack the ability of de novo synthesis of vitamin A, therefore the precursors such as \( \beta \)-carotene from dietary sources are required to meet the physiologic demands (Haskell, 2012). \( \beta \)-carotene is present in date fruits in different amounts depending on the variety and ripening stage (Baliga et al., 2011). The pro-vitamin A activity in the Algerian date fruits range between 0.5 to 11.7 RE/100g, however, the \( \beta \)-carotene content range from 2.5 to 6.4 µg/100g that is much lower than our findings.
(Boudries et al., 2007). Difference could probably be due to the varietal variations. The β-carotene content reported by Gross et al. (1983) was 0.06 and 0.116 mg/100g for Deglet Noor and Hayany cultivars, respectively. There are not much studies regarding the β-carotene content of date fruits. Therefore, scarce data is available. However, Al-Farsi et al. (2005a) studied the total carotenoid content of fresh dates that ranged between 1.31 to 3.03 mg/100g. Present results regarding the β-carotene content may relate to the overall carotenoid content. Epidemiological studies also suggest the disease preventive role of β-carotene particularly against cardiac disorders and malignancies (Gul et al., 2015).

Amongst the studied varieties, Dhaki dates contain the maximum β-carotene content as compared to the others.

4.1.4. Vitamin C

Vitamin C is a water soluble vitamin and an electron donor that accounts for all its beneficial antioxidative functions. Vitamin C quantification was carried out using HPLC. Table 4.3 shows that the treatments exhibit significant variations regarding the Vitamin C content. The results reveal maximum vitamin C content in Zahidi (63.38±0.95 mg/100g) followed by Aseel as 46.58±0.71 mg/100g and Dhaki 24.44±0.34 mg/100g.

Vitamin C is present in significant quantity in date fruits (Al-Farsi and Lee, 2008). The findings of the present study are in corroboration to the results of Mrabet et al. (2008) who delineated 24-46 mg/100g of vitamin C in littoral varieties of Southern Tunisia. However, the results obtained are lower than those reported earlier by Al Juhaimi et al. (2014) who found 97.18 to 145.15 mg/100g vitamin C in Saudi date varieties. The difference in cultivars and conditions may be a reason of variations in the vitamin content. Moreover, the extraction method also effects the determined concentrations. Vitamin C is required the biosynthesis of different hormones and collagen. Scientific evidences correlated the intake of foods rich in vitamin C and reduced risk of CVDs (Li and Schellhorn, 2007). Amongst the studied cultivars, vitamin C content is more in Zahidi and if consumed adequately these dates can serve as a promising source of vitamin C.

4.1.5 Sugars

The sugar profile in the selected date cultivars is displayed in Table 4.4. The F value elicited significant variations regarding the carbohydrate, glucose, fructose and sucrose content. The total carbohydrate content determined through approximate method was highest in the Zahidi variety as 80.91±2.82% followed by Dhaki 77.42±2.51% and Aseel 75.03±2.18%.
### Table 4.3. HPLC quantification of \(\beta\)-Carotene and Vitamin C

<table>
<thead>
<tr>
<th>Vitamins (mg/100g F.W)</th>
<th>Dhaki</th>
<th>Aseel</th>
<th>Zahidi</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta)-Carotene</td>
<td>0.54±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.47±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1377.79**</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>24.44±0.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46.58±0.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.38±0.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2271.91**</td>
</tr>
</tbody>
</table>

Values represent mean±SD; One-way ANOVA followed by Tukey’s HSD multiple comparison tests
* = Significant
**= Highly significant

### Table 4.4. Sugar profiling of selected date cultivars

<table>
<thead>
<tr>
<th>Sugars (%)</th>
<th>Dhaki</th>
<th>Aseel</th>
<th>Zahidi</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Carbohydrates</td>
<td>77.42±2.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.03±2.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.91±2.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.14*</td>
</tr>
<tr>
<td>Reducing Sugars</td>
<td>31.31±0.69&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.90±0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.51±0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>195.05**</td>
</tr>
<tr>
<td>Glucose</td>
<td>12.61±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.10±0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.41±1.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>477.13**</td>
</tr>
<tr>
<td>Fructose</td>
<td>18.70±0.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.80±0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.10±0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>769.11**</td>
</tr>
<tr>
<td>F/G Ratio</td>
<td>1.48±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.84±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.79±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>550.37*</td>
</tr>
<tr>
<td>Non-reducing Sugar (Sucrose)</td>
<td>20.11±0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.22±0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.15±0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>678.78**</td>
</tr>
<tr>
<td>Total Sugars</td>
<td>51.42±0.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.12±0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.66±0.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>171.22*</td>
</tr>
</tbody>
</table>

Values represent mean±SD; One-way ANOVA followed by Tukey’s HSD multiple comparison tests
* = Significant
**= Highly significant
NS = non-significant
The HPLC determination of reducing and non-reducing sugars revealed highest reducing sugars in Zahidi (39.51±0.54%) trailed by Aseel (34.90±0.46%) and Dhaki (31.31±0.69%). Amongst, glucose was present in higher amounts in Dhaki as 12.61±0.15% as compared to Aseel 9.10±0.13% and Zahidi 10.41±1.49% varieties. While, better fructose content was exhibited by Zahidi i.e. 29.10±0.38% followed by Aseel 25.80±0.37% and Dhaki 18.70±0.28%. The fructose to glucose ratio was 1.48±0.02, 2.84±0.04 and 2.79±0.04 in Dhaki, Aseel and Zahidi cultivars, respectively. Maximum amount of sucrose (the non-reducing sugar) was found in the Zahidi variety (26.15±0.37%) followed by Dhaki (20.11±0.29%). Whilst, minimum sucrose content was recorded for Aseel (17.22±0.24%).

The findings of Al-Asmari et al. (2017) regarding the sugars in Saudi date cultivars shows more sugar content in those cultivars as compared to the ones studied. The selected Saudi cultivars contains sucrose 42.85-43.78%, glucose 33.58-34.93% and fructose 22.20-22.67%, while the glucose to fructose ratio varies from 1.48 to 1.57. The difference in the sugar content may be attributed to the physicochemical composition of the varieties. Higher moisture content was also reported in the aforementioned cultivars. A change in the concentration and types of sugars is also associated with the developmental stages. The results are also in harmony with the findings of El-Sohaimy and Hafez (2010) regarding the sugar profile of Egyptian dates. The Egyptian date cultivars contained 73% carbohydrates. The reported dominant sugars were glucose, fructose and sucrose, while lower amounts of xylose, lactose, mannose and lactulose were also quantified.

The results are also in agreement with the findings of Borchani et al. (2010) who reported 79.93 to 88.02% carbohydrates in 11 date cultivars of Tunisia. Amongst the sugars, more reducing sugars were found as compared to sucrose except a few cultivars that contained more non-reducing sugars as Deglet Nour. The glucose and fructose content ranged from 19.85 to 44.79% and 16.54 to 41.17%, respectively. The sucrose content was as low as 0.63% in Touzerzailet variety and as high as 51.14% in the Deglet Nour. Similar findings were obtained by Bouhlali et al. (2015) regarding the Moroccan date cultivars with more reducing sugars. These sugars present in the fruit dates; glucose, fructose and sucrose are easily digestible and are required for energy production and various other metabolic processes. Moreover, Ali et al. (2009) inferred that dates can be considered as low to medium glycemic index foods, thus can be an effective part of healthy diet planning for diabetic, obese or heart patients. The present results also suggest that Zahidi is sweeter than Dhaki and Aseel, hence it can be incorporated in food products as a natural sweetener.
SECTION 2: PHYTOCHEMISTRY
4.2. Phytochemical fingerprinting

The concentration of major phenolic compounds that were determined by employing HPLC analysis are presented in Table 4.5. One hydroxybenzoic acid, *i.e.* gallic acid was identified and quantified in all the three varieties. Highest gallic acid content was found in the Zahidi date variety as 192.28±2.58 mg/100g F.W. trailed by Aseel as 128.23±1.71 mg/100g F.W. Nevertheless, much lower content of gallic acid was obtained in the Dhaki variety *i.e.* 16.31±0.22 mg/100g F.W. Caffeic acid was also present in higher amounts in the Zahidi dates 108.64±1.36 mg/100g F.W. while, 7.21±0.10 mg/100g F.W. was found in Dhaki dates and it was not identified in the Aseel cultivar. Sinapic acid was detected and quantified in Aseel and Zahidi dates only as 2.25±0.03 and 4.40±0.06 mg/100g F.W., respectively. Additionally, *m* -coumaric acid was only quantified in the Zahidi variety as 1.49±0.03 mg/100g F.W. The Dhaki dates contained ferulic acid and cinnamic acid as 10.45±0.13 and 5.05±0.07 mg/100g F.W., respectively. Interestingly, higher amounts of vanillic and chlorogenic acids were found in the Aseel cultivar *i.e.* 64.88±0.84 and 24.66±0.33 mg/100g F.W., respectively.

The flavonoid composition of date fruit is presented in Table 4.6. Amongst the flavonols, kaempferol and quercetin were identified in all the cultivars, while myricetin was only found in the Dhaki variety. The kaempferol content ranged from 31.98 to 49.36 mg/100g F.W. among the cultivars that can be ranked from highest to lowest content as Zahidi>Aseel>Dhaki. Similarly, the maximum quercetin content was obtained in Zahidi (2.84±0.04 mg/100g F.W.) followed by Dhaki (2.23±0.03 mg/100g F.W.) and Aseel (0.79±0.01 mg/100g F.W.). Surprisingly, myricetin was only detected in the Dhaki variety as 28.41±0.39 mg/100g on fresh weight basis. Amongst the flavan-3-ols, catechin and epigallocatechin gallate (EGCG) content was determined. All three varieties contained minor amount of catechins ranging from 0.48±0.01 to 1.30±0.02 mg/100g F.W. with highest content in Aseel and lowest in Zahidi. Nonetheless, the epigallocatechin gallate content was 11.91±0.16, 6.75±0.10 and 6.22±0.08 mg/100g F.W. in Aseel, Zahidi and Dhaki, respectively.

HPLC is an efficient technology for the identification as well as quantification of the polyphenolic constituents present in the plant matrix (Khoddami *et al*., 2013). The components identified and quantified in the present research are in harmony with the previous investigations, however, variations also exist in the type and content of phenolics and flavonoids in different cultivars (Al-Farsi *et al*., 2005a; Mansouri *et al*., 2005). In this
regard, El-Sayed et al. (2015) quantified five phenolic acids i.e. coumaric, ferulic, gallic, hydroxybenzoic and hydroxycinnamic acids along with five flavonoids namely quercetin, catechin, lutein, epicatechin and apigenin in Berne date cultivar. Coumaric acid and quercetin are more in content in the Berne dates as compared to the other phytoceutics. Similar findings were obtained by El-Mousalamy et al. (2016) who obtained p-coumaric, caffeic, ferulic, chlorogenic and sinapic acids along with quercetin, apiginin and luteoline. The results are also in agreement with the findings of El Sohaimy et al. (2015) who evaluated the phenolic profile of Egyptian dates. The authors delineated that Egyptian dates contain more aliphatic antioxidants than the aromatic ones. The major polyphenols characterized in those dates were gallic acid, itaconic acid, catechin, esculetin, ferulic acid, and tannic acid.

The results are also in line with the previous research of Farag et al. (2014) who elucidated the metabolomic fingerprints of 21 Egyptian date varieties. The varieties contained quercetin conjugates, glycosides of apigenin and luteolin and hydroxycinnamic acid conjugates. Similarly, El Arem et al. (2014a) quantified twelve polyphenolic moieties in Delga date extract. The authors reported higher content i.e. 5.50 mg/100 g F.W. of ferulic acid, 4.71 mg/100 g of p-coumaric acid and 4.64 mg/100 g of caffeic acid. The other identified polyphenols include gallic, protocatechuic, m-hydroxybenzoic, chlorogenic, syringic, phenylacetic, catechin and o-coumaric acid. Similar polyphenolic profile has been reported for Algerian dates that contain gallic acid as a main free phenolic followed by p-coumaric and ferulic acid with traces of caffeic acid. In another study, Ragab et al. (2013) investigated few individual phenolics in Ajwa dates from Madinah, Saudi Arabia and concluded that Ajwa dates contain 8.1 mg/kg catechin, 7.2 mg/kg rutin and 5.9 mg/kg caffeic acid. They also stated that these bioactives might also be responsible for the reversal of oxidative damage induced by lead, thus possessing significant tissue protective abilities.

The results show that Zahidi dates are a promising source of gallic acid that has attracted a great deal of nutritionist as well as consumer attention owing to its reported health benefits. Gallic acid possess significant cardioprotective, cholesterol lowering and immunomodulatory potentials (Hsu and Yen, 2007; Kulkarni and Swamy, 2015). Contrarily, some phenolics and flavonoids were not identified in all cultivars as evident from the results. Moreover, significant variations were noted in the content of similar phenolics in different cultivars. Zahidi contained the maximum phenolics and flavonoids, while chlorogenic and vanillic acid were only quantified in Aseel. Interestingly, only Dhaki
### Table 4.5. Phenolic acids in selected date cultivars

<table>
<thead>
<tr>
<th>Phenolic acids (mg/100g F.W.)</th>
<th>Dhaki</th>
<th>Aseel</th>
<th>Zahidi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>16.31±0.22</td>
<td>128.23±1.71</td>
<td>192.28±2.58</td>
</tr>
<tr>
<td>m-coumaric acid</td>
<td>-</td>
<td>-</td>
<td>1.49±0.03</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>10.45±0.13</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>5.05±0.07</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>-</td>
<td>24.66±0.33</td>
<td>-</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>-</td>
<td>64.88±0.84</td>
<td>-</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>-</td>
<td>2.25±0.03</td>
<td>4.40±0.06</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>7.21±0.10</td>
<td>-</td>
<td>108.64±1.36</td>
</tr>
</tbody>
</table>

### Table 4.6. Flavonoids in selected date cultivars

<table>
<thead>
<tr>
<th>Flavonoids (mg/100g F.W.)</th>
<th>Dhaki</th>
<th>Aseel</th>
<th>Zahidi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epigallocatechin gallate</td>
<td>6.22±0.08</td>
<td>11.91±0.16</td>
<td>6.75±0.10</td>
</tr>
<tr>
<td>(+)Catechin</td>
<td>1.07±0.015</td>
<td>1.30±0.02</td>
<td>0.48±0.01</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>31.98±0.40</td>
<td>38.94±0.49</td>
<td>49.36±0.63</td>
</tr>
<tr>
<td>Quercetin</td>
<td>2.23±0.03</td>
<td>0.79±0.01</td>
<td>2.84±0.04</td>
</tr>
<tr>
<td>Myricetin</td>
<td>28.41±0.39</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
dates contain myricetin that is a potent antioxidant. Therefore, different cultivars possess different polyphenols that ultimately affects their antioxidant activity and mode of action against different ailments. Thus, dietary inclusion of such foods having a vista of health promoting biological components is required for an optimal health and well-being.

4.3. *In vitro* antioxidant potential

Total phenolics and flavonoids are secondary plant metabolites responsible for multiple physiological functions owing to their significant antioxidant potential. The phenolic profile of date fruit contains both bound and unbound phenolics, one method for the detection of antioxidant activity seems insufficient due to their different mode of actions. Therefore, the colorimetric quantification of polyphenols and flavonoids was carried and different methods were used to assess the antioxidant activity of the selected date fruit cultivars.

4.3.1 Total phenolic content (TPC)

The total polyphenols assessed through Folin-Ciocalteu based colorimetric method significant variations with respect to varieties as evident from F statistics in Table 4.7. The Zahidi variety possessed significantly higher amount of phenolics as 425.29±17.56 mg GAE/100g than the Aseel and Dhaki dates that contained 263.33±11.38 and 247.84±10.43 mg GAE/100g, respectively.

The results obtained in the present study are comparable to the previous investigations. The total phenolic content ranged from 76.74 to 122.20 mg GAE/100g F.W. in some studied Saudi cultivars (Al-Asmari *et al.*, 2017). However, in present study, higher phenolic contents are found that could be due to several reasons. The main determinants could possibly be the cultivar variations, climatic conditions, soil type, genetic factors and/or growth conditions (Al-Farsi and Lee, 2008). Higher phenolic content *i.e.* 14.80 mg GAE/g was found in Egyptian dates as reported by El Sohaimy *et al.* (2015). Broader range of phenolic content in Egyptian date cultivars *i.e.* 233-1897 mg/100g was reported by Farag *et al.* (2014). The data is also in agreement with the previous findings of Lemine *et al.* (2014) who found total phenolics in fully mature Mauritian dates in the range of 405.5-661.1 mg GAE/100g DM. Likewise, Wu *et al.* (2004) reported higher phenolic content in dates (tow cultivar) *i.e.* around 572-661 mg GAE/100g F.W. The findings are also in confirmation with the previous results of Bouhlali *et al.* (2015) who found the phenolic content of Moroccan cultivars between 331.86 and 537.07 mg GAE/100g. The findings of
Kchaou et al. (2014) are also in corroboration with the present findings. They reported 505.49, 240.38 and 391.94 mg GAE/100g phenolics in Allig, Deglet Nour and Bejo cultivars of Tunisia, respectively. While, the Degla cultivar of Tunisia contains 417 mg GAE/100g phenolics on fresh weight basis (El Arem et al., 2014a). The phenolic content in Algerian dates was assessed by Benmeddour et al. (2013) who delineated that Algerian date cultivars contain 167-709 mg GAE/100g phenolics on fresh weight basis. However, Hasnaoui et al. (2012) reported lower phenolic content ranged from 171.39 to 353.92 mg GAE/100g in the Moroccan date cultivars. Similarly, in Sudanese date cultivars lower TPC values were recorded as 35.82 to 199.34 mg GAE/100g (Mohamed et al., 2014). The discordance in results may also be attributed to the phenolic standards and the units in which data is expressed. Moreover, choice of solvent, solubility and extractability of polyphenols might also affect the end results (Harris and Brannan, 2009).

4.3.2 Flavonoid content

Flavonoids hold considerable importance as antioxidants because of their high redox potential that allows them to act as hydrogen donors, reducing agents and singlet oxygen quenchers (Ignat et al., 2011). The aluminum chloride method was used for the determination of total flavonoids. The selected date cultivars expounded momentous variations regarding the flavonoid content as reflected by the F statistics in Table 4.7. The maximum flavonoid content i.e. 204.45±7.21 mg QE/100g was found in the Dhaki date extract followed by 162.48±7.37 mg QE/100g in Zahidi and 145.97±6.25 mg QE/100g in the Aseel varieties. The results are also in corroboration with the earlier findings of Bouhlali et al. (2015) who reported the flavonoid content between 68.87 and 208.53 mg RE/100g in Moroccan date cultivars. While in other investigation on Moroccan dates, the reported flavonoid content varied from 43.28-84.96 mg QE/100g (Hasnaoui et al., 2012). Likewise, Lemine et al. (2014) reported much lower flavonoid content in fully mature Mauritian dates ranging between 39.5 to 112.5 mg QE/100g D.M. The findings are also in corroboration with the results of Kchaou et al. (2014) who reported 58.92 to 213.76 mg CE/100 g in Tunisian dates. The reported flavonoid content in Degla cultivar of Tunisia was 285.23 mg/100g (El Arem et al., 2014a). The total flavonoid content in Algerian date cultivars range from 11.52 to 225.77 mg QE/100 g F.W. as delineated by Benmeddour et al. (2013). In another study, Mohamed et al. (2014) investigated the flavonoid content in Sudanese date cultivars. They delineated that flavonoid content in Sudanese dates range from 1.74 to 3.39 mg CE/100g.
Table 4.7. Antioxidant indices of date fruit extracts

<table>
<thead>
<tr>
<th>Antioxidant assay</th>
<th>Dhaki extract</th>
<th>Aseel extract</th>
<th>Zahidi extract</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolics (mg GAE/100g)</td>
<td>247.84±10.43\textsuperscript{b}</td>
<td>263.33±11.38\textsuperscript{b}</td>
<td>425.29±17.56\textsuperscript{a}</td>
<td>160.80**</td>
</tr>
<tr>
<td>Total flavonoids (mg QE/100g)</td>
<td>204.45±7.21\textsuperscript{a}</td>
<td>145.97±6.25\textsuperscript{b}</td>
<td>162.48±7.37\textsuperscript{b}</td>
<td>57.50**</td>
</tr>
<tr>
<td>DPPH (%)</td>
<td>79.66±2.06\textsuperscript{b}</td>
<td>84.72±2.76\textsuperscript{ab}</td>
<td>90.48±3.45\textsuperscript{a}</td>
<td>11.01*</td>
</tr>
<tr>
<td>FRAP (µM Fe\textsuperscript{2+}/g)</td>
<td>0.90±0.03\textsuperscript{a}</td>
<td>0.64±0.02\textsuperscript{c}</td>
<td>0.76±0.03\textsuperscript{b}</td>
<td>69.58**</td>
</tr>
<tr>
<td>TEAC (µM Trolox/g)</td>
<td>4.10±0.15\textsuperscript{b}</td>
<td>3.92±0.11\textsuperscript{b}</td>
<td>5.50±0.15\textsuperscript{a}</td>
<td>117.92*</td>
</tr>
<tr>
<td>H\textsubscript{2}O\textsubscript{2} scavenging capacity (%)</td>
<td>87.78±2.90</td>
<td>90.33±2.34</td>
<td>91.76±2.88</td>
<td>1.63\textsuperscript{NS}</td>
</tr>
<tr>
<td>Reducing power (%)</td>
<td>88.23±2.84\textsuperscript{a}</td>
<td>79.45±2.59\textsuperscript{b}</td>
<td>85.70±3.03\textsuperscript{ab}</td>
<td>7.72*</td>
</tr>
</tbody>
</table>

Values represent mean±SD; One-way ANOVA followed by Tukey’s HSD multiple comparison tests

* = Significant

**= Highly significant

\textsuperscript{NS} = non-significant
respectively. The range is much lower than our results, these varieties also contained lower amount of phenolics as well that may perhaps be due to the difference in cultivars, cultivation region and conditions.

4.3.3. 1,1-diphenyl-1-picrylhydrazyl (DPPH) antiradical ability

In the DPPH activity assay the antioxidants are allowed to react to the free DPPH (1,1-diphenyl-1-picrylhydrazyl) radical and its reduction is monitored (Aruoma, 2003). The DPPH activity of the tested date cultivars is shown in Table 4.7. Considerable variations in DPPH activity existed within the cultivars. Means for DPPH radical scavenging potential explicated highest percentage 90.48±3.45% for Zahidi extract, whilst lowest for Dhaki extracts as 79.66±2.06%.

The percent inhibition observed by the Egyptian date extract @ 100 mg was 79.32 that is similar to the present findings (El Sohaimy et al., 2015). The results are in confirmation with the previous results stating significant DPPH activity in date fruit extracts from different regions (Lemine et al., 2014; Bouhlali et al., 2015). The DPPH based antioxidant activity of 21 Omani date cultivars ranged between 40 and 86% as stated by Al-Harrasi et al., (2014). The results are in line with the findings of El Arem et al. (2014a) who reported 89.14% DPPH scavenging activity in Tunisian Delga date extract. In an another investigation, Kchaou et al. (2014) stated that scavenging capacity of date extract against DPPH is dose dependent at 20 mg/mL, the radical scavenging activity of Tunisian dates range between 23.98 and 58.77%. However, in the present study the percent inhibition is calculated only. The authors also reported high correlation between DPPH activity and polyphenolic content. Therefore, higher phenolic and flavonoid content in the studied dates may relate to their high DPPH activity. In their earlier study, Kchaou et al. (2013) reported 57.54 to 90.12% DPPH activity in the methanolic date extracts. Noticeably, the Tunisian Zehdi date extract exhibited 88.24% DPPH activity. The strongest DPPH antiradical efficiency in Algerian date cultivars was noted for the Ghazi cultivar i.e. 86% while minimum capacity was observed in case of Thouri cultivar i.e. 32.4% (Benmeddour et al., 2013). In previously studied Pakistani date varieties, the DPPH activity ranged from 85.75 to 90.96% in the methanolic extracts as outlined by Anjum et al. (2012). The differences in results are probably due to the varietal variations as described
earlier. Moreover, the other determinants of the DPPH activity also include the degree of polymerization and hydroxylation (Moure et al., 2001).

4.3.4. Ferric reducing antioxidant power (FRAP)

FRAP determines the electron donating ability of the date extract that lead to the cessation of the free radical mediated chain reactions. The tested date cultivars explicated momentous variations regarding the FRAP activity as reflected by the F statistics in Table 4.7. The FRAP values for Dhaki, Aseel and Zahidi extracts were 0.90±0.03, 0.64±0.02 and 0.76±0.03 µM Fe^{2+}/g, correspondingly.

The results are in agreement with the previous findings of Bouhlali et al. (2015) who reported that Moroccan date cultivars exhibit significant ferric reducing antioxidant capacity. In another study, Mohamed et al. (2014) studied the antioxidant activity in Sudanese date cultivars using FRAP assay. Varietal differences were reported in case of FRAP ranging between 2.82 and 27.50 mM/100g. One of their peers, Kchaou et al. (2014) examined the FRAP activity of Tunisian dates that varied between 1.96 and 4.98 mM FESO_{4}/100 g. In the present study, Dhaki extract possessed better FRAP ability that may indicate its potential to reduce ferric ions as compared to the other date extracts. Detailed studies focusing on the feric ion reducing potential of Dhaki dates are required for a more conclusive approach.

4.3.5. Trolox equivalent antioxidant capacity (TEAC)

TEAC (trolox equivalent antioxidant capacity) reflects the hydrogen/electron donating relative ability of the antioxidants to scavenge the ABTS radical (Antolovich et al., 2002). Statistical assessment of TEAC values revealed considerable variations between the cultivars. It is evident from Table 4.7 that maximum TEAC was exhibited by the Zahidi extract (5.50±0.15 µM Trolox/g) trailed by Dhaki (4.10±0.15 µM Trolox/g) and Aseel (3.92±0.11 µM Trolox/g).

Considerable TEAC activity was found in Moroccan date cultivars as studied by (Bouhlali et al., 2015). The percent inhibition by Omani date extract against ABTS radical was reported by Singh et al. (2012). The authors delineated that Omani date extract possess significant ABTS inhibition capacity from 84-92%. The results concord to the findings of Biglari et al. (2008) who elucidated the antioxidant potential of date cultivars from Iran. The ABTS activity in various Irani cultivars ranged from 22.83 to 54.61 µM Trolox equivalents/ 100g D.W. In the
present study, trolox was used as standard for the measurement of the antioxidant capacity. The mechanism involves interaction with extract or the trolox (control) that suppresses the absorbance of ABTS radical cation. Thus, the findings clearly indicate the ABTS neutralization potential of the date extracts.

4.3.6. H$_2$O$_2$ scavenging capacity

H$_2$O$_2$ is a weak oxidizing agent that can penetrate rapidly in the cell membrane and cause free radical generation. Foods having H$_2$O$_2$ scavenging potential are highly recommended to reduce its formation, thereby preventing the physiologic system from oxidative damage (Mohamed et al., 2014). The H$_2$O$_2$ scavenging capacity exhibited non-momentous effect of treatments as apparent from the F statistic in Table 4.7. The H$_2$O$_2$ scavenging capacity ranged from 87.78±2.90 to 91.76±2.88% with the order as Zahidi>Aseel>Dhaki.

The findings are in corroboration with the results of Kchaou et al. (2014) who elucidated the H$_2$O$_2$ scavenging activity of Tunisian date varieties. They found 89.99, 80.14, and 50.32% H$_2$O$_2$ quenching capacity of Allig, Bejo and Deglet Nour extracts, respectively. The results are also in line with the work of Benmeddour et al. (2013) who assessed the H$_2$O$_2$ scavenging activity in Algerian date cultivars. Their results showed that date extract significantly scavenged H$_2$O$_2$ and the activity range between 14.9 to 97.5% in different date varieties. The results are contrary to the previous findings of Mohamed et al. (2014) regarding the H$_2$O$_2$ scavenging capacity of Sudanese date cultivars. They stated that Sudanese date extract possessed 38.48 to 49.13% H$_2$O$_2$ scavenging capacity. The scavenging hydroxyl radical activity of the date extract can be expounded by preventing the propagation of lipid peroxidation, thereby playing key role in the reduction of chain reactions owing to the occurrence of polyphenolic moieties in the reaction mixture.

4.3.7. Reducing power

The reactive oxygen species like hydroxyl radicals and superoxide radicals cause oxidative damage that results in the cascade various physiological ailments. The reducing properties of extract are primarily concomitant with the occurrence of certain reductones, having the ability to donate electrons to the free radicals making them stable. These reductones can directly react with the peroxides or their precursors, thereby preventing the formation of peroxides (Wang et al., 2008). Results regarding reducing power assay in table 4.7 shows that momentous
variations among the treatments was found. The reducing power varied among the cultivars with a range of 79.45-85.70%. The results are in accordance with the previous findings regarding the reducing power of date extracts. In this regard, Kchaou et al. (2013) stated that Tunisian date extracts possess significant reducing power that may indicate potential antioxidant capacity of date fruit. Likewise, Benmeddour et al. (2013) also reported considerable reducing power in Algerian date cultivars.

In the present study, Zahidi dates exhibited higher phenolic content, while high flavonoid content was found in the Dhaki variety. Zahidi date extract proved better towards DPPH, TEAC, \( \text{H}_2\text{O}_2 \) scavenging capacities, while Dhaki extract showed better FRAP activity and reducing power; both of which involves the reduction of ferric ions. This can be accredited to the stereo selectivity of the radicals (Yu et al., 2002) or the phenolics and flavonoids towards certain substances. Moreover, better ferric reducing abilities of Dhaki extract could also possibly be due to more flavonoid content as compared to the phenolics or owing to certain potent flavonoids like myricetin. The antioxidant potential of polyphenols is also influenced by their chemical structure, as polymeric polyphenols possess more antioxidant potency than the monomeric ones. Besides, the position and number of hydroxyl group in the benzene ring also determines the antioxidant capacity (Moure et al., 2001). Nevertheless, dates possess significant antioxidant activity that should be exploited in diet-based interventions.
SECTION 3: DATE BASED FUNCTIONAL PRODUCT DEVELOPMENT
4.4. Development of date based halwa

The development of novel products demands sufficient knowledge regarding the consumer preferences along with information regarding ingredient selection, product composition and nutritional value. Halwa is a popular sweet and dense delicacy of Middle Eastern, Asian and North African countries (Mureșan et al., 2014). It is relished in the most parts of the subcontinent as a traditional and exotic dessert. The original halwa is rich in sugar and fat which can pose severe health effects. Therefore, present study is an attempt to utilize the healthful properties of date fruit in the development of one of the most relished conventional dessert. Moreover, the white poison (sugar) is being replaced by dates and the fat content has also been reduced for a shelf stable, nutritional and healthy end product that can be consumed equally by the children and adults. Additionally, it provides instant burst of energy and can be consumed as an energy rich food. All the selected cultivars with their respective extracts were used to develop date based functional halwa.

4.4.1. Compositional characterization

F value for proximate composition (Table 4.8) revealed significant variations among date based halwa samples for ash, crude fat, crude protein and carbohydrate content. While, calorie and moisture content remained non-significant as a function of treatments. Higher calorific value was exhibited by Aseel date based halwa (D2) 410.35±14.77 kcal/100g followed by Aseel date extract based halwa (D2a) 408.85±14.31 kcal/100g. Whilst, the energy value in Zahidi date and extract based halwa was 379.82±12.15 (D3) and 386.02±13.32 kcal/100g (D3a), correspondingly. The Dhaki date extract halwa was lower in calorific value (363.46±15.67 kcal/100g) as compared to Dhaki date based halwa (368.19±11.97 kcal/100g). The reported value for control halwa without dates and their extract was 356.21±12.47 kcal/100g.

The moisture content varied non-momentously amongst the treatments and it ranged from 28.40±0.89 to 32.86±1.35%. The ash content was highest in Zahidi date fruit and extract containing halwa treatments i.e. 2.33±0.08 and 2.10±0.07%, respectively, trailed by Dhaki date extract halwa (1.78±0.06%), Aseel date extract halwa (1.76±0.07%), Aseel date halwa (1.71±0.06%), Dhaki date halwa (1.15±0.04%) and the control traditional halwa (0.84±0.03%). Treatments exhibited significant variations regarding the fat content. The fat content in D0, D1, D1a, D2, D2a, D3 and D3a were 12.68±0.41, 16.72±0.63, 17.96±0.54,
25.14±1.03, 26.93±1.18, 18.75±0.62 and 21.42±0.76%, correspondingly. The protein content also varied momentously as a function of treatments. The maximum protein content was exhibited by the Zahidi date based halwa *i.e.* 16.48±0.58% followed by Zahidi date extract based halwa as 15.32±0.55%, Dhaki date halwa 13.77±0.45%, Dhaki date extract based halwa 12.04±0.37%, Aseel date extract halwa 11.13±0.38% and Aseel date based halwa 10.10±0.39%. However, the traditional halwa was low in protein content (7.80±0.28%) as compared to the date containing treatments. Carbohydrate content was highest in the control group D₀ (50.28±1.72%) followed by D₁a (35.76±1.19%), D₁ (38.09±1.47%), D₂ (33.13±1.12%), D₃ (30.98±1.16%), D₃a (28.30±0.97%) and D₂a (28.30±1.01%).

The results obtained are somewhat contrary with the earlier findings regarding different types of halwa as explained earlier due to high fat content. Generally, the commercial sesame paste based halwa characteristically contains high amount of sugars about 47.7%, fat 32.4%, and proteins 13.7%, however low in dietary fiber 1.5% (*Goulas et al.*, 2007). The halwa supplemented with date fiber concentrates was developed earlier by Elleuch *et al.* (2014) using sesame paste as a base ingredient. The proximate composition of the resultant product was sugars 42.57%, fat 31.89% and protein 14.70%. In this study, the date fiber was added with the intention to utilize the waste product and to improve the nutritional properties and texture stability of the product, while in present study, the aim was to replace sugar with natural sweetener alongside reducing the oil content to achieve a good quality functional product. Somewhat similar ingredients like flour, nuts and date paste were used in the development of date bars having low moisture and fat content than the halwa. The proximate composition of the bars showed that they contain 15.56 to 18.42% moisture, 7.41 to 14.96% crude protein, 5.55 to 8.37% fat, 2.30 to 2.91% ash and 70.85 to 81.12 NFE (*Nadeem et al.*, 2012). Itagi *et al.* (2013) developed four types of multigrain halwa mixes. The moisture content of the cooked halwa varied between 26 to 31%. The carbohydrates ranged from 54 to 58% while the protein content was in range of 5.7-6.3%. The fat content of 8-9% was noted primarily by the addition of clarified butter during cooking.

Date fruit and extract containing halwa is an energy dense product that could be used as an instant source of energy for all age groups. It also contains beneficial natural sugars in more quantity than table sugar along with proteins and fiber. Thus, using this product as a meal for
Table 4.8. Compositional profile of developed date based halwa prototypes

<table>
<thead>
<tr>
<th>Compositional analyses</th>
<th>Date Based Halwa (Treatments)</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$D_0$</td>
<td>$D_1$</td>
</tr>
<tr>
<td>Calories (kcal/100g)</td>
<td>356.21±12.47</td>
<td>368.19±11.97</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>28.40±0.89</td>
<td>30.28±1.11</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.84±0.031&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.15±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>12.68±0.41&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.72±0.63&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>7.80±0.28&lt;sup&gt;f&lt;/sup&gt;</td>
<td>13.77±0.45&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>50.28±1.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.09±1.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values represent mean±SD; Two-way ANOVA followed by Tukey’s HSD multiple comparison tests

* = Significant
** = Highly significant

$D_0$ Control (Traditional Halwa)
$D_1$ Dhaki Date Based Halwa
$D_1a$ Dhaki Date Extract Based Halwa
$D_2$ Aseel Date Based Halwa
$D_2a$ Aseel Date Extract Based Halwa
$D_3$ Zahidi Date Based Halwa
$D_3a$ Zahidi Date Extract Based Halwa
school kids could be a good option for working moms. Moreover, other ingredients such as gram flour, semolina and milk are also good for health. The studies also suggest that gram flour is good for diabetics, thus products variants could also be designed for special groups and can be commercialized as well.

4.4.2. Physicochemical analyses

4.4.2.1. Color

Mean squares for the color tonality of date based halwa prototypes (Table 4.9) showed significant effect of treatment, storage and their interaction on a*, b*, chroma and hue angle except for L*, where treatment and storage intervals affected significantly but their interaction remained non-significant.

The means pertaining to L* value of date based halwa are presented in Fig. 4.1. The values for control (D₀), Dhaki date halwa (D₁), Dhaki date extract halwa (D₁a) Aseel date halwa (D₂) Aseel date extract halwa (D₂a), Zahidi date halwa (D₃) and Zahidi date extract halwa (D₃a) were 59.13±2.03, 45.92±1.71, 52.87±2.07, 45.16±1.68, 53.70±1.81, 44.73±1.51 and 53.05±1.84. Moreover, the storage values also exhibited non-significant decrease in this trait at 0, 7th and 14th day i.e. 53.48±1.85, 50.57±1.74 and 47.90±1.83, correspondingly.

Nonetheless, a* values were considerably affected by the halwa treatments during the storage period. In this vista, a* values for treatments D₀, D₁, D₁a, D₂, D₂a, D₃ and D₃a were 0.79±0.03, 3.09±0.11, 1.15±0.04, 1.87±0.07, 0.92±0.03, 4.24±0.15 and 1.72±0.06, respectively (Fig. 4.1). Across the storage, a* values declined considerably from 1.89±0.05 to 2.07±0.07 (Fig. 4.2).

Similarly, values for b* also showed momentous variations in different functional halwa treatments (Fig. 4.1). The highest value was recorded for control (D₀); 17.10±0.62, followed by Aseel date extract halwa (D₂a); 14.42±0.50, Dhaki date extract halwa (D₁a); 13.81±0.49 and Zahidi date extract halwa (D₃a); 13.40±0.44 whereas, b* value was less in case of date fruit containing treatments i.e. Aseel date halwa (D₂) 8.30±0.34, Dhaki date halwa (D₁) 7.68±0.25 and Zahidi date halwa (D₃) 6.76±0.22. Likewise, significant increase was observed for this parameter with the storage progression. The recorded values for b* were 8.57±0.33, 9.08±0.32 and 17.27±0.58, correspondingly at 0, 7th and 14th day (Fig. 4.2).
Table 4.9. Means squares for color tonality of date based halwa

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Chroma</th>
<th>Hue angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments (A)</td>
<td>6</td>
<td>23.39**</td>
<td>762.79**</td>
<td>471.29**</td>
<td>168.97**</td>
<td>164.29**</td>
</tr>
<tr>
<td>Days (B)</td>
<td>2</td>
<td>15.64**</td>
<td>10.18**</td>
<td>1633.89**</td>
<td>678.11**</td>
<td>420.84**</td>
</tr>
<tr>
<td>A x B</td>
<td>12</td>
<td>0.54NS</td>
<td>185.79**</td>
<td>62.37**</td>
<td>20.84**</td>
<td>130.30**</td>
</tr>
<tr>
<td>Error</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant  
* = Significant  
** = Highly significant
Similar trend was depicted by the chroma values as the highest value was recorded for control (D₀); 17.14±0.65, followed by Aseel date extract halwa (D₂a); 14.48±0.55, Dhaki date extract halwa (D₁a); 14.30±0.35 and Zahidi date extract halwa (D₃a); 13.53±0.46. Whereas, b* value was less in case of date fruit containing treatments i.e. Aseel date halwa (D₂) 8.77±0.31, Dhaki date halwa (D₁) 8.77±0.32 and Zahidi date halwa (D₃) 8.61±0.38. (Fig. 4.1). The storage imparted momentous increase in the chroma results at 0, 7th and 14th day intervals i.e. 9.21±0.34, 10.02±0.33 and 17.46±0.62, correspondingly (Fig. 4.2).

The hue angle illustrated significant variations regarding the date fruit containing treatments and date extract containing treatments. The extract hue angle in the extract containing treatments i.e. D₁a, D₂a and D₃a was 89.12±3.23, 93.16±3.32 and 94.71±4.48, respectively that was in close proximity with the control treatment D₀ 87.49±3.40. While, lower range was obtained for the fruit containing halwa treatments D₁, D₂ and D₃ as 63.89±2.05, 69.69±2.34 and 67.37±2.18, correspondingly (Fig. 4.1). Besides, at 0, 7th and 14th day hue angle results were 74.73±2.70, 70.93±2.68 and 96.62±3.63, respectively (Fig. 4.2).

Color of any food product is the main factor through which the consumers judge its acceptance. The color also helps in perceiving the product taste and flavor, therefore it is of considerable importance for a food scientist and manufacturer. It is evident from the results that the control treatment and the extract containing treatments were lighter in color as compared to the date fruit containing treatments being darker. The color also varies during the storage as result of certain biochemical reactions. The results are in line with the previous findings of Elleuch et al. (2014) who reported 11.8% reduction in lightness (L*) of halwa as result of date fiber supplementation. While, the redness (a*) values were higher than the control. Moreover, they further stated that b* value was significantly reduced up to 22.1% by the addition of date fibers. The trend among the color indicators is similar to the present results. The L*, b* and a* values also varies with respect to the varieties. In a study conducted by Bouaziz et al. (2010), it was found that bread flour made with pit of Deglet Nour dates has higher L* and b* values as compared to the bread containing Allig date seed. Similar color variations were recorded for crumb color. Likewise, Masmoudi et al. (2010) developed reduced sugar jellies using date and lemon by-products. The CIE lab coordinates for the prepared jellies showed that the samples has hue angles between 77.38-82.40° having yellowish brown color, somewhat similar to the
Figure 4.1. Effect of treatments on color tonality of date based halwa

D0 Control (Traditional Halwa); D1 Dhaki Date Based Halwa; D1a Dhaki Date Extract Based Halwa
D2 Aseel Date Based Halwa; D2a Aseel Date Extract Based Halwa; D3 Zahidi Date Based Halwa
D3a Zahidi Date Extract Based Halwa
Figure 4.2. Effect of storage on color tonality of date based halwa

Table 4.10. Mean squares for hardness (N) and water activity (aw) of date based halwa

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Hardness</th>
<th>Water Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments (A)</td>
<td>6</td>
<td>223.87**</td>
<td>14.92**</td>
</tr>
<tr>
<td>Days (B)</td>
<td>2</td>
<td>2520.67**</td>
<td>96.68**</td>
</tr>
<tr>
<td>A x B</td>
<td>12</td>
<td>295.28**</td>
<td>3.56*</td>
</tr>
<tr>
<td>Error</td>
<td>42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = Significant  
** = Highly significant
present results. The results show that extract containing treatments were lighter while fruit containing treatments were in darker tone.

4.4.2.2. Hardness

Hardness (N) is the amount of force required to attain a given deformation of a material or the force necessary to bite the particular food with molars. Mean squares for the hardness of date based halwa treatments showed substantial effect of treatment, storage and their interaction (Table 4.10). Means for hardness in control (D₀), Dhaki date halwa (D₁), Dhaki date extract halwa (D₁ₐ) Aseel date halwa (D₂) Aseel date extract halwa (D₂ₐ), Zahidi date halwa (D₃) and Zahidi date extract halwa (D₃ₐ) were 8.07±0.26, 6.47±0.22, 3.64±0.14, 3.70±0.15, 2.09±0.09, 6.65±0.23 and 8.12±0.27 N, respectively (Fig. 4.3). During the storage trial, minimum value for hardness in halwa samples was observed at 0 day i.e. 2.87±0.11 that momentously increased to 9.58±0.32 N at 14th day.

Hardness increases during storage and is affected by the addition of date fruit as well as extract. Elleuch et al. (2014) developed different types of halwa supplemented with date fiber, emulsifier and sesame testae. The addition of these ingredients to the halwa treatments led to 3.08 to 9.38 fold increase in hardness. However, in the present research the hardness is decreasing in treatments containing extract especially, which may be due to increased water content in the sample while, the date fruit containing treatments are comparatively harder. The hardness values of multigrain halwa ranges from 44.5 to 93.5 N as studied by Itagi et al. (2013). The difference in their findings and the present results might be ascribed to the moisture content in samples as more moisture increases the degree of softness. The base ingredients such as flour also affects the texture of the end product. Likewise, fat content also contributes to a softer texture. Even the type of fat or oil also imparts significant effect on the product texture. Manickavasagan et al. (2011) reported that the hardness varied from 6-9 N among the different halwa treatments. The hardness of halwa containing olive oil was more while that of sunflower oil was much less, comparatively. In the present investigation, hardness increased with respect to storage that could possibly be due to textural variations occurring in the product matrix.

4.4.2.3. Water activity

Mean squares for water activity showed considerable effect of treatments, storage and their interaction (Table 4.10). Means obtained for water activity in halwa prototypes were control (D₀) 0.65±0.02, Dhaki date halwa (D₁) 0.71±0.02, Dhaki date extract halwa (D₁ₐ) 0.74±0.02, Aseel date
Figure 4.3. Effect of storage and treatments on hardness of date based halwa

Figure 4.4. Effect of storage and treatments on water activity of date based halwa
halwa (D₂) 0.73±0.02, Aseel date extract halwa (D₂a) 0.75±0.02, Zahidi date halwa (D₃) 0.75±0.02 and Zahidi date extract halwa (D₃a) 0.76±0.02. During storage, minimum value for water activity was observed at 0 day i.e. 0.65±0.03 that significantly increased to 0.77±0.03 at 14th day (Fig. 4.4).

The water activity refers to the unbound water that can predict the shelf stability of a particular food. Refrigeration conditions are required for foods with high water activity to prevent microbial spoilage. Besbes et al. (2009) studied the water activity of date jam prepared using different Tunisian date varieties. The result showed that varietal variations also effect the water activity of the jam, as it was 0.734, 0.690, and 0.689 for Allig, Deglet Nour and Kentichi cultivars, respectively. Water activity of jellies developed using date and lemon by-products was less than 0.86 i.e. within the safe level from an array of bacteria as reported by (Masmoudi et al., 2010). The water activity in the present study ranges between 0.65-0.77 that indicates that it is prone to spoilage particularly by yeasts or molds, therefore ensuring proper packaging and refrigeration conditions may enhance the shelf life up to 2 weeks.

4.4.2.4. Antioxidant activity

Mean squares for TPC, TFC, DPPH, TEAC and FRAP of functional halwa treatments elucidated significant differences due to treatments and storage. However, TPC, TFC and DPPH exhibited non-momentous variations regarding the interaction effect, while interaction imparted momentous effect on DPPH, TEAC and FRAP assays (Table 4.11). Means regarding the effect of treatments on total phenolic content (TPC), total flavonoid content (TFC), DPPH, TEAC and FRAP are presented in Table 4.12. Treatments differed significantly with respect to date varieties and their extracts. Higher TPC was exhibited by the date fruit containing treatments as D₁, D₂ and D₃ 255.86±8.73, 288.59±11.59 and 297.44±10.63 mg GAE/100g, correspondingly. Whilst, lower values were recorded in the extract containing treatments; D₁a, D₂a and D₃a were 216.73±7.20, 276.61±9.60 and 293.34±10.24 mg GAE/100g, respectively. However, lowest TPC was noted in the control sample (185.67±6.44 mg GAE/100g). Maximum flavonoid content was found in Dhaki date based halwa (106.37±4.25 mg QE/100g) followed by Zahidi date based halwa (103.97±3.79 mg QE/100g), Dhaki date extract based halwa (98.85±3.67 mg QE/100g), Zahidi extract based halwa (97.36±3.90 mg
Table 4.11. Mean squares for antioxidant indices of date based halwa

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>TPC</th>
<th>TFC</th>
<th>DPPH</th>
<th>TEAC</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments (A)</td>
<td></td>
<td>163.93**</td>
<td>93.13**</td>
<td>239.32**</td>
<td>338.16**</td>
<td>24.86**</td>
</tr>
<tr>
<td>Days (B)</td>
<td></td>
<td>8.64**</td>
<td>68.90**</td>
<td>82.09**</td>
<td>137.55**</td>
<td>126.32**</td>
</tr>
<tr>
<td>A x B</td>
<td>12</td>
<td>1.15NS</td>
<td>0.95 NS</td>
<td>0.12NS</td>
<td>3.39*</td>
<td>10.74**</td>
</tr>
<tr>
<td>Error</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant
* = Significant
** = Highly significant

Table 4.12. Effect of treatments on antioxidant indices of date based halwa

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Antioxidant Indices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TPC</td>
</tr>
<tr>
<td>D₀</td>
<td>185.67±6.44&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>D₁</td>
<td>255.86±8.73&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>D₁&lt;sub&gt;a&lt;/sub&gt;</td>
<td>216.73±7.20&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>D₂</td>
<td>288.59±11.59&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>D₂&lt;sub&gt;a&lt;/sub&gt;</td>
<td>276.61±9.60&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>D₃</td>
<td>297.44±10.63&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>D₃&lt;sub&gt;a&lt;/sub&gt;</td>
<td>293.34±10.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values represent mean±SD; Two-way ANOVA followed by Tukey’s HSD multiple comparison tests
D₀ Control (Traditional Halwa); D₁ Dhaki Date Based Halwa; D₁<sub>a</sub> Dhaki Date Extract Based Halwa
D₂ Aseel Date Based Halwa; D₂<sub>a</sub> Aseel Date Extract Based Halwa; D₃ Zahidi Date Based Halwa
D₃<sub>a</sub> Zahidi Date Extract Based Halwa
QE/100g), Aseel date based halwa (89.33±3.09 mg QE/100g), Aseel date extract based halwa (85.88±2.90 mg QE/100g) and control halwa (74.32±2.80 mg QE/100g).

The means relating to effect of treatments on DPPH activity of date-based halwa are also presented in Table 4.12. The recorded results for control (D0), Dhaki date halwa (D1), Dhaki date extract halwa (D1a) Aseel date halwa (D2) Aseel date extract halwa (D2a), Zahidi date halwa (D3) and Zahidi date extract halwa (D3a) were 30.14±1.03, 50.54±1.89, 43.98±1.60, 35.70±1.24, 35.46±1.28, 54.51±1.78 and 50.93±2.01%, respectively. Likewise, values for trolox equivalent antioxidant capacity were also significantly affected by the halwa treatments. In this context, TEAC values for treatments D0, D1, D1a, D2, D2a, D3 and D3a were 0.84±0.03, 1.94±0.06, 1.55±0.05, 1.72±0.06, 1.50±0.04, 1.96±0.07 and 1.59±0.06 µM Trolox/g F.W., respectively. Similarly, values for ferric reducing antioxidant power (FRAP) also showed momentous variations in different functional halwa treatments. The recorded means for FRAP in control (D0), Dhaki date halwa (D1), Dhaki date extract halwa (D1a) Aseel date halwa (D2) Aseel date extract halwa (D2a), Zahidi date halwa (D3) and Zahidi date extract halwa (D3a) were 0.85±0.04, 0.92±0.03, 1.11±0.04, 0.94±0.04, 0.95±0.03, 1.27±0.05 and 1.01±0.02 µmol Fe^{2+}/g, respectively.

Means concerning the effect of storage duration on TPC, TFC, DPPH, TEAC and FRAP are shown in Table 4.13. Treatments differed significantly with respect to storage intervals. The phenolic content reduced during the storage, the obtained results at 0, 7th and 14th day were 266.06±9.78, 257.44±8.59 and 254.02±9.24 mg GAE/100g, respectively. Significant decrease in flavonoid content was noted during the storage from 0 to 14th day as 99.66±3.76 to 87.00±3.22 mg QE/100g, respectively. Moreover, the storage values also exhibited momentous decline for DPPH activity at 0, 7th and 14th day i.e. 48.28±1.60, 44.93±1.61 and 41.14±1.42%, correspondingly. However, across the storage, the results for TEAC reduced considerably from 1.73±0.06 to 1.42±0.05 µM Trolox/g F.W.

The polyphenolic contents in date fruit and extract containing treatments are much better than the control treatment. Comparable results were attained by Manickavasagan et al. (2013) who added dates as a sugar substitute in idli. In the control sample, the TPC was 4.5 mg/100g which was improved to 82.1 mg/100g by the addition of date syrup instead of table sugar. Similar improvement was noticed in the vitamin C content. It also indicated improved antioxidant
Table 4.13. Effect storage on antioxidant indices of date based halwa

<table>
<thead>
<tr>
<th>Antioxidant indices</th>
<th>Storage intervals (days)</th>
<th>0</th>
<th>7</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC</td>
<td>266.06±9.78\textsuperscript{a}</td>
<td>257.44±8.59\textsuperscript{b}</td>
<td>254.02±9.24\textsuperscript{b}</td>
<td></td>
</tr>
<tr>
<td>TFC</td>
<td>99.66±3.76\textsuperscript{a}</td>
<td>94.52±3.48\textsuperscript{b}</td>
<td>87.00±3.22\textsuperscript{c}</td>
<td></td>
</tr>
<tr>
<td>DPPH</td>
<td>48.28±1.60\textsuperscript{a}</td>
<td>44.93±1.61\textsuperscript{b}</td>
<td>41.14±1.42\textsuperscript{c}</td>
<td></td>
</tr>
<tr>
<td>TEAC</td>
<td>1.73±0.06\textsuperscript{a}</td>
<td>1.60±0.05\textsuperscript{b}</td>
<td>1.42±0.05\textsuperscript{c}</td>
<td></td>
</tr>
<tr>
<td>FRAP</td>
<td>1.23±0.04\textsuperscript{a}</td>
<td>1.01±0.04\textsuperscript{b}</td>
<td>0.81±0.03\textsuperscript{c}</td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean±SD; Two-way ANOVA followed by Tukey’s HSD multiple comparison tests

Table 4.14. Mean squares for sensorial attributes of date based halwa

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Color</th>
<th>Appearance</th>
<th>Taste</th>
<th>Texture</th>
<th>Aroma</th>
<th>Overall Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments (A)</td>
<td>6</td>
<td>53.24**</td>
<td>23.20**</td>
<td>109.80**</td>
<td>9.65**</td>
<td>22.38**</td>
<td>18.19**</td>
</tr>
<tr>
<td>Days (B)</td>
<td>2</td>
<td>171.26**</td>
<td>107.35**</td>
<td>102.89**</td>
<td>119.71**</td>
<td>86.93**</td>
<td>78.69**</td>
</tr>
<tr>
<td>A x B</td>
<td>12</td>
<td>6.25**</td>
<td>4.42**</td>
<td>7.44**</td>
<td>2.96*</td>
<td>9.05**</td>
<td>5.55**</td>
</tr>
<tr>
<td>Error</td>
<td>294</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = Significant
** = Highly significant
activity. Likewise, Gad et al. (2010) reported that the addition of date palm syrup in yogurt significantly improves the antioxidant potential. The TPC of plain yogurt was 248 mg GAE/100 mL, while in the date paste added yogurt it increased to 306 mg GAE/100 mL. However, during 12 days storage the phenolic content was reduced. The DPPH and FRAP activities also improved considerably by the addition of date paste in yogurt, while storage resulted in decreased activities. Similar trend was observed in the present study as the phenolic profile and antioxidant activity was higher in all date fruit extract containing treatments as compared to control, whilst storage lead to decreased activities with the passage of time. The reduction in antioxidant activity may be ascribed to the decrease the phenolic and flavonoid content over the storage. That might be because of certain biochemical reactions or breakdown products imparting lower affinity towards reagents or having low antioxidant potential (Klimczak et al., 2007). The total phenolic content in Tunisian dates ranged from 280.6 to 681.8 mg GAE/100g, while in their subsequent jam the TPC ranged from 190.6 to 308.2 mg GAE/100g as studied by Besbes et al. (2009). The decrease in phenolics in the product could essentially be explained by the destructive effect of high temperature conditions and addition of other ingredients.

4.4.3. Hedonic response

Sensory quality and appearance considerably influences the product acceptability, consumer demand and purchasing behavior. Addition of date fruit or extract significantly affects organoleptic properties of the end product, thus need to be evaluated. It is clear from the spider graph in Fig. 4.5 that the appearance of date-based halwa was improved during the storage in all treatments. The maximum panelist ratings were scored by D₁, D₃ and D₃a as 7.27±0.24, 7.21±0.24 and 7.07±0.24, respectively. Whilst, lower ratings were achieved by D₁a, D₀, D₂ and D₂a as 6.82±0.23, 6.77±0.23, 6.69±0.22 and 6.64±0.22, correspondingly. However, during the storage the judges’ preferences showed improvement in appearance of all treatments from 6.58±0.22 at 0 day to 6.93±0.23 at 7th day and 7.26±0.24 at 14th day.

It is apparent from Fig. 4.6 that scores for texture varied from 7.22±0.24 to 6.82±0.23 amongst the treatments. Whilst, storage also influenced the texture scores for date based halwa treatments from 7.33±0.25 to 6.76±0.23 at 0th and 14th day, respectively. Mean squares for hedonic response assessment revealed considerable variances as function of treatments, storage
and their interaction (Table 4.14). It is apparent from the spider graph in Fig 4.7 regarding color of date based halwa treatments that maximum score was attained by Dhaki date halwa (D_1) 7.37±0.25 followed by Zahidi date extract halwa (D_{3a}) 7.16±0.24, Zahidi date halwa (D_3) 7.11±0.24, Dhaki date extract halwa (D_{1a}) 6.83±0.23 Aseel date extract halwa (D_{2a}) 6.70±0.22, control (D_0) 6.45±0.22 and Aseel date halwa (D_2) towards a product with different appearance and taste than the traditional one.6.44±0.22. However, it was observed that with the progression of storage the color scores decreased from 7.22±0.24 to 6.50±0.22 from 0 to 14th day respectively.

Similarly, the aroma scores described in the spider graph (Fig. 4.8) showed that Zahidi date and date extract based treatments i.e. D_3 and D_{3a} attained maximum panelist rating as 7.16±0.24 and 7.22±0.24, correspondingly. However, the aroma scores in other treatments varied from 6.50±0.22 to 6.93±0.23. Storage resulted in significant decline in the aroma values from 7.11±0.24 to 6.62±0.22 at and 0 and 14th day, respectively.

Regarding the taste profile of treatments, Fig. 4.9 shows that maximum preference was given to Zahidi date based halwa 7.36±0.25 trailed by Dhaki date based halwa 7.32±0.24. However, pertaining to the fruit containing treatments Aseel score the lowest panelist ratings 5.88±0.20. Comparing the taste preference of extract based treatments, Zahidi date extract based halwa scored the maximum rating as 7.01±0.23 trailed by Dhaki date extract halwa and Aseel date extract halwa as 6.68±0.22 and 6.56±0.22, respectively. While, the score acquired by the control treatment was 6.83±0.23. Storage resulted a decrease in taste ratings from the initiation to culmination of the study i.e. 7.02±0.23 to 6.49±0.22.

The above results were computed to calculate the overall acceptability of date based halwa treatments (Fig. 4.10). Lowest score was attained by the control treatment (6.84±0.23), that is an indication that the developed product was relished by the panelists more than the traditional halwa. Considering the fruit based treatments D_1, D_2 and D_3 highest score was achieved by the D_3 (7.37±0.25) trailed by D_1 (7.24±0.24) and D_2 (6.99±0.23). Similarly, in case of extract containing treatments, the Zahidi date extract containing treatment D_{3a} scored better i.e. 7.00±0.23 as compared to the Dhaki date extract containing treatment D_{1a} 6.87±0.23 and Aseel date extract containing treatment D_{2a} 6.72±0.22 (Fig. 4.10). Moreover, it was observed that the panelists liked the fruit containing treatments more as compared to the extract ones. But, those
judges who prefer plain textured traditional halwa were more in favor of the extract containing treatments. On the whole, Zahidi date and extract based treatments were preferred followed by the Dhaki date treatments. However, slightly tangy flavor was noticed in Aseel variety based treatments.

Sensory profiling basically comprises of a vista of procedures that are employed to arouse, measure, evaluate and infer the human responses towards the food products that is an important pillar for designing and developing any food product (Awan et al., 2015). Sensory response also determines the consumer acceptability, therefore is of prime importance. In this regard, Al-Shamsi et al. (2013) determined the consumer preferences for Omani halwa through a structured questionnaire. They stated five imperative attributes affecting the acceptability of halwa that include color, appearance, sweetness, mouthfeel and solubility. Elleuch et al. (2014) studied the sensory characteristics of halwa supplemented with date fiber, emulsifier and testae and found better overall acceptability for halwa containing date fiber concentrate along with emulsifier. In the present study, the sensory scores vary with respect to fruit, extract, and the varieties. However, overall rating regarding the date supplemented halwa was good and thus, date fruit and extract can be utilized in the development of a commercial value added product with better nutritional profile. The results are similar with the earlier findings of Manickavasagan et al. (2013) who developed date based idli. Their findings show that date paste or syrup imparts light brown color whereas the chopped dates impart darker color having lower surface smoothness as well. The flavor and taste of chopped dates containing idli attained higher score. In another investigation by Gad et al. (2010) date palm flavored yogurt was analyzed for sensory attributes. The panelists’ ratings were highest for yogurt containing 10% date syrup, thus indicating the product acceptability. In the comparison of date jam with reference jam, date jam (Allig cultivar) scored more with respect to taste, texture and color than the reference jam as mentioned by Besbes et al. (2009). In the present study, the date fruit containing treatments especially Zahidi and Dhaki cultivars scored better than the extract ones that shows acceptance of the consumers.
Figure 4.5. Effect of treatments and storage on appearance of date based halwa

Figure 4.6. Effect of treatments and storage on texture of date based halwa

Figure 4.7. Effect of treatments and storage on color of date based halwa

D0=Control (Traditional Halwa)  
D1=Dhaki Date Based Halwa  
D1a=Dhaki Date Extract Based Halwa  
D2=Aseel Date Based Halwa  
D2a=Aseel Date Extract Based Halwa  
D3=Zahidi Date Based Halwa  
D3a=Zahidi Date Extract Based Halwa
Figure 4.8. Effect of treatments and storage on aroma of date based halwa

Figure 4.9. Effect of treatments and storage on taste of date based halwa

Figure 4.10. Effect of treatments and storage on overall acceptability of date based halwa

D₀ = Control (Traditional Halwa)
D₁ = Dhaki Date Based Halwa
D₁a = Dhaki Date Extract Based Halwa
D₂ = Aseel Date Based Halwa
D₂a = Aseel Date Extract Based Halwa
D₃ = Zahidi Date Based Halwa
D₃a = Zahidi Date Extract Based Halwa
SECTION 4: BIOEFFICACY STUDIES
4.5. Selection of best treatment

Based on the phytochemical *in vitro* analysis and hedonic response of developed products, Zahidi date and its respective extract were selected for the bioevaluation studies with special reference to cardiac and hepatic stress.

4.6. Bioefficacy studies

Bioefficacy assessment of selected date fruit and extract was carried out via model feeding trial to evaluate their effectivity on oxidative stress mediated dysfunctions specifically in heart and liver. However, as a part of safety assessment studies, the effect on the kidney parameters was also investigated. The Sprague Dawley strain of rats was used in the bioevaluation trials due to their suitability under controlled environmental conditions. The rats were divided into two broad categories; one fed on normal diet, whereas the others were administered atherogenic diet to induce oxidative stress. Each module was then further divided on the basis of date fruit and date extract consumption. The formulated groups were G₁ (Normal diet), G₂ (Date fruit + normal diet), G₃ (Date extract + normal diet), G₄ (Atherogenic diet), G₅ (Date fruit + atherogenic diet) and G₆ (Date extract + atherogenic diet). The results are presented in five broad sections depending upon the effect of diets on lipidemic, cardiac, hepatic and renal stress markers. Moreover, the hematological aspects were also studied. The investigated parameters were interpreted statistically to draw a conclusive approach.

4.6.1. Growth performance parameters

F statistic value corresponding to body weight gain depicted non-significant difference as a function of treatments in the normal diet fed group, while feed and drink intakes and feed efficiency ratio showed momentous variations in the same group. However, the F values pertaining to body weight gain, average feed and drink intakes and feed efficiency ratio revealed significant impact of treatment in the atherogenic diet fed groups (Table 4.15). The average feed intake in the normal diet fed groups *i.e.* G₁, G₂ and G₃ was 18.61±0.58, 16.94±0.59 and 18.46±0.60 g/day respectively. The feed intake was more in the atherogenic diet fed group G₄ as 20.41±0.61 g/day that decreased to 19.95±0.72 g/day in the date extract + atherogenic diet fed group (G₆) and the lowest feed intake was observed in G₅ fed on date fruit containing atherogenic diet (17.45±0.66 g/day). The water intake increased significantly in the date fruit and extract administered groups 23.46±0.72 and 24.27±0.89 mL/day, respectively as compared to the
Table 4.15. Effect of diets on growth performance parameters of experimental rats

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental Groups</th>
<th>Growth Performance Parameters</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Body Weight Gain</td>
<td>Average Feed Intake</td>
<td>Average Water Intake</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(12\text{th} wk.)</td>
<td>(g/day)</td>
<td>(mL/day)</td>
</tr>
<tr>
<td>Normal Diet</td>
<td>G₁</td>
<td></td>
<td>173.41±5.72</td>
<td>18.61±0.58\textsuperscript{a}</td>
<td>22.32±0.78\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>G₂</td>
<td></td>
<td>170.96±5.81</td>
<td>16.94±0.59\textsuperscript{b}</td>
<td>23.46±0.72\textsuperscript{ab}</td>
</tr>
<tr>
<td></td>
<td>G₃</td>
<td></td>
<td>165.65±5.30</td>
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<td>24.27±0.89\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td></td>
<td>2.93\textsuperscript{NS}</td>
<td>8.35\textsuperscript{*}</td>
<td>4.85\textsuperscript{*}</td>
</tr>
<tr>
<td>Atherogenic Diet</td>
<td>G₄</td>
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<td>249.54±9.48\textsuperscript{a}</td>
<td>20.41±0.61\textsuperscript{a}</td>
<td>28.08±0.89\textsuperscript{a}</td>
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<tr>
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<td>G₅</td>
<td></td>
<td>214.37±9.22\textsuperscript{b}</td>
<td>17.45±0.66\textsuperscript{b}</td>
<td>25.12±0.77\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>G₆</td>
<td></td>
<td>201.84±5.95\textsuperscript{b}</td>
<td>19.95±0.72\textsuperscript{a}</td>
<td>26.56±0.87\textsuperscript{ab}</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td></td>
<td>16.91\textsuperscript{**}</td>
<td>31.34\textsuperscript{**}</td>
<td>10.19\textsuperscript{**}</td>
</tr>
</tbody>
</table>

Values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests

* = Significant
**= Highly significant
\textsuperscript{NS} = non-significant

G₁ Normal diet
G₂ Date fruit + normal diet
G₃ Date extract + normal diet
G₄ Atherogenic diet
G₅ Date fruit + atherogenic diet
G₆ Date extract + atherogenic diet
normal diet fed control group (22.32±0.78 mL/day). Nevertheless, the rats fed on atherogenic diet (G₄) consumed more water (28.08±0.89 mL/day) that decreased upon the administration of date fruit in G₅ (25.12±0.77 mL/day) and date extract in G₆ (26.56±0.87 mL/day). The body weight gain during the trial showed a declining trend from 173.41±5.72 g in G₁ to 170.96±5.81 and 165.65±5.30 g in G₂ and G₃, respectively. Nonetheless, the administration of atherogenic diet significantly increased the body weight in the atherogenic control i.e. 249.54±9.48 g that decreased by the date fruit enriched atherogenic diet G₅ to 214.37±9.22 g. Maximum decline was observed in date extract fed group G₆ as 201.84±5.95 g. The date fruit and extract containing diets reduced weight up to 1.41 and 4.47%, respectively in rats on normal diet. Whereas, 14.09 and 19.12% reduction was found in the date fruit and date extract supplemented atherogenic rats, respectively.

The feed efficiency ratio (FER) depicts the efficiency of an animal to convert feed mass into body mass. The FER varied significantly in all groups; in case of normal rats, the values were 0.10±0.003, 0.11±0.003, 0.09±0.004 in G₁, G₂ and G₃, respectively. Highest FER was observed in G₄ (0.13±0.005), trailed by G₅ (0.12±0.005) and G₆ (0.11±0.004).

Obesity has become the most prevalent health concern in the industrialized economies due to lifestyle modifications (Ogden et al., 2015). At the cellular level, obesity is characterized by rise in number and size of adipocytes in the adipose tissue. The excessive deposition of adipose tissue is accredited to the imbalance between energy consumption and expenditure (Hsu and Yen, 2007). The atherosclerotic process is triggered by the intake of high cholesterol and saturated fat containing diets. In experimental animals, 1-2% dietary cholesterol is found sufficient to induce obese conditions by disrupting the normal cholesterol mechanism (Ness, 2015).

In the present study, atherogenic diet resulted in significant increase in body weight that reduced by the date fruit and extract consumption. Moreover, non-significant decline in body weight was also observed in the normal rats during the course of study. Hence, the data suggest the anti-obesity potential of date fruit and its extract. The results corroborate to the findings of El Arem et al. (2014a), who observed a non-significant decrease in weight of normal rats due to aqueous date extract supplementation. Similarly, Saafi et al. (2011) recorded non-significant decrease in weight in normal rats administered on Deglet Nour extract. However, in case dimethoate treated rats, the weight significantly decreased that was ameliorated by the date fruit extract treatment. Besides, the food intake remained unchanged in all groups. These pesticides such as dimethoate induced
toxicity more often results in severe weight loss. In this vista, date extract proved to be effective in improving the growth performance parameters.

Gallic acid, being the abundant polyphenol in the selected date variety is considered as an anti-obesity phytoceutic. The results are in line with the earlier findings of Hsu and Yen (2007) who studied the anti-obesity effect of gallic acid on high fat diet fed rats. The analysis of growth parameters showed that 0.1 and 0.2% dietary supplementation of gallic acid significantly decreased the body weight in obese rats. The feed intake was higher in the high fat diet groups as compared to the normal or gallic acid fed groups. Similarly, the rats fed on high fat diet also exhibited higher feed efficiency than the other groups. The results of the present study indicate that date extract is more effective in lowering the body weight as compared to the whole fruit that could possibly be attributed to low sugar content in extract as well as the presence of certain bioactive compounds and soluble fiber (Jakobsdottir et al., 2013; Mohamed, 2014). The feed intake was lower in the date fruit fed group as compared to the others that might be due to the reason that one feels satiety after the consumption of fiber rich foods and ultimately the water consumption is increased. These are just the plausible reasons, however, more vigorous investigations are required to clearly manipulate the targeting mechanisms for a better conclusive approach.

4.6.2. Relative organ weights

The F value regarding the relative organ weights in normal diet fed groups explicated non-significant effect of treatments on liver, heart and left kidney. Considerable effect was observed in the right kidney weights. However, the stressed rats showed momentous impact of treatments for heart and right kidney weight with non-momentous variations in liver and left kidney weights. Mean values for relative liver weight in normal diet fed groups ranged from 3.31±0.12 to 3.38±0.11%, whilst in the atherogenic diet fed groups, the relative liver weight decreased non-significantly from 4.49±0.20 to 4.37±0.14% in G4 and G6, respectively. The heart weight in G1 and G2 remained constant i.e. 0.32±0.01%, while slight decrease was observed in G3 0.30±0.01%. Although, the rats fed on atherogenic diet showed significant decrease in heart weight from 0.36±0.02% in atherogenic control to 0.31±0.01 and 0.29±0.01% in the date fruit and extract fed groups, respectively. Likewise, the kidney weight in normal rats ranged from 0.32±0.01 to
Table 4.16. Effect of diets on relative organ weights of experimental rats

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental Groups</th>
<th>Relative Organ Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Normal Diet</td>
<td>G1</td>
<td>3.38±0.11</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>3.34±0.11</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>3.31±0.12</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>0.35\textsuperscript{NS}</td>
</tr>
<tr>
<td>Atherogenic Diet</td>
<td>G4</td>
<td>4.49±0.20</td>
</tr>
<tr>
<td></td>
<td>G5</td>
<td>4.41±0.15</td>
</tr>
<tr>
<td></td>
<td>G6</td>
<td>4.37±0.14</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>1.47\textsuperscript{NS}</td>
</tr>
</tbody>
</table>

Values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests

* = Significant
** = Highly significant
\textsuperscript{NS} = non-significant

G1 Normal diet
G2 Date fruit + normal diet
G3 Date extract + normal diet
G4 Atherogenic diet
G5 Date fruit + atherogenic diet
G6 Date extract + atherogenic diet
0.35±0.01%. The administration of atherogenic diet resulted in increased kidney weight in the G4 (0.43±0.02%) that reduced considerably G5 and G6 as 0.38±0.01 and 0.32±0.01%, correspondingly. The left kidney weight showed no apparent effect of diet, the values ranged from 0.35±0.01 to 0.37±0.01% in all the groups (Table 4.16).

The results are in corroboration with the findings of Hsu and Yen (2007). These researchers observed significant increase in liver weight in high fat diet fed group, whereas it decreased by administrating 0.1 and 0.2% gallic acid. The decrease in liver weight might be due to the ability of gallic acid to reduce the deposited fat in the adipose tissues. However, no significant variations were observed in the heart and kidney weights in their study. Heart weight was significantly influenced in the present study that might be due to the difference in composition of high fat diet and atherogenic diet.

It is observed that in case of stressed conditions liver weight increases. The results corroborate to the findings of El Arem et al. (2014a). The researchers recorded increased liver weight from 3.16±0.05 g/100g in the control group to 4.11±0.06 g/100g in the hepatotoxic group in 2 months study trial. Nonetheless, the weight reduced to 3.10±0.08 g/100g in the normal rats fed on date extract (4 mL/kg) and 3.80±0.06 g/100g in the hepatotoxic ones administered aqueous date extract. One of the researchers groups, Saafi et al. (2011) found no significant variations in relative liver weights of normal rats fed on date fruit extract (4 mL/kg) for 2 months. However, the liver weights decreased due to subchronic administration of dimethoate (pesticide) that were improved by date extract pretreatment. Date fruit extract significantly reduces the increased kidney weight due to oxidative stress (El Arem et al., 2014c). Al-Yahya et al. (2016) studied the effect of oral Ajwa date extract administration on the heart weight of rats and found that the heart/body weight ratio increased due to ISP treatment in the control group, whereas it significantly decreased in date extract treated groups. The significant increase in hepatic and renal weight in the atherogenic stress induced groups might be as a result of hypertrophy in which glycogen is accumulated in the organs. The fat containing diet results in obesity in rats that is responsible for certain morphological changes in the hepatocytes that may also result in hepatomegaly (Altunkaynak and Ozbek, 2009). However, date fruit and extract seemed to be capable enough of adjusting these modifications probably due to the carbohydrate content and bioactive moieties.
PART 1: SERUM LIPOIDEMIC AND OXIDATIVE STRESS BIOMARKERS
4.6.3. Serum lipidemic and oxidative stress biomarkers

Dyslipidemic conditions involve overproduction or deficiencies of lipoproteins that result in disorders in lipoprotein metabolism. These lipoproteins are divided into four major classes that include chylomicrons - the triglyceride rich particles, very low-density lipoproteins, the cholesterol rich low-density lipoproteins and the high-density lipoproteins. Evidences based insights from the epidemiological, pathological and metabolic studies strongly support the causal relation between atherosclerosis and serum lipoproteins.

4.6.3.1. Serum lipidemic profile

In the present study, the effect of date-supplemented diet was assessed on normal and atherogenic rats. The oxidative stress was induced using high fat atherogenic diet, therefore the effect on the serum lipidemic parameters was specially focused. The F values presented in Table 4.17 reflect that the cholesterol, HDL and VLDL showed non-significant behavior with respect to treatments in normal diet fed groups. However, significant variations were recorded in the atherogenic diet fed ones. The serum triglycerides, LDL and nHDL were significantly affected by the treatments during the study period in all groups.

Cholesterol, a hydrophilic lipid, is a precursor of several hormones, bile acids and vitamin D. It is required by the body from exogenous sources and is synthesized endogenously through mevalonate pathway. The amount is regulated through feedback control mechanisms (Rafieian-Kopaei et al., 2014). Means for normal diet fed groups i.e. G₁, G₂, G₃ depicted non-significant decrease in serum cholesterol level from 80.57±2.49 mg/dL in the control group to 77.63±2.64 mg/dL in the date fruit fed group and 76.95±3.31 mg/dL in the group provided with date extract containing diet. In case of atherogenic rats, significant decline in the serum cholesterol level was noted. The diet elevated the cholesterol levels to 168.34±5.94 mg/dL that decreased to 142.86±4.57 and 137.11±4.94 mg/dL in G₅ (date fruit + atherogenic diet) and G₆ (date extract + atherogenic diet), respectively.

The serum triglycerides also decreased significantly in normal as well as stressed rats. The triglyceride level in G₁ (normal diet) was 64.12±2.05 mg/dL that reduced to 61.74±2.34 mg/dL in G₂ (date fruit + normal diet) and 60.88±2.62 mg/dL in G₃ (date extract + normal diet). In the atherogenic diet fed rats maximum triglyceride level was found in G₄ as 128.15±4.61
mg/dL that reduced in G5 (date fruit + atherogenic diet) and G6 (date extract + atherogenic diet) to 112.74±4.12 and 109.56±3.73 mg/dL, respectively.

Low-density lipoprotein cholesterol (LDL-c) is an imperative risk factor for cardiovascular disparities and primary target for CVD risk reduction strategies (Hoogeveen et al., 2014). The results achieved in the present study revealed significant variations pertaining to the LDL level in all groups. The means for normal diet fed groups presented decreasing trend for LDL in G1 (35.86±1.11 mg/dL) trailed by G2 (32.75±1.05 mg/dL) and G3 (31.71±1.14 mg/dL). Similarly, in the rats fed on atherogenic diet, G4, showed highest LDL (116.28±3.63 mg/dL) that gradually reduced in G5 (91.80±2.97 mg/dL) and G6 (86.07±2.84 mg/dL), respectively.

High density lipoprotein cholesterol is considered as good cholesterol and is found to exert prophylactic potential against coronary heart disease possibly through reverse cholesterol transport mechanism and by reducing the LDL associated oxidative stress (Tehrani et al., 2013). The F values relating to HDL expounded non-significant differences for normal diet fed rats whereas, effect was momentous in the rats fed on atherogenic diet. Means relating to HDL in normal diet fed animals showed that treatments did not alter HDL considerably. Nevertheless, values for G1, G2 and G3 groups were 31.89±0.98, 32.53±1.20 and 33.06±1.06 mg/dL, respectively. Nevertheless, the mean HDL concentration for G4 was 26.43±0.83 mg/dL that improved to 28.51±1.05 mg/dL in G5 and 29.13±1.03 mg/dL in G6.

The F values showed that very low-density lipoproteins (VLDL) in normal diet fed rats differed non-momentously due to the treatments, whereas the VLDL level in the atherogenic rat groups varied significantly as a function of treatments. In the normal diet fed rats, means for VLDL in G1, G2 and G3 groups were 12.82±0.40, 12.35±0.53 and 12.18±0.55 mg/dL respectively. In the atherogenic rats, G4 illustrated highest VLDL level 25.63±0.90 mg/dL, trailed by 22.55±0.86 and 21.91±0.75 mg/dL in G5 and G6, correspondingly.

Non-HDL cholesterol (nHDL) is a predictor of CVD risk indicating the content of atherogenic apolipoprotein B containing lipoproteins (LDLs, VLDLs, and IDLs). The nHDL levels also varied significantly as a function of treatments in normal and oxidative stressed rats. In normal rats, mean values for nHDL were 48.68±1.51, 45.10±1.62 and 43.89±1.40 mg/dL for G1, G2 and G3 groups, respectively. Likewise, in the atherogenic diet fed groups, the mean for nHDL
Table 4.17. Effect of diets on serum lipidemic profile

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental Groups</th>
<th>Serum Lipidemic and Glycemic Parameters (mg/dL)</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
<th>nHDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Diet</td>
<td>G1</td>
<td></td>
<td>80.57±2.49</td>
<td>64.12±2.05</td>
<td>31.89±0.98</td>
<td>35.86±1.11</td>
<td>12.82±0.40</td>
<td>48.68±1.51</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td></td>
<td>77.63±2.64</td>
<td>61.74±2.34</td>
<td>32.53±1.20</td>
<td>32.75±1.05</td>
<td>12.35±0.53</td>
<td>45.10±1.62</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td></td>
<td>76.95±3.31</td>
<td>60.88±2.62</td>
<td>33.06±1.06</td>
<td>31.71±1.14</td>
<td>12.18±0.55</td>
<td>43.89±1.40</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td></td>
<td>2.62NS</td>
<td>4.64*</td>
<td>1.43NS</td>
<td>22.66**</td>
<td>2.90NS</td>
<td>4.70*</td>
</tr>
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<td>Atherogenic Diet</td>
<td>G4</td>
<td></td>
<td>168.34±5.94</td>
<td>128.15±4.61</td>
<td>26.43±0.83</td>
<td>116.28±3.63</td>
<td>25.63±0.90</td>
<td>141.91±4.26</td>
</tr>
<tr>
<td></td>
<td>G5</td>
<td></td>
<td>142.86±4.57</td>
<td>112.74±4.12</td>
<td>28.51±1.05</td>
<td>91.80±2.97</td>
<td>22.55±0.86</td>
<td>114.35±3.89</td>
</tr>
<tr>
<td></td>
<td>G6</td>
<td></td>
<td>137.11±4.94</td>
<td>109.56±3.73</td>
<td>29.13±1.03</td>
<td>86.07±2.84</td>
<td>21.91±0.75</td>
<td>107.98±3.73</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td></td>
<td>49.19**</td>
<td>49.22**</td>
<td>13.17**</td>
<td>99.69**</td>
<td>49.92**</td>
<td>126.75**</td>
</tr>
</tbody>
</table>

Values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests

* = Significant

** = Highly significant

NS = non-significant

G1 Normal diet
G2 Date fruit + normal diet
G3 Date extract + normal diet
G4 Atherogenic diet
G5 Date fruit + atherogenic diet
G6 Date extract + atherogenic diet
Figure 4.11. Percent change in serum lipidemic parameters

<table>
<thead>
<tr>
<th></th>
<th>Date Fruit</th>
<th>Date Extract</th>
<th>Date Fruit</th>
<th>Date Extract</th>
<th>Date Fruit</th>
<th>Date Extract</th>
<th>Date Fruit</th>
<th>Date Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>15.14</td>
<td>18.55</td>
<td>12.02</td>
<td>14.51</td>
<td>21.05</td>
<td>25.98</td>
<td>1.97</td>
<td>3.54</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>4.49</td>
<td>5.05</td>
<td>8.66</td>
<td>11.55</td>
<td>7.30</td>
<td>10.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
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</tbody>
</table>
The percent decrease in serum lipidemic parameters in Sprague Dawley rats due to the administration of the date fruit and date extract supplemented functional diets is depicted in Fig. 4.11. It is apparent that date fruit reduced 3.65% serum cholesterol in normal rats, whereas 15.14% in atherogenic rats, while, the date extract treatment resulted in 4.49 and 18.55% cholesterol reduction in normal and atherogenic rats, respectively. Likewise, triglyceride level decreased by 3.71 and 12.02% by date fruit supplementation in normal and atherogenic rats, correspondingly. The percent decrease in date extract fed groups were 5.05 and 14.51% in normal and stressed rats, respectively. LDL cholesterol reduced by 8.66 and 11.55% in date fruit and extract fed normal rats, correspondingly. Whilst, 21.05 and 25.98% reduction was noted in the atherogenic groups fed on date fruit and extract containing diets, respectively.

High fat diets have been used for decades to model dyslipidemia, obesity and insulin resistance in rats. Studies validate that such dietary regimens result in metabolic syndrome that closely resembles the human metabolic dysfunction and may extend to more severe cardiac complications (Buettner et al., 2006). High dietary cholesterol increases the serum and tissue lipidemic markers that are considered as the major risk factors associated with atherosclerosis. The hepatic cholesterogenesis is suppressed due to excess dietary cholesterol and leads to deposition in the system. In the current study, 1.5% cholesterol, 0.5% cholic acid and 0.1% thiouracil were sufficient to induce atherogenic conditions, particularly by elevating the lipid levels (Bravo et al., 2014).

The present findings pertaining to the lipid lowering potential of date fruit supports the earlier results of Al-Yahya et al. (2016). The authors reported the dyslipidemic potential of Ajwa date fruit variety in isoproterenol (ISP)-induced cardiotoxic rodent model. Lyophilized Ajwa date extract was fed to the diseased animals at two levels i.e. 250 and 500 mg/kg/day during 21 days trial. The increase in lipid profile as result of ISP was significantly ameliorated by the Ajwa extracts in a dose dependent manner. The serum cholesterol level in the ISP control group was 201.66±4.83 mg/dL that reduced to 164.33±5.31 and 128.66±3.83 mg/dL by lyophilized Ajwa extract treatment @ 250 and 500 mg/kg/day, respectively. The reduction was 18 and 35% at the aforementioned doses. The higher dose was found more effective as the triglyceride
level also reduced from 154.33±3.20 to 103.51±3.46 mg/dL @ 500 mg/kg i.e. about 32.92% reduction. Similarly, the LDL level also decreased to about 15.74 and 32.92% at lower and higher dose, respectively. The maximum decrease in VLDL was observed from 142.83±6.20 to 67.86±5.96 mg/dL in ISP control group and 500 mg/kg Ajwa extract treated group, correspondingly. Moreover, similar to our study, the Ajwa date extract supplementation significantly improved the serum HDL level up to 31.97%. The possible cholesterol lowering mechanism suggested by the authors focused on the high fiber content in the fruit along with the presence of bioactives and selenium.

In another study, Khalas date pulp was administered on hamsters along with normal and cholesterol containing diets for 13 weeks. Their findings are in accordance with the current results. In case of normal diet fed rats, no significant effect was observed in cholesterol, LDL, TG and HDL levels. However, the cholesterol containing diet raised the lipid profile considerably, whereas date supplemented diet proved effective in modulating the altered lipid profile. The decrease in serum cholesterol level was about 11.30% in normal and 22.29% in hypercholesterolemic rats fed on date-supplemented diets. Likewise, triglycerides reduced up to 28.60 and 32.24% in normal and cholesterol fed rats, respectively. Significant decrease (55.44%) was noted in the LDL level in the hypercholesterolemic rats, whilst the HDL level increased considerably (24.02%) in the diseased group. Consequently, the study advocates the hypolipidemic potential of date fruit (Alsaif et al., 2007). The difference in the results can be attributed to the date variety and dose. The findings of Rock et al. (2009) are also in close agreement with the present study. They summarized their study by declaring date fruit as an anti-atherogenic nutrient because its consumption significantly decreases the serum triacylglycerol levels substantially by 8 to 15%, alongside decreasing the basal oxidative stress up to 33% in healthy subjects for 4 weeks @ 100 g/day dose. They also reported an increase in PON1 activity associated to HDL by 8%. Positive findings regarding the anti-atherogenic potential of date supplementation were associated to the presence of catechins and dietary fiber.

The present study also validates the findings of Vembu et al. (2012) who deduced that phytochemical constituents of date fruit extract are responsible for preventing high fat diet induced obesity and restores the elevated lipid profile as a result of high dietary fat intake. Plant sterols present in the fruit extract could possibly pose beneficial efficaciousness against

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hypercholesterolemic conditions. The phytosterols reduces the cholesterol absorption by increasing the fecal steroid excretion, thereby reducing the lipid contents (Gylling et al., 2014). Moreover, the flavonoids may increase the activity of lecithin acyltransferase that play an imperative role in regulating the blood lipids. It is an anti-atherogenic enzyme that causes esterification of free cholesterol on HDL particles facilitating reverse cholesterol transport (You et al., 2008).

Evidences support that the hypercholesterolemic conditions are strongly associated with cardiac ailments (Dawber et al., 2015). Clinical trials have demonstrated that reducing the total cholesterol and LDL by 1% decreases the incidence of coronary heart disease by 1.5% (Pediatrics, 1992). The cholesterol lowering mechanism of date fruit particularly Zahidi variety and its extract is most likely to be mediated by the presence of major bioactives as gallic acid, caffeic acid, EGCG, kaempferol and quercetin.

Gallic acid (3, 4, 5-trihydroxybenzoic acid) is known to exhibit certain beneficial biological properties. In the studied date variety (Zahidi), gallic acid is present in abundant quantity that may possibly be responsible for its lipid lowering potentials. The study conducted by Hsu and Yen (2007) demonstrates the effectualness of gallic acid against high fat diet-induced dyslipidemic conditions. Gallic acid supplementation @ 100 mg/kg B.W. for 10 days reduces the total cholesterol level (18.33%), LDL cholesterol (37.87%) and triglycerides (13.26%) whilst, the HDL level increases non-significantly (6.52%), thus indicating strong hypolipidemic potential. Moreover, the serum leptin levels also decreases significantly that is directly associated with the body fat. Leptin may increase the intracellular fatty acids by contributing to hepatic steatosis through altering hepatocyte insulin signaling (Uygun et al., 2000). Hence, the plausible hypocholesterolemic mechanism of gallic acid may focus on modulating the leptin levels by preventing their increase, thereby reducing the body fat content. Moreover, gallic acid inhibits the hepatic cholesterol biosynthesis, stimulates receptor-mediated catabolism of LDL and increases the fecal bile acid secretion. Therefore, it can be inferred that the lipogenesis inhibiting potential of gallic acid is responsible for reducing the triglycerides, LDLs and VLDLs (Kulkarni and Swamy, 2015).

Similarly, several scientific evidences endorse the anti-atherosclerotic potential of EGCG, kaempferol and quercitin (Salvamani et al., 2014). In this vista, Kong et al. (2013) suggested
that 30 and 150 mg/kg kaempferol treatment for six and ten weeks prevents high cholesterol induced atherosclerosis in New Zealand rabbits. It decreases the serum lipids and improves the antioxidant ability, besides down regulating the protein and gene expression of pro-atherogenic biomolecules (MCP-1, E-sel, VCAM-1, ICAM-1). It also reduces the release of IL-1β and TNF-α. A more mechanistic insight to the cholesterol regulating mechanism of date fruit polyphenols may be described through a docking study conducted by Islam et al. (2015). Their research clearly demonstrates that EGCG, kaempferol and quercitin can sterically hinder HMG-CoA binding to substrate by blocking the active site through to the L-domain and cis-loop resulting in substrate cavity, therefore, acting as competitive inhibitors.

Besides, date fruit is rich in dietary fiber. Dietary fiber quantification has not been carried out in the present study but the previous studies suggest dates to be abundant in dietary fiber. The crude fiber analysis shows that selected variety contains the maximum amount. Irrefutable scientific evidences based on cohort studies and meta-analysis positively correlate dietary fiber consumption and reduced risk of CVDs by modifying blood lipid profiles particularly decreasing the LDL cholesterol (Riccioni et al., 2012; Wu et al., 2014).

Conclusively, the increased concentration of serum lipids and lipoprotein fractions foster the development of atherosclerotic conditions. Atherogenic diet containing high amounts of fats and cholesterol contribute to the pathophysiological conditions in various organs of the physiologic system, primarily due to the disturbed lipid moieties. Compelling evidences focus that decreased LDLs and increased HDLs may considerably reduce the risk of major coronary diseases. From the current results, it is intriguing to speculate that Zahidi date fruit and extract administration proves to be beneficial in reducing the biomarkers of lipidemic stress and ultimately lowering the risk of cardiovascular and allied ailments.

4.6.3.2. Atherogenic risk ratios

Several atherogenic ratios have been developed involving simple lipid tests to assess the peril of cardiovascular disorders. Accordingly, the atherogenic index of plasma (AIP) is based on triglycerides and HDL, the atherogenic coefficient (AC) is calculated on the basis of total cholesterol and HDL. Similarly, Castelli risk index one [CRI (I)] also involves cholesterol and HDL as the determinants of cardiac malfunctioning. Whereas, Castelli risk index II [CRI (II)] is another fraction that involves the independent CVD risk factors i.e. HDL and LDL. These
ratios are relied due to their significance of predicting the risk of cardiac stress (Nimmanapalli et al., 2017).

The atherogenic index of plasma reflects the balance between the damaging and restoring lipoproteins i.e. triglycerides and high-density lipoproteins. Significant effect of treatment was recorded in normal as well as atherogenic rats. In case of normal rats, $G_1$ exhibited $0.30\pm0.01$ AIP value that reduced due to date fruit as well as date extract treatment to $0.28\pm0.01$ in $G_2$ and $0.27\pm0.01$ in $G_3$ group. However, concerning the atherogenic rats, considerable increase of $0.69\pm0.02$ was observed in the AIP ratio that decreased in the date fruit treated groups $G_5$ and $G_6$ to $0.60\pm0.01$ and $0.58\pm0.02$, respectively (Table 4.18).

The atherogenic coefficient is a ratio between non-HDL and HDL. The present results suggest significant impact of treatments on the AC values. The recorded ratio was higher in normal diet fed rats ($G_1$) $1.53\pm0.05$ that decreased in the preceding date fruit ($G_2$) and date extract ($G_3$) fed groups to $1.39\pm0.06$ and $1.33\pm0.06$, respectively. Likewise, in the rats fed on atherogenic diet ($G_4$), the AC value was highest ($5.37\pm0.19$), that reduced in $G_5$ ($4.01\pm0.15$) and $G_6$ ($3.71\pm0.13$) upon the administration of date fruit and date extract containing atherogenic diets, correspondingly (Table 4.18).

Castelli’s risk indexes are based on three major biomarkers of dyslipidemia. The current study indicates non-significant effect of treatment on CRI (I) and CRI (II) in normal rats. Nonetheless, atherogenic groups expounded significant effect of treatments on these ratios. The obtained results for CRI (I) are $2.53\pm0.08$, $2.39\pm0.09$ and $2.33\pm0.07$ in $G_1$, $G_2$ and $G_3$. Whereas, $G_4$, $G_5$ and $G_6$ rats exhibited $6.37\pm0.20$, $5.01\pm0.18$ and $4.71\pm0.17$, correspondingly. CRI (II) ranged from $0.96\pm0.03$ to $1.12\pm0.04$ in normal rats and $2.95\pm0.10$ to $4.40\pm0.13$ in the atherogenic ones (Table 4.18).

Atherogenic ratios are potential assessors of CVD risks, higher the ratio more is the risk of developing the cardiovascular ailments and vice versa (Ikewuchi and Ikewuchi, 2009). These lipid ratios are considered better predictors as compared to the single lipid markers owing to their association with a cluster of risk factors that may be somewhat unrelated to the cholesterol metabolism. For instance, the triglyceride to HDL ratio also negatively correlates with the insulin stimulated glucose disposal (Brehm et al., 2004). AIP is the proposed as the predictive
### Table 4.18. Effect of diets on atherogenic risk ratios

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental Groups</th>
<th>Atherogenic Risk Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AIP</td>
</tr>
<tr>
<td>Normal Diet</td>
<td>G1</td>
<td>0.30±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>0.28±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>0.27±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>14.33&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Atherogenic Diet</td>
<td>G4</td>
<td>0.69±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G5</td>
<td>0.60±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G6</td>
<td>0.58±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>52.89&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests.
Where; AIP=log TG/HDL, AC= (TC-HDL)/HDL, CRI (I) =TC/HDL, CRI (II) =LDL/HDL.

* = Significant
** = Highly significant
NS = non-significant

G1 Normal diet
G2 Date fruit + normal diet
G3 Date extract + normal diet
G4 Atherogenic diet
G5 Date fruit + atherogenic diet
G6 Date extract + atherogenic diet
marker for CVD risk focusing plasma atherogenicity based on the logarithm of triglyceride to HDL ratio (Upadhyay, 2015). It also correlates with the size of LDL and HDL particles and rate of fractional cholesterol esterification. Whereas, atherogenic coefficient is a measure of bad cholesterol (LDL, VLDL, IDL) with respect to good cholesterol (HDL). It reflects the atherogenic potential of entire spectrum of lipoproteins, hence, indicating the CVD risk (Nimmanapalli et al., 2017). The Castelli’s risk indexes (I & II), based on three important lipid parameters i.e. TC, HDL and LDL that are the predictors of dyslipidemia associated cardiovascular events. The calculation of these ratios can be helpful in envisaging the disease risk as well as the effectualness of therapeutic intervention (Bhardwaj et al., 2013).

In the present study, it is apparent from the results that all the atherogenic risk ratios in normal rats decreased with the provision of date fruit and extract based diets. However, atherogenic diet elevated the ratios that were ameliorated significantly with date-based diets. Specifically date extract proved to be more anti-atherogenic as compared to date fruit. In this regard, Dobiášová and Frohlich (2001) stated that high AIP value predicts higher risk for atherosclerosis or coronary artery disease. Likewise, Rosolova et al. (2014) found that the lipid modifying therapeutic interventions significantly reduces the AIP in atherogenic dyslipidemic patients from a high CV risk to low risk levels. The cholesterol containing diet increases the CRI (I) and CRI (II) ratio as well. Similar effect was observed by Fidèle et al. (2017) in a recent investigation. The researchers observed non-significant increase in the TC/HDL and LDL/HDL ratio in normocholesterolemic rats. However, the hypercholesterolemic rats treated with atorvastatin showed significant reduction in the ratios in a dose dependent manner.

The effect of dietary bioactives (thymoquinone and limonene) was assessed on the CVD risk ratios of atherogenic rats by Ahmad and Beg (2013). Their findings regarding the effect of bioactives on atherogenic rats corroborate with the present results. The ratios increased in lipidemic groups as compared to the non-lipidemic ones, however, the treatments significantly restored the ratios. It is inferred that lowering the cholesterol and LDL content lowers these ratios, hence decreasing the perils of cardiovascular morbidity and mortality. Nevertheless, it was found through regression analysis that out of the four risk ratios, AIP contributes 30% to the CVD risk analysis followed by CRI (I) 20%, AC 16% and CRI II 13% (Bhardwaj et al., 2013). Still, the ratios are helpful risk determinants and can give a quick risk analyses for further treatment and diet manipulation. In the present study, these ratios predicted that the rats
fed on atherogenic diets are at high risk of developing cardiac complications as compared to the normal ones. Moreover, the date extract treatment proved to more effective in alleviating the CVD risk.

4.6.3.3. Serum oxidative stress indicators

Superoxide dismutase and catalase are the primary endogenous antioxidant enzymes in the line of defense against the venomous effects of oxygen radicals in the cells, that catalyzes the dismutation of superoxide radical to \( \text{H}_2\text{O}_2 \), thereby scavenge the free radicals. The statistical interpretation (F values) in Table 4.19 displays momentous differences in serum superoxide dismutase level of rats as a function of treatments during the bioefficacy assessment trial. Means pertaining to the SOD level in normal rats expounded lowest value for \( G_1 \) (16.15±0.50 U/mL) that gradually improved in \( G_2 \) (17.28±0.54 U/mL) and \( G_3 \) (17.04±0.53 U/mL). Likewise, momentous escalation for this attribute was also reported in the atherogenic diet fed rats in which the SOD concentration declined in \( G_4 \) (8.62±0.30 U/mL) that was improved in date fruit fed group (\( G_5 \)) 12.15±0.43 U/mL, trailed by date extract fed group (\( G_6 \)) 11.97±0.42 U/mL.

It is evident from the graphical representation (Fig. 4.12) that date based diets intensified superoxide dismutase level throughout the study. In this context, normal rats presented 6.54 and 5.22% enhancement in SOD by date fruit and date extract containing diets, correspondingly. Similarly, in the atherogenic diet fed groups, percent increase of 29.05 and 27.99 in SOD was observed through the provision of date fruit and date extract supplemented diets, respectively.

F value in Table 4.19 shows that date based diets imparted significant effect on serum catalase contents (CAT) of rats. In normal rats, mean catalase content for \( G_1 \) (91.47±2.83 U/mL) was lower than that of \( G_2 \) (105.72±3.27 U/mL) and \( G_3 \) (99.82±3.09 U/mL) groups. Likewise, in atherogenic rats, \( G_4 \) group consuming control diet showed lesser catalase value as 41.24±1.45 U/mL that increased to 53.28±1.88 and 50.51±1.78 U/mL in \( G_5 \) and \( G_6 \) groups taking date fruit and date extract containing functional diets, respectively. Fig. 4.12 shows percent increase in serum catalase contents in \( G_2 \) was 13.48%, whilst in \( G_3 \), date extract containing diet resulted in 8.37% increase as compared to control. Similarly, in the atherogenic groups, treatments \( G_5 \) and \( G_6 \) showed 22.60 and 18.35% increment, correspondingly for this trait.
Lipid peroxidation is a free radical and reactive oxygen species-mediated chain of reactions that when initiated leads to severe oxidative damage in serum and tissues. The lipid peroxides are unstable in nature and degrade rapidly into certain sub-products. Malondialdehyde (MDA) is the secondary product of lipid peroxidation and most popular marker of oxidative damage to cells (Grotto et al., 2009). It is obvious from the F values displayed in Table 4.18 that serum MDA was considerably affected by the treatments in all groups. Means regarding MDA (normal rats) indicates the highest value of 2.02±0.06 nM/mL in G₁ that significantly reduced to 1.74±0.05 and 1.53±0.04 nM/mL in G₂ and G₃ groups, respectively. Similarly, in the atherogenic diet fed rats, highest MDA value was observed in G₄ (11.59±0.40 nM/mL) that substantially decreased in G₅ (7.69±0.27 nM/mL) and G₆ (8.26±0.29 nM/mL). Fig. 4.12 shows the percent reduction in serum MDA levels by the administration of date based diets. Date fruit have been found to possess more ameliorative properties as lowered 13.64 and 33.67% lipid peroxidation in normal and atherogenic rats, respectively. While, the rats fed on date extract containing diets showed 12.54 and 28.76% reduction in normal and atherogenic rats, correspondingly.

Similar restorative effect of date fruit on endogenous enzymes and lipid peroxidation was observed by Ramadhas et al. (2014) who studied the effect of 200 mg/kg B.W. oral date fruit extract on lambda cyhalothrin induced toxic rats for 21 days. Date fruit treatment substantially improved the status of endogenous enzymes SOD and CAT in the toxic group by 35.68 and 32.88%, respectively. Lipid peroxidation increased significantly in the toxic group; however, date fruit extract treatment reduced the lipid peroxidation considerably. The reduction in serum lipid peroxidation through date fruit extract administration was also observed in another study conducted by Ragab et al. (2013). Ajwa date extract supplementation (300 mg/kg/day) to lead acetate induced stressed rabbits for 14 days decreased the MDA levels by 4.87%. The researchers also observed marked improvements in serum endogenous enzymes SOD and glutathione peroxidase. The results are also in harmony with the findings of Pushpa and Jayachitra (2015) who studied the effect of ethanolic date fruit extract (200 mg/kg) in normal and triton treated Wistar rats. In normal rats, the serum SOD level increased non-significantly from 10.60±1.04 to 10.69±1.05 U/mg protein, while in triton treated rats SOD level significantly increased from 6.39±0.61 to 9.68±0.98 U/mg protein. The MDA level increased
Table 4.19. Effect of diets on serum oxidative stress indicators

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental Groups</th>
<th>Serum Oxidative Stress Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SOD (U/mL)</td>
</tr>
<tr>
<td>Normal Diet</td>
<td>G1</td>
<td>16.15±0.50&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>17.28±0.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>17.04±0.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>6.75*</td>
</tr>
<tr>
<td>Atherogenic Diet</td>
<td>G4</td>
<td>8.62±0.30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G5</td>
<td>12.15±0.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G6</td>
<td>11.97±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>245.57**</td>
</tr>
</tbody>
</table>

Values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests
* = Significant
**= Highly significant
NS = non-significant
G1 Normal diet
G2 Date fruit + normal diet
G3 Date extract + normal diet
G4 Atherogenic diet
G5 Date fruit + atherogenic diet
G6 Date extract + atherogenic diet

Figure 4.12. Percent change in serum oxidative stress markers
due to triton 5.26±0.60 nmol/mL that significantly reduced by the date extract treatment to 3.45±0.49 nmol/mL.

It is inferred that free radical scavenging potential of date fruit may perhaps be responsible for reducing the lipid peroxidation. Phytochemical moieties possess significant antioxidant potential i.e. capable of inhibiting the production of reactive oxygen species, thereby reducing the associated intracellular oxidative stress (Feng et al., 2001). Moreover, improvement in the endogenous antioxidant enzyme activity is the major approach towards reducing the exogenous stress inducing compounds.
PART 2: CARDIAC STRESS BIOMARKERS
4.6.4. Cardiac stress biomarkers

Biomarkers for cardiac damage include aspartate transaminase (AST), creatine kinase (CK), creatine kinase MB (CK-MB) and lactate dehydrogenase. The assessment involves simple biochemical testing of these markers in serum. While, in tissue SOD, CAT and lipid peroxidation status indicates about the extent of stress in the myocardium.

4.6.4.1. Serum cardiac stress biomarkers

The F statistic values presented in Table 4.20 indicates that serum AST level was substantially affected in normal as well as atherogenic diet fed rats. In the rats fed on normal diet, maximum value for AST was obtained by G₁ as 78.12±2.75 IU/L followed by G₂ 74.54±2.38 IU/L and G₃ 71.39±2.57 IU/L. Besides in the atherogenic diet fed rats, G₄ group showed maximum AST level i.e. 114.47±3.54 IU/L that reduced considerably in G₅ 97.91±3.32 IU/L and G₆ 92.72±3.98 IU/L.

The F values showed non-significant effect of treatment on creatine kinase (CK) in normal diet fed rats, whereas significant differences were recorded for the atherogenic rats. Mean values for CK in normal rats G₁, G₂ and G₃ differed non-substantially i.e. 268.44±9.66, 262.15±9.54 and 258.23±8.77 IU/L, respectively. However, in the groups fed on high cholesterol containing atherogenic diet, CK level increased to 473.51±15.15 IU/L in G₄ group nonetheless, diets containing date fruit (G₅) and date extract (G₆) suppressed the values for this trait to 394.55±14.99 and 381.76±16.41 IU/L, respectively.

Statistical analysis (F value) shows that date based diets impart momentous effect on CK-MB level of rats in the entire bioefficacy assessment. In normal rats, mean CK-MB level for G₁ (85.66±2.68 IU/L) was higher than that of G₂ (80.06±2.93 IU/L) and G₃ (78.46±2.77 IU/L) groups. Likewise, in the atherogenic rats, G₄ group consuming atherogenic diet showed higher CK-MB value as 145.98±4.52 IU/L that decreased to 122.64±4.57 and 119.68±3.82 IU/L in G₅ and G₆ groups taking date fruit and date extract based diets, respectively.

F values revealed significant effect of treatments on LDH in all groups. Mean LDH for normal rat groups; G₁, G₂ and G₃ were 258.74±8.07, 240.05±7.78 and 227.82±7.51 IU/L, respectively. Means pertaining to LDH level in case of stressed rats, showed high value in G₄ (354.86±11.06
IU/L) that decreased due to date fruit and extract supplementation in G5 (298.05±9.53 IU/L) and G6 (274.98±9.89 IU/L), respectively.

It is obvious from Fig. 4.13 that date fruit and extract treatments are effective in restoring the cardiac stress markers. Dietary date fruit supplementation lowered 4.58 and 14.47% AST level in normal and atherogenic rat sera, correspondingly. However, date fruit extract proved to be more effective in lowering the serum AST by 8.61 and 19% in normal and stressed rats, respectively. Likewise, CK levels indicating the muscle functionality also decreased up to 2.34 and 3.80% by date fruit and date extract treatments in normal rats. Whereas, 16.68 and 19.38 percent reduction was recorded in the rats fed on date fruit and date extract containing atherogenic diets, respectively. The cardiac specific CK-MB levels decreased by 6.54 and 8.41% in G2 and G3 groups, respectively. Whilst, more reduction was recorded in the groups fed on atherogenic diet along with date fruit (15.99%) and date extract (18.02%). Similarly, the date fruit and date extract treatment also modulated the serum LDH levels by 7.22 and 11.95% in normal rats and 16.01 and 22.51% in atherogenic rats, respectively.

Cardiovascular events are closely associated with cholesterol rich diets. Atherogenic or high cholesterol diet enhances the cholesterol deposition in tissues in form of cholesterol esters. In this regard, numerous scientific explorations have been focused on pre-clinical and clinical trials employing natural antioxidants as therapeutic agents to ameliorate atherosclerosis induced lipidemic stress. The present results are in congruence with the earlier study of Al-Yahya et al. (2016) who elucidated the cardioprotective ability of Ajwa date extracts on albino male Wistar rats. The oral administration of 500 mg/kg/B.W. lyophilized Ajwa date extracts effectively reduced the cardiac stress markers against an ISP induced cardio-toxic control. The AST, CK and LDH levels reduced to 37.22, 19.19 and 29.26% respectively. However, in our study lower results are reported that may be due to the difference in varietal composition as well as the dose rate. The results obtained are also in substantiation with the results of Ramadhas et al. (2014) pertaining to the amelioration of lambda cyhalothrin induced toxicity via date fruit extract administration. Oral date extract supplementation (200 mg/kg B.W.) for 21 days in lambda cyhalothrin reduced AST and LDH up to 32.5 and 23.43%, respectively.

AST and LDH are important indicators of cardiac as well as hepatic stress. The ameliorative potential of date fruit extract on serum AST and LDH is also consistent with the previous
Table 4.20. Effect of diets on serum cardiac stress markers

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental Groups</th>
<th>Serum Cardiac Stress Markers (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AST</td>
</tr>
<tr>
<td>Normal Diet</td>
<td>G1</td>
<td>78.12±2.75a</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>74.54±2.38ab</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>71.39±2.57b</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>9.03*</td>
</tr>
<tr>
<td>Atherogenic Diet</td>
<td>G4</td>
<td>114.47±3.54a</td>
</tr>
<tr>
<td></td>
<td>G5</td>
<td>97.91±3.32b</td>
</tr>
<tr>
<td></td>
<td>G6</td>
<td>92.72±3.98c</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>60.26**</td>
</tr>
</tbody>
</table>

Values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests
* = Significant
** = Highly significant
NS = non-significant
G1 Normal diet
G2 Date fruit + normal diet
G3 Date extract + normal diet
G4 Atherogenic diet
G5 Date fruit + atherogenic diet
G6 Date extract + atherogenic diet
Figure 4.13. Percent reduction in serum specific cardiac stress markers
results by Saafi et al. (2011) who expounded the effect of Deglet Nour date extract (4 mL/kg) on dimethoate induced oxidative stressed rats for 2 months. Pre and post treatment with date extract significantly restored the damage caused by dimethoate as compared to the stressed group. The results also support the findings of El Arem et al. (2014a) who observed 15.83% reduction in serum AST on dichloroacetic acid induced hepatic damaged rats due to Delga date extract supplementation (4 mL/kg/ B.W.), in the same group, serum LDH also decreased up to 6.61%. The variations might be attributed to the variety, stage and dose rate.

The results also corroborate to the findings of Ragab et al. (2013) who investigated the effect of Ajwa date extracts (300 mg/kg/day) on lead acetate induced oxidative stress. Marked variations in AST and CK-MB were recorded in rabbit sera after 14-day trial. The AST level reduced from 423.3±38.12 to 144.11±50.23 IU/L in date extract administered stressed group. Likewise, serum CK-MB levels decreased up to 38.66% indicating the prophylactic potential of the fruit.

It is apparent from the phytochemical analysis, mentioned in the second section (4.2.), that Zahidi dates contains EGCG that has been reported to reduce cardiac stress. Zhong et al. (2015) studied the effect of EGCG against myocardial infarction. The hypercholesterolemic diet containing 5% cholesterol, 2% sodium cholate, 0.15% thiouracil and 0.4% choline chloride was administered to Wistar rats for 30 days followed by the intraperitoneal EGCG administration @ 100 mg/kg B.W. for the last 15 days. The results showed that EGCG improves the serum lipid profile and cardiac risk ratio. The percent decrease in LDH and AST in the hypercholesterolemic rats injected with EGCG was 42.43 and 29.41%, respectively. Similar findings were obtained by Miltonprabu and Thangapandiyan (2015) regarding the restorative potential of EGCG against fluoride induced cardiotoxicity. Pre-administration of EGCG in the intoxicated rats resulted in 50.77% reduction in AST, 50.82% in CK and 22.68% in CK-MB. EGCG regulates the lipid metabolism by increasing the levels of eNOS and SIRT1 that is responsible for exerting protective effects against CVDs. Moreover, EGCG reduces p-AMPKα in the myocardium that may elucidate the key mechanism of EGCG against tissue lipid deposition. The present research validates the in vivo cardioprotective ability of Pakistani Zahidi date and its extract. Further studies are required to determine the effective dose.
4.6.4.2. Cardiac tissue stress indicators

The antioxidant enzymes; superoxide dismutase and catalase alongwith cardiac lipid peroxidation was examined in the cardiac tissue. It is apparent from the F statistic results presented in the Table 4.21 that treatments explicated momentous variations with respect to superoxide dismutase content (SOD) in the cardiac tissues. In normal rats, means exhibited lowest value for the control G₁ group \(13.28\pm0.45 \text{ U/g}\) that was augmented in the date fruit fed group G₂ \(14.48\pm0.46 \text{ U/g}\) and date extract fed group G₃ \(15.35\pm0.56 \text{ U/g}\). Likewise, substantial increase was recorded in the atherogenic diet fed groups, the lowest value was obtained in the atherogenic control group (G₄) \(5.66\pm0.20 \text{ U/g}\) that was improved in date fruit fed group (G₅) \(8.43\pm0.38 \text{ U/g}\) trailed by date extract fed group (G₆) \(9.18\pm0.31 \text{ U/g}\). It is evident from graphical representation in Fig. 4.14 that date enriched functional diets improved the SOD levels in normal as well as atherogenic rats. Maximum increase in SOD was observed G₆ i.e. 38.34% followed by 32.86% in the G₅. While in case of normal groups, 8.29 and 13.49% reduction was observed in G₂ and G₃ groups, respectively.

Catalase enables \(\text{H}_2\text{O}_2\) disposal by erythrocytes, thus protecting against reactive oxygen species. Statistical analysis in Table 4.21 revealed that dietary date fruit supplementation imparts significant effect on cardiac catalase contents. In normal rats, mean catalase content \(95.6\pm3.11 \text{ U/g}\) was increased by the date fruit treatment in G₂ \(108.87\pm3.39 \text{ U/g}\) and date extract treatment in G₃ \(113.42\pm3.52 \text{ U/g}\) groups. Similar increasing trend was observed in the atherogenic diet fed rat groups where G₄ group consuming cholesterol rich diet showed lesser catalase value as \(40.81\pm1.49\) that was improved in the myocardium of G₅ and G₆ groups i.e. \(59.95\pm2.07\) and \(63.48\pm2.06 \text{ U/g}\), respectively. It is apparent from Fig. 4.14 that catalase contents were increased by 12.19 and 15.71% in normal rats by dietary date fruit and date extract supplementation, correspondingly. Besides, the cardiac catalase content was elevated up to 31.93 and 35.71% in atherogenic rats fed on date fruit and date extract based diets, respectively.

Assessment of lipid peroxidation extent is of considerable importance in understanding the pathologies associated with oxidative stress. In this regard, MDA is a good biomarker (Grotto et al., 2009). F statistical value showed that cardiac malondialdehyde concentrations (MDA) were affected substantially by the treatments in all groups (Table 4.21). In normal rats, the
obtained means showed a diminishing pattern with highest tissue MDA concentration in control group (G₁) 5.15±0.18 (nM/g wet weight) that was decreased by the administration of date fruit containing diet to 4.54±0.17 (nM/g wet weight) and date extract containing diet to 4.48±0.14 nM/g wet weight. In atherogenic diet fed rats, the MDA levels increased significantly in G₄ group 15.35±0.54 nM/g that was decreased to 12.27±0.46 and 11.41±0.44 nM/g by the consumption of date fruit and date extract containing diets, respectively. The percent decrease in cardiac MDA levels is graphically represented in Fig. 4.14. The date fruit diets reduced lipid peroxidation in the cardiac tissues by 11.84 and 20.07% in normal and atherogenic rats, respectively. However, more pronounced decrease was observed by the supplementation of date extract i.e. 13.01 and 25.67% in normal and stressed rats, correspondingly.

Oxidative stress is amongst the major causative factors that associates hyperlipidemia with atherogenesis and myocardial infarction (Lee et al., 2007). It increases the production of free radicals, thereby lowering the cellular antioxidant status and elevating the mitochondrial respiration. SOD and CAT are the enzymatic antioxidants involved in the quenching of free radicals, disposal of superoxide anions and hydrogen peroxides. Whereas, lipid peroxidation initiates chain of reactions that enhances the MDA production (Thiruchenduran et al., 2011). It plays key role in the accumulation of lipids in the cardiac tissue resulting in myocardial membrane damage leading to inactivation of several membrane bound enzymes and impaired membrane functionalities (Thangapandiyan and Miltonprabu, 2013).

The ability of date fruit in improving the oxidative stress biomarkers in cardiac tissue (SOD and CAT) and reducing the cardiac lipid peroxidation (MDA) is also reported in another study on Saudi date variety (Ajwa). The cardiac SOD activity increased up to 34.60 and 47.59% by the oral administration of 250 mg/kg and 500 mg/kg date extract, respectively for 21 days. The increased MDA level as result of ISP injection was lowered to 50.92% by 250 mg/kg lyophilized Ajwa extract and 79.11% through 500 mg/kg extract (Al-Yahya et al., 2016).

EGCG being a prominent polyphenol in the selected Zahidi variety may also be responsible for its affirmative effects against cardiac stress. Zhong et al. (2015) elucidated the effect of EGCG on hypercholestrolemic rats. Their research states that EGCG improves the cardiac enzymatic and non-enzymatic antioxidant status alongside decreasing the lipid peroxidation in
Table 4.21. Effect of diets on stress indicators in cardiac tissue

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental Groups</th>
<th>Cardiac Tissue Stress Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SOD (U/g wet weight)</td>
</tr>
<tr>
<td>Normal Diet</td>
<td>G₁</td>
<td>13.28±0.45&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G₂</td>
<td>14.48±0.46&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>G₃</td>
<td>15.35±0.56&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>20.31**</td>
</tr>
<tr>
<td>Atherogenic Diet</td>
<td>G₄</td>
<td>5.66±0.20&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G₅</td>
<td>8.43±0.38&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>G₆</td>
<td>9.18±0.31&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>F value</td>
<td>364.80**</td>
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</table>

Values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests
* = Significant
** = Highly significant
NS = non-significant
G₁ = Normal diet
G₂ = Date fruit + normal diet
G₃ = Date extract + normal diet
G₄ = Atherogenic diet
G₅ = Date fruit + atherogenic diet
G₆ = Date extract + atherogenic diet

Figure 4.14. Percent change in cardio-specific stress markers
the tissue. In another investigation, Miltonprabu and Thangapandiyan (2015) studied the effect of EGCG on fluoride induced cardiac stress. The results indicated 13.59 and 24.52% improvement in SOD and CAT levels of stressed rats fed on EGCG (40 mg/kg B.W.), respectively. Lipid peroxidation also reduced significantly up to 43.52 by the administration of EGCG to the fluoride induced cardiotoxic rats. Other bioactive moieties present in the date fruit and extract such as quercitin, catechin, gallic acid and kaempferol also possess the potential to alleviate cardiovascular disorders as validated by various studies.

4.6.4.3. Histopathological examination of cardiac tissues

The histopathological examination of rat myocardium of all groups is illustrated in photomicrographs (Fig. 4.15). The cardiac parenchyma in G₁ is indicating condensed and pyknotic nuclei along with mild degree of congestion, while in G₂ and G₃ the cardiac parenchyma is normal in appearance with well-organized nucleus having clear stripes of the myocardial fibers. However, in G₄ the cardiac tissue is indicating marked disorganization with the presence of condensed and pyknotic nuclei along with mild degree of congestion. Mild degree of hemorrhages are also present in the cardiac parenchyma. While, G₅ and G₆ parenchyma indicates mild degree of changes in the cardiac parenchyma with mild congestion. At few places, nuclei of cells are also condensed. However, most of the parenchyma is normal in appearance revealing the ameliorative potential of date fruit.

Similar histopathological changes in the rat myocardium are reported by Al-Yahya et al. (2016) who found that date extract treatment mitigates the ISP-induced changes in the rat myocardium and improves the lesions in the tissues dose dependently. The findings of this research are also consistent with the previous results of Ragab et al. (2013), who studied the effect of Ajwa date extract @ 300 mg/kg/day on lead toxicity induced rabbits for 14 days trial period. The myocardium showed normal structure in the date fed group and control group, while abnormalities such as vascular congestion, swelling and necrosis were recorded in lead toxicity induced group. However, the date extract treatment significantly ameliorated the adverse effects of lead toxicity, thereby reducing cardiac muscle congestion, swelling and necrosis.

High cholesterol containing diet leads to edema, myocardial fiber disruption and neutrophil infiltration. These changes were ameliorated by EGCG treatment in hypercholesterolemic rats.
as studied by Zhong et al. (2015). EGCG has been identified in the studied variety that may be responsible for the positive histological alterations in the present study. Miltonprabu and Thangapandiyan (2015) also observed improved histology in cardiac stress induced rats treated with EGCG probably due to its anti-lipoprooxidative and membrane stabilizing properties.

The results suggest that date fruit extract exerts affirmative effects on the human heart. It ameliorates the cardiac enzymes in serum as well as tissue alongside restoring the tissue lipid peroxidation and histological alterations due to high cholesterol diet intake. Therefore, dietary date fruit incorporation could prove to be cardiophillic in nature, protecting and ensuring proper heart functioning.
Figure 4.15. Histopathological analysis of rat myocardium

G1: Normal rat heart showing slight degree of condensation; G2: Date fruit fed rat showing normal appearance of cardiac fibers without any histological alterations; G3: Date extract diet administered rat tissue represents no alteration with normal myocardium; G4: Atherogenic diet fed rat heart showing cardiac damage along with presence of pyknotic nuclei and hemorrhagic conditions; G5: Date fruit + atherogenic diet fed rat’s heart is reflecting the restorative effect of dates with normal appearance of cardiac tissue; G6: Date extract + atherogenic diet fed rat heart revealing mild degree of alterations in cardiac parenchyma, however, most of it is normal in appearance.
PART 3: HEPATIC STRESS BIOMARKERS
4.6.5 Hepatic stress biomarkers

Liver localized enzymes ALT, ALP and γ-GT along with total, direct and indirect bilirubin was determined in the rat sera to analyze the effect of date fruit and extract containing diets. Superoxide dismutase, catalase and lipid peroxidation was measured in the hepatic tissue. Moreover, histopathological alterations in the tissue were also observed.

4.6.5.1. Serum hepatic stress markers

Alanine aminotransferase (ALT) is a liver localized enzyme. It is present in cytosol and is an important indicator of hepatic damage, as in case of membrane injury, it leaks into the blood stream. It is inferred from the F statistics (Table 4.22) that treatments considerably affected serum ALT in all groups. Declining trend was observed in all groups upon the administration of date-based diets. In normal diet fed rats, higher ALT value (38.12±1.18 IU/L) was noted in G₁ that reduced in G₂ (36.43±1.16 IU/L) and G₃ (35.08±1.13 IU/L) groups. Likewise in stressed rat groups, mean for ALT in G₄ was 72.21±2.55 IU/L that decreased to 59.82±1.91 IU/L in G₅ (date fruit containing atherogenic diet) and 56.72±2.61 IU/L in G₆ (date extract containing atherogenic diet). Fig. 4.16 pinpoints 4.43 and 7.97% reduction in serum ALT levels by the administration of date fruit and date extract containing diets in normal rats, respectively. While, date fruit treatment lowered the ALP levels by 17.16% and date extract treatment reduced up to 21.45% in the atherogenic rats.

It is obvious from the F values that ALP level was substantially affected by treatments in all groups (Table 4.22). Mean ALP values in normal rats for G₁, G₂ and G₃ groups were 164.89±5.11, 156.97±6.74 and 153.64±6.95 IU/L, correspondingly. In oxidative stressed rats, supply of high cholesterol containing diet to animals increased their ALP level to 215.43±7.75 IU/L in G₄ group, whilst its value decreased in G₅ and G₆ groups consuming date fruit and date extract enriched diets to 191.83±7.38 and 183.61±6.24 IU/L, respectively. It is apparent from Fig. 4.16, that date fruit and date extract treatment are effective in ameliorating serum ALP levels in normal rats 4.80 and 6.82% and atherogenic rats 10.95 and 14.77%, respectively.

Gamma glutamyl transferase (γ-GT) is considered as an index of liver dysfunction. The characteristic F statistic value revealed non-momentous effect of treatments on γ-GT content in normal rat sera. Although, momentous impact of treatments was exhibited by atherogenic rats. In normal groups, higher γ-GT value was recorded in the control group G₁ (1.95±0.06
IU/L) as compared to the date fruit fed group G₂ (1.91±0.07 IU/L) and date extract fed group G₃ (1.87±0.06 IU/L). Mean γ-GT value was raised up to 2.49±0.08 IU/L in rats fed on atherogenic diet (G₄). However, diminishing trend was observed by the administration of date fruit (G₅) and date extract (G₆) containing atherogenic diets as 2.15±0.08 and 2.02±0.07 IU/L, respectively. Fig. 4.16 depicts 2.05 and 13.65% reduction in γ-GT levels in normal and stressed rats by the administration of date fruit. More profound decrease in γ-GT was observed by the administration of date extract in normal as well as atherogenic rats as 4.10 and 18.88%, respectively. It is evident from Table 4.22 that the AST/ALT ratio remained non-significant in the entire trial. The ratio ranged from 2.04-2.05 in normal rats and 1.59-1.64 in case of oxidative stress induced rats.

It is deduced from the F values that the effect of treatments was statistically alike in normal diet administered groups, whilst for atherogenic diet fed groups substantial behavior was observed for direct, indirect and total bilirubin. Mean values for direct, indirect and total bilirubin in G₁ were 0.38±0.01, 0.83±0.03 and 1.21±0.04 mg/dL, respectively. While, these values decreased non-momentously in G₂ as 0.36±0.01, 0.81±0.03 and 1.17±0.05 followed by G₃ as 0.36±0.01, 0.82±0.03 and 1.18±0.05 mg/dL, correspondingly. However, higher values were reported for the said traits in the atherogenic diet fed rats as in G₄ the recorded values for direct, indirect and total bilirubin were 0.91±0.03, 0.93±0.03 and 1.84±0.07 mg/dL, respectively. These values were reduced in G₅ as 0.85±0.03 mg/dL for direct bilirubin, 0.86±0.03 mg/dL for indirect and 1.71±0.06 mg/dL for total bilirubin. Whilst, maximum reduction in direct (0.81±0.03 mg/dL), indirect (0.84±0.03 mg/dL) and total bilirubin (1.65±0.06 mg/dL) was recorded in rats fed on date extract containing atherogenic diet. It is apparent from Fig. 4.16 that decrease in total bilirubin was 3.31 and 7.07% by the date fruit supplementation in normal and atherogenic diet fed rats, correspondingly. Nevertheless, the date extract supplemented functional diets decreased 2.48% bilirubin in normal rats and 10.33% in atherogenic rats.

Consumption of adipogenic diet consisting of high fat constituents is known to increase the oxidative stress conditions in the physiologic system. Indeed, such diet is associated with increasing the energy flux leading to lipid accumulation in the peripheral tissues, including liver and muscles, resulting in microvesicularsteatosis. The accumulation of ectopic lipid accelerates the generation of ROS leading to several other metabolic
Table 4.22. Effect of diets on serum hepatic stress markers

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental Groups</th>
<th>Serum Hepatic Stress Markers</th>
<th>Bilirubin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ALT (IU/L)</td>
<td>ALP (IU/L)</td>
</tr>
<tr>
<td>Normal Diet</td>
<td>G1</td>
<td>38.12±1.18</td>
<td>164.89±5.11</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>36.43±1.16</td>
<td>156.97±6.74</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>35.08±1.13</td>
<td>153.64±6.95</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>6.68*</td>
<td>4.53*</td>
</tr>
<tr>
<td>Atherogenic</td>
<td>G4</td>
<td>72.21±2.55</td>
<td>215.43±7.75</td>
</tr>
<tr>
<td>Diet</td>
<td>G5</td>
<td>59.82±1.91</td>
<td>191.83±7.38</td>
</tr>
<tr>
<td></td>
<td>G6</td>
<td>56.72±2.61</td>
<td>183.61±6.24</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>91.25**</td>
<td>48.45**</td>
</tr>
</tbody>
</table>

Values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests

* = Significant
** = Highly significant
NS = non-significant

G1 Normal diet
G2 Date fruit + normal diet
G3 Date extract + normal diet
G4 Atherogenic diet
G5 Date fruit + atherogenic diet
G6 Date extract + atherogenic diet
Figure 4.16. Percent reduction in hepatic stress markers in serum
consequences (Liu et al., 2015). Harmful byproducts of lipid peroxidation and oxidative stress are associated with increased fat deposition in the hepatic tissues, cytokine mediated tissue injury and hyperglycemia. It is believed that they trigger the non-alcoholic fatty liver disease, more often contributing to the progression of steatohepatitis and fibrosis (Carmiel-Haggai et al., 2005). When the hepatocyte membrane is damaged, variety of enzymes that are located in cytosol are released into the blood stream. Therefore, the extent of hepatic damage can be ascertained by the determination of mean activities of serum transaminases and alkaline phosphatase.

The present study suggests that oral administration of date fruit and extract significantly decreases the hepatic stress biomarkers in atherogenic as well as normal rats. These hepatoprotective effects are consistent with the findings of Sheikh et al. (2014) who studied the effect of Ajwa dates on CCl₄ induced hepatotoxicity in rats. The date extract @ 1/g/kg/day was administered on rats through gastric gavage for 4 and 12 weeks study trial. In the four-week trial, ALT reduced from 84.2±6.7 to 73.2 ±5.3 IU/L, whereas in case of 12 week trial, it decreased from 166.4 ±7.3 to 56.7 ±7.1 IU/L. Similarly, the AST levels indicate liver functioning as well as cardiac functioning, the results for AST also showed marked decrease from 133.2 ±13.3 to 112.8 ±13.9 IU/L at four-week trial and 235.6 ±22.0 to 84.4 ±6.5 IU/L at the 12th week. The improvement in liver functioning can be attributed to the chelating effect of Ajwa extract and its possible role as a natural antioxidant and anti-mutagenic agent. The results are also in harmony with earlier research of Ramadhas et al. (2014) who examined the effect of date fruit extract @ 200 mg/kg/day for 21 days on lambda cyhalothrin induced stressed rats. The date extract treatment significantly reduced the hepatic stress markers (ALT and ALP) that were increased due to toxicity. ALT reduced by 41.93% while percent reduction recorded in ALP was 36.52.

Contrarily, El Arem et al. (2014a) obtained less reduction in hepatic parameters as compared to our study. The researchers elucidated the effect of aqueous date extract (Delga - besser stage) @ 4 mL/kg B.W. against dichloroacetic acid induced hepatotoxicity in male Wistar rats for 2 months. In the control group, ALT levels were 58.30±0.53 IU/L that decreased in the date extract fed group to 57.53±0.67 IU/L. However, in the hepatotoxic rats at higher dichloroacetic acid concentration (2 g/L), the levels elevated up to 68.75±0.42 IU/L that were reduced to 62.75±0.62 IU/L in the date extract treated groups. Similarly, the γ GT content varied from
56.67±0.59 to 39.31±0.39 in the hepatotoxic groups. Nonetheless, non-significant increase was observed in the control rats. The difference in results can be due to the variety and stage that determines the antioxidant capacity and phenolic composition of the fruit. The quantified phenolic acids in their study are also in lower quantity as compared to the studied Zahidi cultivar.

The findings are also in harmony with the work of Ragab et al. (2013) who investigated the effectualness of Ajwa date extracts in mitigating lead toxicity. Chronic lead exposure disrupts the redox balance, ultimately causing oxidative stress. Ajwa extract @ 300 mg/kg/day was administered for 14 days on normal rabbits as well as lead acetate exposed ones. Significant reduction in ALT level from 407.31±35.5 to 210.52±51.4 IU/L was noted in the lead acetate exposed rabbits fed on date fruit extract. While, non-significant reduction was observed in ALP and total bilirubin contents from 362±34.01 to 312±37.3 IU/L and from 1.44±0.30 to 1.02±0.09 mg/dL, respectively. The ameliorative potential of date extract in the present study is in line with the findings of Saafi et al. (2011) who delineated that Deglet Nour date extract (4 mL/kg) is effective in modulating hepatic stress biomarkers (ALT, ALP, γ-GT and bilirubin) against dimethoate induced oxidative stress. The results are also in corroboration with the findings of Abdu (2011) who elucidated the effect of dates (Ajwa) against ochratoxin A induced hepatotoxicity. 1 mg/kg Ajwa date extract was administered to rats for 4 weeks. Total bilirubin decreased significantly (25.09%) in the stressed groups pretreated with date extract. Similarly, it also reduced the elevated ALT levels up to 15.91%. Present research is also in agreement with previous work conducted on several other date fruit varieties (Bastway et al., 2008; El-Gazzar et al., 2009).

Catechins present in the investigated Zahidi date cultivar could also possibly protect against hepatic abnormalities. Research conducted by Ramesh et al. (2009) states that catechin administration to Wister rats fed on atherogenic diet significantly reduces AST, ALT, ALP and LDH, perhaps by preventing excessive production of reactive oxygen species and lipid peroxidation, thus maintain the normal concentration of hepatic markers. The increasing activity of serum hepatic stress markers indicate the severity of hepatic injury to a considerable extent. The effectualness of Zahidi date fruit and extract against atherogenic diet induced hepatic stress may be due to the decrease in lipid peroxidation or regeneration of the damaged hepatocytes as evident from histology of hepatic tissues (Saafi et al., 2011; Ragab et al., 2013).
Date fruit extract also exhibits significant anti-inflammatory activity. Aqueous date fruit extract modulates inflammation-signaling pathways by down regulating the COX-1 and COX-2 activity, which initiates the inflammatory processes in the physiologic system (Zhang et al., 2013).

The exact mechanism behind the hepatoprotective effects of dates is not certain. However, different mechanisms can be postulated on the basis of the biologically active compounds present in the flesh and extract exhibiting the potential to ameliorate liver dysfunctioning by aiding in liver regeneration. Similar mechanism is also suggested in another study focusing on the hepatoprotective effects of date palm (Bastway et al., 2008). Liver possesses the ability of regeneration after damage through a series complex interactions between the hepatocytes and non-parenchymal expanding cells like the stellate cell, hematopoietic cells and vascular endothelial cells (Matsumoto et al., 2001; Friedman et al., 2013; Ding et al., 2016). Moreover, the pro-inflammatory and redox sensitive transcription factor nuclear factor-κB play an imperative role in regulation of cellular responses. EGCG present in the date fruit proves to be effective in protecting the hepatocytes from CCl₄ induced liver damage by inhibiting the nitric oxide expression through NF-κB and IKKβ pathway (Chen et al., 2004).

4.6.5.2. Hepatic tissue stress indicators

Endogenous enzymes present in the physiologic system are responsible for their defensive role against free radical mediated oxidative stress. The key components of the natural defense system are superoxide dismutase and catalase.

The statistical analysis in Table 4.23 showed substantial variations in the hepatic superoxide dismutase activity as function of treatments during the entire feed model trial. Means regarding the superoxide dismutase level in normal rats expounded lowest value for G₁ (180.32±6.85 U/g wet weight) that gradually improved in G₂ (192.45±6.58 U/g) and G₃ (203.17±6.44 U/g). Likewise, substantial increase for this attribute was also reported in the atherogenic diet fed rats in which the SOD concentration declined in the control group (G₄) 93.28±3.07 U/g that was improved in date fruit fed group (G₅) 123.21±4.08 U/g trailed by date extract fed group (G₆) 133.64±4.58 U/g. It is evident from Fig. 4.17 that date based diets increased the SOD level throughout the study. In this context, normal rats presented 6.30 and 24.29% improvement in superoxide dismutase level through the provision of date fruit and date extract
containing diets, correspondingly. Similarly, in the atherogenic diet fed groups, increase of 11.25 and 30.20% in SOD was observed by date fruit and date extract supplemented diets, respectively.

Statistical analysis (F value) in Table 4.23 showed that date based diets significantly affected the liver catalase contents (CAT) in all groups. In normal diet fed rats, mean catalase content for G$_1$ (140.47±4.35 U/g) was lower than that of G$_2$ (155.62±4.95 U/g) and G$_3$ (158.77±5.57 U/g). Likewise, in atherogenic rats, G$_4$ consuming control diet showed lesser catalase value as 74.71±2.69 U/g that increased to 89.59±3.07 and 94.43±3.10 U/g in G$_5$ and G$_6$ groups taking date fruit and date extract containing functional diets, respectively. Fig. 4.17 showed percent increase in hepatic catalase contents; in normal rats, date fruit containing diet in G$_2$ led to 9.74% improvement, whilst date extract containing diet in G$_3$ resulted in 11.53% increase in hepatic catalase content as compared to control. Similarly, in the atherogenic groups, treatments G$_5$ and G$_6$ showed 16.61 and 20.88% increase, correspondingly for this parameter.

Formation of malondialdehyde (MDA) is an index of lipid peroxidation that triggers the membrane damage and activates membrane bound enzymes (Naik et al., 2011). It is revealed from the F statistic values in Table 4.23 that hepatic MDA contents were affected significantly by the treatments in all groups. Means regarding hepatic lipid peroxidation (MDA) in normal rats indicated the highest value 25.56±0.91 nM/g in G$_1$ that significantly reduced to 23.11±0.88 and 22.25±0.76 nM/g in G$_2$ and G$_3$, respectively. Likewise, the atherogenic rats exhibited the highest MDA value in G$_4$ (40.65±1.57 nM/g) that substantially suppressed in G$_5$ (31.08±1.17 nM/g) and G$_6$ (29.23±1.11 nM/g). It is apparent from Fig. 4.17 that date fruit and date extract containing diets were effective in mitigating lipid peroxidation in the hepatic tissue. Maximum decrease was observed in the atherogenic diets i.e. 23.54 and 28.09% by date fruit and date extract containing diets, respectively. However, in normal rats 9.59 and 12.95% reduction was observed in the date fruit and date extract fed rats, correspondingly.

Atherogenic diet induced oxidative stress usually leads to the development of non-alcoholic fatty liver disease characterized by the accumulation of triglycerides in liver, resulting in certain consequences involving steatosis, insulin resistance and lipotoxicity. Under most situations, the main oxidative fuel in liver are the fatty acids.
Table 4.23. Effect of diets on stress indicators in hepatic tissues

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental Groups</th>
<th>Hepatic Tissue Stress Markers</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SOD (U/g wet weight)</td>
<td>CAT (U/g wet weight)</td>
<td>MDA (nM/g wet weight)</td>
<td></td>
</tr>
<tr>
<td>Normal Diet</td>
<td>G1</td>
<td>180.32±6.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>140.47±4.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.56±0.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>192.45±6.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>155.62±4.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.11±0.88&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<td>G3</td>
<td>203.17±6.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>158.77±5.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.25±0.76&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>33.09**</td>
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<td>Atherogenic Diet</td>
<td>G4</td>
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<td></td>
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<tr>
<td></td>
<td>G5</td>
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<td></td>
<td>G6</td>
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<td>113.87**</td>
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</tbody>
</table>

Values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests
* = Significant  
** = Highly significant  
NS = non-significant

G1 Normal diet  
G2 Date fruit + normal diet  
G3 Date extract + normal diet  
G4 Atherogenic diet  
G5 Date fruit + atherogenic diet  
G6 Date extract + atherogenic diet

Figure 4.17. Percent change in hepato-specific stress indicators
The excess fatty acids produced as result of calorie-enriched diet are converted to triglycerides and are stored as lipid droplets within the hepatocytes. These lipids accumulates in the cell, due to over nutrition, raises the intracellular level of saturated fatty acids that induce cell malfunctioning and death (Aronis et al., 2005). Consequently, leading to increased fatty acid oxidation and generation of reactive oxygen species, ultimately resulting in antioxidant depletion in the hepatocytes (Videla, 2009). This phenomenon explains the reduction of endogenous antioxidants (SOD and CAT) and increase in lipid peroxidation in the rat hepatic parenchyma administered on high fat containing atherogenic diets.

The effect of date fruit on the rat liver redox status is in line with the previous findings. In this regard, El Arem et al. (2014a) probed the restorative potential of aqueous date fruit extract @ 4 mL/kg B.W. in dichloroacetic acid induced hepatotoxic rat. After two months efficacy study, significant increase was recorded in CAT (20.38%) level in the diseased groups administered on date extract containing drinking water. In the hepatotoxic group, significant increase was observed in the lipid peroxidation (TBARS) that declined considerably in the date extract treated groups. Moreover, the date extract also protected against DNA fragmentation. Similar results were obtained by Saafi et al. (2011) pertaining to the liver antioxidant enzymes. Date extract treatment (4 mL/kg) for 2 months improved the endogenous enzyme status in normal rats non-momentously; while momentous increase in CAT i.e. 12.04% was recorded in date extract treated stressed rats. The hepatic MDA levels increased due to 2-month exposure to dimethoate, whilst the date extract treatment effectively attenuated the MDA formation in liver indicating its prophylactic potential against lipid peroxides.

In another investigation, the effect of date extract (300 mg/kg/day) on CCl₄ intoxicated rats was examined in a 7-day study. The results indicated increased SOD (5.88%) and CAT (13.09%) with significantly reduced lipid peroxidation (24.19%) in the hepatic tissues (Naskar et al., 2009). The findings are also in agreement with other studies carried out on the hepatoprotective effects of date fruit (El-Gazzar et al., 2009). Scientific evidences also confirm the presence of selenium in date fruit up to 0.53 mg/100g (Al-Farsi et al., 2005b; Al-Farsi and Lee, 2008). Selenium is a coenzyme for endogenous antioxidant enzyme glutathione peroxidase. Numerous studies support the restorative potential of date fruit in improving the endogenous enzyme status, particularly focusing on the activity and content of glutathione peroxidase (Saafi et al., 2011). Therefore, this possible potential could be attributed to the
presence of selenium in the fruit as it plays an imperative role in protecting the tissues against oxidative stress mediated diseases. Free radical generation induces hepatotoxicity therefore, therapeutic approaches involving the free radical chelation therapy are required. In this regard, Amara et al. (2011) delineated that selenium and/or vitamin E supplementation in the hepatotoxic rats proves to be effective in alleviating plasma transaminases alongside improving the liver structure. Studies also confirm the presence of Vitamin E in the fruit (Al-Qarawi et al., 2005).

The plausible functional elements in date fruit that are contributing to liver protection could be major phenolics (catechins, gallic acid, caffeic acid and kaempferol), dietary fiber and/or vitamin C. Catechins are believed to pose antioxidant effects on the liver by reducing the ROS and improving the overall redox status of the liver. Ramesh et al. (2009) administered the effect of catechins particularly EGCG on atherosclerotic rats and found significant increase in the hepatic SOD and CAT with concomitant reduction in hepatic MDA as compared to the control atherosclerotic rats. Previous scientific evidences also suggest that EGCG attenuates oxidative stress in liver particularly by decreasing the expression of cytochrome P450 2E1 and MDA formation (Kuzu et al., 2008). Moreover, it also up-regulates the antioxidant enzymes (Tipoe et al., 2009). EGCG ameliorates the liver injury by lowering the concentrations of lipid peroxides, pro-fibrogenic factors and pro-inflammatory mediators (Xiao et al., 2014). Likewise, high vitamin C content in the selected cultivar could also be responsible for improving the redox status by neutralizing the reactive oxygen species, thereby lowering the hepatic stress conditions (Harrison et al., 2003).

4.6.5.3. Histopathological examination of hepatic tissues

Fig. 4.18 shows the photomicrographs of hepatic parenchyma in the studied groups. The hepatic parenchyma in G1 is normal in appearance. Hepatocytes are arranged in hepatic cords. Nuclei of the hepatic cells are also normal in appearance having nucleolus and chromatin material. However, at few places pyknotic nuclei are present. The cells forms cords around the sinusoids and the sinusoidal spaces are normal in appearance. Similarly, the liver section in G2 indicates normal appearance. The hepatocytes are arranged in hepatic cords. Nuclei of hepatocytes are normal in appearance having nucleus, nucleolus and chromatin material, thus indicating that date fruit consumption did not alter the hepatic structure and seems to be
improving the overall tissue architecture. Similar normal characteristics were exhibited by the tissue sections of G₃. Whereas, more profound steatosis with macro-vesicular fat accumulation was visible in the hepatic parenchyma of the atherogenic diet fed group. The hepatic parenchyma is indicating mild to modulate degree of fatty changes. Fat vacuoles are present in the cytoplasm of hepatocytes. Sinusoidal spaces are diminished indicating cell swelling. However, in G₅ mild to modulate degree of cell swelling is present. Nonetheless, nuclei of the hepatocytes at few places are normal, while at other places these are condensed indicating mild ameliorative effect of date fruit. Likewise, in G₆ the hepatic parenchyma is indicating cell swelling. The sinusoidal spaces are diminished. Nuclei of the hepatocytes are condensed. However, the overall structure in G₆ appears better with mild changes as compared to G₄.

In atherogenic diet induced hepatic stress conditions, liver receives large amount of fatty acids that result in steatosis. Date fruit and extract shows ameliorative potential towards atherogenic diet induced hepatic changes. Our results are in accordance to the findings of Sheikh et al. (2014), who evaluated the effect of aqueous date fruit extract on carbon tetrachloride induced hepatic stress, the Masson’s trichrome stained liver sections showed increased amount of fibrous tissues in disease induced groups whereas, date extract treated groups showed decrease in area covered by the collagenous fibers. The authors also reported reduction in histological alterations such as vein and sinusoidal dilation, nuclear pyknosis and necrosis.

The present findings are in line with the earlier results of Hsu and Yen (2007) who indicated positive histopathological changes in the hepatic tissue upon gallic acid administration in high fat diet fed rats. Whereas, the control obese rats showed severe steatosis and fat accumulation in the hepatocytes. One of the researchers groups, Ramadhas et al. (2014) studied the effect of date fruit extract on liver histological parameter in lambda cyhalothrin induced stressed rats. Normal rats administered on date extract containing diet showed normal hepatocyte arrangement in the hepatic cords around the central vein. However, the toxicity-induced group indicated hypertrophy, vacuolization, hyalinization and loss of radial arrangement, while the date extract treated stressed rats exhibited better hepatic histology with orderly arranged pattern of hepatocytes having mild portal inflammation confirming the hepatoprotective potential of the fruit. Similar, protective effects were observed in another research work conducted by El Arem et al. (2014a). The histoarchitecture of animals fed aqueous date extract.
Figure 4.18 Histopathological analysis of rat hepatic parenchyma

- G1: The hepatic parenchyma is normal in appearance.
- G2: Date fruit fed rat showing normal in appearance tissue without any histological alterations.
- G3: Date extract diet administered rat tissue represents no alteration with normal hepatic parenchyma.
- G4: Atherogenic diet fed rats liver mild to modulate degree of fatty changes.
- G5: Date fruit + atherogenic diet fed rat’s liver is reflecting mild to modulate degree of cell swelling.
- G6: Date extract + atherogenic diet fed rat liver revealing mild degree of alterations in hepatic parenchyma.
(4 mL/kg B.W.) for 2 months displayed a classical hepatic structure. Whereas, marked alterations were noted in the dichloroacetic acid induced toxic group, that was improved in date extract treated group. Another researchers group, Ragab et al. (2013) delineated that Ajwa date fruit extract treatment improved the liver histology in lead acetate induced stressed rabbits. Lead toxicity results in hepatic congestion, necrosis, pyknosis, karyolysis, cholestasis, vacuolization and cellular infiltration. Conversely, in the Ajwa extract treated group, mild congestion, slight swelling and minimal focal necrosis was observed indicating positive effects against lead toxicity. Similar observations were noted by Saafi et al. (2011), the microscopic liver examination revealed normal architecture in control and date extract treated rats. Severe changes such as sinusoidal enlargement, congestion, cell infiltration and cytoplasmic vacuolization were observed in the dimethoate induced stressed group. On the other hand, the date extract pre-treated group showed marked improvement in the liver histology having mild inflammation and almost absence of vacuolization and necrosis. Comparable findings regarding the protective role of date fruit on hepatic histopathological conditions are also obtained by Abdu (2011). The disease alleviating role of the date fruit and extract is evident from the improvement in hepatic histoarchitecture.

The present study elicited the therapeutic potential of date fruit and extract against hepatic stress. Better ameliorative properties were exhibited by the extract as compared to the fruit, probably due to better bioavailability of the extract. It seems that hepatoprotective efficacy of date fruit can be accredited to the polyphenolic constituents. The absorption of polyphenols is better in form of extract as it is a more concentrated source. Moreover, the extraction method also determines the nature of metabolites. During absorption, the polyphenolic components undergo several modifications such as conjugation, methylation, glucoronidation etc. Consequently the compounds reaching to the blood and tissue are different metabolites (Archivio et al., 2007). This may also explain that fruit consumed as such would be following different course of absorption as compared to the extract. However, such interactions, resulting metabolites and dosages need to be studied in depth.
PART 4: RENAL STRESS BIOMARKERS
4.6.6. Renal stress biomarkers

The serum biomarkers used to assess the renal health status in the present study were urea, creatinine and glucose. Renal tissue stress indicators (CAT, SOD and MDA) and histopathology was also studied.

4.6.6.1. Serum renal stress biomarkers

The F values showed momentous effect of treatments on serum urea content (Table 4.24). In normal rats, mean serum urea values in G1, G2 and G3 were 10.18±0.32, 9.54±0.31 and 9.72±0.35 mg/dL, respectively. Similarly, in atherogenic rats, means for this trait varied substantially as serum urea level elevated to 18.47±0.65 mg/dL in G4 group that was significantly ameliorated by the date fruit and date extract containing diets as G5 and G6 as 15.63±0.50 and 16.14±0.74 mg/dL, respectively.

Similar trend was noticed regarding the creatinine level, in normal rats the highest creatinine level was observed in G1 (0.51±0.01 mg/dL), whilst the lowest in G2 (0.47±0.02 mg/dL). However, pronounced effect was observed in rats fed on atherogenic diets. The highest creatinine level 0.92±0.03 mg/dL was recorded in G4 that suppressed to 0.79±0.03 and 0.81±0.02 mg/dL in G5 and G6, correspondingly.

Percent reduction in nephrotic stress markers in serum are illustrated in Fig. 4.19. Date fruit showed pronounced effect in serum urea concentration as it was reduced in normal and stressed rats by 6.28 and 15.37%, respectively. However, date fruit extract treatment decreased the urea levels by 4.51% in normal rats and 12.61% in atherogenic diet fed stressed rats. Similar pattern was recorded in case of creatinine concentrations that decreased by 7.84% in date fruit treated normal rats, while 3.92% in date extract treated normal rats. The concentration decreased by 14.13 and 11.96% in date fruit and date extract fed atherogenic rats, respectively.

Date fruits being rich in sugars are considered promoters of blood glucose level. However, in the present study the F values showed that glucose level in date fruit fed groups were not statistically affected by the treatments. However, significant effect was recorded in the atherogenic diet fed groups (Table 4.24). The blood glucose level was 97.41±3.16 mg/dL in the control group (G1) that non-significantly increased in the date fruit fed group (G2) up to 100.28±4.31 mg/dL. However, slight reduction in the glucose level was observed in the date
extract fed group (G₆) i.e. 95.03±3.09. In the stressed rats, the positive control group (G₄) showed elevated levels of serum glucose (113.57±3.79 mg/dL) that substantially reduced upon the administration of date extract along with atherogenic diet in G₆ up to 109.66±3.48 mg/dL. Nonetheless, the consumption of date fruit containing diet elevated the glucose levels to 119.02±3.73 mg/dL, nonetheless it remained under the borderline.

Oxidative stress conditions lead to increased inflammatory activity, lipotoxicity, cytokine production and certain hemodynamic factors that result in certain alterations in the renal tissue (Wahba and Mak, 2007). Increase in pro-inflammatory cytokines, ROS and hyperfiltration results in malfunctioning of glomerular capillary endothelial cells, podocytes and tubular interstitial cells. The increase in free fatty acids due to calorie rich diet, increases free radical production that may affect the podocytes that are the visceral epithelial cells accountable for confining the protein passage from blood to urinary space (Pierine et al., 2015). Measuring the renal function status through the determination of urea and creatinine contents are regarded as reliable markers. Their concentration depends mainly on the glomerular filtration for their excretion (Michael and Sircar, 2010). Moreover, increase in urea concentration may also indicate the liver damage as it is the end product of protein catabolism. Present findings are in corroboration with the earlier results of El Arem et al. (2014c) who expounded the effect of date fruit extract (4 mL/kg) of Delga cultivar on trichloroacetic acid (TCA) induced renal toxicity in Wistar rats. The results showed that TCA administration (2 g/L) for two months significantly increases the creatinine and urea level. However, date fruit treatment significantly reduces the creatinine level up to 37.5% and urea up to 14.2%. The results are also in accordance with the findings of Ragab et al. (2013) who investigated the effect of Ajwa date extracts on lead acetate induced oxidative stress. Ajwa extract @ 300 mg/kg/day was administered for 14 days on normal and stressed rabbits. Non-significant effect of date extract on serum creatinine and urea was recorded in the control rats. However, the stressed rabbits showed elevated creatinine and urea levels that significantly decreased up to 19.78 and 14.54%, respectively through Ajwa date extract supplementation.

The results are also in line with the findings of El-Sayed et al. (2015) who elucidated the effect of Berne date extract (300 mg/kg) against cisplatin induced nephrotic damage in male rats. Berne date extract treatment reduces the serum urea and creatinine levels by 73 and 72%,
Table 4.24. Effect of diets on serum renal stress biomarkers

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental Groups</th>
<th>Serum Renal Stress Markers (mg/dL)</th>
<th>Urea</th>
<th>Creatinine</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Diet</td>
<td>G1</td>
<td></td>
<td>10.18±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.51±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97.41±3.16</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td></td>
<td>9.54±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.47±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.28±4.31</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td></td>
<td>9.72±0.35&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.49±0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>95.03±3.09</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td></td>
<td>4.46&lt;sup&gt;*&lt;/sup&gt;</td>
<td>7.83&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.80&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Atherogenic Diet</td>
<td>G4</td>
<td></td>
<td>18.47±0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.92±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>113.57±3.79&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G5</td>
<td></td>
<td>15.63±0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.79±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>119.02±3.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G6</td>
<td></td>
<td>16.14±0.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.81±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>109.66±3.48&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td></td>
<td>61.31&lt;sup&gt;**&lt;/sup&gt;</td>
<td>43.81&lt;sup&gt;**&lt;/sup&gt;</td>
<td>7.26&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests

* = Significant
** = Highly significant
NS = non-significant
G1 Normal diet
G2 Date fruit + normal diet
G3 Date extract + normal diet
G4 Atherogenic diet
G5 Date fruit + atherogenic diet
G6 Date extract + atherogenic diet

Figure 4.19. Percent reduction in serum renal stress markers
correspondingly. Thereby, indicating improvement in the glomerular filtration rate. The difference in results may be due to the varietal variations and extract dose. Moreover, the renal injury also progresses due to upsurge in the activities of caspase-3 and TNF-α leading to cascade of inflammatory changes. However, the Berne date fruit extract also significantly decreases their activities, thus counteracting the nephrotoxicity.

The results are also in strong agreement with the findings of El-Mousalamy et al. (2016) who explicated the effect of aqueous and ethanolic date fruit extract (5 mL/kg) for 15 weeks in normal and diabetic rats. The increased blood glucose level in the diabetic rats considerably reduced in extract fed groups. Maximum decrease of 73.10% in fasting blood glucose was observed in aqueous date extract administered group. Likewise, 50.68% reduction in serum creatinine and 52.29% in serum urea was recorded in rats treated with aqueous date extract. However, the results for methanolic extract were lower indicating more restorative potential of the aqueous extract.

The renoprotective properties of dates can be accredited to the presence of abundant amount of vitamin C. Several scientific investigations focus on the beneficial role of vitamin C against chemically induced renal stress (Das and Buchner, 2007; Karabulut-Bulan et al., 2008; Ajith et al., 2009). Pretreatment of vitamin C @ 16.6 mg/kg for seven days in nickel induced nephrotoxic mice results in significant reduction in serum urea, uric acid and creatinine along with considerable improvement in the renal histoarchitecture (Kadi and Dahdouh, 2016). It is anticipated that vitamin C enhances the cellular antioxidant potential to counteract the free radical species-mediated oxidative damage. Moreover, the nephroprotective effect could also be attributed to quercetin as it plays an important role in vasodilation and also increases the blood flow in the kidneys (Behling et al., 2006). Quercetin administration also reduces the inflammatory process by inhibiting the NF-κB-p65 activation in cisplatin induced nephrotoxic models (Sánchez-González et al., 2010). Quercetin and vitamin C administration effectively reverses the serum glomerular damage biomarkers \(i.e\). urea and creatinine substantiating their prophylactic effect against renal damage (Nabavi et al., 2012).

4.6.6.2. Renal tissue stress indicators

It is clear from the statistical F values displayed in the Table 4.25 that considerable variations are present in renal SOD activity as function of treatments during the bioassessement trial. Means
regarding the nephrotic SOD in normal rats showed that lowest value was obtained by G₁ (233.68±8.88 U/g) than G₂ (249.76±8.54 U/g) and G₃ (247.15±7.83 U/g). In case of rats fed on atherogenic diet, momentous decrease was noted in G₄ (118.62±3.91 U/g) as compared to the normal rats. However, date fruit and date extract treatments significantly improved the SOD concentration in renal tissues of the atherogenic rats to 142.59±4.63 and 137.33±4.71 U/g, respectively.

It is evident from graphical representation (Fig. 4.20) that date based diets increases the superoxide dismutase level (SOD) in kidneys throughout the study. Maximum improvement in the SOD levels was found in groups fed on date fruit containing diets i.e. 6.44 and 16.81% in normal and stressed rats, respectively. Besides, date extract based diet also increases the renal SOD in normal as well atherogenic rats by 5.45 and 13.62%, respectively.

Statistical analysis (F value) in Table 4.25 shows that date based diets imparted significant effect on catalase contents in the kidney tissues in all groups. In normal rats, mean catalase content for G₁ (268.6±8.33 U/g) was lower than that of G₂ (295.42±9.48 U/g) and G₃ (286.79±10.06 U/g) groups. Likewise, in atherogenic rats, G₄ group consuming control diet showed lesser nephrotic CAT value as 157.35±5.66 U/g that increased to 187.45±6.43 and 190.60±6.26 mg/L in G₅ and G₆ groups taking date fruit and date extract containing functional diets, respectively. Fig. 4.20 depicts percent increase in nephrotic catalase contents; in normal rats, date fruit containing diet led to 9.08% improvement whilst, date extract containing diet resulted in 6.34% increase in renal catalase content as compared to control. Similarly, in atherogenic groups, treatments G₅ and G₆ showed 16.06 and 17.44% increment, correspondingly for this trait.

Cell membranes are susceptible to various peroxidizing reactions, the free radicals react with the polyunsaturated fatty acids and form lipid peroxides that decompose and yield a cascade of biochemical reactions. Malondialdehyde (MDA) is one of the product of such reaction and is determined to know the extent of lipid peroxidation (Ramesh et al., 2009). It is apparent from the statistical analysis (F values) that renal MDA were significantly affected by the treatments in all groups (Table 4.25). Means relating to nephrotic lipid peroxidation assessed in terms of MDA content in normal rats indicated the highest value 50.61±1.80 nM/g in G₁ that significantly reduced to 46.15±1.75 and 47.92±1.63 nM/g in G₂ and G₃ groups, respectively.
Table 4.25. Effect of diets on stress indicators in renal tissues

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental Groups</th>
<th>Renal Tissue Stress Markers</th>
<th>SOD (U/g wet weight)</th>
<th>CAT (U/g wet weight)</th>
<th>MDA (nM/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Diet</td>
<td>G1</td>
<td></td>
<td>233.68±8.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>268.6±8.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.61±1.80&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td></td>
<td>249.76±8.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>295.42±9.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.15±1.75&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td></td>
<td>247.15±7.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>286.79±10.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.92±1.63&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td><strong>F value</strong></td>
<td></td>
<td>7.48**</td>
<td>10.04**</td>
<td>12.31*</td>
</tr>
<tr>
<td>Atherogenic Diet</td>
<td>G4</td>
<td></td>
<td>118.62±3.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>157.35±5.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.67±2.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G5</td>
<td></td>
<td>142.59±4.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>187.45±6.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.03±2.30&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G6</td>
<td></td>
<td>137.33±4.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>190.6±6.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.28±2.48&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td><strong>F value</strong></td>
<td></td>
<td>62.72**</td>
<td>49.75**</td>
<td>42.18**</td>
</tr>
</tbody>
</table>

Values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests

* = Significant
** = Highly significant
NS = Non-significant

G1 Normal diet
G2 Date fruit + normal diet
G3 Date extract + normal diet
G4 Atherogenic diet
G5 Date fruit + atherogenic diet
G6 Date extract + atherogenic diet

Figure 4.20. Percent change in reno-specific stress indicators
Likewise, in the atherogenic rats, highest MDA value was observed in G₄ (74.67±2.86 nM/g) that substantially suppressed in G₅ (61.03±2.30 nM/g) and G₆ (65.28±2.48 nM/g). Percent decrease in renal MDA content is shown in Fig. 4.20 that illustrates 8.81 and 18.27% reduction in lipid peroxidation in renal parenchyma by the administration of date fruit and date extract containing normal diets. Likewise, percent reductions in MDA contents up to 5.32 and 12.58% were found in normal and stressed rats relied on date extract containing diets, correspondingly.

In agreement with the previous investigations, the study clearly shows the reduction in renal SOD and CAT and increase in MDA due to the consumption of atherogenic diet. However, date fruit and extract treatment significantly restores these traits. The primary mechanism that links the progression of kidney disease, oxidative stress and inflammation involves the activities of oxygen-derived radicals that results in kidney injury. Superoxide and hydroxyl radicals interact with nephrotic components causes oxidation of amino acids, increased lipid peroxidation and reduced membrane viability that may also lead to certain harmful DNA mutations (Kao et al., 2010). Present findings are in line with the earlier results of El Arem et al. (2014) who elucidated the effect of Delga date fruit extract (4 mL/kg) on TCA induced renal toxicity in Wistar rats. The TCA administration (2 g/L) for 2 months resulted in decreased SOD and CAT with increased lipid peroxidation similar to our results. However, date fruit extract treatment reversed the antioxidant enzyme levels and alleviated the lipid peroxidation in the renal tissue. The results are also in corroboration with the findings of El-Sayed et al. (2015) who reported significant improvements in the SOD and CAT levels and reduction in MDA by the administration of Berne date fruit extract to cisplatin induced renotoxic rats, indicating the protective abilities of date fruit extract against renotoxicity.

The results also corroborates to the findings of El-Mousalamy et al. (2016) who expounded the influence of aqueous and ethanolic date fruit extract (5 mL/kg) administration through gastric gavage for 15 weeks in normal and diabetic rats. The results revealed significant improvement in the SOD and catalase content in diabetic rats administered on date extracts. Moreover, the treatment also decreased the MDA contents in kidney tissue considerably.

Quercetin and vitamin C administration improves the renal redox status and mitigates the lipid peroxidation in fluoride induced stressed rats (Nabavi et al., 2012). The reactive oxygen species have been implicated in the initiation as well as progression of renotoxicity. Quercetin
is multifunctional molecule, being an efficient scavenger of reactive oxygen species and metal chelator, it improves the oxidant-antioxidant imbalance. Thus, these biomolecules in date fruit might be responsible for its renoprotective abilities.

4.6.6.3. Histopathological examination of renal tissues

The photomicrographs are displayed in Fig. 4.21. The hematoxylin and eosin staining showed normal architecture of renal parenchyma in G₁ indicating normal urinary space in the glomeruli. Tubular epithelial cells are also normal. However, at few places, few condensed nuclei are present. Mild to modulate degree of congestion is also present. The renal parenchyma in G₂ is normal in appearance. The renal tissue in G₃ is signifying the appearance of normal tubular epithelium. Nuclei of the tubular cords are having nucleus with nucleolus and chromatic material. However, at few places condensed nuclei are present. Mild degree of congestion is also present. In the G₄, renal parenchyma is indicating severe necrotic changes indicated by pyknotic nuclei of the tubular epithelial cell throughout the renal parenchyma. Modulate degree of congestion is also present. Whereas, the renal parenchyma in G₅ is indicating mild degree of congestion and mild degree of necrotic changes. However, urinary space is clear and dilated. Nuclei are also condensed to pyknotic. While, the G₆ renal tissue is indicating mild degree of pyknotic changes along with mild degree of congestion.

The results of the present investigation are in line with the earlier findings of Ragab et al. (2013) who studied the effect of Ajwa date extract @ 300 mg/kg/day on lead toxicity induced rabbits for 14 days trial period. Normal rabbits fed on date fruit extract exhibited the normal classical renal structure. Nevertheless, lead exposure resulted in renal congestion, cloudy swelling, interstitial hemorrhages and focal tubular necrosis, while the Ajwa extract treated group showed less swelling, mild congestion and absence of interstitial and tubular hemorrhages. Similar findings were obtained by El Arem et al. (2014). The researchers’ explicated positive histopathologic alterations in the date fruit extract treated TCA intoxicated rats. Control group showed normal glomerulus appearance surrounded by epithelial capsule having urinary space in between. Besides, the intoxicated rats showed significant alterations such as congestion in capillary loops, glomerular atrophy with mild dilation of Bowman’s space. The date extract treatment restored the histological alterations by reducing the glomerular hypertrophy, tubular degeneration, dilation and congestion.
Figure 4.21. Histopathological analysis of rat renal parenchyma

G1: In Control group renal parenchyma is indicating normal urinary space in glomeruli along with mild to moderate degree of congestion. 
G2: Date fruit fed rat kidney showing normal appearance of renal parenchyma, few condensed nuclei are also present. 
G3: Date extract administered rat tissue is indicating normal appearance of tubular epithelium. 
G4: Atherogenic diet fed rats renal parenchyma is indicating severe necrotic changes throughout the renal parenchyma. 
G5: Date fruit + atherogenic diet fed rat renal parenchyma is indicating mild degree of necrotic changes. 
G6: Date extract + atherogenic diet fed rat renal parenchyma is indicating mild degree of pyknotic changes.
Moreover, glomerular atrophy and leukocyte infiltration was diminished. Similar findings were obtained by El-Mousalamy et al. (2016) who reported improved morphology and redox status of kidney tissues due to date extract supplementation in diabetic rats. The extract treatment restored the glomerular sclerosis index, arterial injury and tubular interstitial injury. The current histological findings are also in harmony with the results of El-Sayed et al. (2015) who examined positive changes in the cisplatin toxified renal tissue due to Berne date extract treatment. Vitamin C administration to nephrotoxic mice showed improved histological structure with minimal necrotic changes, tubular generation and infiltration indicating the renoprotective effect of vitamin C (Kadi and Dahdouh, 2016). Date fruit being rich in vitamin C could also be renoprotective owing to its presence. In this regard, Ali and Abdu (2011) observed improvement in the renal proximal and distal tubules. Studies do validate the renoprotective effect of date extract particularly. However, the present study suggests that date fruit administration is more ameliorative as compared to the extract. Therefore, further investigations involving detailed study of renal biomarkers and metabolite behavior in the physiologic system is required to draw a conclusive approach.
PART 5: HEMATOLOGICAL ASPECTS
4.6.7. Hematological aspects

Hematology is the study that involves the diagnosis, treatment and prevention of ailments related to blood. In the present research, the hematological aspects are studied to assess whether they are affected by the diets, and to what extent, either positively or negatively. Dates being rich in certain nutrients and bioactives are believed to pose beneficial effects on the blood parameters as well. The studied parameters are described under:

4.6.7.1. Erythrocyte indices

The red blood cells carry oxygen and take away the waste from the physiologic system. It is deduced from the statistical interpretation in Table 4.26 for red blood cell indices that the treatments imparted non-substantial effect on normal groups, whereas significant effect was noted in the stress-induced groups. Means for normal rats presented an increasing trend for RBC from 7.12±0.26 M/uL in control group (G\textsubscript{1}) to 7.35±0.27 and 7.22±0.24 M/uL in date fruit (G\textsubscript{2}) and date extract (G\textsubscript{3}) fed groups, respectively. Whereas, atherogenic groups showed a substantial rise that ranged from 6.34±0.25 to 7.21±0.28 M/uL. However, all the values were within the acceptable limits.

The F values (Table 4.26) for hemoglobin showed substantial effect of treatments on stressed rats. However, normal rat groups were affected non-momentously. In this connection, the recorded means for normal rats were 14.04±0.42 (G\textsubscript{1}), 14.48±0.46 (G\textsubscript{2}) and 14.35±0.46 (G\textsubscript{3}) g/dL. In the stress-induced groups, the hemoglobin level was 13.33±0.48 g/dL in the atherogenic control group. That was significantly improved by date fruit containing diet to 14.96±0.46 and date extract containing diet to 14.65±0.44 mg/dL.

It is apparent from the F statistics that hematocrit values were non-momentously affected as function of dietary treatments in normal rats. Nevertheless, substantial changes were noted in atherogenic rats (Table 4.26). Means for this trait in G\textsubscript{1}, G\textsubscript{2} and G\textsubscript{3} (normal rats) were 39.18±1.33, 37.40±1.61 and 38.21±1.33%, respectively. Likewise in atherogenic diet fed groups, hematocrit was higher in G\textsubscript{4} (42.50±1.62%) that was significantly increased by date supplemented diets; G\textsubscript{5} (40.21±1.33%) and G\textsubscript{6} (39.25±1.22%).

The F statistics in Table 4.26 expounded substantial variations in the MCV because of treatments in all the groups. Groups relied on normal diet presented momentous changes in
Table 4.26. Effect of diets on erythrocyte indices

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental Groups</th>
<th>Erythrocyte Indices</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hemoglobin (g/dL)</td>
<td>Hematocrit (%)</td>
<td>Total RBC count (M/uL)</td>
<td>MCV (fL)</td>
<td>MCH (PG)</td>
<td>MCHC (g/DL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1</td>
<td>14.04±0.42</td>
<td>39.18±1.33</td>
<td>7.12±0.26</td>
<td>55.03±1.93</td>
<td>19.72±0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G2</td>
<td>14.48±0.46</td>
<td>37.40±1.61</td>
<td>7.35±0.27</td>
<td>50.88±1.78</td>
<td>19.70±0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G3</td>
<td>14.35±0.46</td>
<td>38.21±1.33</td>
<td>7.22±0.24</td>
<td>52.91±2.01</td>
<td>19.88±0.63</td>
</tr>
<tr>
<td>Normal</td>
<td>F value</td>
<td></td>
<td>1.08NS</td>
<td>3.49NS</td>
<td>1.12NS</td>
<td>3.87*</td>
<td>0.29NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G4</td>
<td>13.33±0.48</td>
<td>42.5±1.62a</td>
<td>6.34±0.25b</td>
<td>67.03±2.28a</td>
<td>21.03±0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G5</td>
<td>14.96±0.46a</td>
<td>40.21±1.33b</td>
<td>7.21±0.28a</td>
<td>55.77±1.95b</td>
<td>20.75±0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G6</td>
<td>14.65±0.44a</td>
<td>39.25±1.22b</td>
<td>7.08±0.24a</td>
<td>55.44±1.96b</td>
<td>20.69±0.69</td>
</tr>
<tr>
<td>Atherogenic</td>
<td>F value</td>
<td></td>
<td>14.05**</td>
<td>11.49*</td>
<td>23.37**</td>
<td>70.48**</td>
<td>0.24NS</td>
</tr>
</tbody>
</table>

Values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests

* = Significant
** = Highly significant
NS = non-significant

G1 Normal diet
G2 Date fruit + normal diet
G3 Date extract + normal diet
G4 Atherogenic diet
G5 Date fruit + atherogenic diet
G6 Date extract + atherogenic diet
MCV for G₁, G₂ and G₃ as 55.03±1.93, 50.88±1.78 and 52.91±2.01 fL, respectively. Similarly, in the groups relied on atherogenic diet, MCV values were 67.03±2.28, 55.77±1.95 and 55.44±1.96 fL for G₄, G₅ and G₆.

Mean corpuscular hemoglobin values (Table 4.26) indicated non-significant variations with respect to treatments in all groups. In normal rats, the obtained values for the said trait were 19.72±0.75, 19.70±0.68 and 19.88±0.63 PG in G₁, G₂ and G₃. 20.69±0.69 to 21.03±0.62 PG.

The F value for mean corpuscular hemoglobin concentration (MCHC) was reported to exhibit significant variations due to treatment in all groups (Table 4.26). It can be seen that MCHC values were 35.83±1.40, 38.72±1.47 and 37.57±1.22 in G₁, G₂ and G₃, respectively. Significant increase in MCHC was found in the stressed groups, the control group G₄ had 31.36±0.94 g/dL MCHC that was increased to 37.20±1.38 and 37.32±1.46 g/dL in date fruit fed stressed group G₅ and date extract fed stressed group G₆, correspondingly.

The present findings are in harmony with earlier research of Ramadhas et al. (2014) who examined the effect of date fruit extract @ 200 mg/kg/day for 21 day on hematological parameters of normal and lambda cyhalothrin induced toxic rats. The RBC count in normal rats was 8.65±0.27 M/µL that increased non-significantly to 8.87±0.23 M/µL in the date extract fed rats. The RBC count decreased significantly in the toxic group to 5.59±0.31 M/µL. However, it was considerably improved (7.33±0.65 M/µL) through the date extract treatment. Date extract treatment also increased the hemoglobin content from 16.75±0.45 to 16.98±0.9 g/dL in normal rats and 9.54±0.09 to 14.76±1.3 in the toxicity induced rats. Non-significant improvement was noticed in the MCV, MCH and MCHC levels in the normal groups. Whereas, in lambda cyhalothrin induced toxic rats the levels declined. Nonetheless, substantially improvement in the date extract treated groups was recorded. Significant increase in the hemoglobin content agrees with the previous investigations regarding the anti-anemic properties of date fruit and its role in hemopoiesis.

Likewise, Onuh et al. (2012) also reported similar findings regarding the hemoglobin and MCH values in Wistar rats fed on date extract containing diets. The hemoglobin significantly improved by the administration of aqueous as well as methanolic date fruit extract in a dose dependent manner. In another study by Orabi and Shawky (2014) it was found that date seed significantly increases the hemoglobin, MCH and MCHC concentrations, while no significant
difference was noticed regarding the RBCs. The results are also in line with the work of Uzun and Kalender (2013) who reported non-significant effect of catechin and quercitin against chlorpyrifos induced toxicity on red blood cells, hemoglobin, hematocrit, MCH, MCV and MCHC. However, in the present study effect on erythrocyte parameters especially hemoglobin may be attributed to the presence of iron in date fruit and can also propose the anemic properties of date fruit that need to be studied further. Other biological molecules may also be responsible but requires extensive research to determine a proper mechanism.

4.6.7.2. Leucocyte count

The leucocytes or white blood cells play key role in helping the body to fight off the infections. The F values (Table 4.27) regarding the total white blood cell count (WBC) pinpoints substantial effect of treatments in atherogenic groups. However, normal rat groups were statistically alike. In this connection, the observed means in normal rats were 6.59±0.20 (G₁), 6.25±0.24 (G₂) and 6.43±0.22 (G₃) K/UL. Similar decreasing trend was found in stressed groups for G₄ (11.48±0.40 K/UL) and G₅ (11.57±0.42 K/UL) as compared to G₄ (12.95±0.44 K/UL).

The F statistic values in Table 4.27 showed inconsiderable impact of treatments on neutrophil content during the rodent experimental modeling. In normal rats, the observed differences in the neutrophils were 18.51±0.68, 18.24±0.67 and 18.18±0.65% for G₁, G₂ and G₃ groups, correspondingly. Similarly, atherogenic groups also presented non-momentous decline for G₄, G₅ and G₆ groups; 21.47±0.72, 20.36±0.75 and 19.90±0.68% respectively.

The statistical F values in displayed in the Table 4.27 presented trivial variations in lymphocyte count due to treatments in all groups. Means for normal rats revealed that treatments behaved statistically alike in the groups; G₁ (80.45±2.82%), G₂ (81.02±2.43%) and G₃ (81.14±3.08%). However, treatments (stressed rats) showed non-substantial increment in lymphocytes that ranged from 74.67±3.21 to 75.12±2.63 and 76.24±2.80% for G₄, G₅ and G₆, respectively.

It is inferred from the F statistics in Table 4.27 that no momentous changes were imparted by the treatments regarding the monocytes in normal rats. Nevertheless, momentous changes were recorded in the atherogenic ones. Means pertaining to the monocytes in normal rats elucidated non-significant variations among the groups G₁, G₂ and G₃ ranging from 0.85±0.03
Table 4.27. Effect of diets on leucocyte count

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental Groups</th>
<th>Total WBC count</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Eosinophils</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Diet</td>
<td>G1</td>
<td>6.59±0.20</td>
<td>18.51±0.68</td>
<td>80.45±2.82</td>
<td>0.85±0.03</td>
<td>1.41±0.05</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>6.25±0.24</td>
<td>18.24±0.67</td>
<td>81.02±2.43</td>
<td>0.86±0.03</td>
<td>1.44±0.05</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>6.43±0.22</td>
<td>18.18±0.65</td>
<td>81.14±3.08</td>
<td>0.85±0.03</td>
<td>1.42±0.05</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>2.80NS</td>
<td>0.40NS</td>
<td>0.13NS</td>
<td>0.19NS</td>
<td>0.40NS</td>
<td>-</td>
</tr>
<tr>
<td>Atherogenic Diet</td>
<td>G4</td>
<td>12.95±0.44a</td>
<td>21.47±0.72</td>
<td>74.67±3.21</td>
<td>1.10±0.04a</td>
<td>0.94±0.03b</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>G5</td>
<td>11.48±0.40b</td>
<td>20.36±0.75</td>
<td>75.12±2.63</td>
<td>0.99±0.03b</td>
<td>1.01±0.04a</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>G6</td>
<td>11.57±0.42b</td>
<td>19.90±0.68</td>
<td>76.24±2.80</td>
<td>0.98±0.03b</td>
<td>0.99±0.03a</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>18.78**</td>
<td>6.06NS</td>
<td>0.78NS</td>
<td>5.52*</td>
<td>7.73*</td>
<td>-</td>
</tr>
</tbody>
</table>

Values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests

* = Significant  
** = Highly significant  
NS = non-significant 

G1 Normal diet  
G2 Date fruit + normal diet  
G3 Date extract + normal diet  
G4 Atherogenic diet  
G5 Date fruit + atherogenic diet  
G6 Date extract + atherogenic diet
to 0.86±0.03%. However in case of atherogenic rats, mean values for this trait in G₄ (1.10±0.04%) was decreased in G₅ (0.99±0.03%) and G₆ (0.98±0.03%) groups. It is obvious from the F statistics in Table 4.27 that treatments behaved statistically alike regarding the eosinophils in normal rats. While, significant variations were noted in the atherogenic ones. Means pertaining to the eosinophils in normal rats showed non-momentous variations among the groups G₁, G₂ and G₃ as 1.41±0.05, 1.44±0.05 and 1.42±0.05%, respectively. However in case of atherogenic rats, mean values for this trait in G₄ (0.94±0.03%) was increased in G₅ (1.01±0.04%) and G₆ (0.99±0.03%) groups.

The present findings are in agreement with earlier results of Ramadhas et al. (2014) who studied the influence of date fruit extract treatment (@ 200 mg/kg/day for 21 days) on leucocyte count of normal and lambda cyhalthrin induced toxic rats. Non-significant decrease in WBC count from 7.87±0.56 to 7.54±0.65 K/UL was recorded in normal rats, whereas significant decline from 9.45±1.34 to 8.75±0.98 K/UL was observed in toxicity-induced rats similar to our findings. However, the neutrophils reduced non-significantly in the normal rats, while increased in the toxic rats. The other parameters including lymphocytes, monocytes, eosinophils and basophils showed trivial differences in all groups. The results indicate that date fruit may have some stimulatory effect on the bone marrow for hemopoietic activities.

Comparable findings were also reported by Onuh et al. (2012) who elucidated the effect of methanolic and aqueous date extract on total and differential white blood cell count. Likewise, Uzun and Kalender (2013) evaluated the effect of quercetin and catechin on hematological alterations in rats treated with chlorpyrifos. The researchers observed significant increase in WBC count in the chlorpyrifos treated group about 15.64 K/IU that reduced to 9.74 and 9.96 K/IU by the simultaneous administration of catechin and quercetin, respectively. The findings concord the present study as the WBC count increased in the atherogenic rats and decreased in rats fed on date containing diets.

Hematopoiesis is the process of forming the blood components from the stem cells (hematopoietic) in the bone marrow. There are fat tissues present in the bone marrow that may store certain lipophilic compounds as xenobiotics (Merhi et al., 2010). The increase in the leucocyte count in case of diseased animal as in the atherogenic rats might be indicating the activation of immune system and cellular defense mechanism.
4.6.7.3. Thrombocytes – Platelet count

Thrombocytes/platelets are the second most abundant cells after erythrocytes that are responsible for mediating hemostatic functions and wound healing (Ferdous and Scott, 2015). The F values pertaining to the platelet count in Table 4.28 expounded non-momentous effect of treatments in the entire trial. Means for platelet count (normal rats) in groups G₁, G₂ and G₃ were 573.86±21.81, 568.24±25.57 and 568.16±17.04 K/UL, correspondingly. As far as atherogenic groups are concerned, similar pattern was noticed for G₄ (597.17±20.30 K/UL), G₅ (592.34±19.01 K/UL) and G₆ (590.74±19.32 K/UL) groups.

Scientific investigations demonstrates the imperative role of platelets in various inflammatory functions and immune responses. Under normal physiological conditions, the platelets are formed from megakaryocytes within 2-3 days in rats and 4 to 6 days in humans (Machlus and Italiano, 2013). The results are in line with the previous research of Onuh et al. (2012) who elucidated the haemopoietic activity of date fruit extract and found dose dependent effect of aqueous and methanolic date extract on the platelet count. They found increased platelet count in DMSO treated group that reduced dose dependently by the date extract administration. The present results are also in harmony with the earlier findings of Uzun and Kalender (2013) who studied the effect of catechin and quercitin on chlorpyrifos induced toxicity. Non-momentous effect was found in case of normal rats, while the platelets increased in diseased group from 685.21 to 890.61 K/UL. However, the catechin and quercitin treatments reduced the platelet count to 787.73 and 770.11 K/UL, respectively.

The non-significant increase in thrombocyte count in the atherogenic rats in the present study may also indicate liver damage or nephrotic syndrome (Tkaczyk and Baj, 2002) resulting in increased production of platelets to cope with the injury. This condition was ameliorated as evident from the decrease in platelets in date fruit and extract fed groups, indicating their effectualness.

4.6.7.4. Inflammatory marker – Erythrocyte sedimentation rate (ESR)

ESR is the rate at which the erythrocytes sediment in a specific one hour time period. It is a non-specific indicator of inflammation. Statistical inferences (F values) regarding the erythrocyte sedimentation rate in Table 4.28 revealed non-considerable variations as function of treatments during entire course of study. In normal rats, means for ESR were 2.48±0.09, 2.48±0.09, 2.48±0.09, respectively.
Table 4.28. Effect of diets on thrombocytes, ESR and clotting profile

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental Groups</th>
<th>Thrombocytes (K/UL)</th>
<th>Inflammatory Marker (mm after 1st hour)</th>
<th>Clotting Profile (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Diet</td>
<td>G1</td>
<td>573.86±21.81</td>
<td>2.48±0.09</td>
<td>14.57±0.55</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>568.24±25.57</td>
<td>2.46±0.07</td>
<td>14.59±0.44</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>568.16±17.04</td>
<td>2.44±0.08</td>
<td>14.62±0.60</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>0.31&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.27&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.17&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Atherogenic Diet</td>
<td>G4</td>
<td>597.17±20.30</td>
<td>3.15±0.11</td>
<td>14.02±0.53</td>
</tr>
<tr>
<td></td>
<td>G5</td>
<td>592.34±19.01</td>
<td>3.01±0.12</td>
<td>14.19±0.49</td>
</tr>
<tr>
<td></td>
<td>G6</td>
<td>590.74±19.32</td>
<td>2.98±0.12</td>
<td>14.18±0.43</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>0.14&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>6.05&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.43&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests
* = Significant
** = Highly significant
<sup>NS</sup> = non-significant
G1 Normal diet
G2 Date fruit + normal diet
G3 Date extract + normal diet
G4 Atherogenic diet
G5 Date fruit + atherogenic diet
G6 Date extract + atherogenic diet
2.46±0.07 and 2.44±0.08 (mm after 1st hour) in groups G₁, G₂ and G₃, correspondingly whereas, means for oxidative stressed rats also showed non-significant differences in the groups; G₄ (3.15±0.11 mm after 1st hour), G₅ (3.01±0.12 mm after 1st hour) and G₆ (2.98±0.12 mm after 1st hour).

ESR is influenced by serum cholesterol concentrations resulting in increased content (Choi and Pai, 2004). The results are in general agreement with the findings of Mohamed and Al-Okbi (2004) who probed the anti-inflammatory effect of date extracts in adjuvant arthritis. The methanolic date extract proved effective in ameliorating the ESR and plasma fibrinogen. ESR increased from 1.25 mm/hour in normal rats to 4.38 mm/hour in arthritic control that reduced to 1.63 mm/hour by the methanolic date extract. The results of the present study also indicate slight degree of inflammation through increased ESR in atherogenic rats that is also supported by histopathological findings. Thus, the results show non-momentous efficacious role of date fruit and extract against inflammation.

4.6.7.5. Clotting profile – Prothrombin time

Prothrombin time is used to assess liver functioning as it indicates the formation of blood clotting factors made by the liver (Thapa and Walia, 2007). The statistical values in Table 4.28 presented non-substantial impact of treatments on prothrombin time during the bioevaluation studies. In normal rats, the noted variations in prothrombin time were 14.57±0.55, 14.59±0.44 and 14.62±0.60 seconds for G₁, G₂ and G₃ groups, correspondingly. Similarly, atherogenic groups also presented non-momentous increase for G₄, G₅ and G₆ groups; 14.02±0.53, 14.19±0.49 and 14.18±0.43 seconds, respectively. Hepatic injuries result in reduced formation and activation of certain blood clotting factors due to the compromised metabolic activities in liver (Singh et al., 2011a). Prothrombin time is the marker of liver biosynthetic capacity that is disturbed in chronic cases. In the present study, no statistically significant variations were recorded regarding this parameter.

CHAPTER 5

SUMMARY

The nutraceutical advancement has altered the dietary patterns with more emerging disease ameliorative interventions. It has evoked a paradigm shift based on the plausibility of employing nature-based therapeutics to prevent numerous malfunctions. The food contains bioactive entities that interact with human physiology in various ways. The use of functional/designer foods in the dietary regime proves to be pragmatic approach to ensure better health and well-being. In this regard, date fruit (*Phoenix dactylifera*) is a promising source of nutrients and nutraceutics. The mandate of the present research was to probe the restorative potential of dates against atherogenic diet induced cardiac and hepatic stress. In this milieu, the study was divided into four modules; in the first phase locally available date varieties were ascertained for their compositional characteristics. Further, the bioactives were quantified using HPLC and the antioxidant activity was assessed. In the third module, the varieties and their respective extracts were utilized in the development of date-based halwa. The physicochemical behavior, storage stability and hedonic response of the developed product were also determined. Finally, in the last part of the study, the bioefficacy assessment of the date fruit and extract was carried out on normal and atherogenic diet induced oxidative stressed rats. Subsequently, the resultant data was inferred statistically to determine the level of significance.

Nutritional profile of the selected date cultivars was assessed in the first phase of the study. The results indicated that Aseel cultivar contains highest moisture (17.71±0.67%) trailed by Dhaki (15.54±0.54%) and Zahidi (10.50±0.39%). More protein was present in the Zahidi cultivar as 4.60±0.18% followed by Dhaki and Aseel as 4.22±0.15 and 3.72±0.14%, respectively. However, the crude fat content was higher in Aseel and Zahidi dates *i.e.* 2.61±0.08 and 2.08±0.07%, correspondingly as compared to Dhaki (1.28±0.04%). The fiber content was highest in the Zahidi cultivar (7.19±0.27%) followed by Dhaki (6.47±0.23%) and Aseel (4.84±0.17%). The ash content was recorded as 1.54±0.06, 0.94±0.03 and 1.91±0.07% in Dhaki, Aseel and Zahidi, respectively. Mineral content determination showed that among the mineral elements, potassium is dominant in all the selected varieties. Highest potassium content was found in Zahidi (870.83±32.39 mg/100g), followed by Dhaki (640.23±24.97
mg/100g) and Aseel (577.30±17.90 mg/100g) on fresh weight basis. Calcium was also high in Zahidi as 96.86±3.18 mg/100g trailed by Aseel (58.42±2.04 mg/100g) and Dhaki (37.19±1.30 mg/100g). Similar trend was observed in case of sodium, as highest content of 8.49±0.31 mg/100 g was found in Zahidi and lowest in Dhaki (3.27±0.10 mg/100 g). Iron was also higher in Zahidi as compared to the other two cultivars. The recorded iron contents for Dhaki, Aseel and Zahidi were 1.98±0.07, 1.58±0.04 and 3.23±0.11 mg/100g, respectively. Besides, β-carotene content was more in Dhaki and Zahidi as 0.54±0.01 and 0.47±0.01 mg/100g, respectively and less in Aseel (0.32±0.01 mg/100g). Whereas, vitamin C content was highest in Zahidi (63.38±0.95 mg/100g) followed by Aseel as 46.58±0.71mg/100g and Dhaki 24.44±0.34 mg/100g. Date fruits are considered rich source of sugars. The sugar profile of selected cultivars was assessed chromatographically and results revealed highest reducing sugars in Zahidi (39.51±0.54%), trailed by Aseel (34.90±0.46%) and Dhaki (31.31±0.69%). Glucose was present in higher amounts in Dhaki as compared to Aseel and Zahidi. While, better fructose content was exhibited by Zahidi followed by Aseel and Dhaki. Maximum amount of sucrose was found in Zahidi variety (26.15±0.37%) trailed by Dhaki (20.11±0.29%) and Aseel (17.22±0.24%).

In the second phase, the phytochemical constituents were quantified using HPLC followed by the assessment of in vitro antioxidant activity. In this regard, it was found that the cultivars contain a myriad of biologically active constituents responsible for its disease preventive and ameliorative properties. Amongst, gallic acid was identified and quantified in all the three varieties. Highest gallic acid content was found in the Zahidi dates as 192.28±2.58 mg/100g F.W. trailed by Aseel (128.23±1.71 mg/100g F.W.) and Dhaki (16.31±0.22 mg/100g F.W.). Caffeic acid was also present in high amounts in the Zahidi dates 108.64±1.36 mg/100g F.W., while, 7.21±0.10 mg/100g F.W. was found in Dhaki. However, it was not identified in the Aseel cultivar. Sinapic acid was detected and quantified in Aseel and Zahidi dates only as 2.25±0.03 and 4.40±0.06 mg/100g F.W., respectively. Additionally, m-coumaric acid was only quantified in the Zahidi variety as 1.49±0.03 mg/100g F.W. Dhaki dates contained ferulic acid and cinnamic acid as 10.45±0.13 and 5.05±0.07 mg/100g F.W., respectively. Interestingly, higher amounts of vanillic and chlorogenic acid were present in the Aseel cultivar i.e. 64.88±0.84 and 24.66±0.33 mg/100g F.W., respectively. Amongst the flavonols, kaempferol and quercitin were identified in all the cultivars, while myricetin was only found in the Dhaki
variety. The kaempferol content ranged from 31.98 to 49.36 mg/100g F.W. among the cultivars. Similarly, the maximum quercetin content was obtained in Zahidi (2.84±0.04 mg/100g F.W.), followed by Dhaki (2.23±0.03 mg/100g F.W.) and Aseel (0.79±0.01 mg/100g F.W.). Surprisingly, myricetin was only detected in the Dhaki variety as 28.41±0.39 mg/100 g F.W. Amongst the flavan-3-ols, catechin and epigallocatechin gallate (EGCG) content was also determined. All the three varieties contained catechins ranging from 0.48±0.01 to 1.30±0.02 mg/100g F.W. with highest content in Aseel and lowest in Zahidi. Nonetheless, the epigallocatechin gallate content was 11.91±0.16, 6.75±0.10 and 6.22±0.08 mg/100g F.W. in Aseel, Zahidi and Dhaki, respectively.

The total polyphenols assessment in the selected date cultivars showed that Zahidi variety possesses significantly higher amount of phenolics as 425.29±17.56 mg GAE/100g than Aseel and Dhaki varieties as 263.33±11.38 and 247.84±10.43 mg GAE/100g, respectively. However, the maximum flavonoid content i.e. 204.45±7.21 mg QE/100g was found in the Dhaki date extract followed by 162.48±7.37 mg QE/100g in Zahidi and 145.97±6.25 mg QE/100g in the Aseel extracts. Means for DPPH antiradical ability explicated highest percentage 90.48±3.45% for Zahidi extract, whilst lowest for Dhaki extracts as 79.66±2.06%. The FRAP values for Dhaki, Aseel and Zahidi extracts were 0.90±0.03, 0.64±0.02 and 0.76±0.03 μM Fe²⁺/g F.W., correspondingly. Regarding the TEAC assay, Zahidi extract showed maximum potential as 5.50±0.15 μM Trolox/g trailed by Dhaki (4.10±0.15 μM Trolox/g) and Aseel (3.92±0.11 μM Trolox/g). The H₂O₂ scavenging ability of the date extracts ranged from 87.78±2.90 to 91.76±2.88%. Similarly, the reducing power also varied among the cultivars with a range of 79.45-85.70%.

All the selected cultivars with their respective extracts were used in the development of date based functional halwa. Higher calorific value was exhibited by Aseel date based halwa (D₂) 410.35±14.77 kcal/100g followed by Aseel date extract based halwa (D₂a) 408.85±14.31 kcal/100g. Whilst, the energy value in Zahidi date and extract based halwa was 379.82±12.15 (D₃) and 386.02±13.32 kcal/100g (D₃a), correspondingly. The Dhaki date extract halwa was lower in calorific value (363.46±15.67 kcal/100g) as compared to Dhaki date based halwa (368.19±11.97 kcal/100g). The reported value for control halwa without date fruit and extract was 356.21±12.47 kcal/100g.
The moisture content varied non-momentously amongst the treatments and ranged from 28.40±0.89 to 32.86±1.35%. The ash content was highest in Zahidi date fruit and extract containing halwa *i.e.* 2.33±0.08 and 2.10±0.07%, respectively, while lowest in Dhaki date halwa (1.15±0.04%) and the control traditional halwa (0.84±0.03%). The fat content varied between 12.68±0.41 and 26.93±1.18% in the treatments. Maximum protein content was exhibited by the Zahidi date based halwa *i.e.* 16.48±0.58% followed by Zahidi date extract based halwa as 15.32±0.55%, whilst lowest in the traditional halwa (7.80±0.28%). The color tonality of date-based halwa showed significant variations amongst the treatments during storage. The means pertaining to L* value ranged between 44.73±1.51 and 59.13±2.03 among the developed prototypes. The lighter color was observed for the control and extract-containing treatments however, the fruit containing treatments exhibited darker color tonality. Likewise, a* values for treatments; D₀, D₁, D₁a, D₂, D₂a, D₃ and D₃a were 0.79±0.03, 3.09±0.11, 1.15±0.04, 1.87±0.07, 0.92±0.03, 4.24±0.15 and 1.72±0.06, respectively. Similarly, the highest b* value was recorded for control (D₀) 17.10±0.62 and lowest for Zahidi date halwa (D₃) 6.76±0.22. The hardness assessed using texture analyzer showed that hardness in D₀, D₁, D₁a, D₂, D₂a, D₃ and D₃a were 8.07±0.26, 6.47±0.22, 3.64±0.14, 3.70±0.15, 2.09±0.09, 6.65±0.23 and 8.12±0.27 N, respectively. Hardness increased during the storage with minimum at 0 day *i.e.* 2.87±0.11 that significantly increased to 9.58±0.32 N at the 14th day. Likewise, the water activity in halwa prototypes varied between 0.65±0.01 to 0.76±0.02 with minimum water activity at 0 day (0.65±0.02) and maximum at 14th day (0.77±0.01).

Regarding the *in vitro* antioxidant capacity, higher TPC was exhibited by the date fruit based treatments, whilst, lower values were recorded in the extract containing halwa samples with minimum in the control. The flavonoid content varied between 103.97±3.79 and 74.32±2.80 mg QE/100g with maximum in Dhaki date based halwa. Maximum DPPH antiradical activity was exhibited by the Zahidi date and extract containing treatments as compared to others. Likewise, values for TEAC for treatments; D₀, D₁, D₁a, D₂, D₂a, D₃ and D₃a were 0.84±0.03, 1.94±0.06, 1.55±0.05, 1.72±0.06, 1.50±0.04, 1.96±0.07 and 1.59±0.06 µM Trolox/g F.W., respectively. Similarly, ferric reducing antioxidant power also showed momentous variations in different functional halwa treatments ranging between 0.85±0.04 and 1.27±0.05 µmol Fe²⁺/g, correspondingly.
Hedonic assessment of the developed halwa prototypes was conducted to determine their acceptability and consumer response. Appearance, texture, color, aroma and taste were the studied sensory attributes and by using this information, the overall acceptability ratings were computed. Maximum scores for overall acceptability were attained by the Zahidi date and its respective extract containing halwa treatments.

On account of phytochemical in vitro analyses and hedonic response of the developed halwa, Zahidi date and its extract were selected for the bioevaluation studies with special reference to cardiac and hepatic stress. Regarding the bioefficacy assessment, the average feed and water intakes were recorded on daily basis and feed efficiency ratio was calculated. The date fruit and extract containing diets reduced the body weight in normal rats up to 1.41 and 4.47%, respectively. Whereas, atherogenic rats showed 14.09 and 19.12% reduction in body weight through date fruit and date extract supplemented diets, correspondingly. The oxidative stress induced rats showed momentous impact of treatments for heart and right kidney weight. However, non-momentous variations in liver and left kidney weights were noted.

In the present study, the effect of date-supplemented diets was assessed on normal and stressed rats. Oxidative stress was induced using atherogenic diet, therefore the effect on the serum lipidemic parameters was especially focused. The results showed that date fruit reduced 3.65% serum cholesterol in normal rats, whereas 15.14% in atherogenic rats, while, the date extract treatment resulted in 4.49 and 18.55% reduction in normal and atherogenic rats, respectively. Likewise, triglyceride levels reduced by 3.71 and 12.02% by date fruit supplemented diet in normal and atherogenic rats, correspondingly. The percent decrease in date extract fed groups were 5.05 and 14.51% in normal and stressed rats, respectively. LDL cholesterol reduced by 8.66 and 11.55% in date fruit and date extract fed normal rats, correspondingly. Whilst, 21.05 and 25.98% reductions were noted in the atherogenic groups fed on date fruit and date extract containing diets, respectively.

To assess the extent of cardiac risk in the subjects, several atherogenic ratios have been developed based on the serum lipid parameters. In case of normal rats, the control (G1) exhibited 0.30±0.01 atherogenic index of plasma that reduced due to date fruit as well as date extract treatments to 0.28±0.01 and 0.27±0.01, respectively. However, regarding the atherogenic rats, increase of 0.69±0.02 was observed in the AIP ratio that decreased in the
treated groups; G5 and G6 to 0.60±0.01 and 0.58±0.02, correspondingly. The recorded ratio for atherogenic coefficient was higher in normal diet fed rats (G1) 1.53±0.05 that decreased in the preceding date fruit (G2) and date extract (G3) fed groups to 1.39±0.06 and 1.33±0.06, respectively. Likewise, in the rats fed on atherogenic diet (G4), the AC value was highest (5.37±0.19), that reduced in G5 (4.01±0.15) and G6 (3.71±0.13) upon simultaneous administration of date fruit and date extract containing atherogenic diets. The obtained results for CRI (I) were 2.53±0.08, 2.39±0.09 and 2.33±0.07 in G1, G2 and G3, respectively in case of normal diet fed rats. Whereas, G4, G5 and G6 rats exhibited 6.37±0.20, 5.01±0.18 and 4.71±0.17, correspondingly. CRI (II) ranged from 0.96±0.03 to 1.12±0.04 in normal and 2.95±0.10 to 4.40±0.13 in the atherogenic rats.

The date fruit and extract-based diets intensified serum superoxide dismutase level throughout the study. In this context, normal rats presented 6.54 and 5.22% enhancement in SOD by date fruit and date extract containing diets, correspondingly. Similarly, in the atherogenic diet fed groups, increase of 29.05 and 27.99% in SOD was observed in G4 and G5, respectively. Date fruit containing diet (G2) led to 13.48% improvement in the serum catalase content, whilst date extract containing diet (G3) resulted in 8.37% increase as compared to control. Likewise, in atherogenic groups, treatments; G5 and G6 showed 22.60 and 18.35% increment, correspondingly for this trait. Date fruit has been found to possess more ameliorative properties, lowered lipid peroxidation by 13.64 and 33.67% in normal and atherogenic rats, respectively. While, the rats fed on date extract-containing diets showed 12.54 and 28.76% reductions in normal and atherogenic rats, correspondingly.

The cardiac stress indicators in serum as well as tissues were determined to evaluate the effectualness of Zahidi date fruit and extract. In this regard, dietary date fruit supplementation lowered AST level by 4.58 and 14.47% in normal and atherogenic rat sera, accordingly. Nevertheless, date fruit extract was found to be more effective in lowering the serum AST by 8.61 and 19.00% in normal and stressed rats, respectively. Likewise, CK levels indicating the muscle functionality also decreased up to 2.34 and 3.80% by date fruit and date extract treatments in normal rats, respectively. Whereas, 16.68 and 19.38 percent reductions were recorded in the rats fed on date fruit and date extract containing atherogenic diets, respectively. The cardiac specific CK-MB levels decreased by 6.54 and 8.41% in G2 and G3, respectively. Whilst, more reduction was recorded in the groups fed on atherogenic diet along with date fruit.
(15.99%) and date extract (18.02%). Similarly, the date fruit and extract-based treatments also modulated the serum LDH levels by 7.22 and 11.95% in normal rats and 16.01 and 22.51% in the atherogenic rats, correspondingly. The cardiac tissue antioxidant status improved by the administration of date based functional diets. The respective diets also reduced the lipid peroxidation in the myocardium and ameliorated the histoarchitecture. Maximum increase in cardiac SOD was observed in G₆ i.e. 38.34% followed by 32.86% in G₅. Whilst, in case of normal groups, 8.29 and 13.49% reductions were observed in G₂ and G₃, respectively. Likewise, catalase activity increased by 12.19 and 15.71% in normal rats by dietary date fruit and date extract supplementation, correspondingly. Besides, the cardiac catalase level was raised up to 31.93 and 35.71% in atherogenic rats fed on date fruit and date extract based diets, respectively. The date fruit diets reduced lipid peroxidation in the cardiac tissues by 11.84 and 20.07% in normal and atherogenic rats, correspondingly. However, more pronounced decrease was observed by the supplementation of date extract i.e. 13.01 and 25.67% in normal and oxidative stressed rats, respectively.

To analyze the effect of diets on liver, certain hepatic parameters were ascertained. The results showed 4.43 and 7.97% reduction in serum ALT levels by the administration of date fruit and date extract containing diets in normal rats, respectively. While, date fruit treatment lowered the ALP levels by 17.16% and date extract treatment reduced up to 21.45% in the atherogenic rats. Moreover, γ-GT levels reduced to about 2.05 and 13.65% in normal and oxidative stressed rats by the administration of date fruit, respectively. More decrease in γ-GT was observed by the administration of date extract in normal as well as atherogenic rats as 4.10 and 18.88%, respectively. Likewise, percent decrease in total bilirubin was 3.31 and 7.07% by the date fruit supplementation in normal and atherogenic diet fed rats, correspondingly. However, the date extract decreased 2.48% of bilirubin in normal and 10.33% in atherogenic rats. Regarding the hepatic redox status, normal rats presented 6.30 and 24.29% improvement in superoxide dismutase level through date fruit and date extract containing diets, correspondingly. Similarly, in the atherogenic diet fed groups, increase of 11.25 and 30.20% in SOD was observed by date fruit and date extract supplemented diets, respectively. The hepatic catalase levels G₂ showed 9.74% improvement, whilst G₃ group exhibited 11.53% increase in catalase activity as compared to control. Likewise, in the atherogenic groups, G₅ and G₆ showed 16.61 and 20.88% increase, correspondingly for this parameter. Date fruit and date extract containing diets were
effective in mitigating lipid peroxidation in hepatic tissues. Maximum decrease was observed in the atherogenic diets i.e. 23.54 and 28.09% by date fruit and date extract supplementation, respectively. However, in normal rats 9.59 and 12.95% reductions were observed in the date fruit and date extract fed rats, correspondingly. Moreover, positive alterations were observed in the hepatic histoarchitecture.

Date fruit showed pronounced effect in serum urea concentration as it reduced in normal and oxidative stressed rats by 6.28 and 15.37%, respectively. However, date extract supplemented diet reduced the urea levels by 4.51% in normal rats and 12.61% in atherogenic diet fed oxidative stressed rats. Similar pattern was recorded for creatinine concentration that decreased by 7.84% in date fruit treated normal rats and 3.92% in date extract treated normal rats. The concentration decreased by 14.13 and 11.96% in date fruit and date extract fed atherogenic rats, respectively. Date based diets increased the superoxide dismutase level in kidneys throughout the study. Maximum improvement in the SOD levels was found in groups fed on date fruit containing diets i.e. 6.44 and 16.81% in normal and oxidative stressed rats, respectively. Besides, date extract based diet also increased the nephrotic SOD in normal as well atherogenic rats by 5.45 and 13.62%, respectively. In normal rats, date fruit containing diet led to 9.08% improvement in nephrotic catalase whilst, date extract containing diet resulted in 6.34% increase in renal catalase as compared to control. Similarly, in atherogenic groups, treatments; G5 and G6 showed 16.06 and 17.44% increment, correspondingly for this trait. Regarding renal MDA content, 8.81 and 18.27% reduction was observed by the administration of date fruit and date extract containing normal diets, correspondingly. Likewise, percent reduction in MDA contents were up to 5.32 and 12.58% in normal and oxidative stressed rats fed on date extract containing diets, correspondingly. The renal histology also showed positive influence of date based functional diets.

In a nutshell, date fruit containing optimal mix of health benefitting nutrients can be an ideal substitute for a range of value added designer products. In the current research, date fruit containing functional diets proved effectual in the modulation of blood lipids, cardiac, hepatic and renal parameters. Concisely, date based value added foods have the capacity to curtail the menace of some metabolic syndromes such as dyslipidemia, hyperglycemia, hepatotoxicity, nephrotoxicity, obesity and cardiovascular ailments. Hence, dietary date fruit supplementation should be encouraged among the masses to ensure optimal health.
RECOMMENDATIONS

- Fruits like dates containing plethora of nutrients should be promoted in dietary therapy
- Varietal variations need to be explored, those possessing better functional profile like Zahidi require further extensive studies in relation to phytoceutics
- Date fruit supplementation particularly Zahidi cultivar should be used as an effective strategy to reduce oxidative stress mediated dysfunctions
- Health care professionals should recommend the consumption of Zahidi dates for optimal cardiac and hepatic health
- Further assessment requires integration of multiple biomarkers to tailor cost effective date based therapeutic interventions for lifestyle related disorders *i.e.* cardiac and hepatic complications
- Clinical trials should be planned for intellectual meticulousness pertaining to the prevention and amelioration of CVDs by employing date based products
- Metabolomics involving novel biomarkers are required to provide insight regarding multifaceted biochemical pathways underlying nutritional effects of date fruit
- Multi-sectoral participatory approaches should be encouraged for designing integrated need-based research projects focusing on existing lifestyle and dietary perils
- Community awareness programs fostering nutritional understanding of date based foods with special reference to disease prevention ought to be launched among the masses
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# APPENDIX I

## FORMULATION OF DATE HALWA

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control (%)</th>
<th>Date Fruit (%)</th>
<th>Date Extract (%)</th>
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</thead>
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<tr>
<td>Clarified Butter</td>
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<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Gram Flour</td>
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<td>10.0</td>
</tr>
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<td>10.0</td>
</tr>
<tr>
<td>Nuts</td>
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<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
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<tr>
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</tr>
<tr>
<td>Date Extract**</td>
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<td>-</td>
<td>50.0</td>
</tr>
</tbody>
</table>

- *Dates: All varieties were added on dry matter basis in respective samples, after soaking them in water and milk*
- **350 g dates were soaked in 100 mL water to obtain the extract which was around 500 mL**
### SENSORY RESPONSE PROFORMA

**Designer Date Halwa**

**Name of Judge:** …………………
**Age:** ………………………..
**Gender:** ……………………….
**Designation:** ……………………

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<tr>
<th>Trait</th>
<th>D₀</th>
<th>D₁</th>
<th>D₁a</th>
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<td>Overall acceptability</td>
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</table>

**Comments/Suggestions:** ________________________________________________________________

**Scale for Evaluation**
- Extremely poor: 1
- Very poor: 2
- Poor: 3
- Below fair above poor: 4
- Fair: 5
- Below good above fair: 6
- Good: 7
- Very good: 8
- Excellent: 9

**Signature:** ………………………
**Date:** ………………………
<table>
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<tr>
<th>Ingredients (g/kg)</th>
<th>G_1</th>
<th>G_2</th>
<th>G_3</th>
<th>G_4</th>
<th>G_5</th>
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</table>

* the extract obtained from 200 g dates was equal to 330 mL extract approximately and it was added in diet and kneaded to form pellets for rats.