Investigation of Hepatoprotective Activity of Herbal Constituents

A Thesis submitted for the Degree of Doctor of Philosophy

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Dedicated To My Family
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Summary

The aqueous-methanolic extracts from indigenous plants and some of the pure compounds were subjected to preliminary screening for their possible protective effects against lethal dose of paracetamol (1 g/Kg) in mice. The hepatoprotective effect was studied further in rats, using paracetamol- and carbon tetrachloride (CCl₄)-induced hepatotoxicity as *in vivo* model. The cytoplasmic enzymes (i.e., GOT, GPT and ALP) are released into serum upon hepatocellular damage and the serum values of such enzymes are reliable indices to assess the extent of hepatic damage. The enzyme values were found to be high in toxin treated animals, whereas the serum enzyme values in the animals pretreated with plant materials were found to be significantly lower than those of the toxic control group and similar to the normal values indicating hepatoprotective effect.

To resolve whether the hepatoprotective effect was mediated through inhibition of microsomal drug metabolizing enzymes (MDME), the plant material was co-administered with pentobarbital (PB) to observe possible prolongation in pentobarbital-induced sleep in mice. The test material that was found to prolong the PB sleep were further subjected to strychnine-induced toxicity in mice to clarify whether the prolongation in PB sleep was solely due to inhibitory action on MDME or central depressive activity.

Since some known calcium channel blockers (CCBs) also exhibit hepatoprotective action and the facility for measuring CCBs-activity was available in the laboratory, the plant materials were also tested for this activity by using isolated tissue experiments (i.e. rabbit jejunum). Plant material with inhibitory action on the spontaneous contraction of jejunum was further tested against the high K⁺ (>30 mM)-induced spastic contractions as well as in the Ca²⁺-free medium.
The post-treatment (curative) experiments were performed in selected cases where
the test material was found to be inhibitory against MDME and was helpful in deciding
whether the hepatoprotective activity was solely due to MDME inhibition or some other
mechanism was also in operation.

*Artemisia absinthium* (shoots), *Berberis aristata* (shoot and fruits) and berberine
(an alkaloid from *Berberis aristata*) were found to be protective against both paracetamol-
and CCl₄-induced liver damage and also showed MDME inhibitory activities. These were
also capable to show curative effect against paracetamol but unable to mitigate the
toxicity due to CCl₄, suggesting that beside MDME inhibitory activity other mechanisms
may also be involved in the hepatoprotective response.

*Artemisia maritima* (shoots), *Artemisia scoparia* (shoots), *Cichorium intybus*
(shoots and seeds), *Cyperus scariosus* (rhizomes), *Rubia cordifolia* (roots) extracts, and
pure compounds from plants (caffeic acid, esculetin, quercetin and rutin) were able to
provide the hepatoprotection against both paracetamol and CCl₄ but were not inhibitory
to MDME. The above mentioned plant extracts, quercetin as well as *Artemisia
absinthium* extract exhibited Ca²⁺ channel blocking activities, hence this property may
partly be responsible for the observed hepatoprotection.

The plant materials were found to be relatively safe in acute toxicity testing in
mice and this study rationalizes the folklore use of plants in the hepatobiliary disorders.
ABBREVIATIONS

ALP  Alkaline phosphatase
CCB  Calcium channel blockers
CCl₄  Carbon tetrachloride
GOT  Glutamate oxaloacetate transaminase
GPT  Glutamate pyruvate transaminase
hr(s)  Hour(s)
IMAC  Intra mitochondrial membrane anion channels
IU  International units
Kg  Kilo gram
L  Liter
MDME  Microsomal drug metabolizing enzymes
μM  Micro molar
mg  Milli grams
mL  Milli liter
mM  Milli molar
NAPQI  \(\text{N-}\text{Acetyl-p-}
\text{benzoquinoneimine}\)
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11.36 Effect of *Rubia cordifolia* root extract on CCl₄-induced elevation of serum transaminase levels in rats

XXII
1. INTRODUCTION
GENERAL INTRODUCTION

The advancements in medicine and public health measures have made possible, the worldwide decline in the incidence and prevalence of infectious liver diseases (i.e., viral, bacterial, rickettsial, fungal and parasitic). However, the chemical-induced diseases are increasing paradoxically in recent years to become a major health problem in near future. The general population, especially industrial workers are likely to be affected by the carcinogenic hazards from a vast array of chemical structures (xenobiotics). The toxicological important xenobiotics may get access into the body as environmental contaminants (food, water and air) or are administered inadvertently with therapeutic and suicidal intention.

1.1 Xenobiotics

The natural food stuffs provides all the essential nutrients required for the growth, maintenance and reproduction, yet a number of chemicals are added to dietary items in the course of production, processing, packing and distribution (Miller, 1992). The fertilizers, growth promoters, pesticides and herbicides are being used to increase the agricultural crop production and residual amounts of which are likely to be present in the finished food items (Jackson et al., 1986; Miller, 1992). The chemicals under the titles of vitamins, minerals, antioxidants, antimycotics and antimicrobials are added deliberately to dietary items in an attempt to improve/preserve the desirable nutritive qualities in a time span likely to be spent in distribution channel, whereas flavorants, colorants, thickeners and emulsifiers are incorporated in food to enhance its aesthetic appearance (Borzelleca et al., 1983). In addition, certain chemicals are considered unavoidable food contaminants because they are either leached from material used in manufacturing
of processing, packing and eating utensils or are known pollutants of water and air (Bidlack and Riewbow, 1989).

Pure water is not available naturally and water considered fit for human consumption is contaminated with trace amounts of both organic and inorganic substances (Hills, 1979; Kanarek and Young, 1982). The human and industrial waste materials disposed into the nearest stream have greatly increased the waste burden of the natural water sources used for drinking purpose (Dowty et al., 1975; Munson et al., 1982). The contamination of water source with pesticides and herbicides is also common through 'run-off' from agricultural lands (Buhler et al., 1973; Kaligore and Akesson, 1980).

The atmosphere in industrial area is continuously polluted by volatile industrial products and the vapor concentration of many organic solvents (i.e., hydrocarbons, chlorinated hydrocarbons, alcohols, ethers, esters and ketones) is approaching many hundreds or thousands of parts per million capable to be absorbed systemically through inhalation (Browning, 1965).

1.2 Biotransformation

Several tissues (i.e., kidney, gut and endocrine glands, etc.) participate in a complementary manner for the elimination of endogenous and exogenous compounds but liver remains to be quantitatively most active in this regard.

The liver is concerned with metabolic derivatisation of small non-polar molecules, thus increasing its polarity and hence help excretion in urine. Similarly large molecules (in excess of 400 dalton) carrying polar or hydrophilic groups can be eliminated efficiently through biliary pathway (Millburn, 1970). The biotransformation function is accomplished by liver enzymes which oxidize,
reduce, hydrolyze or conjugate the xenobiotics (i.e., pollutants and drugs) and endogenous metabolites (Gillette, 1966; Kappas and Alvares, 1975). The main drug-metabolizing system resides in the microsomal fraction of the hepatocytes (smooth endoplasmic reticulum) and are induced non-specifically by many lipid-soluble substances (i.e., alcohol, barbiturates, anesthetics etc.).

The oxidation-reduction and hydrolytic reactions often generate metabolically active sites such as carboxyl, epoxide, or hydroxyl groups in the parent compounds, which serve as acceptor of acetate, amino acids, sulphate, glucuronic acid and glutathione in synthetic reactions catalyzed by transferases.

1.3 Toxic metabolites formation

The biotransformation reactions may occur in such a way that reaction products become more reactive as compared to the parent compounds. Such metabolic reactions usually include oxidation, reduction, and/or conjugation processes to produce a chemical entity that may be reactive intermediate or that rearranges to the unstable reactive intermediate.

1.3.1 Oxidative processes

Several compounds, or their metabolites are reactive electrophilic species capable to interact with nucleophilic sites on tissue components, i.e., sulphydryl (HS-) group of glutathione and cysteine, or the amino (NH₂-) or hydroxyl (OH-) groups present in DNA, RNA and protein. This covalent interaction with tissue macromolecules is thought to be a key factor in the toxic cell injury (Gillette, 1974).

Paracetamol is a safe analgesic in therapeutic doses (Meredith and Goulding, 1980) and only under abnormal conditions does it result in
hepatotoxicity (Hinson, 1980). Three main pathways, i.e., sulfation, glucuronidation and oxidation are involved in paracetamol metabolism (Clement et al., 1984; Siegers et al., 1984), but only oxidative pathway generates toxic metabolites (Hinson et al., 1983). The estimated percentage ratio of glucuronide, sulfate and oxidative conjugates in adult human urine are expressed as 60, 35 and 5 respectively (Miller et al., 1976; Alan et al., 1977).

Following large doses, the sulfate pool become exhausted before much of the compound is biotransformed, while the glucuronidation pathway is rate limited (Hinson, 1983; Sipes and Gandolfi, 1992), leaving a large amount of the parent compound to be metabolized by cytochrome P-450 system (Potter et al., 1973).

The paracetamol is oxidized to N-hydroxy paracetamol, which is subsequently converted to a quinone resonance form N-acetyl-p-benzoquinoneimine (NAPQI) (Corcoran et al., 1980). NAPQI is normally detoxified by endogenous glutathione stores but the excessive production on paracetamol overdose may deplete glutathione stores (Jollow, 1980), allowing the reactive intermediate to interact with cellular macromolecules (Prescott, 1983; Linden and Rumack, 1984).

1.3.2 Reductive processes

The carbon tetrachloride (CCl₄) like other halogenated alkanes (i.e., chloroform, dichloromethane, bromotrichloro-methane, etc.) undergoes cytochrome P-450 catalyzed reductive dehalogenation and liberates trichloromethyl (CCl₃) (Recknagel and Glende, 1973).

Several cellular macromolecules such as lipids, nucleic acids, proteins and polysaccharides are susceptible to CCl₃ attack by hydrogen abstraction or addition reactions. Reaction of CCl₃ with poly-unsaturated fatty acids in membrane lipids
(RH) releases lipid free radicals (R) and hence initiates the chain reaction process of lipid peroxidation. The lipid free radicals (R) react with molecular oxygen (O₂) to generate lipid peroxy radicals (R-O-O·), which in turn abstract hydrogen atom from neighboring lipid molecule and generate another lipid free radical.

Alternatively, the free radicals may attack the methylene bridges of unsaturated fatty acid side chains of microsomal lipids causing morphological alterations in endoplasmic reticulum, causing loss to drug metabolizing enzymes, glucose-6-phosphatase activity, protein synthesis, and lipoprotein egress (Recknagel and Glende, 1973).

1.4 Calcium and toxic cell death

The cytosolic free calcium (Ca²⁺) concentration in mammalian hepatocytes is maintained at very low level (≈ 0.1 μM) as compared to the extracellular fluid concentration (≈ 1.3 mM) and the passive Ca²⁺ influx along the electrochemical gradient is compensated by active efflux (Ca²⁺/Mg²⁺-ATPase) and sequestration in storage sites (i.e., endoplasmic reticulum, mitochondria and calcium binding proteins) (Carafoli, 1987).

Sulphydryl (HS-) groups in protein structure (cysteine residues) are vital for the catalytic activity of a wide variety of enzymes (including those involved in cytosolic Ca²⁺ homeostasis) and modification of which may result in the inactivation of the corresponding enzyme (Mitchell et al., 1974). The HS- groups are acting as nucleophilic center and serve as a target site for arylation species. The toxic species (i.e., NAPQI, lipid peroxides, quinones, etc.) cause modification of critical HS- groups on Ca²⁺ regulatory proteins actively engaged in the maintenance of cytosolic Ca²⁺ homeostasis, with their subsequent inability to
control raised intracellular Ca\(^{2+}\) level (Beilomo et al., 1985; Tsokos-Kuhn et al., 1988). This impairment of Ca\(^{2+}\) homeostatic process may initiate cytotoxic sequence of events leading ultimately to cell death (van Kuijk et al., 1987).

1.4.1 Calcium and membrane functions

The Ca\(^{2+}\)-dependent alteration in the functional integrity of the plasma membrane is probably a key initial event in the pathogenesis of liver cell necrosis (Farber, 1975; Schanne et al., 1979). The cell surface blebs formation is regarded as an outcome of the cytoskeletal network disruption due to activation of certain Ca\(^{2+}\)-dependent catabolic enzymes (van Kuijk et al., 1987).

Phospholipase A\(_2\) is a Ca\(^{2+}\)-dependent lipolytic enzyme capable to release free fatty acids from peroxidized membrane as a part of the detoxication process, however, uncontrolled activation may lead to wide spread plasma membrane damage and thus generation of lysophospholipid and arachidonic acid. The accelerated phospholipid degradation from plasma membrane is an important event in hepatic injury (Farber and Young, 1981; James et al., 1982), because so many hepatic functions are disrupted following alteration in membranous phospholipid composition (Coleman, 1973). The lysophospholipids are cytotoxic agents, whereas the arachidonic acid metabolites are mediators of inflammatory and allergic reactions. The Ca\(^{2+}\)-dependent phospholipase C activation would also contribute toward phospholipid degradation causing not only the membrane function disruption (Lamb and Schwartz, 1982), but also impair the ability of mitochondria and endoplasmic reticulum to sequester Ca\(^{2+}\), leading ultimately to liver cell necrosis (Schanne et al., 1979).
1.4.2 Calcium and energy crisis

Most of the cellular energy requirements are fulfilled by ATP hydrolysis, which is made persistently available through oxidative phosphorylation in mitochondria. However, the raised intracellular Ca\(^{2+}\) level causes fatal perturbation in cellular energy status (Orrenius et al., 1989), which can be attributed to enhanced ATPase activity associated with cytoplasmic ion extrusion pumps (Aldridge, 1981), active mitochondrial Ca\(^{2+}\) sequestration of (Moore et al., 1988), and progressively diminished ATP formation by mitochondria following Ca\(^{2+}\) uptake (Landon et al., 1986).

The mitochondrial Ca\(^{2+}\) uptake requires extrusion of proton (H\(^+\)) to maintain the overall electroneutrality across the inner mitochondrial membranes (Becker et al., 1980; Akerman and Nicholls, 1983). The amount of Ca\(^{2+}\) that can be accumulated by this way is limited and is compensated by concomitant permeation of anions into the mitochondrial matrix via an inner mitochondrial membrane anion channel (IMAC) (Beavis and Garlid, 1987). Mitochondrial inner membranes contain specific binding sites for Ca\(^{2+}\) channel blockers (i.e., 1,4-dihydropyridine, phenyl-alkylamine and benzothiazines) associated with IMAC (Zerning et al., 1990). Calcium channel blockers thus prevent mitochondrial Ca\(^{2+}\) overload through modulation of anion influx via IMAC and hence protect the cellular viability through ATP conservation (Zerning, 1990).

1.5 Cellular protective measures

The formation of toxic metabolites is not always associated with adverse cellular events and can be detoxified effectively provided there is balance between the rate of formation and rate of detoxification. This critical balance can be
disturbed, either by the enhanced production of reactive intermediates or diminished capacity for their detoxification and then the toxic metabolites formation can lead to cellular injury. The balance between the formation and detoxification of reactive metabolites is abolished following enzyme induction, excessive xenobiotics intake, disease, starvation and concomitant drug therapy, etc.

The glutathione peroxidase and α-tocopherol (vitamin E) are able to detoxify the free radicals (l lipid peroxides and peroxy radicals), whereas hydroxyalkenals are eliminated by glutathione S-transferases catalyzed glutathione conjugation and cell death is averted until the level of reduced glutathione (GSH) falls substantially (Anundi et al., 1979; Sies et al., 1980). The cellular GSH level is replenished continuously by the NADPH-dependent GSSG-reductase by reducing back GSSG to GSH unless the rate of GSH oxidation to GSSG exceeds the capacity of GSSG-reductase (Meister, 1982).

1.6 Natural products and modern therapy

Health and well-being have been a subject of man's primary concern since time immemorial. From his early experiments with herbs and plants growing in his environment and treatment of disease, man was eventually able to establish empirical systems of medicine. The use of plants, plant extracts or pure compounds isolated from natural products to treat disease is a therapeutic modality, which has stood the test of time even if much of the science behind such therapy is still in its infancy. Phytochemical examination of plants and animal cure available to earlier civilizations has often shown that these contained active principles, responsible for therapeutic success. Presently, synthetic drugs outnumber those of natural origin in modern medicine, and research into the
Table 1.1

Plant Based Drugs (Oo, 1991).

<table>
<thead>
<tr>
<th>Plants</th>
<th>Drugs</th>
<th>Current Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Atropa belladona</em></td>
<td>atropine</td>
<td>antispasmodic, mydriatic, pre-anaesthetic agent, antidote for anticholinesterase/mushroom poisoning</td>
</tr>
<tr>
<td><em>Catharanthus roseus</em></td>
<td>vinblastine, vincristine</td>
<td>anti-neoplastic agent</td>
</tr>
<tr>
<td><em>Cephaellis ipecacuanha</em></td>
<td>emetine</td>
<td>treatment of amoebiasis, emetic</td>
</tr>
<tr>
<td><em>Chondrodendron torontosum</em></td>
<td>tubocurarine</td>
<td>skeletal muscle relaxant</td>
</tr>
<tr>
<td><em>Cinchona succirubra</em></td>
<td>quinidine, quinine</td>
<td>antiarrhythmic, antimalarial</td>
</tr>
<tr>
<td><em>Chelchicum autumnale</em></td>
<td>colchicine</td>
<td>anti-gout</td>
</tr>
<tr>
<td><em>Digitalis ionata</em></td>
<td>digoxin</td>
<td>treatment of congestive heart failure, supraventricular arrhythmias</td>
</tr>
<tr>
<td><em>Digitalis purpurea</em></td>
<td>digitoxin</td>
<td></td>
</tr>
<tr>
<td><em>Ephedra sinica</em></td>
<td>ephedrine</td>
<td>nasal decongestant</td>
</tr>
<tr>
<td><em>Hyoscyamus niger</em></td>
<td>hyoscine</td>
<td>anti-spasmodic, pre-anaesthetic agent, antidote for anticholinesterases/mushroom poisoning</td>
</tr>
<tr>
<td><em>Papaver somniferum</em></td>
<td>codeine, morphine, papaverine</td>
<td>suppression of cough, analgesic, antiarrheal smooth muscle relaxant</td>
</tr>
<tr>
<td><em>Physostigma venenosum</em></td>
<td>physostigmine</td>
<td>treatment of glaucoma, atropine poisoning</td>
</tr>
<tr>
<td><em>Pilocarpus microphyllus</em></td>
<td>pilocarpine</td>
<td>miotic, treatment of glaucoma</td>
</tr>
<tr>
<td><em>Rauwolfia serpentina</em></td>
<td>reserpine</td>
<td>anti-hypertensive</td>
</tr>
<tr>
<td><em>Strophanthus gratus</em></td>
<td>ouabain</td>
<td>treatment of congestive heart failure</td>
</tr>
</tbody>
</table>
isolation and pharmacology of natural products now lags far behind that of synthetic drugs. Nevertheless, there are still many drugs which have their origin in natural products derived from animal or vegetable source (Table 1.1). Indeed today many, if not most, pharmacological classes of drugs include a natural product prototype and morphine, digoxin, quinine, atropine, reserpine, physostigmine, pilocarpine, vinblastine, vincristine, artemisinin and taxol are a few examples of what medicinal plants have given us in the past.

There has been a resurgence of scientific interest in medicinal plants during the past 20 years, being rekindled by the world-wide importance of medicinal plants and crude drugs in traditional medicine. Moreover, empirical studies on medicinal plants revealed the fact that for a plant extract to be active clinically, it is not necessary for the active component to be isolated and the structure established. A large number of crude plant extracts are now being utilized in naturopathic remedies in addition to the purified natural substances.

A large proportion of the human population is on herbal remedies, however, only a limited number of plants have been investigated pharmacologically. The inherent biological complexity in plants makes it imperative to evaluate the safety, efficacy and quality during development of plant-based drugs.

1.6.1 *Herbal products and hepatoprotection*

Liver is the principal organ related to metabolism and excretion of a wide variety of environmental pollutants and also therapeutic agents. These xenobiotic either directly or indirectly (after bioactivation) may exert deleterious effects upon the structural as well as functional integrity of this vital organ, which are exhibited clinically as inflammatory, non-inflammatory or degenerative hepatic disorders.
In modern medicine, symptomatic relief is achieved through corticosteroids or immunosuppressive agents without any modification in the course of disease. On the other hand, herbal remedies support natural healing phenomena through blocking the progression of the degenerative pathological processes.

A number of herbal remedies (with claimed liver protecting activity) are marketed throughout the world, however, the informations regarding scientific validation of these claims with a few exception are not available. The aim of this study was to test the hepatoprotective effect of various indigenous plants used traditionally for their usefulness in liver disorders. The hepatoprotective effect of a given herbal material is screened by the available in vivo and in vitro test model systems. These test models system are having their own limitations and are feasible only with hepatotoxin producing reproducible type of hepatic injury (predictable: paracetamol and CCl₄, etc.), capable to be quantified conveniently. The magnitude of toxic effect is assessed by a suitable parameters, e.g., by the serum activity of certain intracellular enzymes i.e., alkaline phosphatase, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) (Antonov and Malchevski, 1980; Mihas et al., 1981) or by recording the increase in pentobarbital sleeping time or by the histological examination of the liver. The substance under test is then given together with the toxic dose of the hepatotoxin and if the toxic effect is blocked, the substance under test is considered to be effective (Handa et al., 1986). The in vitro toxicity model uses primary cultured hepatocytes, being intoxicated with any of the predictable type hepatotoxins, however, the in vivo model was to be used in the present study due to lack of cell culture facilities.

The general method used for this study is presented in chapter 2, whereas
subsequent chapters are assigned to plants separately: each containing plant
description, ethnopharmacological background, pharmacological studies along with
results and discussion. The general discussion/conclusion of the combined result
is presented at the end and bibliography is included in each chapter for the sake
of convenience.

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2. GENERAL EXPERIMENTAL
GENERAL EXPERIMENTAL

2.1 Plant selection

The plants included in the studies for hepatoprotective activity were selected after review of native literature as well as through personal interviews of the traditional healers. In the following studies the plant materials were collected with the help of an expert person in herbal trade followed by authentication by taxonomists.

2.2 Extraction of the plant materials

The plant material was extracted with 80% aqueous-methanol because at that concentration it extracts most of the constituents and also inhibit growth of the majority of the microbes (Brain and Turner, 1975).

Besides the pharmacological studies, it was planned initially to perform phytochemical studies (in collaboration with a chemist) on a limited number of plants involving activity-directed fractionation, leading ultimately to the isolation of pure compounds. However, this planning faced several limitations. The *in vitro* model (Hikino, 1987) that is considered economical and quick for repeated frequent biological testing required for the isolation process was not available due to limited facilities. Consequently, the *in vivo* model was used for hepatoprotective screening which requires a large number of animal to monitor each isolated fraction for its possible activity and this practice was not feasible.

In addition, it was extremely difficult to maintain the interest of the collaborating chemists because they are more enthusiastic for novel structure rather than the novel activities of known compounds and the plant found active in the preliminary study were those which were already well characterized
phytochemically.

Alternatively, we opted to increase the number of plants screened for the pharmacological activity and to incorporate the commercially available pure compounds already reported to be present in plants, found active during hepatoprotective screening.

2.3 Pharmacological materials and animals

The following reference materials were obtained from the following sources. paracetamol (acetaminophen, 4-hydroxy acetanilide), potassium chloride, carbon tetrachloride (CCl₄), ketamine hydrochloride, pentobarbital sodium, methylcellulose and verapamil chloride (Sigma Chemicals Company St. Louis, MO, USA), Calcium chloride (E. Merck, Darmstadt, F.R. Germany), pentothal sodium (Abbott Laboratories, Pakistan) and olive oil (P. Sasso e Figili, Oneglia, Italy).

Paracetamol and CCl₄ were suspended in 1% methylcellulose (50 mg/mL) and olive oil (20% V/V) respectively, whereas all other drugs were dissolved in distilled water.

Swiss male mice (20-25 g), male albino Wistar rats (200-250 g), and New Zealand white rabbits (2-3 Kg) of either sex were obtained from the Animal House of The Aga Khan University. The plastic cages (47 x 34 x 18 cm³) lined with saw dust (renewed after every 48 h.), maintained at 23-25°C were used to accommodate rats (5/cage) & mice (10/cage) and had free access to water and feed.

The doses of test substances mentioned are based on the preliminary studies as the minimum effective doses.
2.4 Lethality study in mice

Preliminary experiments were performed on mice to assess the protective effect of plant extract against lethal dose of paracetamol (1 g/Kg), as it was found optimal for lethality studies in preliminary experiments. Animals were divided into 2 groups of 10 animals each. One group was treated orally with plant extract (usually 500 mg/Kg) followed after one hr by oral administration of paracetamol (1 g/Kg). The second group served as a control and received same treatment except that normal saline (0.9 % NaCl) was administered instead of plant extract. The mortality was observed for 24 hrs post-administration of paracetamol.

2.5 Induction of hepatic injury.

Hepatic injury in rats was induced separately by oral administration of paracetamol or CCl₄ and the control animals received an equal volume of respective vehicles.

In preliminary experiments, 640 mg/Kg of paracetamol and 1.5 mL/Kg of CCl₄ were found to be respective toxic doses capable to cause significant liver damage (without any lethality), which is monitored by raised serum glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and in some experiments alkaline phosphatase (ALP) enzymes.

2.6 Multiple dose pre-treatment in rats

Rats were divided into 3 groups of 10 animals each. Group 1 served as vehicle control and received normal saline (10 mL/Kg) and vehicle (1 % methylcellulose; 13 mL/Kg) orally. Group 2 was given 4 doses of normal saline
at 12 hrs interval and paracetamol was administered orally 1 hr post-treatment of the last dose. Group 3 was treated similar to that of group 2, except that test material (usually 500 mg/Kg) dissolved in 10 mL saline was administered instead of saline.

In a parallel study on 3 similar groups of rats (n=10), normal saline (10 mL/Kg) and vehicle (olive oil; 7.5 mL/Kg) were administered orally to vehicle control group, whereas, remaining 2 groups were treated similar to the study mentioned above except that paracetamol was replaced by ('Cl).

Animals were anaesthetized with ketamine (100 mg/Kg, i.m.) 24 hr after the last treatment and blood (3 mL) was collected by cardiac puncture using sterile disposable syringes. Serum was separated by centrifugation (3000 r.p.m., for 15 min) and serum GOT, GPT or ALP were estimated on the same day spectrophotometrically using Merck diagnostic kits.

2.7 *Multiple dose post-treatment in rats*

The test was performed in selected cases where the plant material showed microsomal drug metabolizing enzymes (MDME) inhibitory activity by increased pentobarbital-induced sleeping time as well as enhanced strychnine lethality in mice and was exploited to decide whether the hepatoprotection was mediated through MDME inhibition only or some other mechanism(s) is/are also involved.

The hepatoprotective effect in cases, where the plant material showing preventive effect as well as MDME inhibition but lacks any curative effect, can be assigned to the interference with the metabolism of toxin(s) into reactive products. However, in cases where the test substance also exhibited curative effect, the protective effect is not entirely dependent upon the MDME inhibition and some
other mechanism(s) is/are also in operation (see respective discussion(s) for details).

Rats were divided into 3 groups of 10 animals each. Group 1 received a single oral dose of vehicle (1% methyl-cellulose; 13 mL/Kg) at 0 hr, followed by 3 doses of normal saline (10 mL/Kg; oral) at 6 hrs intervals (i.e., 6th, 12th, and 18th hr) and blood samples were collected at 24 hrs. In group 2, the same treatment schedule was followed except that the vehicle was replaced by paracetamol (640 mg/Kg; orally). Group 3 was treated similar to group 2, except that extract dissolved in saline was substituted for saline.

In an other study on 3 similar groups of rats (n=10), same protocol was followed except the toxin (paracetamol) was replaced by CCl₄ and olive oil served as vehicle. All other procedures i.e., blood collection, serum separation and enzyme estimation were performed as mentioned above.

2.8 Pentobarbital-induced sleeping time in mice

The duration of pentobarbital-induced sleep in intact animals is considered as a reliable index for the activity of hepatic microsomal drug metabolizing enzymes (MDME) (Conney, 1967). Pentobarbital is metabolized by the hepatic MDME to inactive metabolites and any drug with inhibitory effect on MDME is likely to prolong pentobarbital-induced sleeping time (Fujimoto et al., 1960).

The effect of plant extract on pentobarbital-induced sleeping time was studied in mice as described by Montilla and colleagues (1990). Animals were divided into 2 groups of 10 animals each. Group 1 received normal saline (10 mL/Kg), while group 2 was given plant material in a single oral dose and pentobarbital (75 mg/Kg, i.p.) was then administered after 1 hr to both the groups.
The prolongation of pentobarbital-induced sleeping time is usually considered due to the inhibition of the microsomal drug metabolizing capacity, but potentiation of pentobarbital sleep can also be achieved by CNS depressing drugs without alteration in MDME activity (Shin, 1989). To rule out the possibility of CNS depressant activity, strychnine-induced lethality test was performed as detailed below.

2.9 Strychnine-induced lethality in mice

The strychnine lethality test was performed in selected cases where extract showed prolongation of pentobarbital sleep and was used to decide whether the plant extract mediated potentiation of pentobarbital sleep is due to enzyme inhibitory action or sedative effect. The strychnine is a substrate for MDME (Adamson and Fouts, 1959) and most known inhibitors of MDME increase the toxicity of strychnine through potentiation of its CNS stimulant activity (Kato, 1968). If the combination of hepatoprotective agent with the sub-lethal dose of strychnine results in lethality it is suggestive of the potentiating effect of the plant material and confirms its inhibitory effect upon MDME.

Animals were divided into 2 groups of 10 mice each. One group was given vehicle (1% methylcellulose; 10 mL/Kg; orally) followed after 1 hr by sub-lethal dose of strychnine (0.4 mg/Kg). The animals in group 2 were given similar treatment except vehicle was replaced by plant material. The animals were monitored for next 2 hrs to count mortalities.

2.10 CCl₄-induced prolongation of pentobarbital sleep interval

The CCl₄-induced prolongation of pentobarbital sleeping time was
performed in those cases where the test material itself was unable to prolong pentobarbital sleeping time. The hepatocytic exposure to CCl₄ causes damage to MDME reducing drug metabolizing capacity of the liver, resulting in prolongation of pentobarbital-induced sleeping time (Javatilaka et al., 1990). The preventive effect of a substance against CCl₄-induced prolongation of pentobarbital-sleeping time can be used to confirm its protective effect against CCl₄-induced damage to hepatocytes including MDME.

To assess the effect of plant material on CCl₄-induced prolongation of pentobarbital sleeping time, 2 groups of mice each containing 10 animals were added to the study and were treated as follows: Group 1 received 4 doses of normal saline orally at 12 hrs intervals and CCl₄ was administered as a bolus dose (1.5 mL/Kg) 1 hr post-treatment of the last dose of saline followed after 24 hrs by pentobarbital (75 mg/Kg, i.p.) Animals in group 2 were treated similar to group 1 except that plant material was substituted for normal saline.

2.11 Evaluation of Ca²⁺ channel blocking activity

Ca²⁺ is involved in final outcome of the toxic liver damage and Ca²⁺-channel blockers are capable to provide hepato-protection (Moore et al., 1985; Landon et al., 1986). This test was performed towards the later stages of the study on plant materials as well as pure compounds to rule out the possible mechanism of action.

Rabbits starved for 24 hrs were killed by cervical dislocation and exsanguininated. Segments of jejunum about 2 cm length were mounted in 20 ml tissue bath containing Kreb's-Henseleit solution, maintained at 37°C and bubbled with a gas mixture of 95 % O₂ and 5 % CO₂. A preload of 1.0 g was applied and
the tissues were allowed to equilibrate for one hour before addition of any drug. The spontaneous contractions were recorded isotonically via T-3 isotonic transducer on Bioscience MD recorder.

The smooth muscle contractile response is dependent upon the increased free cytosolic Ca$^{2+}$ concentration (Karaki and Weiss, 1988), due either to influx and receptor-operated via voltage-dependent/Ca$^{2+}$ channels or its release from intracellular stores in sarcoplasmic reticulum (van Breemen et al., 1982). The spontaneous intestinal movements are regulated by periodic depolarization due to rapid Ca$^{2+}$ influx via voltage dependent calcium channels (VDCs) appears as action potential (Brading, 1981). Verapamil, a standard Ca$^{2+}$ channel blocker (Whit and Bradford, 1986; Triggle and Rampe, 1989) is capable to exert inhibitory effect on spontaneous contraction by restricting Ca$^{2+}$ entry via VDCs. The high K$^+$ (> 30 mM) is known to cause smooth muscle contraction through Ca$^{2+}$ ingress (Bolton, 1979) and any substance that relaxes high K$^+$-induced contraction is considered to block VDCs (Hamilton et al., 1986; Deitmer et al., 1992).

The spontaneous movements in rabbit jejunum are abolished in Ca$^{2+}$ free bathing fluid, but are resumed on Ca$^{2+}$ supplementation. When, Ca$^{2+}$ addition fails to restore such phasic movements in the presence of test substance, it is considered as the test substance has rendered the added Ca$^{2+}$ unavailable to contractile elements in cytosol (Brading, 1981).

2.12 Acute toxicity

Different groups of 5 mice each were given graded doses of plant extract (0.5-4 g/Kg, orally) and were kept under constant observation for 6 hrs to note any behavioral changes and mortality was recorded 24 hrs after drug administration.
2.13 **Statistical analysis**

The results are expressed as Mean ± Standard error of means (S.E.M.) and all statistical comparisons were made by means of Student's t-test and $P<0.05$ was regarded as a significant.

2.14 **References**


3. *Artemisia absinthium*
3 *Artemisia absinthium*

3.1 *Summary*

Effect of aqueous-methanolic extract of *Artemisia absinthium*, (Compositae) was investigated against paracetamol- and CCl₄-induced hepatic damage. Paracetamol produced 100% mortality at the dose of 1 g/Kg in mice while pretreatment of animals with plant extract (500 mg/Kg) reduced the death rate to 20%. Pretreatment of rats with plant extract (500 mg/Kg, orally twice daily for 2 days) prevented \( P < 0.01 \) the paracetamol (640 mg/Kg) as well as CCl₄ (1.5 mL/Kg)-induced rise in serum transaminases (GOT and GPT). Post-treatment with three successive doses of extract (500 mg/Kg, 6 hourly) was able to restrict the hepatic damage induced by paracetamol \( P < 0.01 \) but was unable to modify CCl₄-induced hepatotoxicity \( P > 0.05 \). Plant extract (500 mg/Kg) caused significant prolongation \( P < 0.05 \) in pentobarbital (75 mg/Kg)-induced sleep as well as increased strychnine-induced lethality in mice suggestive of inhibitory effect on microsomal drug metabolizing enzymes (MDME). Moreover, the plant extract also exhibited Ca²⁺-channel blocking activity in isolated tissue experiments and hence the observed hepatoprotective effect of *Artemisia absinthium* can be attributed to the MDME inhibitory and Ca²⁺ channel blocking activities.
3.2 Plant Description/Literature survey

*Artemisia absinthium* Linn. (Family: Compositae), commonly known as "Wormwood" or "Vilayatí afsanteen" is a perennial herb growing wild in northern hilly area of Pakistan (Haq, 1983). The plant is also commonly grown in the west and recognized for its medicinal value particularly in the hepato-biliary complaints and in helminth infections (Keviele, 1991). The herbal material (leaves and flowering tops) is regarded as anthelmintic, antiseptic, febrifuge and stomachic in indigenous system of medicine and has been employed successfully to alleviate chronic fever, dyspepsia and hepato-biliary ailments (Nadkarni, 1976; Said, 1982).

The plant has undergone extensive phytochemical investigations and the presence of a variety of chemical constituents such as ascorbic acid (Klyshev and Alyukina, 1971; Slepetsys, 1975), flavonoids (Hoffmann and Herrmann, 1982), carotenoids (Sergeeva and Zakharova, 1977), tannins (Slepetsys, 1975) and lignans (Greger and Hofer, 1980) have been identified. Similarly, the phytopharmacological evaluation showed the presence of anti-inflammatory (Sommer et al., 1965), antipyretic (Ikram et al., 1987), antifertility (Rao et al., 1988), antibacterial (Kaul et al., 1976), antifungal (Maruzzella et al., 1960), anthelmintic (Caius and Mahasker, 1920), antimollusk (Gurevich, 1948), anti-amoebic (Tahir et al., 1991) and antimalarial (Hernandez et al., 1990; Zafar et al., 1990) activities.

3.3 Materials and Methods

3.3.1 Plant extract

*Artemisia absinthium* (aerial parts) were purchased from local herbal store
and authenticated with the help of a botanist at The University of Karachi. The plant material was powdered and macerated in 80% aqueous-methanol for one week with occasional shaking. The extract was filtered and concentrated to dark greenish brown residue under reduced pressure on a rotary evaporator, with approximate yield of 8%.

3.3.2 Animal studies

The effect of shoot extract was studied on the paracetamol-induced lethality; paracetamol- and CCl₄-induced hepatotoxicity (pre- and post-treatment); pentobarbital-induced sleep duration; strychnine lethality by the methods described in the general experimental section (Chapter 2).

3.4 Results

3.4.1 Effect on paracetamol-induced lethality

Paracetamol at the dose of 1 g/Kg induced 100% lethality in mice. In a group of animals pretreated with plant extract (500 mg/Kg), the same dose of paracetamol killed only 2 out of 10 animals resulting in 80% protection against lethal effect of paracetamol (Table 3.1).

3.4.2 Preventive effect on paracetamol-induced toxicity

Control (saline + vehicle) serum values of GOT and GPT in rats were found to be 98 ± 11 and 39 ± 08 IU/l., respectively (Fig. 3.1; Appendix 1), while a toxic dose of paracetamol (640 mL/Kg) raised significantly ($P < 0.01$), the respective serum enzyme values to 1424 ± 454 and 741 ± 217. Group 3 was pretreated with plant extract (500 mg/Kg, orally, twice daily for 2 days) to
Table 3.1
Effect of *Artemisia absinthium* extract on paracetamol-induced lethality in mice (n= 10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Lethality</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Paracetamol</td>
<td>10/10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(10 mL/Kg + 1 g/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Extract + Paracetamol</td>
<td>02/10</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>(500 mg/Kg + 1 g/Kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Saline/extract and paracetamol were given orally.
Fig. 3.1 Preventive effect of *Artemisia absinthium* on paracetamol-induced elevation of serum transaminase levels in rats.
determine its effect on paracetamol-induced rise in serum enzymes. The serum values of transaminases in pretreated group were found to be 85 ± 18 (GOT) and 34 ± 08 (GPT), which are significantly lower ($P < 0.01$) than the values of toxic control and were similar to the control values ($P > 0.05$).

3.4.3 Preventive effect on CCl$_4$-induced toxicity

In another set of experiments, the normal values of serum GOT and GPT in rats were found to be 106 ± 15 and 45 ± 11 IU/L respectively (Fig. 3.2; Appendix 2), which were raised significantly ($P < 0.05$) to respective values of 494 ± 155 and 305 ± 83 after administration of a toxic dose of CCl$_4$ (1.5 mL/Kg). However, pretreatment of animals with plant extract (500 mg/Kg, orally, twice daily for 2 days) returned the serum GOT and GPT values to 139 ± 18 and 76 ± 27 IU/L respectively, which were significantly lower ($P < 0.05$) than values of toxic control and were close to the control values ($P > 0.05$).

3.4.4 Curative effect on paracetamol-induced toxicity

Control (saline + vehicle) serum values of GOT and GPT in rats were found to be 108 ± 22 and 42 ± 11 IU/L ($n=10$) respectively, (Fig. 3.3; Appendix 3), while a toxic dose of paracetamol (640 mg/Kg) raised significantly ($P < 0.01$), the respective serum enzyme values to 1125 ± 281 and 833 ± 195. The treatment with the plant extract (500 mg/Kg, orally) was started to group 3 animals (6 hrs after paracetamol administration) to evaluate its curtailing effect upon the paracetamol-induced progression of hepatic damage duly monitored by serum transaminases. The serum values of enzymes in post-treated group were found to be 167 ± 28 (GOT) and 88 ± 26 (GPT), which were significantly lower.
Fig. 3.2 Preventive effect of *Artemisia absinthium* on carbon tetrachloride-induced elevation of serum transaminase levels in rats.
Fig 3.3 Curative effect of *Artemisia absinthium* on paracetamol-induced elevation of serum transaminase levels in rats.
than the serum values of toxic control group ($P<0.01$) and were comparable to the normal values ($P>0.05$).

3.4.5 Post-treatment of extract and $\text{CCl}_4$-induced toxicity

The administration of toxic dose of $\text{CCl}_4$ (1.5 mL/Kg; orally) raised significantly ($P<0.05$), the serum values of GOT and GPT to $511 \pm 165$ and $353 \pm 101$ U/L respectively, compared to respective control values of $95 \pm 13$ and $48 \pm 10$ (Fig. 3.4; Appendix 4). The group 3 animals were treated with multiple doses of plant extract, following $\text{CCl}_4$ intoxication. The serum values in the treated group were found to be $523 \pm 94$ (GOT) and $415 \pm 123$ (GPT), which were similar to the values of toxic control group ($P>0.05$) and higher than that of normal values ($P<0.05$).

3.4.6 Effect on pentobarbital-induced sleep

Effect of plant extract on pentobarbital sleeping time was studied in mice and the results are shown in Table 3.2. Pentobarbital at a dose of 75 mg/Kg, i.p., caused sleep in mice for a period of $81 \pm 02$ min (mean $\pm$ S.E.M, $n=10$), whereas the sleeping time in the group of animals pretreated with plant extract was found to be $117 \pm 16$ min, which was significantly higher than that in the control group ($P<0.05$).

3.4.7 Interaction with strychnine

Pre-treatment with a single oral dose (500 mg/Kg) of plant extract 1 hr prior to strychnine administration potentiated the effect of strychnine causing almost 60% mortality rate (Table 3.3).
Fig. 3.4 Effect of *Artemisia absinthium* post-treatment on carbon tetrachloride-induced elevation of serum transaminase levels in rats.
Table 3.2

Effect of *Artemisia absinthium* extract on pentobarbital sleeping time in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sleeping Time</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>(Vehicle + Pentobarbital)</td>
<td>81 ± 02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(10 mL/Kg + 75 mg/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>(Extract + Pentobarbital)</td>
<td>117 ± 16</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>(500 mg/Kg + 75 mg/Kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 10 determinations. Salines/Extract was given orally, while pentobarbital was given intraperitoneally.
Table 3.3
Effect of *Artemisia absinthium* extract on strychnine-induced lethality in mice (n = 10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Lethality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>(Vehicle + Strychnine)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(10 mL/Kg + 0.4 mg/Kg)</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>(Extract + Strychnine)</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>(500 mg/Kg + 0.4 mg/Kg)</td>
<td></td>
</tr>
</tbody>
</table>

Salines/Extract was given orally, while strychnine was given intraperitoneally.
3.4.8 \textit{Ca}^{2+} \textit{Channel blocking activity}

Plant extract at the concentration of 0.1-1 mg/mL caused a concentration-dependent inhibition of spontaneous movements of rabbit jejunum as shown in Fig. 3.5. This effect was reversible as the tissue regained its spontaneous activity after washing the tissue several times with the fresh bathing fluid.

K$^+$ at the concentration of 40 mM caused a sustained tonic contraction which allowed to obtain concentration-dependent inhibitory responses (Fig. 3.5). Addition of plant extract at 0.1 mg/mL, caused a slight inhibition, while at 0.3 mg/mL, it produced almost 50% relaxant effect. The K$^+$-induced contractile response was completely abolished by the next higher concentration of plant extract (1 mg/mL).

When the bathing fluid was replaced with Ca$^{2+}$ free Kreb's solution, the spontaneous movements of the tissue were abolished within one minute (Fig. 3.6). Addition of Ca$^{2+}$ (15-25 \textmu M) restored spontaneous activity in a concentration-dependent manner. Plant extract was also tested in Ca$^{2+}$ free Kreb's solution for its effect on Ca$^{2+}$ responses. Supplementation of Ca$^{2+}$ (25-50 \textmu M) failed to restore spontaneous movements of the tissue in the presence of extract (0.3 mg/mL).

The plant extract up to an oral dose of 3 g/Kg was found devoid of any lethal effect and no apparent behavioral change was observed.

3.5 Discussion

Paracetamol- and CCl$_4$-induced hepatic injuries are commonly used models for the screening of hepatoprotective drugs (Slater, 1965; Plaa and Hewitt, 1982) and the extent of hepatic damage is assessed by the level of released cytoplasmic transaminases (GOT and GPT) in circulation (Chenoweth and Hake, 1962; Sallie
Fig. 3.5. A representative tracing showing effect of alcoholic extract of *Artemisia absinthium* (AA) on spontaneous movements (upper panel) and on $\text{K}^+$-induced contraction (lower panel) in the isolated rabbit jejunum preparation.
Fig. 3.6  A representative tracing showing effect of Ca^{++} supplementation in the absence (upper panel) and presence of alcoholic extract of *Artemisia absinthium* (AA) (lower panel) on spontaneous movements of rabbit jejunum, suspended in Ca^{++} free kreb's solution.
et al., 1991). The plant extract when administered prophylactically, exhibited protection against both paracetamol- and CCl₄-induced liver injuries as manifested by the reduction in toxin-mediated rise in serum transaminases in rats as well as protection against lethal dose of paracetamol in mice.

Both paracetamol and CCl₄ share a common property to be converted into their respective reactive metabolites N-acetyl-p-benzoquinoneimine (NAPQI) and halogenated free radicals (HFR) by hepatic cytochrome P-450 (Packer et al., 1978; van de Straat et al., 1987). The massive production of reactive species may lead to depletion of protective physiological moieties (glutathione and α-tocopherol, etc.), ensuing wide-spread propagation of the alkylation as well as peroxidation, causing damage to the macromolecules in vital biomembranes (Pesh-Imam and Recknagel, 1977; Aldridge, 1981; Moore et al., 1985).

The inhibitors of microsomal drug metabolizing enzymes (MDME) can impair the bio-activation of paracetamol and CCl₄ into their respective reactive species and thus provide protection against the prevailing hepatocellular damage (Castro et al., 1974; Nelson et al., 1980). Since the MDME inhibitory activity is reported to be common in medicinal plants (Shin, 1989), the plant extract was subjected to pentobarbital sleep study to see if it also exhibits inhibitory effect on MDME. The pretreatment of animals with plant extract resulted in the prolongation of pentobarbital sleeping time (P<0.05), therefore, it is not unreasonable to speculate that the plant extract might contain MDME inhibitory constituents that cause hepatoprotection. However, sleep potentiation of pentobarbital can also be achieved by CNS depressing drugs without alteration in MDME activity (Shin, 1989). Strychnine toxicity test was performed to see whether the plant extract mediated potentiation of pentobarbital sleep is due to
enzyme inhibitory action or sedative effect. The observed mortality at the sub-lethal dose of strychnine is suggestive of the potentiating effect of the plant extract and confirms its inhibitory effect upon MDME.

It is reported earlier that the compounds with methylene-dioxynbenzene group are likely to exhibit inhibitory effect on MDME (Anders, 1968). The literature survey revealed the presence of sesarmin in this plant (Ahmed et al., 1986) and interestingly, this compound carries methylene-dioxynbenzene group in its structure (Greger and Hofer, 1980), thus suggesting that the possible inhibitory effect of plant extract on MDME may be due to the presence of sesarmin as a plant constituent. The exact mode of hepatoprotective action of the plant extract may be speculative at this stage but these results indicate that the possible presence of enzyme inhibitory effect may be responsible for the hepatoprotective effect of plant extract.

The inhibitors of MDME can provide protection against the hepatotoxicity only when they are given before the metabolic activation of the hepatotoxin and fails to provide any protection after generation of reactive metabolites. Following ingestion, paracetamol and CCl4 are metabolized to their respective reactive species within 6 hrs (Bramanti et al., 1978; Akintonwa and Essien, 1990) and hepatotoxicity can be monitored by measuring serum transaminases at 24 hrs. The plant extract treatment started 6 hrs after the paracetamol administration inhibited the spread of hepatic damage as manifested by insufficient release of cytoplasmic transaminases.

The observed curative effect against paracetamol may be attributed to the reported presence of flavonoids (Hoffmann and Hermann, 1982), ascorbic acid (Klyshev and Alyukina, 1971; Slepetsy, 1975), carotenoids (Sergeeva and
Zakharova, 1977), Tannins (Slepetsy, 1975) and lignans (Greger and Hofer, 1980) among the plant constituents. The flavonoids are known to be antioxidants (Torel et al., 1986; Faurè et al., 1990), free radical scavengers (Bors and Saran, 1987; Husain et al., 1987) and anti-lipoperoxidant (Younes and Siegers, 1981; Robak et al., 1986; Ratty and Das, 1988) leading to hepatoprotection (Kiso et al., 1984; Handa et al., 1986). Similarly, ascorbic acid serves as antioxidant (Demopoulos, 1973; Bus and Gibson, 1984), inhibits covalent binding of NAPQI to vital macromolecules (Lake et al., 1981) and consequently can minimize toxic damage (Harman, 1985). Moreover, carotenoids are also reputed to be antioxidants (Kläui, 1982) and thus showing anti-hepatotoxic activity (Oshima et al., 1984). Furthermore, the hepatoprotective potential of tannins (Hikino et al., 1985) as well as lignans (Faurè et al., 1990) is also well documented.

The crude extract of Artemisia absinthium exhibited Ca^{2+} channel blocking activity in isolated tissue experiments. Calcium contents in the liver cells are increased during the process of experimental hepatic damage (Moore et al., 1985) and calcium channel blocking drugs are capable to ameliorate the paracetamol- and CCl_{4}-induced hepatoxotoxicity (Landon et al., 1986; Thibault et al., 1991). Similarly, the presence of calcium channel blocking in the crude extract of Artemisia absinthium might have also contributed to its hepatoprotective activity.

However, the anomalous observation due to inability of extract treatment to curtail progression of hepatic damage after CCl_{4} activation can partly be justified on the basis of reported facts. The paracetamol toxicity following NAPQI generation is chiefly due to oxidative stress and can effectively be ameliorated by antioxidants (Harman, 1985), whereas, the hepatic damage due to HFR may be due to lipid peroxidation (Bus and Gibson, 1979) as well as alkylation (Dugterom
et al., 1988). The possible presence of antioxidant and anti-lipoperoxidant activities to protect against NAPQI, can only inhibit the lipid peroxidation process but are unable to prevent the alkylation process due to HFR (Poli et al., 1989).

The crude extract of *Artemisia absinthium* affords protection against paracetamol and CCl<sub>4</sub>-induced liver damage in rats as well as in mice when given before the metabolic activation of toxins. However, the protection against the metabolically generated reactive species is effective only against the paracetamol. The hepatoprotective action may be mediated through inhibition of hepatic MDME, calcium channel blocking activity and/or presence of certain antioxidants. The plant material is safe as is obvious by the lack of any symptom of acute toxicity at an oral dose of as high as 4 g/Kg and has been also reported safe after chronic treatment (Schmahl, 1956). Thus, this study provides scientific basis for the traditional use of *Artemisia absinthium* plant in hepato-biliary diseases.

### 3.6 References


Poli, G., Cheeseman, K. H., Biasi, F., Chiarpotto, E., Dianzani, M. U.,


Proceeding of the Second International Symposium, ed. by E. B.

Lee, K. H. Shin, S. S. Kang and Y. N. Han, pp. 176-195. Natural Products Research Institute, Seol National University, Seol.


4. *Artemisia maritima*
4 Artemisia maritima

4.1 Summary

Effect of aqueous-methanolic extract of Artemisia maritima (Compositae) was investigated against paracetamol- and CCl₄-induced hepatic damage. Paracetamol produced 100% mortality at the dose of 1 g/Kg in mice while pretreatment of animals with plant extract (500 mg/Kg) reduced the death rate to 20%. Pretreatment of rats with plant extract (500 mg/Kg, orally twice daily for 2 days) prevented \( P < 0.01 \) the paracetamol (640 mg/Kg) as well as CCl₄ (1.5 mL/Kg)-induced rise in serum transaminase (GOT and GPT) levels. Plant extract (500 mg/Kg) was found devoid of inhibitory effect on microsomal drug metabolizing enzymes (MDME) as suggested by the observed lack of any increase in pentobarbital (75 mg/Kg)-induced sleep. However, it prevented the CCl₄-induced prolongation in pentobarbital sleeping time in mice, confirming its protective effect against CCl₄. Moreover, the plant extract exhibited Ca²⁺-channel blocking activity in isolated tissue experiments and the observed hepatoprotective can be viewed as an outcome of Ca²⁺-channel blocking effect. The plant material was devoid of any acute toxicity symptom up to an oral dose of as high as 3 g/Kg and this study rationalizes the traditional use of Artemisia maritima plant in hepato-biliary disorders.
4.2 Plant Description

*Artemisia maritima*, Linn. (Syn: *A. brevifolia* Wall., *A. herba alba* Asso., *A. kurramensis* Qazalbash; Family: Compositae) locally known as ‘Afsanteen-ul-bahr’ (Nadkarni, 1976), is a bitter aromatic herb or low shrub with much divided leaves and inconspicuous flowers born on numerous small heads (Dey, 1980). It grows abundantly in the high altitudes of NWFP and Baluchistan provinces of Pakistan (Nadkarni, 1976).

The plant is known to possess antibacterial (Yasliphe et al., 1979), anthelmintic (Sareen et al., 1961; Khafagy et al., 1971), antidiabetic (Twaij and Al-Badr, 1983) and antifertility (Sareen et al., 1961) activities. Moreover, it has folkloric reputation to be used in jaundice (Baquir, 1989) and in intermittent as well as remittent fever (Kirtikar and Basu, 1918).

4.3 Materials and Methods

4.3.1 Plant extract

*Artemisia maritima* (aerial parts) was collected from rural area near Quetta and authenticated with the help of a botanist at The University of Karachi and the sample submitted in the herbarium of the University. The plant materia. was powdered and macerated in 80% aqueous-methanol for one week with occasional shaking. The extract was filtered and concentrated to dark greenish brown residue under reduced pressure on a rotary evaporator, with approximate yield of 15%.

4.3.2 Animal Studies

The effect of shoot *...* extract was studied on the paracetamol-induced lethality; paracetamol- and CCl₄-induced hepatotoxicity; pentobarbital-
induced sleep duration and CCl₄-induced prolongation of pentobarbital sleeping time; spontaneous movements as well as K⁺-induced contractions of rabbit jejunum by the methods described in the general experimental section (chapter 2).

4.4 Results

4.4.1 Effect on paracetamol-induced lethality

Paracetamol at the dose of 1 g/Kg induced 100% lethality in mice. In a group of animals pretreated with plant extract (500 mg/Kg), the same dose of paracetamol killed only 2 out of 10 animals resulting in 80% protection against lethal effect of paracetamol (Table 4.1).

4.4.2 Effect on paracetamol-induced hepatotoxicity

Control (saline + vehicle) serum values of GOT and GPT in rats were found to be 87 ± 12 and 31 ± 05 IU/L (n=10) respectively (Fig. 4.1; Appendix 5), while a toxic dose of paracetamol (640 mg/Kg) raised significantly (P<0.001), the respective serum transaminase values to 1529 ± 172 and 904 ± 116 IU/L. Group 3 was pretreated with plant extract (500 mg/Kg, orally, twice daily for 2 days) to determine its effect on paracetamol-induced rise in serum enzymes. The serum enzyme values in pretreated group were found to be 112 ± 10 (GOT) and 47 ± 11 (GPT), which were significantly lower (P<0.001), than the values of toxic control and were similar to the control values (P>0.05).

4.4.3 Effect on CCl₄-induced hepatotoxicity

The estimated values of serum GOT and GPT in control (saline + vehicle) group of rats were found to be 92 ± 18 and 35 ± 09 IU/L (n=10) respectively
Table 4.1
Effect of *Artemisia maritima* extract on paracetamol-induced lethality in mice (n = 10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Lethality</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Paracetamol (10 mL/Kg + 1 g/Kg)</td>
<td>10/10</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>Extract + Paracetamol (500 mg/Kg + 1 g/Kg)</td>
<td>02/10</td>
<td>80</td>
</tr>
</tbody>
</table>

Saline/extract and paracetamol were given orally.
Fig. 4.1 Effect of *Artemisia maritima* on paracetamol-induced elevation of serum transaminases in rats
(Fig. 4.2; Appendix 6), which were raised significantly \( P < 0.01 \) to respective values of 463 ± 122 and 366 ± 58 after administration of a toxic dose of CCl\(_4\) (1.5 mL/Kg). However, pretreatment of animals with plant extract (500 mg/Kg, orally, twice daily for 2 days) returned the serum GOT and GPT values to 105 ± 29 and 53 ± 17 IU/L respectively, which were significantly lower \( P < 0.05 \) than values of toxic control and were close to the normal values \( P > 0.05 \).

4.4.4 Effect on pentobarbital-induced sleep

Effect of plant extract on pentobarbital sleeping time as well as on CCl\(_4\)-induced prolongation of pentobarbital sleeping time was studied in mice and the results are shown in Table 4.2. Pentobarbital at a dose of 75 mg/Kg, i.p., caused sleep in mice for a period of 145 ± 06 min (mean ± S.E.M, \( n = 10 \)). Pentobarbital sleeping time in the group of animals pretreated with plant extract was found to be 151 ± 10 min, which is similar to that in the control group \( P > 0.05 \).

4.4.5 Effect on CCl\(_4\)-induced pentobarbital sleep increase

Pretreatment of animals with CCl\(_4\) prolonged the pentobarbital sleeping time to 223 ± 15 min, the value that is significantly higher \( P < 0.001 \) than that of control (Table 4.2). However, prior treatment of animals with plant extract returned this CCl\(_4\)-induced prolongation of pentobarbital sleeping time to 154 ± 16 min, which was close to the control sleeping time \( P > 0.05 \).

4.4.6 Ca\(^{2+}\) channel blocking activity of plant extract

Plant extract at the concentration of 0.1–1 mg/mL caused a concentration-
Fig 4.2 Effect of *Artemisia maritima* on carbon tetrachloride-induced elevation of serum transaminases in rats.

*P < 0.001
Table 4.2
Direct effect of *Artemisia maritima* extract on pentobarbital sleeping time as well as on \( \text{CCl}_4 \)-induced prolongation of pentobarbital sleeping time in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sleeping time</th>
<th>( P )-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Pentobarbital</td>
<td>145 ± 06</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(10 mL/Kg + 75 mg/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Extract + Pentobarbital</td>
<td>151 ± 10</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>(500 mg/Kg + 75 mg/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Saline + ( \text{CCl}_4 ) + Pentobarbital</td>
<td>223 ± 15*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(10 mL/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Extract + ( \text{CCl}_4 ) + Pentobarbital</td>
<td>154 ± 16*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>(500 mg/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 10 determinations. Saline/Extract/\( \text{CCl}_4 \) was given orally, while pentobarbital was given intraperitoneally.

* Compared to group 1 (control).

\* Compared to group 3.
RABBIT JEJUNUM

Artemisia maritima (mg/ml)

Fig. 4.3. A representative tracing showing effect of alcoholic extract of Artemisia maritima (AM) on spontaneous movements (upper panel) and on K⁺-induced contraction (lower panel) in the isolated rabbit jejunum preparation.
4.4. A representative tracing showing effect of Ca\(^{++}\) supplementation in the absence and presence of alcoholic extract of *Artemisia maritima* (AM) on spontaneous movements of isolated rabbit jejunum suspended in Ca\(^{++}\) free kreb's solution.
dependent inhibition of spontaneous movements of rabbit jejunum as shown in Fig. 4.3. This effect was reversible as the tissue regained its spontaneous activity after washing the tissue several times with the fresh bathing fluid.

K⁺ at the concentration of 40 mM caused a sustained tonic contraction which allowed to obtain concentration-dependent inhibitory responses (Fig. 4.3). Addition of plant extract at 0.1 mg/mL, caused a slight inhibition, while at 0.3 mg/mL, it produced almost 50% relaxant effect. The K⁺-induced contractile response was completely abolished by the next higher concentration of plant extract (1 mg/mL).

When the bathing fluid was replaced with Ca²⁺ free Kreb's solution, the spontaneous movements of the tissue were abolished within one minute (Fig. 4.4). Addition of Ca²⁺ (10-30 μM) restored spontaneous activity in a concentration-dependent manner. Plant extract was also tested in Ca²⁺ free Kreb's solution for its effect on Ca²⁺ responses. Supplementation of Ca²⁺ (30-50 μM) failed to restore spontaneous movements of the tissue in the presence of extract (1 mg/mL).

The plant extract up to an oral dose of 3 g/Kg was found devoid of any lethal effect and no apparent behavioral change was observed.

4.5 Discussion

The plant extract exhibited hepatoprotection against both paracetamol and CCl₄-induced liver injuries as manifested by reduction in toxins-mediated rise in serum transaminases (GOT and GPT) in rats. Similarly, pretreatment of animals with of plant extract afforded 80% protection against lethal dose of paracetamol and also prevented CCl₄-induced prolongation of pentobarbital sleeping time in mice.
Paracetamol and CCl₄-induced liver injuries are commonly used models for the screening of hepatoprotective drugs (Slater, 1965; Strubelt et al., 1974; Plaa and Hewitt, 1982). The rise in serum levels of transaminases (GOT and GPT) has been attributed to the damaged structural integrity of the liver (Chenoweth and Hake, 1962) because these are cytoplasmic in location and are released into circulation after cellular damage (Sallie et al., 1991). Paracetamol and CCl₄ are converted to their reactive metabolites (N-acetyl-p-benzoquinoneimine and halogenated free radicals respectively) by their respective specific isozyme of cytochrome P-450 (Packer et al., 1978; van de Straat et al., 1987). Physiologically important protective mechanisms involving both vitamin E (α-tocopherol) and glutathione (GSH) are available to curtail progression of cellular damage (Potter et al., 1974). However, the massive production of reactive species may lead to depletion of protective moieties, ensuing wide-spread propagation of the alkylation as well as peroxidation, causing damage to both lipids and proteins present in biomembranes of microsomes and mitochondria (Rao and Rechkagel, 1969; Pesh-Iman and Rechneagel, 1977; Aldridge, 1981). These bio-activation mediated cytotoxic effects are subject of active research and the toxic manifestations can effectively be minimized by cytochrome P-450 inhibitors (Castro et al., 1974; Tredger et al., 1985; Beyeler et al., 1988), GSH precursors (Ferreira et al., 1974; Prescott et al., 1979), free radical scavengers (Mailing et al., 1974), antioxidants (Gazzard et al., 1974; Koester-Albercht et al., 1979; Yasuda et al., 1980), sulfhydryl agents (Strubelt et al., 1974; Mark et al., 1990) and Ca²⁺ channel blockers (Landon et al., 1986; Thibault et al., 1991; Gilani and Janbaz, 1993).

The crude extract of Artemisia maritima plant used in this study seems to
preserve the structural integrity of the hepato-cellular membrane. This was evident from the protection provided to mice against lethal dose of paracetamol as well as significant reduction in the paracetamol and CCl₄-induced rise in serum GOT and GPT levels in rats. To see whether the plant extract has inhibitory effect on hepatic microsomal drug metabolizing enzymes (MDME), it was administered with pentobarbital to mice and possible change in the duration of sleep was recorded. The fact that the plant extract did not prolong pentobarbital sleeping time (P > 0.05) suggests that it is devoid of inhibitory effect on hepatic MDME such as cytochrome P-450 and its hepatoprotective effect is mediated perhaps through other mechanism(s).

Calcium content in the liver cells are increased during the process of experimental hepatic damage (Farber et al., 1982; Moore et al., 1985; Landon et al., 1986; Tsokos-Kuhn, 1989) and calcium channel blocking drugs i.e., nifedipine, diltiazem and verapamil were found to inhibit the development of hepatic damage, induced by different hepatotoxins including paracetamol and CCl₄ (Landon et al., 1986; Thibault et al., 1991). Similarly, hepatoprotective activity of the plant extract, against paracetamol and CCl₄-induced liver damage may be attributed to its Ca²⁺ channel blocking activity as evidenced by the inhibitory effect of plant extract on spontaneous activity as well as K⁺-induced contractions in rabbit jejunum. Since, contractions induced by high K⁺ (> 30 mM) are mediated through extracellular Ca²⁺ influx through voltage-dependent slow calcium channels (Bolton, 1979; Godfrained et al., 1986; Deitmer et al., 1992) and inhibitory effect of plant extract on such contractions may be due to calcium channel blockade. This was confirmed when Ca²⁺ supplementation was failed to restore spontaneous movements of tissue because due to treatment with plant extract, the required Ca²⁺
for contraction was rendered unavailable to tissue (Bolton, 1979). Thus protective
effect of crude extract against paracetamol- and CCl₄-induced liver damage may
be an outcome of the presence of Ca²⁺ channel blocker(s).

The results presented in this study are indicative of the fact that the crude
extract of *Artemisia maritima* affords protection against paracetamol and CCl₄-
induced liver damage in rats as well as mice. This hepatoprotective activity of
plant extract is not mediated through inhibition of hepatic MDME and can be
attributed to the possible presence of Ca²⁺ channel blockers constituent(s). The
plant material is safe as is obvious by lack of any acute toxicity symptom up to an
oral dose of as high as 3 g/Kg. Thus, this study rationalizes the traditional use of
*Artemisia maritima* plant in hepato-biliary disorders.

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5. *Artemisia scoparia*
5 Artemisia scoparia

5.1 Summary

The hepatoprotective activity of *Artemisia scoparia* extract was investigated against paracetamol and CCl₄-induced hepatic damage. Paracetamol produced 100% mortality at the dose of 1 g/Kg in mice while pretreatment of animals with plant extract (150 mg/Kg) reduced the death rate to 20%. Pretreatment of rats with plant extract (150 mg/Kg) as well as pure compounds i.e., caffeic acid (6 mg/Kg), quercetin (10 mg/Kg) and rutin (20 mg/Kg), prevented (*P* < 0.05) the paracetamol (640 mg/Kg) and also CCl₄ (1.5 mL/Kg)-induced rise in serum transaminase (GOT and GPT) levels. The plant extract and pure compounds were found devoid of any inhibitory effect on microsomal drug metabolizing enzymes (MDME) as suggested by the observed lack of increase in pentobarbital (75 mg/Kg)-induced sleep at these doses. However, they prevented the CCl₄-induced prolongation in pentobarbital sleeping time in mice, confirming their protective effect against CCl₄. In addition, the plant extract also exhibited Ca²⁺-channel blocking (CCB) activity in isolated tissue experiments. Similarly, the plant constituents are known to possess CCB (quercetin) and antioxidant (caffeic acid, quercetin and rutin) properties and may be contributing toward the protective effects exhibited by the individual components as well as plant extract.
5.2 Plant Description/Literature Survey

*Artemisia scoparia* Thunb. (*Compositae*) is a perennial herb found throughout the Pakistan (Baquar, 1989). The plant has the folkloric reputation as a febrifuge, diuretic and antispasmodic (Chin, 1951). Moreover, infusion made from whole plant has been traditionally used to treat jaundice and other liver disorders (Perry, 1980). Extensive phytochemical work on the plant material has revealed the presence of a galaxy of herbal constituents including flavonoids i.e., quercetin, rutin and kaempferol etc. (Ryakhovskya and Alyukina, 1971), phenolic compounds i.e., chlorogenic acid, caffeic acid (Hu *et al.*, 1965), pyrogallol (Maksudov *et al.*, 1962) and vanillin (Stefanovic *et al.*, 1973), carotenoids and L-ascorbic acid (Solov’eva *et al.*, 1970).

thyrotropic (Aufmkolk et al., 1936) and anti-steroidogenesis (Kojima et al., 1985; Solano et al., 1988) activities. It inhibits some of the key enzymes like xanthine oxidase (Chang et al., 1994), 5-lipoxygenase (Boot et al., 1985; Murota et al., 1985; Murota and Koshihara, 1985), glutathione-S-transferase (Senjo et al., 1988), dopa decarboxylase (Rosei, 1987) and dihydrofolate reductase (Strehl et al., 1994). Moreover, it exhibits hepatoprotective activity against CCl₄-induced toxic liver damage both in vitro (Adzet et al., 1987) and in vivo (Perez-Alvarez et al., 1993) conditions.

Quercetin is one of the bioflavonoid distributed universally in plants and is ingested as part of the diet (Singleton, 1981). It exhibits a wide variety of biological activities including anti-inflammatory (Alcaraz and Jimenez, 1988; Loggia et al., 1988; Taguchi et al., 1983), anti-allergy (Middleton and Drzewiecki, 1984; Gabor, 1986), anti-oxidant (Chen et al., 1990; Negre-Salvayre and Salvayre, 1992; Nakayama, 1994; Oyama et al., 1994), free radical scavenging (Bors and Saran, 1987; Afanas’ev et al., 1989; Chen et al., 1990), anti-platelet (Beretz et al., 1981; Landolfi et al., 1984; Chung et al., 1993), anti-tumor (Scambia et al., 1990; Deschner et al., 1991; Deschner et al., 1993; Ramanathan et al., 1994), anti-ulcer (Zhelyazkov and Georgieva, 1973; Martin et al., 1993), anti-diarrhoeal (Meli et al., 1990; Galvez et al., 1993), anti-mutagenesis (Buening et al., 1981; Huang et al., 1981; Wargovich et al., 1985; Alldrich et al., 1986), vasodilatory (Durate et al., 1993a; Durate et al., 1993b; Durate et al., 1994) activities and also causes impairment of natural killer cells function (Bray and Brahmi, 1986; Leung and Ip, 1986).
Some of the above mentioned activities are explainable due to the limited availability of intracellular Ca$^{2+}$ (Morales and Lozoya, 1994), either due to restricted influx (Fewtrell and Gompertes, 1977; Morales and Lozoya, 1994) or impaired release from storage site (Shoshan et al., 1980), whereas others are based upon the inhibition of some enzymes of key importance, such as protein kinase C (Gschwendt et al., 1983; Hofmann et al., 1988; Ferriola et al., 1989), cyclic AMP-phosphodiesterase (Beretz et al., 1978; Beretz et al., 1980; Nilaido et al., 1982), Xanthine oxidase (Ito et al., 1986), glutathione S-transferase (Das et al., 1984), NADPH-oxidase (Tauber et al., 1984), cyclooxygenase/tipoxxygenase (Yoshimoto et al., 1983; Swies et al., 1984; Moroney et al., 1988; Robak et al., 1988; Ferrandiz and Alcaraz, 1991).

Quercetin-3-rutinoside (rutin), exhibits anti-inflammatory (Alcaraz and Jimenez, 1988), anti-oxidant (Chen et al., 1990, Negre-Salvayre et al., 1991; Grinberg et al., 1994; Kozlov et al., 1994), free radical scavenging (Torel et al., 1986; Chen et al., 1990; Hanasaki et al., 1994), anti-platelet (Robbins, 1971; Chung et al., 1993), anti-tumor (Deschner et al., 1991; Deschner et al., 1993; Ura et al., 1993), anti-diarrheal (Di-Carlo et al., 1993), and vasorelaxing (Chung et al., 1993) activities. It inhibits cyclooxygenase (Baumann et al., 1980; Corvazier and Macloux, 1985), angiotensin II (Altinkurt and Abacioglu, 1980) and prostaglandin E$_2$ (Altinkurt and Abacioglu, 1980) activities, while it enhances the endogenous release of histamine (Juchmas-Ferir and Lecompte, 1971) and 5-hydroxytryptamine (Juchmas-Ferir and Lecompte, 1971; Yildizoglu-Arn et al., 1991).
The studies pertaining to the evaluation of the protective effect of the plant extract as well as pure compounds (i.e., caffeic acid, quercetin and rutin) were carried out at different occasions, consequently separate control group had to be included in each case.

5.3 Materials and Methods

5.3.1 Plant extract and commercially available constituents.

Whole Artemisia scoparia plants were collected during the month of April 1991 from rural area around Thatta District in the province of Sind, Pakistan and authenticated with the help of a botanist at The University of Karachi. The plant material was shade dried, powdered and macerated in 80 % aqueous methanol for one week with occasional shaking. The extract was filtered and concentrated to dark greenish brown residue under reduced pressure on a rotary evaporator, with approximate 8% yield.

Some of the commercially available plant constituents (i.e., caffeic acid, quercetin and rutin) were procured from Sigma Chemicals Company, St. Louis, Mo, USA, and tested for possible hepatoprotective activity.

5.3.2 Animal studies

The effect of plant extract was studied on the paracetamol-induced lethality; paracetamol- and CCl₄-induced hepatotoxicity; pentobarbital-induced sleep duration and CCl₄-induced prolongation of pentobarbital sleeping time; spontaneous movements as well as K⁺-induced contractions of rabbit jejunum by the methods
described in the general experimental section (chapter 2). The above mentioned experiments were also repeated for pure compounds (i.e., caffeic acid, quercetin and rutin), except paracetamol-induced lethality and experiments on isolated rabbit jejunum.

5.4 Results

5.4.1 Effect of extract on paracetamol-induced lethality

Paracetamol at the dose of 1 g/Kg induced 100% lethality in mice. In a group of animals pretreated with plant extract (150 mg/Kg), the same dose of paracetamol killed only 2 out of 10 animals resulting in 80% protection against lethal effect of paracetamol (Table 5.1).

5.4.2 Effect of extract on paracetamol-induced hepatotoxicity

Control (saline + vehicle) serum values of GOT and GPT in rats were found to be 80 ± 10 and 38 ± 09 IU/L respectively (Fig. 5.1; Appendix 7), while a toxic dose of paracetamol (640 mg/Kg) raised significantly the respective serum enzyme values to 1528 ± 310 ($P < 0.001$) and 904 ± 261 ($P < 0.01$). Group 3 was pretreated with plant extract to determine its effect on paracetamol-induced rise in serum enzymes (Appendix 7). The serum values of transaminases in pretreated group were found to be 85 ± 11 (GOT) and 23 ± 06 (GPT), which are significantly lower ($P < 0.001$ and $P < 0.01$ respectively) than the values of toxic control and were similar to the control values ($P > 0.05$).
Table 5.1
Effect of *Artemisia scoparia* extract on paracetamol-induced lethality in mice (n = 10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Lethality</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Paracetamol (3 mL/Kg + 1 g/Kg)</td>
<td>10/10</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>Extract + Paracetamol (150 mg/Kg + 1 g/Kg)</td>
<td>02/10</td>
<td>80</td>
</tr>
</tbody>
</table>

Saline/extract and paracetamol were given orally.
Fig. 5.1 Effect of *Artemisia scoparia* on paracetamol-induced elevation of serum transaminase levels in rats.
5.4.3 Effect of caffeic acid on paracetamol-induced hepatotoxicity

Control (saline + vehicle) serum values of GOT and GPT in rats were found to be 88 ± 15 and 51 ± 17 IU/L respectively (Fig. 5.2; Appendix 8), while a toxic dose of paracetamol (640 mg/Kg) raised significantly the respective serum enzyme values to 938 ± 284 (P<0.01) and 727 ± 211 (P<0.01). Group 3 was pretreated with caffeic acid (6 mg/Kg) to determine its effect on paracetamol-induced rise in serum enzymes (Appendix 8). The serum values of transaminases in pretreated group were found to be 138 ± 28 (GOT) and 54 ± 13 (GPT), which are significantly lower (P<0.05 and P<0.01 respectively) than the values of toxic control and were similar to the control values (P>0.05).

5.4.4 Effect of quercetin on paracetamol-induced hepatotoxicity

Control (saline + vehicle) serum values of GOT and GPT in rats were found to be 89 ± 13 and 41 ± 10 IU/L respectively (Fig. 5.3; Appendix 9), while a toxic dose of paracetamol (640 mg/Kg) raised significantly the respective serum enzyme values to 813 ± 158 (P<0.001) and 475 ± 124 (P<0.01). Group 3 was pretreated with quercetin (10 mg/Kg) to determine its effect on paracetamol-induced rise in serum enzymes (Appendix 9). The serum values of transaminases in pretreated group were found to be 105 ± 11 (GOT) and 46 ± 09 (GPT), which are significantly lower (P<0.001 and P<0.01 respectively) than the values of toxic control and were similar to the control values (P>0.05).
Fig. 5.2 Effect of caffeic acid on paracetamol-induced serum transaminase levels in rats.
Fig. 5.3 Effect of quercetin on paracetamol-induced serum transaminase levels in rats.
5.4.5 Effect of rutin on paracetamol-induced hepatotoxicity

Control (saline + vehicle) serum values of GOT and GPT in rats were found to be 118 ± 16 and 39 ± 07 IU/L respectively (Fig. 5.4; Appendix 10), while a toxic dose of paracetamol (640 mg/Kg) raised significantly the respective serum enzyme values to 1013 ± 258 (P < 0.01) and 686 ± 219 (P < 0.01). Group 3 was pretreated with rutin (20 mg/Kg) to determine its effect on paracetamol-induced rise in serum enzymes (Appendix 10). The serum values of transaminases in pretreated group were found to be 145 ± 22 (GOT) and 61 ± 15 (GPT), which are significantly lower (P < 0.01 and P < 0.05 respectively) than the values of toxic control and were similar to the control values (P > 0.05).

5.4.6 Effect of extract on CCl₄-induced hepatotoxicity

The estimated values of serum transaminases (GOT and GPT) in control (saline + vehicle) group of rats were found to be 106 ± 15 and 26 ± 04 IU/L respectively (Fig. 5.5; Appendix 11), which were raised significantly (P < 0.05) to the respective values of 395 ± 110 and 258 ± 61 after administration of a toxic dose of CCl₄ (1.5 mL/Kg). However, pretreatment of animals with plant extract returned the serum GOT and GPT values to 93 ± 05 and 27 ± 03 IU/L respectively, which are significantly lower (P < 0.05) than values of toxic control and were close to the normal values (P > 0.05).

5.4.7 Effect of caffeic acid on CCl₄-induced hepatotoxicity

The estimated values of serum transaminases (GOT and GPT) in control
Fig. 5.4 Effect of rutin on paracetamol-induced serum transaminase levels in rats.
Fig. 5.5 Effect of *Artemisia scoparia* on carbon tetrachloride-induced serum transaminase levels in rats.
(saline + vehicle) group of rats were found to be 102 ± 12 and 45 ± 09 IU/L respectively (Fig. 5.6; Appendix 12), which were raised significantly \((P<0.01)\) to the respective values of 982 ± 277 and 605 ± 181 after administration of a toxic dose of CCl₄ (1.5 mL/Kg). However, pretreatment of animals with caffeic acid (6 mg/Kg) returned the serum GOT and GPT values to 158 ± 32 and 87 ± 28 IU/L respectively, which are significantly lower \((P<0.05)\) than values of toxic control and were close to the normal values \((P>0.05)\).

5.4.8 Effect of quercetin on CCl₄-induced hepatotoxicity

The estimated values of serum transaminases (GOT and GPT) in control (saline + vehicle) group of rats were found to be 96 ± 14 and 49 ± 09 IU/L respectively (Fig. 5.7; Appendix 13), which were raised significantly \((P<0.05)\) to the respective values of 465 ± 132 and 381 ± 116 after administration of a toxic dose of CCl₄ (1.5 mL/Kg). However, pretreatment of animals with quercetin (10 mg/Kg) returned the serum GOT and GPT values to 123 ± 25 and 59 ± 17 IU/L respectively, which are significantly lower \((P<0.05)\) than values of toxic control and were close to the normal values \((P>0.05)\).

5.4.9 Effect of rutin on CCl₄-induced hepatotoxicity

The estimated values of serum transaminases (GOT and GPT) in control (saline + vehicle) group of rats were found to be 111 ± 13 and 40 ± 10 IU/L respectively (Fig. 5.8; Appendix 14), which were raised significantly \((P<0.05)\) to the respective values of 853 ± 252 and 551 ± 196 after administration of a
Fig. 5.6 Effect of caffeic acid on carbon tetrachloride-induced serum transaminase levels in rats.
Fig. 5.7 Effect of quercetin on carbon tetrachloride-induced serum transaminase levels in rats.
Fig. 5.8 Effect of rutin on carbon tetrachloride-induced serum transaminase levels in rats.
toxic dose of CCl₄ (1.5 mL/Kg). However, pretreatment of animals with rutin (20 mg/Kg) returned the serum GOT and GPT values to 153 ± 27 and 64 ± 24 IU/L respectively, which are significantly lower (P<0.05) than values of toxic control and were close to the normal values (P>0.05).

5.4.10 Effect of extract on pentobarbital-induced sleep

Effect of plant extract on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time was studied in mice and the results are shown in Table 5.2. Pentobarbital at a dose of 75 mg/Kg, i.p., caused sleep in mice for a period of 141 ± 02 min (Mean ± S.E.M, n=10). Pentobarbital sleeping time in the group of animals pretreated with plant extract was found to be 140 ± 02 min which is similar to that in the control group (P>0.05).

5.4.11 Effect of caffeic acid on pentobarbital-induced sleep

Effect of caffeic acid on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time was studied in mice and the results are shown in Table 5.3. Pentobarbital at a dose of 75 mg/Kg, i.p., caused sleep in mice for a period of 131 ± 05 min (Mean ± S.E.M, n=10). Pentobarbital sleeping time in the group of animals pretreated with caffeic acid was found to be 136 ± 04 min which is similar to that in the control group (P>0.05).
Table 5.2
Direct effect of *Artemisia scoparia* extract on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sleeping time</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Pentobarbital (3 mL/Kg + 75 mg/Kg)</td>
<td>141 ± 02</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Extract + Pentobarbital (150 mg/Kg + 75 mg/Kg)</td>
<td>140 ± 02</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>3.</td>
<td>Saline + CCl₄ + Pentobarbital (3 mL/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>212 ± 19*</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>4.</td>
<td>Extract + CCl₄ + Pentobarbital (150 mg/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>144 ± 06**</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 10 determinations. Saline/extract/CCl₄ was given orally, while pentobarbital was given intraperitoneally.

* Compared to group 1 (control).

** Compared to group 3.
Table 5.3
Direct effect of caffeic acid on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sleeping time</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Pentobarbital (1.5 mL/Kg + 75 mg/Kg)</td>
<td>131 ± 05</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Caffeic acid + Pentobarbital (6 mg/Kg + 75 mg/Kg)</td>
<td>136 ± 04</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>3.</td>
<td>Saline + CCl₄ + Pentobarbital (3 mL/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>234 ± 19°</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4.</td>
<td>Caffeic acid + CCl₄ + Pentobarbital (150 mg/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>157 ± 17°</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 10 determinations. Saline/caffeic acid/CCl₄ was given orally, while pentobarbital was given intraperitoneally.

* Compared to group 1 (control).

** Compared to group 3.
5.4.12 Effect of quercetin on pentobarbital-induced sleep

Effect of quercetin on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time was studied in mice and the results are shown in Table 5.4. Pentobarbital at a dose of 75 mg/Kg, i.p., caused sleep in mice for a period of 127 ± 07 min (Mean ± S.E.M., n=10). Pentobarbital sleeping time in the group of animals pretreated with quercetin was found to be 139 ± 08 min which is similar to that in the control group (P>0.05).

5.4.13 Effect of rutin on pentobarbital-induced sleep

Effect of rutin on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time was studied in mice and the results are shown in Table 5.5. Pentobarbital at a dose of 75 mg/Kg, i.p., caused sleep in mice for a period of 144 ± 09 min (Mean ± S.E.M., n=10). Pentobarbital sleeping time in the group of animals pretreated with rutin was found to be 154 ± 12 min which is similar to that in the control group (P>0.05).

5.4.14 Extract and CCl₄-induced pentobarbital sleep increase

Pretreatment of animals with CCl₄ prolonged the pentobarbital sleeping time to 212 ± 19 min, the value that is significantly higher (P<0.005) than that of control (Table 5.2). However, prior treatment of animals with extract returned this CCl₄-induced prolongation of pentobarbital sleeping time to 144 ±
Table 5.4
Direct effect of quercetin on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sleeping time</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Pentobarbital (5 mL/Kg + 75 mg/Kg)</td>
<td>127 ± 07</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Quercetin + Pentobarbital (10 mg/Kg + 75 mg/Kg)</td>
<td>139 ± 08</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>3.</td>
<td>Saline + CCl₄ + Pentobarbital (5 mL/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>234 ± 24*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4.</td>
<td>Quercetin + CCl₄ + Pentobarbital (10 mg/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>136 ± 32**</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 10 determinations. Saline/quercetin/CCl₄ was given orally, while pentobarbital was given intraperitoneally.

* Compared to group 1 (control).

** Compared to group 3.
Table 5.5
Direct effect of rutin on pentobarbital sleeping time as well as on $\text{CCl}_4$-induced prolongation of pentobarbital sleeping time in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sleeping time</th>
<th>$P$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Pentobarbital (4 mL/Kg + 75 mg/Kg)</td>
<td>144 ± 0.5</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Rutin + Pentobarbital (20 mg/Kg + 75 mg/Kg)</td>
<td>154 ± 12</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>3.</td>
<td>Saline + $\text{CCl}_4$ + Pentobarbital (4 mL/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>250 ± 29*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>4.</td>
<td>Rutin + $\text{CCl}_4$ + Pentobarbital (20 mg/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>158 ± 18**</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

* Each value represents the mean ± S.E.M. of 10 determinations. Saline/rutin/$\text{CCl}_4$ was given orally, while pentobarbital was given intraperitoneally.

* Compared to group 1 (control).

** Compared to group 3.
06 min, which is significantly lower \((P < 0.01)\) than the value of group 3 and close to the normal sleeping time \((P > 0.05)\).

### 5.4.15 Caffeic acid and CCl₄-induced pentobarbital sleep increase

Pretreatment of animals with CCl₄ prolonged the pentobarbital sleeping time to 234 ± 19 min, the value that is significantly higher \((P < 0.001)\) than that of control (Table 5.3). However, prior treatment of animals with caffeic acid returned this CCl₄-induced prolongation of pentobarbital sleeping time to 157 ± 17 min, which is significantly lower \((P < 0.01)\) than the value of group 3 and close to the normally sleeping time \((P > 0.05)\).

### 5.4.16 Quercetin and CCl₄-induced pentobarbital sleep increase

Pretreatment of animals with CCl₄ prolonged the pentobarbital sleeping time to 236 ± 24 min, the value that is significantly higher \((P < 0.001)\) than that of control (Table 5.4). However, prior treatment of animals with quercetin returned this CCl₄-induced prolongation of pentobarbital sleeping time to 136 ± 32 min, which is significantly lower \((P < 0.05)\) than the value of group 3 and close to the normally sleeping time \((P > 0.05)\).

### 5.4.17 Rutin and CCl₄-induced pentobarbital sleep increase

Pretreatment of animals with CCl₄ prolonged the pentobarbital sleeping time to 250 ± 29 min, the value that is significantly higher \((P < 0.01)\) than that of control (Table 5.5). However, prior treatment of animals with rutin
returned this CCl₄-induced prolongation of pentobarbital sleeping time to 158 ± 18 min, which is significantly lower (P < 0.05) than the value of group 3 and close to the normally sleeping time (P > 0.05).

5.4.18 Ca²⁺ channel blocking activity

Plant extract at the concentration of 0.1-1 mg/mL caused a concentration-dependent inhibition of spontaneous movements of rabbit jejunum as shown in Fig. 5.9. This effect was reversible as the tissue regained its spontaneous activity after washing the tissue a couple of times with the fresh bathing fluid.

Verapamil, a standard calcium channel blocker (White and Bradford, 1986; Triggle and Ramp, 1989) was also included in the study for comparison, which caused a similar dose-dependent (0.3-3 μM) inhibitory response and spontaneous movements were restored after repeated washings (10-15 min.).

Potassium at a concentration of 40 mM caused a sustained tonic contraction which allowed to obtain concentration-dependent inhibitory responses (Fig. 5.10). Addition of the plant extract at 0.1 mg/mL, caused a slight inhibition, while at 0.3 mg/mL, it produced almost 50% relaxant effect. The next higher concentration (1 mg/mL) completely suppressed contractile effect of K⁺. Similarly, verapamil also relaxed K⁺-induced contraction in a dose dependent manner (1-3 μM).

When the bathing fluid was replaced with Ca²⁺ free Kreb’s solution, the spontaneous movements of the tissue were abolished within 1 min. (Fig. 5.11). Addition of Ca²⁺ (10-50 μM) restored spontaneous activity in a concentration-dependent manner.
Fig. 5.9. A representative tracing showing inhibitory effects of *Artemisia scoparia* (AS) and verapamil on spontaneous movements of isolated rabbit jejunum. Triangles represent the time at which the drugs were added to the tissue bath (10 ml) and normal saline (0.1 ml) was administered at respective times in upper panel (control).
Fig. 5.10. A representative tracing showing comparison of *Artemisia scoparia* (AS) and verapamil for their inhibitory effects on isolated rabbit jejunum precontracted with $K^+$. 
Fig. 5.11. A representative tracing showing effect of Ca++ supplementation on isolated rabbit jejunum (in Ca++ free kreb’s solution. Effect of pretreatment with Artemisia scoparia (AS) or verapamil (V) on Ca++ supplementation is also shown.
The plant extract and verapamil were also tested in Ca\textsuperscript{2+} free Kreb's solution for their effect on Ca\textsuperscript{2+} responses. Calcium failed to restore spontaneous movements of the tissue in the presence of the plant extract (0.3 mg/mL) or verapamil (1 \textmu M).

The plant extract up to an oral dose of 3 g/Kg was found devoid of any lethal effect and no apparent behavioral change was observed.

5.5 Discussion

The hydro-methanolic extract of Artemisia scoparia as well as pure compounds (caffeic acid, quercetin and rutin) exhibited hepatoprotection against both paracetamol and CCl\textsubscript{4}-induced liver injuries as manifested by reduction in toxins-mediated rise in serum transaminases (GOT and GPT) in rats. Pretreatment of animals with plant extract afforded 80% protection against lethal dose of paracetamol, whereas both plant extract and pure compounds were found capable to prevent CCl\textsubscript{4}-induced prolongation of pentobarbital sleeping time in mice.

Liver injuries induced by paracetamol and CCl\textsubscript{4} are commonly used models for the screening of hepatoprotective drugs (Slater, 1965; Strubelt et al. 1974; Plaa and Hewitt, 1982). The rise in serum levels of transaminases (GOT and GPT) has been attributed to the damaged structural integrity of the liver (Chenoweth and Hake, 1962) because these are cytoplasmic in location and are released into circulation after cellular damage (Sallie et al., 1991). Paracetamol and CCl\textsubscript{4} are converted to their reactive metabolites (N-acetyl-p-benzoquinoneimine and halogenated free radicals respectively) by their respective specific isozyme of
cytochrome P-450 (Packer et al., 1978; van de Straat et al., 1987). Physiologically important protective mechanisms involving both vitamin E (α-tocopherol) and glutathione (GSH) are available to curtail progression of cellular damage (Potter et al., 1974). However, the massive production of reactive species may lead to depletion of protective moieties, ensuing wide-spread propagation of the alkylation as well as peroxidation, causing damage to both lipids and proteins present in biomembranes of microsomes and mitochondria (Rao and Recknagel, 1969; Peshimam and Recknagel, 1977; Aldridge, 1981). These bio-activation mediated cytotoxic effects are subject of active research and the toxic manifestations can effectively be minimized by cytochrome P-450 inhibitors (Castro et al., 1974; Tredger et al., 1985; Beyeler et al., 1988), GSH precursors (Ferreyra et al., 1974; Prescott et al., 1979), free radical scavengers (Mailing et al., 1974), antioxidants (Gazzard et al., 1974; Koester-Albercht et al., 1979; Yasuda et al., 1980), sulfhydryl agents (Strubelt et al., 1974; Mark et al., 1990) and Ca²⁺ channel blockers (Landon et al., 1986; Thibault et al., 1991).

The plant extract and pure compounds seem to preserve the structural integrity of the hepatocellular membranes as was evident from the significant reduction in the paracetamol and CCl₄-induced rise in serum GOT and GPT levels in rats, and is confirmed by prevention of the CCl₄-induced prolongation of pentobarbital-sleeping time in mice. The fact that the test materials were unable to prolong pentobarbital sleeping time (P > 0.05) suggests that these are devoid of inhibitory effect on hepatic MDME such as cytochrome P-450 and their hepatoprotective effect is mediated perhaps through some other mechanism(s).
The observed protective effect of plant extract against paracetamol and CCl₄ can be attributed to the reported presence of flavonoids (Ryakhovskaya and Alyukina, 1971), L-ascorbic acid (Solov’eva et al., 1970), carotenoids (Solov’eva et al., 1970) and phenolic compounds (Hu et al., 1965) among the plant constituents. The flavonoids are known to be anti-oxidants (Torel et al., 1986; Faurè et al., 1990), free radical scavengers (Bors and Saran, 1987; Husain et al., 1987) and anti-lipoperoxidant (Younes and Siegers, 1981; Robak et al., 1986; Ratty and Das, 1988) leading to hepatoprotection (Kiso et al., 1984; Handa et al., 1986). Similarly, ascorbic acid serves as anti-oxidant (Demopoulos, 1973; Bus and Gibson, 1984), inhibits covalent binding of NAPQI to vital macromolecules (Lake et al., 1981) and consequently can minimize toxic damage (Harman, 1985). Moreover, carotenoids are also reputed to be antioxidants (Kläui, 1982) and thus showing anti-hepatotoxic activity (Oshima et al., 1984). Furthermore, the phenolic compounds are reported to have antioxidant and free radical scavenging potential (Zhou and Zheng, 1991) and can prevent peroxidative damage to biomembranes in hepatocytes (Liu et al., 1992).

The caffeic acid is one of the known plant constituents (Hu et al., 1965) and exhibited hepatoprotective activity. The hepatoprotective activity of caffeic acid against CCl₄-induced damage is already reported (Adzet et al., 1987; Perez-Alvarez et al., 1993) and the present study confirms those findings but also extend it against paracetamol-induced hepatic damage. The hepatoprotective potential of caffeic acid against experimental liver damage can be viewed as an outcome of its anti-oxidant property (Kimura et al., 1985; Nakayama, 1994).
Moreover, quercetin and its 3-rutinoside derivative (rutin) being member of flavonoid family have also been identified from this plant (Ryakhovskya and Alyukina, 1971) and the observed hepatoprotective activity can be attributed to their anti-oxidant (Chen et al., 1990; Negre Salvayre et al., 1991; Oyama et al., 1994) and free radical scavenging (Torel et al., 1986; Afnas'eve et al., 1989; Hanasaki et al., 1994) properties.

The cytosolic free calcium concentration in hepatocytes is known to be increased during experimental liver damage (Farber et al., 1982; Moore et al., 1985; Landon et al., 1986; Tsokos-Kuhn, 1989) and calcium channel blocking drugs (i.e., nifedipine, diltiazem and verapamil) are also capable to provide protection against paracetamol- and CCl₄-induced hepatopathy (Landon et al., 1986; Thibault et al., 1991). Quercetin exhibited CCB activity (data not shown as this was repeatedly reported by Fewtrell and Gompertes, 1977; Morales and Lozoya, 1994) and may also have contributed toward hepatoprotective activity. Thus, it is logical to think for a plausible link between the proposed hepatoprotective activity and Ca²⁺ channel blocking activity of plant extract.

Moreover, the plant extract suppressed the spontaneous movements as well as tonic contractions of rabbit jejunum and thus exhibited its smooth muscle relaxant (spasmolytic) activity. The spontaneous movements of rabbit jejunum are inhibited by the methanolic extract of Artemisia scoparia and can be accounted for the interference either with the depolarization process or with the Ca²⁺ influx through VDCs and this is further substantiated by the fact that quercetin being one of the plant constituent is reported to exhibit the same property (Shoshan et al.,
1980; Morales and Lozoya, 1994).

The results presented so far clearly indicate that the crude extract of Artemisia scoparia and pure compounds (caffeic acid, quercetin and rutin) afford protection against paracetamol and CCl₄-induced liver damage in rats as well as mice and this action is not through inhibition of hepatic MDME. The observed hepatoprotective effect of plant can be attributed to the known activities of its constituents (i.e., anti-oxidant, free radical scavenging and Ca²⁺ channel blockade). The plant material is safe as is obvious by lack of any symptom of acute toxicity at an oral dose of as high as 4 g/Kg. Thus, this study rationalizes the traditional use of Artemisia scoparia plant in hepato-biliary diseases.

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6. Berberis aristata
6. *Berberis aristata*

6.1 Summary

The hepatoprotective activity of *Berberis aristata* extracts (shoots and fruits) and pure compound (berberine) were investigated against paracetamol- and carbon tetrachloride-induced hepatic damage. Paracetamol produced 100% mortality at the dose of 1 g/Kg in mice, while pretreatment of animals with shoot and fruit extracts (500 mg/Kg; orally) reduced the death rate to 30 and 10% respectively. Pretreatment of rats with four oral doses of plant extracts (500 mg/Kg) and berberine (4 mg/Kg) at 12 hrs interval, prevented \( P < 0.05 \) the paracetamol (640 mg/Kg) and CCl\(_4\) (1.5 mL/Kg)-induced rise in serum enzymes (GOT and GPT). Post-treatment with three successive doses of plant extracts (500 mg/Kg) and berberine (4 mg/Kg) at 6 hrs interval (started 6 hrs after toxins administration), restricted the paracetamol-induced hepatic damage \( P < 0.05 \), but CCl\(_4\)-induced hepatotoxicity remained unaltered \( P > 0.05 \). The same dose of plant extracts as well as berberine resulted in significant prolongation \( P < 0.01 \) in pentobarbital (75 mg/Kg)-induced sleep and also potentiated the strychnine-induced lethality in mice, suggestive of inhibitory effect on microsomal drug metabolizing enzymes (MDME). These results indicate that the crude extracts of *Berberis aristata* (shoots and fruits) and berberine exhibit hepatoprotective action partly through MDME inhibitory action and validates the traditional use of plant in hepatic damage.
6.2 Plant Description

*Berberis aristata* DC. (Berberidaceae) locally known as ‘Zarishk’ is a shrub found in the northern mountainous regions of Pakistan and India (Nadkarni, 1976). The plant material (leaves, stem and roots) is regarded as stomachic, astringent, antiperiodic, antipyretic and diaphoretic in indigenous systems of medicine (Unani and Ayurvedic) and has been employed successfully to alleviate jaundice, periodic neuralgia, intermittent fever, painful micturition and enlargement of spleen (Saíd, 1982). The crude drug as a sole agent or as a part of recipes (containing multiple ingredients) has proved its efficacy in the management of allergic conjunctivitis (Athneria *et al.*, 1987), acne vulgaris (Chattopadhya, 1991), rheumatic pain (Sharma, 1991), premature ejaculation (Misra *et al.*, 1980), leucorrhoea (Mukherjee and Banerjee, 1989), inflammation (Misra, 1991), fungal infection (Nair *et al.*, 1989), coccidiosis (Guha *et al.*, 1991), viral hepatitis (Singh *et al.*, 1991) and in toxic liver damage (Gilani and Janbaz, 1992, Bhaumik and Sharma, 1993; Sharma *et al.*, 1993).

Moreover, the edible fruits of *Berberis aristata* are consumed largely both in fresh and dried form and exhibits hypocholestraemic activity (Kamran and Ahmad, 1992). The phytochemical investigation on plant material revealed the presence of berberine (Chandra and Todaria, 1983), anthocyanins (Chandra and Todaria, 1983; Vereskovskii and Shapiro, 1985), flavonoids (Shapiro *et al.*, 1983; Vereskovskii and Shapiro, 1986), β-carotene (Chandra and Todaria, 1983; Shapiro *et al.*, 1983), catechins (Shapiro *et al.*, 1983), mono- and polysaccharides (Martynov *et al.*, 1984; Karitonova, 1986)) and ascorbic acid (Shapiro *et al.*, 1983; Kharitonova, 1986) among the plant constituents.

Berberine is an alkaloid isolated from *Berberis aristata* (Chandra and Todaria, 1983) and exhibited some important biological activities such as anil-
bacterial (Ukita et al., 1949; Aoyama, 1951; Tomita and Watanabe, 1951; Johnson et al., 1952; Lambin and Bernard, 1953; Geonya and Progressov, 1961; Amin et al., 1969), anti-yeast (Müsel and Sokolova, 1959), anti-leishmaniasis (Devi, 1929; Gupta and Dikshit, 1929; Gupta, 1930; Bennett, 1935), anti-inflammatory (Yasukawa et al., 1991; Akhter et al., 1977), anti-muscarnic (Mercier, 1937; Tsai and Ochillo, 1988; Tsai and Ochillo, 1991), hypotensive (Suzuki, 1939; Honda et al., 1960b), anti-anaemic (Kudo, 1953), chologogue (Velluda et al., 1958; Turova et al., 1964), anti-tumor (Honda et al., 1960a; Hano and Akashi, 1964), anti-diarrhoeal (Mekawi, 1966; Bhide et al., 1969; Akhter et al., 1979; Eaker and Sninsky, 1989) activities. Moreover, it decreases the intestinal secretions (Sack and Froehlich, 1982; Tai et al., 1981), while ACTH secretion is increased (Liu et al., 1966).

The studies pertaining to the evaluation of the protective effect of the shoots and fruits extract as well as berberine were carried out at different occasions, consequently separate control group was used in each case.

6.3 Materials and Methods

6.3.1 Plant extract

Berberis aristata shoots were collected during July 1991 from "Northern Area" of Pakistan, whereas fruits were purchased from local herbal store and authenticated with the help of a botanist at The University of Karachi. The fruits were crushed directly, while shoots were powdered subsequent to shade drying. The powdered materials were macerated separately in 80% aqueous-methanol for one week with occasional shaking. The extract was filtered and concentrated to a dark greenish brown residue (shoots) and thick dark pinkish brown syrup (fruits) under reduced pressure on a rotary evaporator, with approximate yield of the
concentrated extracts were 7.5% and 21% respectively.

Berberine was procured from Sigma Chemicals Company St. Lious, Mo, USA and tested for possible hepatoprotective activity.

6.3.2 Animal studies
The effects of shoot and fruit extracts were studied on the paracetamol-induced lethality; preventive effect paracetamol- and CCl₄-induced hepatotoxicity; curative effect on paracetamol and CCl₄-induced hepatotoxicity; pentobarbitai-induced sleep duration and strychnine lethality by the methods described in general method section (chapter 2). The above mentioned experiments were also repeated for berberine, except preliminary experiments on paracetamol-induced lethality.

6.4 Results
6.4.1 Plants extracts and paracetamol-induced lethality
Paracetamol at the dose of 1 g/Kg induced 100% lethality in mice. In a group of animals pretreated with shoot extract (500 mg/Kg), the same dose of paracetamol killed only 3 out of 10 animals resulting in 70% protection against lethal effect of paracetamol, whereas, same dose of fruit extract exhibited 90% protection (Table 6.1).

6.4.2 Shoot extract pretreatment and paracetamol toxicity
Control (saline + vehicle) serum values of ALP, GOT and GPT in rats were found to be 203 ± 17, 97 ± 10 and 27 ± 04 IU/L respectively (Fig. 6.1; Appendix 15), while a toxic dose of paracetamol (640 mg/Kg) raised significantly (P<0.001), the respective serum enzyme values to 361 ± 35, 1447 ± 182 and 899 ± 201. Group 3 was pretreated with plant shoots extract (500 mg/Kg, orally,
Table 6.1

Effect of *Berberis aristata* shoot and fruit extracts on paracetamol-induced lethality in mice (n=10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Lethality</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Paracetamol</td>
<td>10/10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(10 mL/Kg + 1 g/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Shoot extract + Paracetamol</td>
<td>03/10</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>(500 mg/Kg + 1 g/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Fruit extract + Paracetamol</td>
<td>01/10</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>(500 mg/Kg + 1 g/Kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Saline/extracts and paracetamol were given orally.
Fig. 6.1 Preventive effect of *Beris aristata* shoots on paracetamol-induced elevation of serum enzymes in rats.

\[ *P < 0.01 \]
\[ **P < 0.001 \]
twice daily for 2 days) to determine its effect on paracetamol-induced rise in serum enzymes. The serum values of enzymes in pretreated group were found to be 162 ± 25 (ALP), 230 ± 157 (GOT) and 169 ± 134 (GPT), which are significantly lower ($P<0.01$) than the values of toxic control and were similar to the control values ($P>0.05$).

6.4.3 *Fruit extract pretreatment and paracetamol toxicity*

Control (saline + vehicle) serum values of ALP, GOT and GPT in rats were found to be 219 ± 21, 80 ± 10 and 38 ± 09 IU/L respectively (Fig. 6.2; Appendix 16), while a toxic dose of paracetamol (640 mg/Kg) raised significantly ($P<0.01$), the respective serum enzyme values to 312 ± 21, 1534 ± 249 and 430 ± 104. Group 3 was pretreated with plant fruits extract (500 mg/Kg, orally, twice daily for 2 days) to determine its effect on paracetamol-induced rise in serum enzymes. The serum values of enzymes in pretreated group were found to be 207 ± 16 (ALP), 102 ± 12 (GOT) and 45 ± 06 (GPT), which are significantly lower ($P<0.01$) than the values of toxic control and were similar to the control values ($P>0.05$).

6.4.4 *Berberine pretreatment and paracetamol toxicity*

Control (saline + vehicle) serum values of ALP, GOT and GPT in rats were found to be 176 ± 05, 75 ± 04 and 38 ± 03 IU/L respectively (Fig. 6.3; Appendix 17), while a toxic dose of paracetamol (640 mg/Kg) raised significantly ($P<0.01$), the respective serum enzyme values to 259 ± 10, 582 ± 95 and 243 ± 79. Group 3 was pretreated with berberine (4 mg/Kg, orally, twice daily for 2 days) to determine its effect on paracetamol-induced rise in serum enzymes. The serum values of enzymes in pretreated group were found to be 182 ± 10 (ALP),
Fig. 6.2 Preventive effect of *Berberis aristata* fruits on paracetamol-induced elevation of serum enzymes in rats.
Fig. 6.3 Preventive effect of berberine on paracetamol-induced elevation of serum enzymes.
67 ± 11 (GOT) and 37 ± 05 (GPT), which are significantly lower ($P < 0.01$) than the values of toxic control and were similar to the control values ($P > 0.05$).

6.4.5 Shoot extract pretreatment and CCl₄ hepatotoxicity

The values of serum ALP, GOT and GPT in control (saline + vehicle) group of rats were found to be 237 ± 13, 99 ± 22 and 35 ± 10 IU/L respectively (Fig. 6.4; Appendix 18), which were raised significantly ($P < 0.05$) to respective values of 291 ± 17, 541 ± 164 and 320 ± 94 after administration of a toxic dose of CCl₄ (1.5 mL/Kg). However, pretreatment of animals with plant shoots extract (500 mg/Kg, orally, twice daily for 2 days) returned the serum ALP, GOT and GPT values to 222 ± 18, 134 ± 21 and 76 ± 37 IU/L respectively, which are significantly lower ($P < 0.05$) than values of toxic control and were close to the control values ($P > 0.05$).

6.4.6 Fruit extract pretreatment and CCl₄ toxicity

The values of serum ALP, GOT and GPT in control (saline + vehicle) group of rats were found to be 215 ± 16, 89 ± 09 and 41 ± 14 IU/L respectively (Fig. 6.5; Appendix 19), which were raised significantly ($P < 0.05$) to respective values of 303 ± 18, 480 ± 150 and 296 ± 80 after administration of a toxic dose of CCl₄ (1.5 mL/Kg). However, pretreatment of animals with plant fruits extract (500 mg/Kg, orally, twice daily for 2 days) returned the serum ALP, GOT and GPT values to 224 ± 21, 100 ± 18 and 62 ± 12 IU/L respectively, which are significantly lower ($P < 0.05$) than values of toxic control and were close to the control values ($P > 0.05$).
Fig. 6.4 Preventive effect of Berberis aristata shoots on carbon tetrachloride-induced elevation of serum enzymes in rats.
Fig. 6.5 Preventive effect of *Boerhaavia diffusa* fruits on carbon tetrachloride-induced elevation of serum enzymes in rats.
6.4.7 Berberine pretreatment and CCl₄ toxicity

The values of serum ALP, GOT and GPT in control (saline + vehicle) group of rats were found to be 207 ± 18, 109 ± 12 and 39 ± 16 IU/L respectively (Fig. 6.6; Appendix 20), which were raised significantly ($P < 0.05$) to respective values of 275 ± 24, 734 ± 191 and 415 ± 76 after administration of a toxic dose of CCl₄ (1.5 mL/Kg). However, pretreatment of animals with berberine (4 mg/Kg, orally, twice daily for 2 days) returned the serum ALP, GOT and GPT values to 219 ± 20, 144 ± 28 and 76 ± 25 IU/L respectively, which are significantly lower ($P < 0.05$) than values of toxic control and were close to the control values ($P > 0.05$).

6.4.8 Shoot extract post-treatment and paracetamol toxicity

Control (saline + vehicle) serum values of ALP, GOT and GPT in rats were found to be 229 ± 21, 103 ± 19, and 38 ± 15 IU/L ($n=10$) respectively (Fig. 6.7; Appendix 21), while a toxic dose of paracetamol (640 mg/Kg) raised significantly ($P < 0.01$), the respective serum enzyme values to 346 ± 25, 1180 ± 197 and 751 ± 159. The treatment with the plant shoots extract (500 mg/Kg, orally) was started to group 3 animals (6 hrs after paracetamol administration) to evaluate its curtailing effect upon the paracetamol-induced progression of hepatic damage duly monitored by serum enzymes. The serum values of enzymes in post-treated group were found to be 253 ± 30 (ALP), 118 ± 34 (GOT) and 53 ± 27 (GPT), which were significantly lower than the serum values of toxic control group ($P < 0.05$) and were comparable to the normal values ($P > 0.05$).

6.4.9 Fruit extract post-treatment and paracetamol toxicity

Control (saline + vehicle) serum values of ALP, GOT and GPT in rats
Fig. 6.6 Preventive effect of berberine on carbon tetrachloride-induced elevation of serum enzymes.
Fig. 6.7 Curative effect of *Berberis aristata* shoots on paracetamol-induced elevation of serum enzymes in rats.
were found to be 222 ± 26, 95 ± 11 and 36 ± 13 IU/L (n = 10) respectively (Fig. 6.8; Appendix 22), while a toxic dose of paracetamol (640 mg/Kg) raised significantly (P<0.01), the respective serum enzyme values to 331 ± 23, 1430 ± 217 and 483 ± 125. The treatment with the plant fruits extract (500 mg/Kg, orally) was started to group 3 animals (6 hrs after paracetamol administration) to evaluate its curtailing effect upon the paracetamol-induced progression of hepatic damage duly monitored by serum enzymes. The serum values of enzymes in post-treated group were found to be 247 ± 27 (ALP), 70 ± 17 (GOT) and 38 ± 08 (GPT), which were significantly lower than the serum values of toxic control group (P<0.05) and were comparable to the normal values (P>0.05).

6.4.10 Berberine post-treatment and paracetamol toxicity

Control (saline + vehicle) serum values of ALP, GOT and GPT in rats were found to be 198 ± 13, 93 ± 16 and 48 ± 10 IU/L (n = 10) respectively (Fig. 6.9; Appendix 23), while a toxic dose of paracetamol (640 mg/Kg) raised significantly (P<0.01), the respective serum enzyme values to 361 ± 19, 996 ± 228 and 495 ± 133. The treatment with berberine (4 mg/Kg, orally) was started to group 3 animals (6 hrs after paracetamol administration) to evaluate its curtailing effect upon the paracetamol-induced progression of hepatic damage duly monitored by serum enzymes. The serum values of enzymes in post-treated group were found to be 210 ± 14 (ALP), 138 ± 29 (GOT) and 52 ± 15 (GPT), which were significantly lower than the serum values of toxic control group (P<0.01) and were comparable to the normal values (P>0.05).

6.4.11 Shoot extract post-treatment and CCl₄ toxicity

The administration of a toxic dose of CCl₄ (1.5 mL/Kg; orally) raised
Fig 6.8 Curative effect of *Berberis aristata* fruits on paracetamol-induced elevation of serum enzymes in rats.
Fig. 6.9 Curative effect of berberine on paracetamol-induced elevation of serum enzymes.
significantly ($P < 0.05$), the serum values of ALP, GOT and GPT to 327 ± 18, 572 ± 192 and 343 ± 121 IU/L respectively, compared to respective control values of 215 ± 18, 105 ± 18 and 42 ± 16 (Fig. 6.10; Appendix 24). The group 3 animals were treated with multiple doses of plant shoots extract, following CCl₄ intoxication and the serum values in the treated group were found to be 297 ± 32 (ALP), 394 ± 131 (GOT) and 278 ± 38 (GPT), which were similar to the values of toxic control group ($P > 0.05$) and higher than that of normal values ($P < 0.05$).

6.4.12 *Fruit extract post-treatment and CCl₄ toxicity*

The administration of a toxic dose of CCl₄ (1.5 mL/Kg; orally) raised significantly ($P < 0.05$), the serum values of ALP, GOT and GPT to 297 ± 13, 602 ± 212 and 273 ± 96 IU/L respectively, compared to respective control values of 193 ± 16, 91 ± 08 and 35 ± 14 (Fig. 6.11; Appendix 25). The group 3 animals were treated with multiple doses of plant fruits extract, following CCl₄ intoxication and the serum values in the treated group were found to be 321 ± 40 (ALP), 185 ± 14 (GOT) and 189 ± 33 (GPT), which were similar to the values of toxic control group ($P > 0.05$) and higher than that of normal values ($P < 0.05$).

6.4.13 *Berberine post-treatment and CCl₄ toxicity*

The administration of a toxic dose of CCl₄ (1.5 mL/Kg; orally) raised significantly ($P < 0.05$), the serum values of ALP, GOT and GPT to 268 ± 26, 911 ± 237 and 375 ± 92 IU/L respectively, compared to respective control values of 201 ± 14, 99 ± 17 and 46 ± 12 (Fig. 6.12; Appendix 26). The group 3 animals were treated with multiple doses of berberine (4 mg/Kg), following CCl₄ intoxication and the serum values in the treated group were found to be 239 ± 23 (ALP), 644 ± 198 (GOT) and 262 ± 73 (GPT), which were similar to the values
Fig 6.10 Post-treatment with Berberis aristata shoots and carbon tetrachloride-induced elevation of serum enzymes in rats.
Fig. 6.11  Post-treatment with *Berberis aristata* fruits and carbon tetrachloride-induced elevation of serum enzymes in rats.
Fig. 6.12 Effect of berberine post-treatment on carbon tetrachloride-induced elevation of serum enzymes.
of toxic control group \((P > 0.05)\) and higher than that of normal values \((P < 0.05)\).

6.4.14 **Shoot extract and pentobarbital sleeping time**

Pentobarbital at a dose of 75 mg/Kg, i.p., caused sleep in mice for a period of 85 ± 09 min (mean ± S.E.M, \(n=10\)), whereas the sleeping time in the group of animals pretreated with plant shoots extract was found to be 137 ± 14 min, which was significantly higher than that in the control group \((P < 0.01)\) (Table 6.2).

6.4.15 **Fruit extract and pentobarbital sleeping time**

Pentobarbital at a dose of 75 mg/Kg, i.p., caused sleep in mice for a period of 78 ± 16 min (mean ± S.E.M, \(n=10\)), whereas the sleeping time in the group of animals pretreated with plant extract was found to be 143 ± 12 min, which was significantly higher than that in the control group \((P < 0.01)\) (Table 6.3).

6.4.16 **Berberine and pentobarbital sleeping time**

Pentobarbital at a dose of 75 mg/Kg, i.p., caused sleep in mice for a period of 71 ± 02 min (mean ± S.E.M, \(n=10\)), whereas the sleeping time in the group of animals pretreated with berberine was found to be 92 ± 09 min, which was significantly higher than that in the control group \((P < 0.05)\) (Table 6.4).

6.4.17 **Shoot extract and strychnine lethality**

In preliminary experiments, the median lethal dose \((L.D_{50})\) of strychnine was found to be 0.9 mg/Kg, whereas 0.4 mg/Kg was proved as a sub-lethal dose. However, pre-treatment with a single dose (500 mg/Kg) of plant shoots extract 1 hr prior to strychnine administration, potentiated the effect of strychnine causing
Table 6.2
Effect of *Berberis aristata* shoot extract on pentobarbital sleeping time in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sleeping time</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vehicle + Pentobarbital (10 mL/Kg + 75 mg/Kg)</td>
<td>85 ± 09</td>
<td>—</td>
</tr>
<tr>
<td>2.</td>
<td>Extract + Pentobarbital (500 mg/Kg + 75 mg/Kg)</td>
<td>137 ± 14</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 10 determinations. Salines/Extract was given orally, while pentobarbital was given intraperitoneally.
Table 6.3

Effect of *Berberis aristata* fruit extract on pentobarbital sleeping time in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sleeping time</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>(Vehicle + Pentobarbital)</td>
<td>78 ± 16</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(10 mL/Kg + 75 mg/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>(Extract + Pentobarbital)</td>
<td>143 ± 12</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td></td>
<td>(500 mg/Kg + 75 mg/Kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 10 determinations. Salines/Extract was given orally, while pentobarbital was given intraperitoneally.
Table 6.4
Effect of berberine on pentobarbital sleeping time in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sleeping time</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vehicle + Pentobarbital (1 mL/Kg + 75 mg/Kg)</td>
<td>71 ± 02</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Berberine + Pentobarbital (4 mg/Kg + 75 mg/Kg)</td>
<td>92 ± 09</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 10 determinations. Salines/berberine was given orally, while pentobarbital was given intraperitoneally.
almost 60% mortality rate (Table 6.5).

6.4.18 Fruits extract and strychnine lethality

When the sub-lethal dose of strychnine (0.4 mg/Kg) was administered following treatment with a single dose (500 mg/Kg) of plant fruits extract, potentiated effect of strychnine causing almost 50% mortality rate was observed (Table 6.5).

6.4.19 Berberine and strychnine lethality

When the sub-lethal dose of strychnine (0.4 mg/Kg) was administered following treatment with a single dose (4 mg/Kg) of berberine, potentiated effect of strychnine causing almost 100% mortality rate was observed (Table 6.6).

The plant extract up to an oral dose of 4 g/Kg was found devoid of any lethal effect and no apparent behavioral change was observed.

6.5 Discussion

Paracetamol- and CCl₄-induced hepatic injuries are commonly used models for the screening of hepatoprotective drugs (Slater, 1965; Plaa and Hewitt, 1982) and the extent of hepatic damaged is assessed by the level of released cytoplasmic alkaline phosphatase (ALP) and transaminases (GOT and GPT) in circulation (Chenoweth and Hake, 1962; Sallie et al., 1991).

The prophylactic administration of Berberis aristata extracts (shoots and fruits) and berberine, exhibited protection against both paracetamol- and CCl₄-induced liver injuries as manifested by the reduction in toxin-mediated rise in serum enzymes in rats. The extract also showed protection against lethal dose of paracetamol in mice.
Table 6.5

Effect of *Berberis aristata* shoot and fruit extracts on strychnine-induced lethality in mice (*n* = 10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Lethality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vehicle + Strychnine</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(10 mL/kg + 0.4 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Shoot extract + Strychnine</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>(500 mg/kg + 0.4 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Fruit extract + Strychnine</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>(500 mg/kg + 0.4 mg/kg)</td>
<td></td>
</tr>
</tbody>
</table>

Salines/Extract was given orally, while strychnine was given intraperitoneally.
Table 6.6

*Effect of berberine on strychnine-induced lethality in mice (n = 10).*

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Lethality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vehicle + Strychnine</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(1 mL/Kg + 0.4 mg/Kg)</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Berberine + Strychnine</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>(4 mg/Kg + 0.4 mg/Kg)</td>
<td></td>
</tr>
</tbody>
</table>

Salines/berberine was given orally, while strychnine was given intraperitoneally.
Both paracetamol and CCl₄ share a common property to be converted into their respective reactive metabolites N-acetyl-p-benzoquinonimine (NAPQI) and halogenated free radicals (HFR) by hepatic cytochrome P-450 (Facker et al., 1978; van de Straat et al., 1987). The massive production of reactive species may lead to depletion of protective physiological moieties (glutathione and α-tocopherol, etc.), ensuing wide-spread propagation of the alkylation as well as peroxidation, causing damage to the macromolecules in vital biomembranes (Pesh-Imam and Recknagel, 1977; Aldridge, 1981). The inhibitors of microsomal drug metabolizing enzymes (MDME) can impair the bioactivation of paracetamol and CCl₄ into their respective reactive species and thus provide protection against the prevailing hepatocellular damage (Castro et al., 1974; Nelson et al., 1980).

The plant extracts and berberine were subjected to pentobarbital sleep study to evaluate its possible inhibitory effect on MDME (Shin, 1989). The duration of pentobarbital-induced sleep is a reliable index for the activity of hepatic MDME (Conney, 1967) and the inhibitors of MDME are likely to prolong pentobarbital-induced sleeping time (Fujimoto et al., 1960). The observed increase in pentobarbital-induced sleeping time (P<0.01) following extracts and berberine treatment, suggested that MDME inhibitory activity might be responsible for hepatoprotection. However, pentobarbital-induced sleep duration can also be increased by CNS depressing drugs without any effect on MDME activity (Shin, 1989). The sedative and MDME inhibitory activities can be differentiated by means of strychnine toxicity test, where MDME inhibitors potentiate the famous convulsive activity, by preventing its metabolic inactivation (Kato, 1968). The observed increase in mortality rate on administration of the sub-lethal dose of strychnine is thus confirmation of the presence of MDME inhibitory in plant extracts (shoots and fruits) and berberine.
The compounds with methylenedioxyber-zene (MDB) group are known inhibitors of MDME (Anders, 1968) and berberine is a MDB group carrying alkaloids (Anonymous, 1989) reported to be present in this plant (Chandra and Todaria, 1983). The observed inhibitory effect of plant extracts upon MDME may be due to the presence of berberine and other similar compounds as plant constituents. The exact mode of hepatoprotective action is likely to be speculative at this stage but the possible presence of MDME inhibitory activity in plant extract may be responsible for its hepatoprotective activity.

The inhibitors of MDME can provide protection against the hepatotoxicity only when they are given before the metabolic activation of the hepatotoxin and fails to provide any protection after generation of reactive metabolites (Castro et al., 1974; Neison et al., 1980). Following ingestion, paracetamol and CCl₄ are metabolized to their respective reactive species within six hours (Bramanti et al., 1978; Akintonwa and Essien, 1990) and hepatotoxicity can be monitored by measuring serum transaminases at 24 hrs. The plant extracts (shoots and fruits) extract and berberine treatment started 6 hrs after the paracetamol administration inhibited the spread of hepatic damage as manifested by insufficient release of cytoplasmic enzymes.

The observed curative effect of plant extracts against paracetamol can be attributed to the reported presence of berberine as a plant constituent (Chandra and Todaria, 1983) because the berberine mediated curative effect was similar to that of the plant extracts. In addition, the other plant constituents such as flavonoids (Shapiro et al., 1983; Vereskovskii and Shapiro, 1985), ascorbic acid (Shapiro et al., 1983; Karitnova, 1986), β-carotene (Chandra and Todaria, 1983), and Tannins (Shapiro et al., 1983) may also have contributed toward the observed hepatoprotective activity. The flavonoids are antioxidants (Torel et al., 1986;
Faurè et al., 1990), free radical scavengers (Bors and Saran, 1987; Husain et al., 1987) and anti-lipoperoxidant (Younes and Siegers, 1981; Robak et al., 1986; Ratty and Dae, 1988) leading to hepatoprotection (Kiso et al., 1984; Handa et al., 1986). Similarly, ascorbic acid serves as antioxidant (Demopoulos, 1973; Bus and Gibson, 1984), inhibits covalent binding of NAPQI to vital macromolecules (Lake et al., 1981) and consequently can minimize toxic damage (Harman, 1985). Moreover, carotenoids are also reputed to be antioxidants (Kläui, 1982) and thus showing anti-hepatotoxic activity (Oshima et al., 1984). Furthermore, the hepatoprotective potential of tannins (Hikino et al., 1985) is also well documented.

The curative effect exhibited by berberine against paracetamol-induced hepatic damage is difficult to explain, however, the reported choleretic (Velluda et al., 1958; Turova et al., 1964) property might have some beneficial role to play.

However, the anomalous observation due to inability of plant extracts and berberine treatment to curtail progression of hepatic damage after CCl₄ activation can partly be justified on the basis of reported facts. The paracetamol toxicity following NAPQI generation is chiefly due to oxidative stress and can effectively be ameliorated by antioxidants (Harman, 1985), whereas, the hepatic damage due to HFR may be due to lipid peroxidation (Bus and Gibson, 1979) as well as alkylation (Dogterom et al., 1988). The possible presence of antioxidant and anti-lipoperoxidant activities in plant extracts effective against NAPQI, can only inhibit the lipid peroxidation process but were unable to prevent the alkylation process due to HFR (Poli et al., 1989).

The crude extracts of Berberis aristata (shoots and fruits) and berberine afforded protection against paracetamol and CCl₄-induced liver damage when given before the metabolic activation of toxins. However, the protection against the metabolically generated reactive species is effective only against the paracetamol.
The hepatoprotective action of extracts may be mediated through inhibition of hepatic MDME, free radical scavengers, antioxidants and/or anti-lipoperoxidants; whereas berberine is likely to possess MDME inhibitory and choleretic properties. The plant material is safe as is obvious by the lack of any symptom of acute toxicity at an oral dose of as high as 4 g/kg. Thus, this study provides scientific basis for the traditional use of Berberis aristata shoots and fruits in hepatobiliary disorders.

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7. *Cichorium intybus*
7. *Cichorium intybus*

7.1 *Summary*

The hepatoprotective activity of *Cichorium intybus* extracts (shoots and seeds) and a pure compound (esculetin) were investigated against paracetamol and CCl₄-induced hepatic damage. Paracetamol produced 100% mortality at the dose of 1 g/Kg in mice while pretreatment of animals with shoots (1 g/Kg) and seeds extract (500 mg/Kg) reduced the death rate to 40% and 30% respectively. Oral administration of paracetamol (640 mg/Kg) and CCl₄ (1.5 mL/Kg) produced liver damage in rats as manifested by significant rise (P < 0.05) in serum enzyme values (ALP, GPT and GPT). Pre-treatment of rats with four oral doses of shoots (600 mg/Kg) and seeds (500 mg/Kg) extract as well esculetin (6 mg/Kg) at 12 hrs interval, prevented the paracetamol (640 mg/Kg) and CCl₄ (1.5 mL/Kg)-induced rise in serum enzymes (ALP, GOT and GPT). At these doses the plant extracts and pure compound were found devoid of any inhibitory effect on microsomal drug metabolizing enzymes (MDME) as suggested by the observed lack of increase in pentobarbital (75 mg/Kg)-induced sleep. However, they prevented the CCl₄-induced prolongation in pentobarbital sleeping time in mice, confirming their protective effect against CCl₄. Moreover, both the shoots and seeds extracts exhibited the Ca²⁺ channel blocking (CCB) activities in *in vitro* experiments on isolated rabbit jejunum. So, the observed the hepatoprotective activity of the plant extracts may be mediated through their CCB and known anti-inflammatory activities of esculetin may be contributing toward the hepato-protection.
7.2 Plant Description/Literature Survey

*Cichorium intybus*, Linn. (Compositae) locally known as "Wild Kasni", is a small-to-medium sized perennial herb growing wild in the Punjab and N.W.F.P. provinces of Pakistan (Nadkarni, 1976). It is mostly used abroad as a salad plant and the roasted root is an important adulterant of coffee (Nadkarni, 1976). Extensive phytochemical work on the plant material revealed the presence of vitamins i.e., ascorbic acid (Gallot, 1941) retinol, thiamine, riboflavin and niacin (Mac-Gillivary et al., 1952), carotenoids (Wills et al., 1986), inulin (Kroeber, 1950), sesquiterpenes i.e., cichorin, esculetin, esculin, lactucin and lactacopicrin (Hee, 1965), hydroxycinnamic acids (Scarpati and Orienti, 1958; Dem’yarenko and Dranik, 1972) and zinc (Bertrand and Benzon, 1928). The plant constituents also exhibit inhibitory activity against some strains of bacteria and fungi when tested in vitro (Sproston et al., 1948). Moreover, it shows laxative (Caravaggi and Manfredi, 1937), choleretic (Grabe and Heidz, 1935), cytotoxic (Seto, et al., 1988) and hypocholesteremic (Kaur et al., 1991) activities.

The plant is also considered, as an indigenous source of medicine to have stomachic, carminative, diuretic, anti-asthmatic and anti-inflammatory actions (Said, 1982). Furthermore, plant seeds are used in biliary disorders including jaundice and are ingredient of several recipes prescribed by traditional healers to cure hepato-biliary complaints (Said, 1982).

Esculetin is one of the coumariins found in *Cichorium intybus* (Hee, 1965) and is known to possess analgesic (Tubaro et al., 1988), anti-inflammatory (Tajima et al., 1986; Loggia et al., 1988; Tubaro et al., 1988), anti-tumor (Nakadate et al., 1984; Noguchi et al., 1993; Kitagawa and Noguchi, 1994), anti-arrhythmic (Riedel and Mest, 1987; Riedel et al., 1988; Mest et al., 1988), anti-
steroidogenic (Hata et al., 1987; Solano et al., 1988) properties. It inhibits various enzymes such as cyclooxygenase (Sekiya et al., 1982; Kimura et al., 1985; De-Clerck and Xhonneux, 1986; Simmet et al., 1987), 5-lipoxygenase (Neichi et al., 1983; Panossian, 1984; Kimura et al., 1985; Leung and Ip, 1986), 12-lipoxygenase (Morita et al., 1983; Neichi et al., 1983; Flatman et al., 1986), xanthine oxidase (Chang et al., 1994), catechol-O-methyl transferase (Veser, 1987), and NADPH oxidase (Ozaki et al., 1986). Moreover, the modified arachidonic acid metabolism is reflected in the inhibition of a number of important biological activities such as activation of natural killer cells against tumor cells (Leung and Ip, 1986), target cells lysis by natural killer cells (Leung et al., 1986), generation of O2 radical by neutrophils (Ozaki et al., 1986), interleukin-2 production (Kato et al., 1986), interleukin-2 dependent lymphocytes proliferation (Hata et al., 1987), mouse myoblast fusion (Steiner et al., 1984), interleukin 1α and interleukin 1β mediated inhibition of DNA synthesis and cell division in mouse osteoblasts (Ohmori et al., 1988).

The studies pertaining to evaluation of protective effect of the shoot, seed extracts and esculentin were carried at different occasions, so separate control groups had to be included in each case. Moreover, the dose of shoot extract used in hepato-protective studies was higher (600 mg/Kg) compared to seed extract in this study or usual dose in other plants (500 mg/Kg). This was because the 500 mg/Kg dose of shoot extract was unable to show clear hepatoprotection and consequently, the dose has to be increased and discussed in the discussion section.
7.3 Materials and Methods

7.3.1 Plant extract

*Cichorium intybus* shoots (aerial parts) were collected during march 1991 from rural area of Okara district in the province of Punjab, whereas seeds were purchased from local herbal store and authenticated with the help of a botanist at The University of Karachi. Seeds were powdered directly, while shoots were subjected to shade drying and powdered subsequently. The powdered materials were macerated separately in 80% aqueous-methanol for one week with occasional shaking. The extracts were filtered and concentrated to dark green residue (shoots) and dark greenish brown oily paste (seeds) under reduced pressure on a rotary evaporator, with approximate yield of the concentrated extract were 8.5% and 10% respectively. The esculetin was purchased from Sigma chemical company St. Louis, Mo, USA.

7.3.2 Animal studies

The effects of plant extracts (shoot and seed) were studied on the paracetamol-induced lethality; paracetamol- and CCl₄-induced hepatotoxicity; pentobarbital-induced sleep duration and CCl₄-induced prolongation of pentobarbital sleeping time; spontaneous movements as well as K⁺-induced contractions of rabbit jejunum by the methods described in general experimental section (chapter 2). The above mentioned experiments were also repeated for esculetin except, paracetamol-induced lethality in mice and *in vitro* experiments on rabbit jejunum.
7.4 Results

7.4.1 Effect on paracetamol-induced lethality

Paracetamol at the dose of 1 g/Kg induced 100% lethality in mice. In a group of animals pre-treated with shoot extract (1 g/Kg), the same dose of paracetamol killed only 4 out of 10 animals resulting in 60% protection against lethal effect of paracetamol, whereas one half the dose of seed extract (500 mg/Kg) exhibited 70% protection (Table 7.1).

7.4.2 Shoot extract and paracetamol-induced toxicity

Control (saline + vehicle) serum values of ALP, GOT and GPT in rats were found to be 231 ± 12, 97 ± 10 and 26 ± 02 IU/L (n=10) respectively (Fig. 7.1 ; Appendix 27), while a toxic dose of paracetamol (640 mg/Kg) raised significantly (P<0.001), the respective serum enzyme values to 410 ± 24, 1447 ± 182 and 899 ± 201 IU/L. Group 3 was pre-treated with shoots extract (600 mg/Kg, orally, twice daily for 2 days) to determine its effect on paracetamol-induced rise in serum enzymes. The serum enzyme values in pre-treated group were found to be 224 ± 21 (ALP), 487 ± 301 (GOT) and 77 ± 13 (GPT), which are significantly lower (P<0.05), than the values of toxic control and were similar to the control values (P>0.05).

7.4.3 Seed extract and paracetamol-induced toxicity

Control (saline + vehicle) serum values of ALP, GOT and GPT in rats were found to be 198 ± 15, 76 ± 07 and 39 ± 09 IU/L (n=10) respectively
Table 7.1
Effect of *Cichorium intybus* shoot and seed extracts on paracetamol-induced lethality in mice (*n* = 10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Lethality</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Paracetamol (20 mL/Kg + 1 g/Kg)</td>
<td>10/10</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>Shoot extract + Paracetamol (1 g/Kg + 1 g/Kg)</td>
<td>4/10</td>
<td>60</td>
</tr>
<tr>
<td>3.</td>
<td>Seed extract + Paracetamol (500 mg/Kg + 1 g/Kg)</td>
<td>3/10</td>
<td>70</td>
</tr>
</tbody>
</table>

Saline/extract and paracetamol were given orally.
**Fig. 7.1** Effect of *Cichorium intybus* shoots on paracetamol-induced elevation of serum enzymes in rats.
(Fig. 7.2; Appendix 28), while a toxic dose of paracetamol (640 mg/Kg) raised significantly \( P < 0.01 \), the respective serum enzyme values to 393 ± 28, 767 ± 215 and 692 ± 191 IU/L. Group 3 was pre-treated with seeds extract (500 mg/Kg, orally, twice daily for 2 days) to determine its effect on paracetamol-induced rise in serum enzymes. The serum enzyme values in pre-treated group were found to be 228 ± 16 (ALP), 68 ± 10 (GOT) and 41 ± 08 (GPT), which are significantly lower \( P < 0.01 \), than the values of toxic control and were similar to the control values \( P > 0.05 \).

7.4.4 Esculetin and paracetamol-induced toxicity

Control (saline + vehicle) serum values of ALP, GOT and GPT in rats were found to be 219 ± 10, 84 ± 12 and 33 ± 07 IU/L (n=10) respectively (Fig. 7.3; Appendix 29), while a toxic dose of paracetamol (640 mg/Kg) raised significantly \( P < 0.05 \), the respective serum enzyme values to 262 ± 14, 1210 ± 242 and 735 ± 223 IU/L. Group 3 was pre-treated with esculetin (6 mg/Kg, orally, twice daily for 2 days) to determine its effect on paracetamol-induced rise in serum enzymes. The serum enzyme values in pre-treated group were found to be 204 ± 18 (ALP), 137 ± 41 (GOT) and 71 ± 34 (GPT), which are significantly lower \( P < 0.05 \), than the values of toxic control and were similar to the control values \( P > 0.05 \).

7.4.5 Shoot extract and CCl₄-induced toxicity

The estimated values of serum ALP, GOT and GPT in control (saline +
Fig. 7.2 Effect of *Cichorium intybus* seeds on paracetamol-induced elevation of serum enzymes in rats
Fig. 7.3 Effect of esculetin on paracetamol-induced elevation of serum enzymes in rats.
vehicle) group of rats were found to be 176 ± 05, 95 ± 11 and 42 ± 13 IU/L (n= 10) respectively (Fig. 7.4; Appendix 30), which were raised significantly (P<0.01) to respective values of 276 ± 13, 532 ± 89 and 263 ± 74 after administration of a toxic dose of CCl₄ (1.5 mL/Kg). However, pretreatment of animals with shoots extract (600 mg/Kg, orally, twice daily for 2 days) returned the serum ALP, GOT and GPT values to 173 ± 11, 127 ± 2) and 63 ± 15 IU/L respectively, which are significantly lower (P<0.05) than values of toxic control and were close to the normal values (P>0.05).

7.4.6 Seed extract and CCl₄-induced toxicity

The estimated values of serum ALP, GOT and GPT in control (saline + vehicle) group of rats were found to be 215 ± 16, 79 ± 18 and 49 ± 10 IU/L (n= 10) respectively (Fig 7.5; Appendix 31), which were raised significantly (P<0.01) to respective values of 312 ± 20, 503 ± 98 and 407 ± 109 after administration of a toxic dose of CCl₄ (1.5 mL/Kg). However, pretreatment of animals with seeds extract (500 mg/Kg, orally, twice daily for 2 days) returned the serum ALP, GOT and GPT values to 222 ± 27, 114 ± 23 and 68 ± 14 IU/L respectively, which are significantly lower (P<0.05) than values of toxic control and were close to the normal values (P>0.05).

7.4.7 Esculetin and CCl₄-induced toxicity

The estimated values of serum ALP, GOT and GPT in control (saline + vehicle) group of rats were found to be 198 ± 13, 103 ± 15 and 48 ± 12 IU/L
Fig. 7.4 Effect of *Cichorium intybus* shoots on carbon tetrachloride-induced elevation of serum enzymes in rats.
Fig. 7.5 Effect of Cichorium intybus seeds on carbon tetrachloride-induced elevation of serum enzymes in rats.
(n = 10) respectively (Fig 7.6; Appendix 32), which were raised significantly
(P < 0.05) to respective values of 267 ± 25, 868 ± 249 and 576 ± 159 after
administration of a toxic dose of CCl₄ (1.5 mL/Kg). However, pretreatment of
animals with esculetin (6 mg/Kg, orally, twice daily for 2 days) returned the serum
ALP, GOT and GPT values to 207 ± 12, 133 ± 23 and 85 ± 27 IU/L
respectively, which are significantly lower (P < 0.05) than values of toxic control
and were close to the normal values (P > 0.05).

7.4.8 Shoots extract and pentobarbital-induced sleep

Effect of shoots extract on pentobarbital sleeping time as well as on CCl₄-
induced prolongation of pentobarbital sleeping time was studied in mice and the
results are shown in Table 7.2. Pentobarbital at a dose of 75 mg/Kg, i.p., caused
sleep in mice for a period of 111 ± 12 min (Mean ± S.E.M, n = 10).
Pentobarbital sleeping time in the group of animals pre-treated with shoot extract
(600 mg/Kg) was found to be 117 ± 09 min, which is similar to that in the control
group (P > 0.05).

7.4.9 Seeds extract and pentobarbital-induced sleep

Effect of seeds extract on pentobarbital sleeping time as well as on CCl₄-
induced prolongation of pentobarbital sleeping time was studied in mice and the
results are shown in Table 7.3. Pentobarbital at a dose of 75 mg/Kg, i.p., caused
sleep in mice for a period of 140 ± 11 min (Mean ± S.E.M, n = 10).
Pentobarbital sleeping time in the group of animals pre-treated with seeds extract

187
Fig. 7.6 Effect of esculetin on carbon tetrachloride-induced elevation of serum enzymes in rats.
Table 7.2

**Effect of *Cichorium intybus* shoots extract on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time in mice.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sleeping time</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline + Pentobarbital (10 mL/Kg + 75 mg/Kg)</td>
<td>111 ± 12</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Extract + Pentobarbital (600 mg/Kg + 75 mg/Kg)</td>
<td>117 ± 09</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>3</td>
<td>Saline + CCl₄ + Pentobarbital (10 mL/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>232 ± 23’</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>Extract + CCl₄ + Pentobarbital (600 mg/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>139 ± 18**</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 10 determinations. Salines/Extract/CCl₄ was given orally, while pentobarbital was given intraperitoneally.

* Compared to group 1 (control).

** Compared to group 3.
Table 7.3
Direct effect of *Cichorium intybus* seeds extract on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sleeping time</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Pentobarbital (10 mL/Kg + 75 mg/Kg)</td>
<td>140 ± 11</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Extract + Pentobarbital (&lt;500 mg/Kg + 75 mg/Kg)</td>
<td>128 ± 14</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>3.</td>
<td>Saline + CCl₄ + Pentobarbital (10 mL/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>272 ± 17*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4.</td>
<td>Extract + CCl₄ + Pentobarbital (500 mg/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>160 ± 20**</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 10 determinations. Salines/Extract/CCl₄ was given orally, while pentobarbital was given intraperitoneally.

* Compared to group 1 (control).

** Compared to group 3.
(500 mg/Kg) was found to be 128 ± 14 min, which is similar to that in the control group \( (P > 0.05) \).

7.4.10 Esculetin and pentobarbital-induced sleep

Effect of esculetin on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time was studied in mice and the results are shown in Table 7.4. Pentobarbital at a dose of 75 mg/Kg, i.p., caused sleep in mice for a period of 132 ± 15 min (Mean ± S.E.M, \( n = 10 \)). Pentobarbital sleeping time in the group of animals pre-treated with esculetin (6 mg/Kg) was found to be 139 ± 17 min, which is similar to that in the control group \( (P > 0.05) \).

7.4.11 Shoots extract and CCl₄-induced sleep increase

Pretreatment of animals with CCl₄ prolonged the pentobarbital sleeping time to 232 ± 23 min, the value that is significantly higher \( (P < 0.001) \) than that of the control value (Table 7.2). However, prior treatment of animals with shoots extract (600 mg/Kg) returned this CCl₄-induced prolongation of pentobarbital sleeping time to 139 ± 18 min, which is significantly lower than group 3 animals \( (P < 0.01) \) and close to the sleeping time of control group \( (P > 0.05) \).

7.4.12 Seeds extract and CCl₄-induced sleep increase

Pretreatment of animals with CCl₄ prolonged the pentobarbital sleeping time to 272 ± 17 min, the value that is significantly higher \( (P < 0.001) \) than that of the control value (Table 7.3). However, prior treatment of animals with seeds extract
Table 7.4
Direct effect of esculetin on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sleeping time</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Pentobarbital (3 mL/Kg + 75 mg/Kg)</td>
<td>132 ± 15</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Esculetin + Pentobarbital (6 mg/Kg + 75 mg/Kg)</td>
<td>139 ± 17</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>3.</td>
<td>Saline + CCl₄ + Pentobarbital (3 mL/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>242 ± 20*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4.</td>
<td>Esculetin + CCl₄ + Pentobarbital (6 mg/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>151 ± 16**</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 10 determinations. Salines/esculetin/CCl₄ was given orally, while pentobarbital was given intraperitoneally.

* Compared to group 1 (control).

** Compared to group 3.
(500 mg/Kg) returned this CCl₄-induced prolongation of pentobarbital sleeping time to 160 ± 20 min, which is significantly lower than group 3 animals and close to the sleeping time of control group ($P > 0.05$).

### 7.4.13 Esculetin and CCl₄-induced sleep increase

Pre-treatment of animals with CCl₄ prolonged the pentobarbital sleeping time to 242 ± 23 min, the value that is significantly higher ($P < 0.001$) than that of the control value (Table 7.4). However, prior treatment of animals with esculetin (6 mg/Kg) returned this CCl₄-induced prolongation of pentobarbital sleeping time to 151 ± 16 min, which is significantly ($P < 0.01$) lower than group 3 animals and close to the sleeping time of control group ($P > 0.05$).

### 7.4.14 Plant extracts and Ca²⁺ channel blocking activity

Shoot extract at the concentration of 0.3-3 mg/mL caused a concentration-dependent inhibition of spontaneous movements of rabbit jejunum as shown in Fig. 7.7. The seed extract also caused similar inhibitory effect on spontaneous movements but at much lower concentrations (0.1-0.3 mg/mL). This inhibitory effect of both shoot and seed extracts were reversible as the tissue regained its spontaneous activity after washing the tissue several times with the fresh bathing fluid.

Potassium at the concentration of 40 mM caused a sustained tonic contraction which allowed to obtain concentration-dependent inhibitory responses (Fig. 7.7). Addition of shoot or seed extract produced concentration-dependent
Fig. 7.7. A representative tracing showing inhibitory effect of alcoholic extract of *Cichorium intybus* shoot on spontaneous movements (upper panel) of isolated rabbit jejunum. The seed extract had similar activity but at lower doses (not shown). The comparison of inhibitory effects of *Cichorium intybus* shoot and seed extract against K⁺-induced contractions is shown in the middle and lower panels.
Fig. 7.8. A representative tracing showing comparison of inhibitory effects of alcoholic extract of *Cichorium intybus* shoot and seed at different doses agonist Ca$^{++}$ supplementation in Ca$^{++}$ free kreb solution. The upper panel shows the control effect of Ca$^{++}$ supplement in the presence of normal saline.
RABBIT JEJUNUM

--- Ca++ free kreb's solution ---

--- Ca++ free kreb's solution ---

--- Ca++ free kreb's solution ---

--- Ca++ free kreb's solution ---

--- Cl shoot (3 mg/ml) ---

--- Cl seed (0.3 mg/ml) ---

--- Cl shoot (3 mg/ml) ---

--- Cl seed (0.3 mg/ml) ---
inhibition of $K^+$-induced contractions, and the seed extract was found approximately 10 time more potent than the shoot extract.

In another set of experiments, these extracts were also tested for their inhibitory effect against $Ca^{2+}$ supplementation. When the bathing fluid was replaced with $Ca^{2+}$ free Kreb's solution, the spontaneous movements of the tissue were abolished within one minute (Fig. 7.8). Addition of $Ca^{2+}$ restored spontaneous activity in a concentration-dependent manner (15-25 μM). Supplementation of calcium failed to restore spontaneous movements of the tissue in the presence of shoot extract (3 mg/mL) or seed extract (0.3 mg).

The plant extracts (shoots and seeds) up to an oral dose of 3 g/Kg were found devoid of any lethal effect and no apparent behavioral changes were observed.

7.4 Discussion

Paracetamol- and CCl₄-induced hepatic injuries are commonly used models for hepatoprotective drug screening (Slater, 1965; Plaa and Hewitt, 1982). The elevated serum enzyme levels has been attributed to the damaged structural integrity of the liver (Chenoweth and Hake, 1962), because these are cytoplasmic in location and are released into circulation after cellular damage (Sallie et al., 1991).

Paracetamol and CCl₄ are converted to their reactive metabolites i.e., N-acetyl-p-benzoquinoneimine (NAPQI) and halogenated free radicals (HFR) respectively by their respective specific isozyme of cytochrome P-450.
al., 1978; van de Straat et al., 1987). Physiologically important protective mechanisms involving both vitamin E (α-tocopherol) and reduced glutathione (GSH) are available to curtail progression of cellular damage (Potter et al., 1974). However, the massive production of reactive species may lead to depletion of protective moieties, ensuing wide-spread propagation of the alkylation as well as peroxidation, causing damage to both lipids and proteins present in biomembranes of microsomes and mitochondria (Rao and Recknagel, 1969; Pesh-Imam and Recknagel, 1977; Aldridge, 1981). These bio-activation mediated cytotoxic effects are subject of active research and the toxic manifestations can effectively be minimized by cytochrome P-450 inhibitors (Castro et al., 1974; Tredger et al., 1985; Beyeler et al., 1988), GSH precursors (Ferreira et al., 1974; Prescott et al., 1979), free radical scavengers (Mailing et al., 1974), antioxidants (Gazzard et al., 1974; Koester-Albercht et al., 1979; Yasuda et al., 1980), sulfhydryl agents (Strubelt et al., 1974; Mark et al., 1990) and Ca²⁺ channel blockers (Landon et al., 1986; Thibault et al., 1991; Gilani and Janbaz, 1993).

The plant extracts (shoots and seeds) and esculetin seem to preserve the structural integrity of the hepato-cellular membrane. This was evident from the significant reduction in paracetamol and CCl₄-induced rise in serum ALP, GOT and GPT levels in rats and inhibition of CCl₄-induced prolongation of pentobarbital sleeping time in mice. The seeds extract was proved to be more potent than the shoot extract in providing hepato-protection.

The plant extracts were administered to mice along with pentobarbital to see whether the plant extracts exhibit inhibitory effect on hepatic microsomal drug
metabolizing enzymes (MDME) and possible changes in sleep duration were recorded. The fact that the plant extracts did not prolong pentobarbital sleeping time ($P > 0.05$) suggests that it is devoid of inhibitory effect on hepatic MDME such as cytochrome P-450 and hence hepatoprotective effect is/are mediated through some other mechanism(s).

The observed protective effect of plant extracts against paracetamol and CCl$_4$ can be attributed to the reported presence of esculetin (Hee, 1965), ascorbic acid (Gallot, 1941), carotenoids (Willis et al., 1986), esculetin (Hee, 1965) and zinc (Bertrand and Benz, 1928) among the plant constituents. Esculetin, a known anti-inflammatory (Tajima et al., 1986; Loggia et al., 1988; Tubaro et al., 1988) and inhibitor of natural killing process (Leung et al., 1986) has exhibited hepatoprotection. The ascorbic acid serves as an antioxidant (Demopoulos, 1973; Bus and Gibson, 1984), inhibits covalent binding of NAPQI to vital macromolecules (Lake et al., 1981) and consequently can minimize toxic damage (Harman, 1985). The carotenoids are also reputed to be antioxidants (Klæui, 1982) and thus showing antihepatotoxic activity (Oshima et al., 1984). Similarly, zinc induces hepatic metallothioneins synthesis (Nakamura et al., 1982), which can scavenge chemically reactive species and hence provide protection against toxic liver damage (Clark and Lui, 1986).

The plant extracts (shoots and seeds) was found to inhibit $K^+$-induced contractions of rabbit jejunum. Since, contractions induced by high $K^+$ ($>30$ mM) are mediated through extracellular Ca$^{2+}$ influx through voltage-dependent slow calcium channels (Bolton, 1979) and inhibitory effect of plant extracts on such
contractions may be due to calcium channel blockade.

The cytosolic free calcium concentration in hepatocytes is known to be increased during the process of experimental liver damage (Farber et al., 1982; Moore et al., 1985) and calcium channel blocking drugs (i.e., nifedipine, diltiazem and verapamil) are capable to provide protection against paracetamol- and CCl₄-induced hepatopathy (Landon et al., 1986; Thibault et al., 1991). The protective effect of extracts against paracetamol- and CCl₄-induced liver damage may be attributed to the presence of Ca²⁺ channel blocker(s) and this is further strengthened with the observation that both hepatoprotective and CCB activities are concentrated more in the seed extract than in the extract derived from shoots. The dose-dependent relaxation response with shoot extract was observed at the concentration of 1-3 mg/mL, whereas the similar activity of seed extract was observed at a smaller concentration (0.3-1 mg/mL). Similarly, the seed extract provided 70% protection against the paracetamol-induced lethality at a dose (500 mg/Kg) one half to that of shoot extract (1000 mg/Kg), which afforded only 60% protection. Furthermore, the degree of hepatoprotection provided by the seed extract (500 mg/Kg) was greater than that observed with the shoot extract even at larger dose (600 mg/Kg). Similarly, seed extract was also found to be more potent in causing inhibition of CCl₄-induced prolongation of pentobarbital sleeping time in mice (Table 7.2-7.3).

The plant material is safe as is obvious by lack of any symptom of acute toxicity at an oral dose of as high as 3 g/Kg and this study rationalizes the traditional use of Cichorium intybus seeds and shoots in hepato-biliary diseases.
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8. *Cyperus scariosus*
8. *Cyperus scariosus*

8.1 *Summary*

The hepatoprotective activity of aqueous-methanolic extract of *Cyperus scariosus* was investigated against paracetamol and CCl₄-induced hepatic damage. Paracetamol produced 100% mortality at the dose of 1 g/Kg in mice while pretreatment of animals with plant extract (500 mg/Kg) reduced the death rate to 30%. Paracetamol at the dose of 640 mg/Kg produced liver damage in rats as manifested by the rise in serum levels of alkaline phosphatase (ALP), GOT and GPT to $430 \pm 68$, $867 \pm 305$ and $732 \pm 212$ IU/L ($n=10$) respectively, compared to respective control values of $202 \pm 36$, $59 \pm 14$ and $38 \pm 07$. Pretreatment of rats with plant extract (500 mg/Kg) lowered significantly ($P<0.05$) the respective serum ALP, GOT and GPT levels to $192 \pm 31$, $63 \pm 09$ and $35 \pm 08$. The hepatotoxic dose of CCl₄ (1.5 mL/Kg; orally) raised serum ALP, GOT and GPT levels to $328 \pm 30$, $493 \pm 102$ and $357 \pm 109$ IU/L ($n=10$) respectively, compared to respective control values of $177 \pm 21$, $106 \pm 15$ and $47 \pm 12$. The same dose of plant extract (500 mg/Kg) was able to prevent significantly ($P<0.05$), the CCl₄-induced rise in serum enzymes and the estimated values of ALP, GOT and GPT were $220 \pm 30$, $207 \pm 95$ and $75 \pm 38$ respectively. The plant extract also prevented CCl₄-induced prolongation in pentobarbital sleeping time confirming hepatoprotective activity. Moreover, the plant extract exhibited Ca²⁺ channel blocking activity in *in vitro* experiments on rabbit jejunum. The observed hepatoprotective activity is seemed to be mediated through Ca²⁺ channel blocking activity.
8.2 Plant Description

*Cyperus scariosus*, Br. (Syn: *C. pertenuis*, *Roxb.*; family: Cyperaceae) is a delicate grass, growing luxuriously in damp places of eastern and southern parts of Indo-Pak subcontinent (Kirtikar and Basu, 1918). The brown colored plant rhizomes has a folkloric reputation as cordial, tonic, emmenagogue, vermifuge, diuretic, diaphoretic and desiccant (Kirtikar and Basu, 1918; Watt, 1972; Said, 1982). It remained to be an important component of several prescriptions used in native system of medicine to treat a variety of diseases including diarrhea, epilepsy, gonorrhoea, syphilis and liver damage (Kirtikar and Basu, 1918; Watt, 1972; Said, 1982). The essential oil obtained after steam distillation of rhizomes and roots of the plant has its value in perfumery (Kahol, 1987), and is also known to possess antibacterial (Laharia and Rao, 1979), antifungal (Desmukh and Jain, 1985) as well as plant growth-regulating properties (Kalsi et al., 1980). We have recently shown that the crude extract of plant tubers exhibits antihypertensive and spasmolytic activities possibly through calcium channel blocking action (Gilani, et al., 1994a).

Phytochemical studies revealed the presence of sesquiterpenes (Nerali and Chakravarti, 1969; Nerali, et al., 1970), steroidal saponin (Bhatt, et al., 1982), aurone (Bhatt, et al., 1984) and substituted hydrocarbons (Neville, et al., 1968; Uppal, 1984). However, scientific studies on its usefulness in liver damage is lacking and in the present investigation, plant extract was tested against paracetamol as well as carbon tetrachloride (CCl₄)-induced liver injuries to validate the folkloric use of *Cyperus scariosus* in hepatic damage.
8.3 Materials and Methods

8.3.1 Plant extract

*Cyperus scariosus* tubers were purchased from local herbal store and authenticated with the help of a botanist at The University of Karachi. The plant material was powdered and macerated in 80% aqueous-methanol for one week with occasional shaking. The extract was filtered and concentrated to dark brown residue under reduced pressure on a rotary evaporator, with approximate yield of 8%.

8.3.2 Animal studies

The effect of shoot and seed extracts was studied on the paracetamol-induced lethality; paracetamol- and CCl₄-induced hepatotoxicity; pentobarbital-induced sleep duration and CCl₄-induced prolongation of pentobarbital sleeping time; spontaneous movements as well as K⁺-induced contractions of rabbit jejunum by the methods described general method section (chapter 2).

8.4 Results

8.4.1 Effect on paracetamol-induced lethality

Paracetamol at the dose of 1 g/Kg induced 100% lethality in mice. In a group of animals pre-treated with tuber extract (500 mg/Kg), the same dose of paracetamol killed only 3 out of 10 animals resulting in 70% protection against lethal effect of paracetamol (Table 8.1).

8.4.2 Effect on paracetamol-induced hepatotoxicity

Control (saline + vehicle) serum values of ALP GOT and GPT in rats were
Table 8.1
Effect of *Cyperus scariosus* rhizomes extracts on paracetamol-induced lethality in mice (*n* = 10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Lethality</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Paracetamol</td>
<td>10/10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(10 mL/Kg + 1 g/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Extract + Paracetamol</td>
<td>3/10</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>(500 mg/Kg + 1 g/Kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Saline/extract and paracetamol were given orally.
found to be 202 ± 36, 59 ± 14 and 38 ± 07 IU/L (n = 10) respectively (Fig. 8.1; Appendix 33), while a toxic dose of paracetamol (640 mg/Kg) raised significantly (P<0.05), the respective serum enzyme values of 430 ± 68, 867 ± 305 and 732 ± 212 IU/L. Group 3 was pre-treated with crude extract (500 mg/Kg, orally, twice daily for 2 days) to determine its effect on paracetamol-induced rise in serum enzymes. The serum enzymes values in pre-treated group were found to be 192 ± 31 (ALP), 63 ± 09 (GOT) and 35 ± 08 (GPT), which are lower (P<0.05) than the values of toxic control and were similar to the control values (P>0.05).

8.4.3 Effect on CCl₄-induced hepatotoxicity

The estimated values of serum ALP, GOT and GPT in control (saline + vehicle) group of rats were found to be 177 ± 21, 106 ± 15 and 47 ± 12 IU/L (n = 10) respectively (Fig. 8.2; Appendix 34), which were raised significantly (P<0.05) to respective values of 326 ± 30, 493 ± 102 and 357 ± 109 after administration of a toxic dose of CCl₄ (1.5 mL/Kg). However, pretreatment of animals with plant extract (500 mg/Kg, orally, twice daily for 2 days) returned the serum ALP, GOT and GPT values to 220 ± 30, 207 ± 90 and 75 ± 38 IU/L respectively, which are significantly lower (P<0.05) than the values of toxic control and were close to the control values (P>0.05).

8.4.4 Effect on pentobarbital-induced sleep

Effect of tuber extract on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time was studied in mice and the results are shown in Table 8.2. Pentobarbital at a dose of 75 mg/Kg, i.p., caused
Fig. 8.1 Effect of *Cyperus scariosus* rhizomes extract on paracetamol-induced elevation of serum enzymes in rats.
Fig. 8.2 Effect of *Cyperus scariosus* rhizomes on carbon tetrachloride-induced elevation of serum enzymes in rats.
sleep in mice for a period of 177 ± 12 min (Mean ± S.E.M., n = 10). Pentobarbital sleeping time in the group of animals pre-treated with test material was found to be 189 ± 17 min, which is similar to that in the control group (P > 0.05).

8.4.5 Effect on CCl₄-induced pentobarbital sleep increase

Pretreatment of animals with CCl₄ prolonged the pentobarbital sleeping time to 267 ± 21 min, the value that is significantly higher (P < 0.01) than that of control (Table 8.2). However, prior treatment of animals with crude extract returned this CCl₄-induced prolongation of pentobarbital sleeping time to 184 ± 23 min, which was significantly less (P < 0.01) than group 3 value and close to the control sleeping time (P > 0.05).

8.4.6 Ca²⁺ channel blocking effect

Crude extract at the concentration of 0.1-1 mg/mL caused a concentration-dependent inhibition of spontaneous movements of rabbit jejunum as shown in Fig. 8.3. This effect was reversible as the tissue regained its spontaneous activity after washing the tissue several times with the fresh bathing fluid.

K⁺ at the concentration of 40 mM caused a sustained tonic contraction which allowed to obtain concentration-dependent inhibitory responses (Fig. 8.3). Addition of plant extract at 0.1 mg/mL, caused a slight inhibition, while at 0.3 mg/mL, it produced almost 50% relaxant effect, whereas next higher concentration (1 mg/mL) completely suppressed the contractile effect.

When the bathing fluid was replaced with Ca²⁺ free Kreb’s solution, the spontaneous movements of the tissue were abolished within one minute (Fig. 8.4).
Table 8.2

Effect of *Cyperus scariosus* rhizomes extract on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sleeping time</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Pentobarbital (10 mL/Kg + 75 mg/Kg)</td>
<td>177 ± 12</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Extract + Pentobarbital (500 mg/Kg + 75 mg/Kg)</td>
<td>189 ± 17</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>3.</td>
<td>Saline + CCl₄ + Pentobarbital (10 mL/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>267 ± 12*</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>4.</td>
<td>Extract + CCl₄ + Pentobarbital (500 mg/Kg + 1.5 mL/Kg + 75 mg/Kg)</td>
<td>184 ± 23*</td>
<td>&gt;0.01</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 10 determinations. Salines/Extract/CCl₄ was given orally, while pentobarbital was given intraperitoneally.

* Compared to group 1 (control).

* Compared to group 3.
Fig. 8.3. A representative tracing showing effect of alcoholic extract of *Cyperus scariosus* (CS) on spontaneous movements (upper panel) and on K⁺-induced contraction (lower panel) in the isolated rabbit jejunum preparation.
Fig. 8.4. A representative tracing showing effect of Ca\(^{++}\) supplementation in the absence (upper panel) and presence of alcoholic extract of *Cyperus scariosus* (CS) (lower panel) on spontaneous movements of rabbit jejunum, suspended in Ca\(^{++}\) free kreb's solution.
Addition of Ca^{2+} (10-30 µM) restored spontaneous activity in a concentration-dependent manner. Plant extract was also tested in Ca^{2+} free Kreb's solution for its effect on Ca^{2+} responses. Supplementation of Ca^{2+} (30-50 µM) failed to restore spontaneous movements of the tissue in the presence of extract (1 mg/mL).

The plant extract up to an oral dose of 3 g/Kg was found devoid of any lethal effect and no apparent behavioral change was observed.

8.5 Discussion

The aqueous-methanolic extract of *Cyperus scariosus* exhibited hepatoprotection against both paracetamol and CCl₄-induced liver injuries as manifested by reduction in toxin-mediated rise in serum transaminases in rats. Similarly, pretreatment of animals with plant extract afforded 70% protection against lethal dose of paracetamol and also prevented CCl₄-induced prolongation of pentobarbital sleeping time in mice.

Liver injuries induced by paracetamol and CCl₄ are commonly used models for the screening of hepatoprotective drugs (Slater, 1965; Plaa and Hewitt, 1982). The rise in serum levels of ALP, GOT and GPT has been attributed to the damaged structural integrity of the liver (Chenoweth and Hake, 1962), because these are cytoplasmic in location and are released into circulation after cellular damage (Sallie et al., 1991). Paracetamol and CCl₄ are converted to their reactive metabolites (N-acetyl-p-benzoquinoneimine and halogenated free radicals respectively) by their respective specific isozyme of cytochrome P-450 (Packer et al., 1978; van de Straat et al., 1987). Physiologically important protective mechanisms involving both vitamin E (α-tocopherol) and glutathione are available to curtail progression of cellular damage (Potter et al., 1974). However, the
massive production of reactive species may lead to depletion of protective moieties, ensuing wide-spread propagation of the alkylation as well as peroxidation, causing damage to both lipids and proteins present in biomembranes of microsomes and mitochondria (Pesh-Imam and Recknagel, 1977; Aldridge, 1981).

To see whether the plant extract has inhibitory effect on hepatic microsomal drug metabolizing enzymes (MDME), it was administered with pentobarbital to mice and possible change in the duration of sleep was recorded. The duration of pentobarbital-induced sleep in intact animals is considered as a reliable index for the activity of hepatic MDME (Conney, 1967). Pentobarbital is metabolized by the hepatic MDME to inactive metabolites and a drug with inhibitory effect on MDME is expected to prolong pentobarbital sleep time. The fact that the plant extract did not prolong pentobarbital sleeping time ($P > 0.05$) suggests that it is devoid of inhibitory effect on hepatic MDME such as cytochrome P-450.

The damage conferred by CCl$_4$ to hepatocytes as well as hepatic MDME causes loss of drug metabolizing capacity of the liver, resulting in prolongation of pentobarbital-induced sleeping time (Javatilaka et al., 1990). Pretreatment of animals with plant extract prevented the CCl$_4$-induced prolongation of pentobarbital-sleeping time confirming its protective effect against CCl$_4$-induced damage to hepatocytes including MDME. The possible mechanism of the protective effect of Cyperus scariosus would be rather speculative at this stage, however, it is clear from the results of sleeping time study that the protective effect is not mediated through inhibition of hepatic MDME.

The test material has exhibited Ca$^{2+}$ channel blocking activity in isolated tissue experiments and cytosolic Ca$^{2+}$ concentration of hepatocytes are known to
be increased during experimental damage (Moore et al., 1985; Tsokos-Kuhn, 1989). The known calcium channel blocking drugs, i.e., nifedipine, diltiazem and verapamil were found to inhibit the progression of hepatic damage, induced by different hepatotoxins including paracetamol and CCl₄ (Landon et al., 1986; Thibault et al., 1991). Similarly, protective effect of the tuber extract against paracetamol and CCl₄-induced liver damage can be attributed to its Ca²⁺ channel blocking activity, though direct evidence of this mechanism is lacking.

The plant material is safe as is obvious by lack of any symptom of acute toxicity at an oral dose of as high as 3 g/Kg. Thus, this study rationalizes the traditional use of Cyperus scariosus rhizomes in hepato-biliary diseases and liver protective activity is seemed to be associated with Ca²⁺ channel blocking activity.

§7 References


Hamdard Foundation Press, Karachi.


9. *Rubia cordifolia*
9. *Rubia cordifolia*

9.1 *Summary*

The hepatoprotective activity of aqueous-methanolic extract of *Rubia cordifolia* (Rubiaceae) was investigated against paracetamol and CCl₄-induced hepatic damage. Paracetamol produced 100 % mortality at the dose of 1 g/Kg in mice while pretreatment of animals with plant extract (500 mg/Kg) reduced the death rate to 30 %. Paracetamol at the dose of 640 mg/Kg produced liver damage in rats as manifested by the rise in serum levels of GOT and GPT to 1447 ± 182 and 899 ± 201 IU/L ($n=10$) respectively, compared to respective control values of 97 ± 10 and 36 ± 11. Pretreatment of rats with plant extract (500 mg/Kg) lowered significantly ($P<0.005$) the respective serum GOT and GPT levels to 161 ± 48 and 73 ± 29. Similarly, hepatotoxic dose of CCl₄ (1.5 mg/Kg; orally) raised the serum transaminases (GOT and GPT) levels to 422 ± 102 and 354 ± 74 IU/L respectively compared to respective control values of 99 ± 15 and 29 ± 08. The same dose of plant extract (500 mg/Kg) was able to prevent significantly ($P<0.01$) the CCl₄-induced rise in serum enzymes and the estimated values of GOT and GPT were 95 ± 09 and 33 ± 07 respectively. The same dose of roots extract (500 mg/Kg) prevented CCl₄-induced prolongation in pentobarbital sleeping time, thus confirming its hepatoprotectivity. Moreover, the roots extract also exhibited Ca²⁺ channel blocking effect in *in vitro* experiments on isolated rabbit jejunum and this activity is considered to be responsible for the observed hepatoprotectivity.
9.2 Plant Description

*Rubia cordifolia* Linn. (Family: Rubiaceae; Synonymy: *R. purpuria* DC.) locally known as "*Manjit*" is a perennial climbing herb growing wild in northern hilly areas of Pakistan (*Baqar*, 1989). The plant has been employed to dye cotton, silk and wooly garments *since ancient times* (Reinking, 1939; Farnsworth, 1951). Dried roots are considered to be *useful* in alleviating dropsy, paralysis, jaundice, amenorrhea and visceral obstructions (*Nadkarni*, 1976). Plant is also known to exhibit antineoplastic (*Itojawa*, 1985; *Itokawa*, 1989), antibacterial (Gaw and Wang, 1949) and antiviral (*Jin et al.*, 1989) properties. Moreover, it exerts uricolytic (*Decaux*, 1949) and nephrolytic (*Nikolov et al.*, 1960) activities and thus promotes disintegration as well as elimination of urinary stone (*Keller and Görlich*, 1944). We have recently reported the possible presence of calcium channel blocking constituent(s), which may explain its *antilithiatic* and diuretic activities (*Gilani et al.*, 1994). Furthermore, this plant is used as an ingredient of a native recipe "Qurse-rewand", being prescribed by traditional healers to cure chronic diseases and trauma of liver (*Said*, 1982). However, scientific studies on its usefulness in liver damage is lacking and in the present investigation plant extract was tested against paracetamol as well as carbon tetrachloride (*CCL4*)-induced liver injuries to validate the folkloric use of *Rubia cordifolia* in hepatic damage.

9.3 Materials and Methods

9.3.1 Plant extract

*Rubia cordifolia* roots were purchased from local herbal store and authenticated with the help of a botanist at The University of Karachi. The plant
material was powdered and macerated in 80% aqueous-methanol for one week with occasional shaking. The extract was filtered and concentrated to dark reddish brown residue under reduced pressure on a rotary evaporator, with approximate 11% yield.

9.3.2 Animal studies

The effect of shoot and seed extracts was studied on the paracetamol-induced lethality; paracetamol- and CCl₄-induced hepatotoxicity; pentobarbital-induced sleep duration and CCl₄-induced prolongation of pentobarbital sleeping time; spontaneous movements as well as K⁺-induced contractions of rabbit jejunum by the methods described in general method section (chapter 2).

9.4 Results

9.4.1 Effect on paracetamol-induced lethality

Paracetamol at the dose of 1 g/Kg induced 100% lethality in mice. In a group of animals pretreated with plant extract (500 mg/Kg), the same dose of paracetamol killed only 3 out of 10 animals resulting in 70% protection against lethal effect of paracetamol (Table 9.1).

9.4.2 Effect on paracetamol-induced hepatotoxicity

Control (saline + vehicle) serum values of GOT and GPT in rats were found to be 97 ± 10 and 36 ± 11 IU/L respectively (Fig. 9.1; Appendix 35), while a toxic dose of paracetamol (640 mg/Kg) raised significantly (P < 0.001) the respective serum enzyme values to 1447 ± 182 and 899 ± 201. Group 3 was pretreated with plant extract (500 mg/Kg, orally, twice daily for 2 days) to
Table 9.1
Effect of *Rubia cordifolia* roots extract on paracetamol-induced lethality in mice (n = 10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Lethality</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Paracetamol</td>
<td>10/10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(10 mL/Kg + 1 g/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Extract + Paracetamol</td>
<td>03/10</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>(500 mg/Kg + 1 g/Kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Saline/extract and paracetamol were given orally.
Fig. 9.1 Effect of *Rubia cordifolia* extract on paracetamol-induced elevation of serum transaminase levels in rats.
determine its effect on paracetamol-induced rise in serum enzymes. The serum
values of transaminases in pretreated group were found to be $161 \pm 48$ (GOT) and
73 $\pm$ 29 (GPT), which are significantly lower ($P < 0.001$) than the values of toxic
control and were similar to the control values ($P > 0.05$).

9.4.3 Effect on CCl$_4$-induced hepatotoxicity

The estimated values of serum transaminases (GOT and GPT) in control
(saline + vehicle) group of rats were found to be $99 \pm 15$ and $29 \pm 08$ IU/L
respectively (Fig. 9.2; Appendix 36), which were raised significantly ($P < 0.01$)
to respective values of $422 \pm 102$ and $354 \pm 74$ after administration of a toxic
dose of CCl$_4$ (1.5 mL/Kg).

However, pretreatment of animals with plant extract (500 mg/Kg, orally,
twice daily for 2 days) returned the serum GOT and GPT values to $95 \pm 09$ and
33 $\pm$ 07 IU/L respectively, which are significantly lower ($P < 0.01$) than values
of toxic control and were close to the control values ($P > 0.05$).

9.4.4 Effect on pentobarbital-induced sleeping time

Effect of plant extract on pentobarbital sleeping time as well as on CCl$_4$-
induced prolongation of pentobarbital sleeping time was studied in mice and the
results are shown in Table 9.2. Pentobarbital at a dose of 75 mg/Kg, i.p., caused
sleep in mice for a period of $144 \pm 03$ min (Mean $\pm$ S.E.M, $n=10$). Pentobarbital sleeping time in the group of animals pretreated with plant extract
was found to be $150 \pm 07$ min which is similar to that in the control group
($P > 0.05$).
Fig. 9.2 Effect of *Rubia cordifolia* extract on carbon tetrachloride-induced elevation of serum transaminase levels in rats.
Table 9.2
Direct effect of *Rubia cordifolia* roots extract on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sleeping time</th>
<th>P-Value</th>
</tr>
</thead>
</table>
| 1.    | Saline + Pentobarbital  
(10 mL/Kg + 75 mg/Kg) | 144 ± 03 | - |
| 2.    | Extract + Pentobarbital  
(500 mg/Kg + 75 mg/Kg) | 150 ± 07 | >0.05 |
| 3.    | Saline + CCl₄ + Pentobarbital  
(10 mL/Kg + 1.5 mL/Kg + 75 mg/Kg) | 219 ± 17* | <0.001 |
| 4.    | Extract + CCl₄ + Pentobarbital  
(500 mg/Kg + 1.5 mL/Kg + 75 mg/Kg) | 149 ± 16* | <0.01 |

Each value represents the mean ± S.E.M. of 10 determinations. Saline/Extract/CCl₄ was given orally, while pentobarbital was given intraperitoneally.

* Compared to group 1 (control).

* Compared to group 3.
9.4.5 Effect on CCl₄-induced pentobarbital sleep increase

Pretreatment of animals with CCl₄ prolonged the pentobarbital sleeping time to 219 ± 17 min, the value that is significantly higher (P < 0.001) than that of control (Table 9.2). However, prior treatment of animals with plant extract returned this CCl₄-induced prolongation of pentobarbital sleeping time to 149 ± 16 min, which is significantly less than group 3 value (P < 0.01) and close to the control sleeping time (P > 0.05).

9.4.6 Ca²⁺ channel blocking activity

Plant root extract at the concentration of 0.1-1 mg/mL caused a concentration-dependent inhibition of spontaneous movements of rabbit jejunum as shown in Fig. 9.3. This effect was reversible as the tissue regained its spontaneous activity after washing the tissue a couple of times with the fresh bathing fluid.

K⁺ at the concentration of 40 mM caused a sustained tonic contraction which allowed to obtain concentration-dependent inhibitory responses (Fig. 9.3). Addition of root extract at 0.1 mg/mL caused a slight inhibition, while at 0.3 mg/mL, it produced almost 50% relaxant effect and next higher concentration (1 mg/mL) completely suppressed K⁺-induced contractile effect.

When the bathing fluid was replaced with Ca²⁺ free Kreb’s solution, the spontaneous movements of the tissue were abolished within one minute (Fig. 9.4). Addition of Ca²⁺ (15-25 μM) restored spontaneous activity in a concentration-dependent manner.

Root extract was also tested in Ca⁺ free Kreb’s solution for its effect on Ca²⁺ responses and Ca²⁺ failed to restore spontaneous movements of the tissue in
RABBIT JEJUNUM

Rubia cardifolia

Fig. 9.3. A representative tracing showing effect of alcoholic extract of Rubia cordifolia on spontaneous movements (upper panel) and on K⁺-induced contraction (lower panel) in the isolated rabbit jejunum preparation.
Fig. 9.4. A representative tracing showing effect of Ca\(^{++}\) supplementation in the absence (upper panel) and presence of alcoholic extract of *Rubia cordifolia* (lower panel) on spontaneous movements of rabbit jejenum, suspended in Ca\(^{++}\) free kreb's solution.
the presence of root extract (1 mg/mL).

The plant extract up to an oral dose of 4 g/Kg was found devoid of any lethal effect and no apparent behavioral change was observed.

9.5 Discussion

The aqueous-methanolic extract of *Rubia cordifolia* exhibited hepatoprotection against both paracetamol and CCl₄-induced liver injuries as manifested by reduction in toxins-mediated rise in serum transaminases (GOT and GPT) in rats. Similarly, pre-treatment of animals with the plant extract afforded 70% protection against lethal dose of paracetamol and also prevented CCl₄-induced prolongation of pentobarbital sleeping time in mice.

Liver injuries induced by paracetamol and CCl₄ are commonly used models for the screening of hepatoprotective drugs (Slater, 1965; Strubelt et al., 1974; Plaa and Hewitt, 1982). The rise in serum levels of transaminases (GOT and GPT) has been attributed to the damaged structural integrity of the liver (Chenoweth and Hake, 1962) because these are cytoplasmic in location and are released into circulation after cellular damage (Sallie et al., 1991). Paracetamol and CCl₄ are converted to their reactive metabolites (N-acetyl-p-benzoquinoneimine and halogenated free radicals respectively) by their respective specific isozyme of cytochrome P-450 (Packer et al., 1978; van de Straat et al., 1987). Physiologically important protective mechanisms involving both vitamin E (α-tocopherol) and glutathione (GSH) are available to curtail progression of cellular damage (Potter et al., 1974). However, the massive production of reactive species may lead to depletion of protective moieties, ensuing wide-spread propagation of the alkylation as well as peroxidation, causing damage to both lipids and proteins present in
biomembranes of microsomes and mitochondria (Rao and Recknagel, 1969; Pesh-Imam and Recknagel, 1977; Aldridge, 1981). These bio-activation mediated cytotoxic effects are subject of active research and the toxic manifestations can effectively be minimized by cytochrome P-450 inhibitors (Castro et al., 1974; Tredger et al., 1985; Beyeler et al., 1988), GSH precursors (Ferreyra et al., 1974; Prescott et al., 1979), free radical scavengers (Mailing et al., 1974), antioxidants (Gazzard et al., 1974; Koester-Albercht et al., 1979; Yasuda et al., 1980), sulfhydryl agents (Strubelt et al., 1974; Mark et al., 1990) and Ca\textsuperscript{2+} channel blockers (Landon et al., 1986; Thibault et al., 1991; Gilani and Janbaz, 1993).

The crude extract of *Rubia cordifolia* roots used in this study seems to preserve the structural integrity of the hepatocellular membrane. This was evident from the protection provided to mice against lethal dose of paracetamol as well as significant reduction in the paracetamol and CCl\textsubscript{4}-induced rise in serum GOT and GPT levels in rats. To see whether the plant extract has inhibitory effect on hepatic microsomal drug metabolizing enzymes (MDME), it was administered with pentobarbital to mice and possible change in the duration of sleep was recorded. The fact that the plant extract did not prolong pentobarbital sleeping time (*P > 0.05*) suggests that it is devoid of inhibitory effect on hepatic MDME such as cytochrome P-450 and its hepatoprotective effect is mediated perhaps through other mechanism(s).

Calcium content in the liver cells are increased during the process of experimental hepatic damage (Farber et al., 1982; Moore et al., 1985; Landon et al., 1986; Tsokos-Kufin, 1989) and calcium channel blocking drugs i.e., nifedipine, diltiazem and verapamil were found to inhibit the development of
hepatic damage, induced by different hepatotoxins including paracetamol and CCl₄ (Landon et al., 1986; Thibault et al., 1991). Similarly, hepatoprotective activity of the root extract, against paracetamol and CCl₄-induced liver damage may be attributed to its Ca²⁺ channel blocking activity as evidenced by the inhibitory effect of root extract on spontaneous activity as well as K⁺-induced contractions in rabbit jejunum. Since, spontaneous activity and contractions induced by high K⁺ (> 40 mM) are mediated through extracellular Ca²⁺ influx through voltage-dependent slow calcium channels (Bolton, 1979) and inhibitory effect of plant extracts on such contractions may be due to calcium channel blockade.

The cytosolic free calcium concentration in hepatocytes is known to be increased during the process of experimental liver damage (Farber et al., 1982; Moore et al., 1985) and calcium channel blocking drugs (i.e., nifedipine, diltiazem and verapamil) are capable to provide protection against paracetamol- and CCl₄-induced hepatopathy (Landon et al., 1986; Thibault et al., 1991). Thus protective effect of root extract against paracetamol- and CCl₄-induced liver damage may be an outcome of the presence of Ca²⁺ channel blocker(s).

The results presented in this study are indicative of the fact that the crude extract of Rubia cordifolia affords protection against paracetamol and CCl₄-induced liver damage in rats as well as mice. This hepato-protective activity of plant tissue is not mediated through inhibition of hepatic MDME and can be attributed to the possible presence of Ca²⁺ channel blockers constituent(s).

The plant material is safe as is obvious by lack of any acute toxicity symptom up to an oral dose of as high as 4 g/Kg. Thus, this study rationalizes the traditional use of Rubia cordifolia plant in hepato-biliary disorders.
9.6 References


10. GENERAL DISCUSSION
10. General Discussion

The liver being the carrier of a great variety of rather non-specific enzymes is recognized as the major organ for all phases of intermediary metabolism (Brauer, 1956; Brauer, 1963). The unique anatomical placement interposed it between the portal and systemic circulations and hence exposed to chemicals of diverse nature entered via the portal supply during oral absorption. The hepatic metabolism of exogenous compounds can modify their inherent pharmacological and toxicological properties and may generate toxic metabolites capable to attack macromolecules (i.e., proteins and DNA) in the liver.

The chemical-induced liver damage can not be healed by mere removal of the toxins and good remedies are indeed required to stop the progression of the lesion. Modern medicine offers limited success in providing effective cure and there is a severe need to develop new drugs capable to heal toxic liver damage. In traditional medicine a number of medicinal plants are claimed to be effective and are used successfully to alleviate multiple liver disorders.

Although the folkloric uses of indigenous drugs are supported by a long history of human experience, yet these medicinal plant are needed to be evaluated scientifically to render them wide-spread acceptability.

The hepatotoxin-induced hepatic damage in small animals is a simple assay procedure, which can mimic the sequence of events occurring in human hepatitis caused by the toxins of similar nature. Paracetamol- and CCl₄-induced hepatic injuries are commonly used models for the screening of hepatoprotective drugs and the extent of hepatic damage is assessed by the levels of released cytoplasmic enzymes (Chenoweth and Hake, 1962; Sallie et al., 1991).
In therapeutic doses, paracetamol is safely eliminated via sulphation and glucuronidation pathways (Clement et al., 1984; Siegers et al., 1984), however, large doses cause the major fraction of the compound to be metabolized through cytochrome P-450, resulting in production of N-hydroxy paracetamol (Potter et al., 1973; Hinson, 1983), which is subsequently converted to a toxic quinone resonance form N-acetyl-p-benzoquinoneimine (NAPQI) (Corcoran et al., 1980). NAPQI is detoxified normally by endogenous glutathione but excessive production may deplete glutathione stores (Jollow, 1980), allowing the reactive intermediate to interact with cellular macromolecules (Prescott, 1983; Linden and Rumack, 1984).

Carbon tetrachloride, a halogenated hydrocarbon used extensively in industry and this wide-spread application has increased the human health hazards risk due to dietary sources contamination. The CCl₄, like other halogenated alkanes (i.e., chloroform, dichloromethane, bromotrichloromethane, etc.) undergoes cytochrome P-450 catalyzed reductive dehalogenation and thus liberates trichloromethyl (CCl₃) free radicals (Recknagel and Glende, 1973).

The ·CCl₃ attacks several cellular macromolecules (i.e., lipids, nucleic acids, proteins and polysaccharides) by hydrogen abstraction or addition reactions. Interaction of ·CCl₃ with poly-unsaturated fatty acids in membrane lipids releases lipid free radicals (R) and hence initiates the chain reaction process of lipid peroxidation. The R reacts with molecular oxygen (O₂) to generate lipid peroxy radicals (ROO ·), which in turn abstracts a hydrogen atom from neighboring lipid molecules and releases other R(s). Alternatively, the free radicals may attack the methylene bridges of unsaturated fatty acid side-chains of microsomal lipids causing morphological alteration in endoplasmic reticulum, loss to drug
metabolizing enzymes, reduced glucose-6-phosphatase activity, impaired protein synthesis and defected lipoprotein egress (Recknagel and Gleide, 1973).

The inhibition of microsomal drug metabolizing enzymes (MDME) can impair the bio-activation of paracetamol and CCl₄ into their respective reactive species and hence provide protection against the prevailing hepatocellular damage (Castro et al., 1974; Nelson et al., 1980). Amongst the plants proved to be hepatoprotective (Table 10.1), the crude extracts of Artemisia absinthium (shoots) and Berberis aristata (shoots and fruits) were found to enhance the pentobarbital-induced sleeping time as well as strychnine-induced lethality in mice and hence the observed hepatoprotectivity is seemed to be mediated through MDME inhibitory activities. The compounds containing methylene-dioxybenzene groups are likely to reveal inhibitory effect on MDME (Anders, 1968) and hence play an important role in anti-hepatotoxic activities (Hikino et al., 1984; Hikino and Kiso, 1988). The reported methylene-dioxybenzene compounds in Artemisia absinthium and Berberis aristata were sesartemin (Ahmad et al., 1986) and berberine (Chandra and Todaria, 1983) respectively. Sesartemin is not available commercially, whereas berberine was found to be hepatoprotective with MDME inhibitory mechanism (Table 10.2).

The presence of MDME inhibitory activity in Artemisia absinthium and Berberis aristata extracts as well as berberine assign them a prophylactic role against paracetamol- and CCl₄-induced hepatotoxicity by preventing their biotransformation into respective reactive forms. However, these extracts and berberine also showed curative potential against paracetamol-induced liver damage, when the treatment was started after the metabolic activation. The exhibition of curative potential against paracetamol-induced liver damage by these extracts,
Table 10.1
Hepato-protective effect of indigenous medicinal plants against paracetamol- and CCl₄-induced hepatotoxicity.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Parts used</th>
<th>Dose* mg/Kg</th>
<th>Hepatotoxin</th>
<th>Possible Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Paracetamol</td>
<td>CCl₄</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Artemisia absinthium</td>
<td>Shoot</td>
<td>500</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Artemisia maritima</td>
<td>Shoot</td>
<td>500</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Artemisia scoparia</td>
<td>Shoot</td>
<td>150</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Berberis aristata</td>
<td>Shoot, Fruit</td>
<td>500, 500</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cichorium intybus</td>
<td>Shoot, Seed</td>
<td>600, 500</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Cyperus scariosus</td>
<td>Rhizome</td>
<td>500</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Ruta cordifolia</td>
<td>Root</td>
<td>500</td>
<td>+</td>
<td>ND</td>
</tr>
</tbody>
</table>

+ : Activity present.
- : Activity absent.
ND : Activity not determined.

Ca²⁺ : Calcium channel blocking activity was studied in *in vitro* conditions upto the dose of 3 mg/mL.

MDME : Microsomal drug metabolizing enzymes inhibitory activity.

* : The doses mentioned represent the in vivo studies (hepatoprotective activity in rats and MDME inhibition in mice).
Table 10.2
Hepato-protective effect of pure compounds against paracetamol and CCl₄-induced hepatotoxicity.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Sources</th>
<th>Doses**</th>
<th>Hepatotoxin</th>
<th>Possible Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/Kg</td>
<td>µM/Kg</td>
<td>Paracetamol</td>
</tr>
<tr>
<td>Berberine</td>
<td><em>Berberis aristata</em></td>
<td>4</td>
<td>11</td>
<td>+</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td><em>Artemisia scoparia</em></td>
<td>6</td>
<td>34</td>
<td>+</td>
</tr>
<tr>
<td>Esculetin</td>
<td><em>Cichorium intybus</em></td>
<td>6</td>
<td>34</td>
<td>+</td>
</tr>
<tr>
<td>Quercetin</td>
<td><em>Artemisia scoparia</em></td>
<td>10</td>
<td>30</td>
<td>+</td>
</tr>
<tr>
<td>Rutin</td>
<td><em>Artemisia scoparia</em></td>
<td>20</td>
<td>30</td>
<td>+</td>
</tr>
</tbody>
</table>

+ : Activity is present.
- : Activity is Absent.

MDME: Microsomal drug metabolizing enzyme inhibition.

Ca²⁺ : Calcium channel blocking activity was studied in in vitro conditions up to the dose of 3 mg/mL.

* : Known Ca²⁺ channel blocking activity (Fewtrell and Gomperts, 1977; Shoshan et al., 1980; Morales and Lozoya, 1994).

** : The doses mentioned represent the in vivo studies (hepatoprotective in rats and MDME inhibition in mice).
revealed the fact that beside the MDME inhibition some other protective mechanism(s) were also involved. The observed curative effect against paracetamol can be attributed to the reported presence of flavonoids (Hoffmann and Hermann, 1982), ascorbic acid (Klyshev and Alyukina, 1971; Slepetys, 1975), carotenoids (Sergeeva and Zakharova, 1977), tannins (Slepetys, 1975) and lignans (Greger and Hofer, 1980) among the plant constituents in *Artemisia absinthium*, whereas berberine (Chandra and Todaria, 1983), flavonoids (Shapiro et al., 1983; Vereskovskii and Shapiro, 1986), ascorbic acid (Shapiro et al., 1983; Karitonova, 1986), β-carotene (Chandra and Todaria, 1983), and tannins (Shapiro et al., 1983) are reported constituents of *Berberis aristata*. Berberine exhibited curative effect in this study, while flavonoids are known to be antioxidants (Torel et al., 1986; Faurè et al., 1990), free radical scavengers (Bors and Saran, 1987; Husain et al., 1987) and anti-lipoperoxidant (Younes and Siegers, 1981; Robak et al., 1986; Ratty and Das, 1988) leading to hepatoprotection (Kiso et al., 1984; Handa et al., 1986). Similarly, ascorbic acid serves as antioxidant (Demopoulos, 1973; Bus and Gibson, 1984), inhibits covalent binding of NAPQI to vital macromolecules (Lake et al., 1981) and consequently can minimize toxic damage (Harman, 1985). Moreover, carotenoids are also reputed to be antioxidants (Kläui, 1982) and thus showing anti-hepatotoxic activity (Oshima et al., 1984). Furthermore, the hepatoprotective potential of tannins (Hikino et al., 1985) as well as lignans is also well documented (Faurè et al., 1990).

The *Artemisia absinthium* (shoots) and *Berberis aristata* (shoots and fruits) extracts as well as berberine were unable to mitigate the hepatic damage after CCl₄ activation and this anomalous observation can partly be justified on the basis of reported facts. The paracetamol toxicity following NAPQI generation is chiefly
due to oxidative stress and can effectively be ameliorated by antioxidants (Harman, 1985), whereas, the hepatic damage due to HFR may be due to lipid peroxidation (Bus and Gibson, 1979) as well as alkylation (Dogterom et al., 1988). The antioxidant and anti-lipoperoxidant activities of plant constituents are proved effective against the NAPQI through inhibition of the lipid peroxidation processes, whereas these were unable to prevent the alkylation process due to HFR (Poli et al., 1989).

The crude extracts of *Artemisia maritima* (shoots), *Artemisia scoparia* (shoots), *Cichorium intybus* (shoots and seeds), *Cyperus scariosus* (rhizomes) and *Rubia cordifolia* (roots) as well as pure compounds, i.e., caffeic acid, esculetin, quercetin and rutin exhibited hepatoprotection. The crude extracts of the above mentioned plants were also found to exhibit Ca$^{2+}$ channel blocking activities in isolated tissue experiments and among the pure compounds quercetin is well known to share this property (Few·rell and Gomperts, 1977; Shoshan et al., 1980; Morales and Lozoya, 1994). The observed hepatoprotective activity of plant extracts is likely to be mediated through their proposed Ca$^{2+}$ channel blocking activities. As the Ca$^{2+}$ channel blockers may interfere with the mitochondrial Ca$^{2+}$ uptake by impairment of the overall electroneutrality across the inner mitochondrial membranes (Becker et al., 1980; Akerman and Nicholls, 1983) because the amount of Ca$^{2+}$ that can be accumulated is compensated by concomitant permeation of anions into the mitochondrial matrix via mitochondrial inner membrane anion channel (IMAC) (Beavis and Garlid, 1987). The availability of specific binding sites for Ca$^{2+}$ channel blockers (CCB) on mitochondrial inner membranes in association with IMAC (Zerning et al., 1990) may allow the CCB to prevent mitochondrial Ca$^{2+}$ overload via modulation of anion influx via IMAC
and hence ATP conservation (Zerning, 1990) and is further confirmed by the 
reported inhibition of paracetamol- and CCl₄-induced progression of hepatic 
damage by some standard CCBs, i.e., nifedipine, diltiazem and verapamil (Landon 
et al., 1986; Thibault et al., 1991). In addition to the known CCB activity 
(Fewtrell and Gomperts, 1977; Shoshan et al., 1980; Morales and Lozoya, 1994), 
some other reported properties of quercetin, i.e., anti-oxidant (Chen et al., 1990; 
Negre-Salvayre and Salvayre, 1992; Oyama et al., 1994) and free radical 
scavenging (Bors and Saran, 1987, Afanas’eve et al., 1989; Chen et al., 1990) 
may also be responsible for the observed hepatoprotection. Similarly, the pure 
compounds like caffeic acid, esculetin and rutin although found devoid of Ca²⁺ 
channel blocking activity, yet their hepatoprotective potential can be assigned to 
their reported anti-oxidant (Laranjinha et al., 1994; Grinberg et al., 1994; Kozlov 
et al., 1994), free radical scavenging (Hanasaki et al., 1994) and anti-
inflammatory (Ozaki et al., 1986; Tajima et al., 1986; Alcaraz and Jimenez, 1988) 
properties.

References

Afanas’eve, J. B., Dorozhko, A. J., Brodskii, A. V., Kostyuk, U. A. and 
mechanism of inhibitory action of rutin and quercetin in lipid 

Soc. Pak. 8, 277-296.

Akerman, K. E. O., and Nicholls, D. G., (1983). Physiological and 
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metyrapone in the treatment of acetaminophen toxicity in mice. *Toxicology* 17, 73-81.


11. APPENDICES
Appendix 1.

Preventive effect of *Artemisia absinthium* extract on paracetamol-induced elevation of serum transaminase levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (10 mL/Kg + 13 mL/Kg)</td>
<td>98 ± 11</td>
<td>39 ± 08</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + Paracetamol (10 mL/Kg + 640 mg/Kg)</td>
<td>1424 ± 454'</td>
<td>741 ± 217'</td>
</tr>
<tr>
<td>3.</td>
<td>Extract + Paracetamol (500 mg/Kg + 640 mg/Kg)</td>
<td>85 ± 18'</td>
<td>34 ± 08'</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of plant extract (500 mg/Kg) at 12 hrs intervals before paracetamol administration.

* P < 0.01; Compared to group 1.

' P < 0.01; Compared to group 2.
**Appendix 2.**

Preventive effect of *Artemisia absinthium* extract on CCl₄-induced elevation of serum transaminase levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (10 mL/Kg + 7.5 mL/Kg)</td>
<td>106 ± 15</td>
<td>45 ± 11</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + CCl₄ (10 mL/Kg + 1.5 mL/Kg)</td>
<td>494 ± 155*</td>
<td>305 ± 83**</td>
</tr>
<tr>
<td>3.</td>
<td>Extract + CCl₄ (500 mg/Kg + 1.5 mL/Kg)</td>
<td>139 ± 18*</td>
<td>76 ± 27*</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of plant extract (500 mg/Kg) at 12 hrs intervals before CCl₄ administration.

* P < 0.05, ** P < 0.01; Compared to group 1.

* P < 0.05; Compared to group 2.
Appendix 3.
Curative effect of *Artemisia absinthium* extract on paracetamol-induced elevation of serum transaminase levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vehicle + Saline</td>
<td>108 ± 22</td>
<td>42 ± 11</td>
</tr>
<tr>
<td></td>
<td>(13 mL/Kg + 10 mL/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Paracetamol + Saline</td>
<td>112 ± 281 *</td>
<td>833 ± 195 **</td>
</tr>
<tr>
<td></td>
<td>(640 mg/Kg + 10 mL/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Paracetamol + Extract</td>
<td>167 ± 28 *</td>
<td>88 ± 26 *</td>
</tr>
<tr>
<td></td>
<td>(640 mg/Kg + 500 mg/Kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received three doses of plant extract (500 mg/Kg) at 6 hr intervals started 6 hrs after paracetamol administration.

* P < 0.01, ** P < 0.001; Compared to group 1.

* P < 0.01; Compared to group 2.
Appendix 4.

Effect of *Artemisia absinthium* extract post-treatment on CCl₄-induced elevation of serum transaminase levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vehicle + Saline (7.5 mL/Kg + 10 mL/Kg)</td>
<td>95 ± 13</td>
<td>48 ± 10</td>
</tr>
<tr>
<td>2.</td>
<td>CCl₄ + Saline (1.5 mL/Kg + 10 mL/Kg)</td>
<td>511 ± 165*</td>
<td>353 ± 101**</td>
</tr>
<tr>
<td>3.</td>
<td>CCl₄ + Extract (1.5 mL/Kg + 500 mg/Kg)</td>
<td>523 ± 94†</td>
<td>415 ± 123‡</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received three doses of plant extract (500 mg/Kg) at 6 hr intervals started 6 hrs after CCl₄ administration.

* P < 0.05, ** P < 0.01; Compared to group 1.
† P > 0.05; Compared to group 2.
Appendix 5.
Effect of *Artemisia maritima* extract on paracetamol-induced elevation of serum transaminase levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (10 mL/Kg + 13 mL/Kg)</td>
<td>87 ± 12</td>
<td>31 ± 05</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + Paracetamol (10 mL/Kg + 640 mg/Kg)</td>
<td>1529 ± 172&quot;</td>
<td>904 ± 136&quot;</td>
</tr>
<tr>
<td>3.</td>
<td>Extract + Paracetamol (500 mg/Kg + 640 mg/Kg)</td>
<td>112 ± 10&quot;</td>
<td>47 ± 11&quot;</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of plant extract (500 mg/Kg) at 12 hrs intervals before paracetamol administration.

* P<0.001; Compared to group 1.
* P<0.001; Compared to group 2.
Appendix 6.

Effect of *Artemisia maritima* extract on CCl₄-induced elevation of serum transaminase levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (10 mL/Kg + 7.5 mL/Kg)</td>
<td>92 ± 18</td>
<td>35 ± 09</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + CCl₄ (10 mL/Kg + 1.5 mL/Kg)</td>
<td>463 ± 122*</td>
<td>366 ± 58**</td>
</tr>
<tr>
<td>3.</td>
<td>Extract + CCl₄ (500 mg/Kg + 1.5 mL/Kg)</td>
<td>105 ± 25*</td>
<td>53 ± 17**</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of plant extract (500 mg/Kg) at 12 hrs intervals before CCl₄ administration.

* P < 0.01, ** P < 0.001; Compared to group 1.

* P < 0.05, ** P < 0.001; Compared to group 2.
Appendix 7.

Effect of *Artemisia scoparia* extract on paracetamol-induced elevation of serum transaminase levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (3 mL/Kg + 13 mL/Kg)</td>
<td>80 ± 10</td>
<td>38 ± 09</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + Paracetamol (3 mL/Kg + 640 mg/Kg)</td>
<td>1528 ± 310&quot;</td>
<td>904 ± 261'</td>
</tr>
<tr>
<td>3.</td>
<td>Extract + Paracetamol (150 mg/Kg + 640 mg/Kg)</td>
<td>85 ± 11&quot;</td>
<td>23 ± 06'</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of plant extract (150 mg/Kg) at 12 hrs intervals before paracetamol administration.

* P < 0.01, ** P < 0.001; Compared to group 1

* P < 0.01, ** P < 0.001; Compared to group 2
Appendix 8.
Effect of caffeic acid on paracetamol-induced elevation of serum transaminase levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle</td>
<td>88 ± 15</td>
<td>51 ± 17</td>
</tr>
<tr>
<td></td>
<td>(1.5 mL/Kg + 13 mL/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Saline + Paracetamol</td>
<td>938 ± 284'</td>
<td>727 ± 211'</td>
</tr>
<tr>
<td></td>
<td>(1.5 mL/Kg + 640 mg/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Caffeic acid + Paracetamol</td>
<td>138 ± 28'</td>
<td>54 ± 13''</td>
</tr>
<tr>
<td></td>
<td>(6 mg/Kg + 640 mg/Kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of caffeic acid (6 mg/Kg) at 12 hrs intervals before paracetamol administration.

* P < 0.01; Compared to group 1 (control).

* P < 0.05, ** P < 0.01; Compared to group 2
Appendix 9.
Effect of quercetin on paracetamol-induced elevation of serum transaminase levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (5 mL/Kg + 13 mL/Kg)</td>
<td>89 ± 13</td>
<td>41 ± 10</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + Paracetamol (5 mL/Kg + 640 mg/Kg)</td>
<td>813 ± 158&quot;</td>
<td>475 ± 124&quot;</td>
</tr>
<tr>
<td>3.</td>
<td>Quercetin + Paracetamol (10 mg/Kg + 640 mg/Kg)</td>
<td>105 ± 11&quot;</td>
<td>46 ± 09&quot;</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of quercetin (10 mg/Kg) at 12 hrs intervals before paracetamol administration.

' P < 0.01, " P < 0.001; Compared to group 1

* P < 0.01, ** P < 0.001; Compared to group 2
Appendix 10.

Effect of rutin on paracetamol-induced elevation of serum transaminase levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (4 mL/Kg + 13 mL/Kg)</td>
<td>$118 \pm 16$</td>
<td>$39 \pm 07$</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + Paracetamol (4 mL/Kg + 640 mg/Kg)</td>
<td>$1013 \pm 258^*$</td>
<td>$686 \pm 219^*$</td>
</tr>
<tr>
<td>3.</td>
<td>Rutin + Paracetamol (20 mg/Kg + 640 mg/Kg)</td>
<td>$145 \pm 22^{''}$</td>
<td>$61 \pm 15^*$</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of rutin (20 mg/Kg) at 12 hrs intervals before paracetamol administration.

* $P < 0.01$; Compared to group 1 (control).

$^* P < 0.05$, $^{''} P < 0.01$; Compared to group 2
Appendix 11.
Effect of *Artemisia scoparia* extract on CCl₄-induced elevation of serum transaminase levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle</td>
<td>106 ± 15</td>
<td>26 ± 04</td>
</tr>
<tr>
<td></td>
<td>(3 mL/Kg + 7.5 mL/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Saline + CCl₄</td>
<td>395 ± 110*</td>
<td>258 ± 61**</td>
</tr>
<tr>
<td></td>
<td>(3 mL/Kg + 1.5 mL/Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Extract + CCl₄</td>
<td>93 ± 05*</td>
<td>27 ± 03**</td>
</tr>
<tr>
<td></td>
<td>(150 mg/Kg + 1.5 mL/Kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of plant extract (150 mg/Kg) at 12 hrs intervals before CCl₄ administration.

* P < 0.05, ** P < 0.01; Compared to group 1

* P < 0.05, ** P < 0.01; Compared to group 2
Appendix 12.

Effect of caffie acid on CCl₄-induced elevation of serum transaminase levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (1.5 mL/Kg + 7.5 mL/Kg)</td>
<td>102 ± 12</td>
<td>45 ± 09</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + CCl₄ (1.5 mL/Kg + 1.5 mL/Kg)</td>
<td>982 ± 277*</td>
<td>605 ± 181*</td>
</tr>
<tr>
<td>3.</td>
<td>Caffeic acid + CCl₄ (6 mg/Kg + 1.5 mL/Kg)</td>
<td>158 ± 32*</td>
<td>87 ± 28*</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of caffie acid (6 mg/Kg) at 12 hrs intervals before CCl₄ administration.

\* P<0.01; Compared to group 1 (control).

\* P<0.05, " P<0.01; Compared to group 2.
Appendix 13.
Effect of quercetin on CCl₄-induced elevation of serum transaminase levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (5 mL/Kg + 7.5 mL/Kg)</td>
<td>96 ± 14</td>
<td>49 ± 09</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + CCl₄ (5 mL/Kg + 1.5 mL/Kg)</td>
<td>465 ± 132*</td>
<td>381 ± 116*</td>
</tr>
<tr>
<td>3.</td>
<td>Quercetin + CCl₄ (10 mg/Kg + 1.5 mL/Kg)</td>
<td>123 ± 25*</td>
<td>59 ± 17*</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses quercetin (10 mg/Kg) at 12 hrs intervals before CCl₄ administration.

* P < 0.05; Compared to group 1 (control).

* P < 0.05; Compared to group 2.
Appendix 14.
Effect of rutin on CCl4-induced elevation of serum transaminase levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (4 mL/Kg + 7.5 mL/Kg)</td>
<td>111 ± 13</td>
<td>40 ± 10</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + CCl4 (4 mL/Kg + 1.5 mL/Kg)</td>
<td>853 ± 252&quot;</td>
<td>551 ± 196'</td>
</tr>
<tr>
<td>3.</td>
<td>Rutin + CCl4 (20 mg/Kg + 1.5 mL/Kg)</td>
<td>153 ± 27'</td>
<td>64 ± 24'</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of rutin (20 mg/Kg) at 12 hrs intervals before CCl4 administration.

* P < 0.05, ** P < 0.01: Compared to group 1.
* * P < 0.05; Compared to group 2.
Appendix 15.
Preventive effect of *Berberis aristata* shoot extract on paracetamol-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
</table>
| 1.    | Saline + Vehicle  
(10 mL/Kg + 13 mL/Kg)  | 203 ± 17 | 87 ± 10 | 27 ± 02 |
| 2.    | Saline + Paracetamol  
(10 mL/Kg + 640 mg/Kg)  | 361 ± 35* | 1447 ± 182* | 899 ± 201* |
| 3.    | Extract + Paracetamol  
(500 mg/Kg + 640 mg/Kg)  | 162 ± 25** | 230 ± 157** | 169 ± 134** |

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of plant shoots extract (500 mg/Kg) at 12 hrs intervals before paracetamol administration.

* P < 0.001; Compared to group 1 (control).

* P < 0.01, ** P < 0.001; Compared to group 2.
Appendix 16.
Preventive effect of *Berberis aristata* fruits extract on paracetamol-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline + Vehicle</td>
<td>219 ± 21</td>
<td>80 ± 10</td>
<td>38 ± 09</td>
</tr>
<tr>
<td></td>
<td>(10 mL/Kg + 13 mL/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Saline + Paracetamol</td>
<td>312 ± 21*</td>
<td>153 ± 249**</td>
<td>430 ± 104*</td>
</tr>
<tr>
<td></td>
<td>(10 mL/Kg + 640 mg/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Extract + Paracetamol</td>
<td>207 ± 16**</td>
<td>102 ± 12**</td>
<td>45 ± 06*</td>
</tr>
<tr>
<td></td>
<td>(500 mg/Kg + 640 mg/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of plant fruits extract (500 mg/Kg) at 12 hrs intervals before paracetamol administration.

* P < 0.01, ** P < 0.001; Compared to group 1.

* P < 0.01, ** P < 0.001; Compared to group 2.
Appendix 17.
Preventive effect of berberine on paracetamol-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle</td>
<td>176 ± 05</td>
<td>75 ± 04</td>
<td>38 ± 03</td>
</tr>
<tr>
<td></td>
<td>(1 mL/Kg + 13 mL/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Saline + Paracetamol</td>
<td>259 ± 10'</td>
<td>582 ± 95'</td>
<td>243 ± 79'</td>
</tr>
<tr>
<td></td>
<td>(1 mL/Kg + 640 mg/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Berberine + Paracetamol</td>
<td>182 ± 10''</td>
<td>67 ± 11''</td>
<td>37 ± 05'</td>
</tr>
<tr>
<td></td>
<td>(4 mg/Kg + 640 mg/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of berberine (4 mg/Kg) at 12 hrs intervals before paracetamol administration.

' P < 0.001; Compared to group 1 (control).

* P < 0.01, ** P < 0.001; Compared to group 2.
Appendix 18.
Preventive effect of *Berberis aristata* shoot extract on CCl₄-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Saline + Vehicle</em> (10 mL/Kg + 7.5 mL/Kg)</td>
<td>237 ± 13</td>
<td>99 ± 22</td>
<td>35 ± 10</td>
</tr>
<tr>
<td>2.</td>
<td><em>Saline + CCl₄</em> (10 mL/Kg + 1.5 mL/Kg)</td>
<td>291 ± 17°</td>
<td>451 ± 164°</td>
<td>328 ± 94°</td>
</tr>
<tr>
<td>3.</td>
<td><em>Extract + CCl₄</em> (500 mg/Kg + 1.5 mL/Kg)</td>
<td>222 ± 18°</td>
<td>144 ± 21°</td>
<td>76 ± 37°</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of plant shoots extract (500 mg/Kg) at 12 hrs intervals before CCl₄ administration.

* P < 0.05, ** P < 0.01; Compared to group 1 (control).

* P < 0.05; Compared to group 2.
Appendix 19.

Preventive effect of *Berberis aristata* fruits extract on CCl₄-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
</table>
| 1.    | Saline + Vehicle  
(10 mL/Kg + 7.5 mL/Kg) | 215 ± 16 | 89 ± 09 | 41 ± 14 |
| 2.    | Saline + CCl₄  
(10 mL/Kg + 1.5 mL/Kg) | 303 ± 18" | 480 ± 150° | 296 ± 80" |
| 3.    | Extract + CCl₄  
(500 mg/Kg + 1.5 mL/Kg) | 224 ± 21° | 100 ± 18° | 62 ± 12" |

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of plant fruits extract (500 mg/Kg) at 12 hrs intervals before CCl₄ administration.

* P < 0.05, ** P < 0.01; Compared to group 1.

* P < 0.05, ** P < 0.01; Compared to group 2.
Appendix 20.
Preventive effect of berberine on CCl₄-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (1 mL/Kg + 7.5 mL/Kg)</td>
<td>207 ± 18</td>
<td>109 ± 12</td>
<td>39 ± 16</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + CCl₄ (1 mL/Kg + 1.5 mL/Kg)</td>
<td>275 ± 24&quot;</td>
<td>734 ± 191'</td>
<td>415 ± 76&quot;</td>
</tr>
<tr>
<td>3.</td>
<td>Berberine + CCl₄ (1 mg/Kg + 1.5 mL/Kg)</td>
<td>219 ± 204'</td>
<td>144 ± 28'</td>
<td>76 ± 25&quot;</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of berberine (4 mg/Kg) at 12 hrs intervals before CCl₄ administration.

* P < 0.05, ** P < 0.01; Compared to group 1.

* P < 0.05, ** P < 0.01; Compared to group 2.
Appendix 21.
Curative effect of *Berberis aristata* shoot extract on paracetamol-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vehicle + Saline (13 mL/Kg + 10 mL/Kg)</td>
<td>229 ± 21</td>
<td>103 ± 19</td>
<td>38 ± 15</td>
</tr>
<tr>
<td>2.</td>
<td>Paracetamol + Saline (640 mg/Kg + 10 mL/Kg)</td>
<td>346 ± 25'=</td>
<td>1180 ± 197''</td>
<td>751 ± 159''</td>
</tr>
<tr>
<td>3.</td>
<td>Paracetamol + Extract (640 mg/Kg + 500 mg/Kg)</td>
<td>253 ± 30'</td>
<td>118 ± 34''</td>
<td>53 ± 27''</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received three doses of plant shoots extract (500 mg/Kg) at 6 hrs intervals, started 6 hrs after paracetamol administration.

* P < 0.01, ** P < 0.001; Compared to group 1.

* P < 0.05, ** P < 0.001; Compared to group 2.
Appendix 22.
Curative effect of *Berberis aristata* fruit extract on paracetamol-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vehicle + Saline (13 mL/Kg + 10 mL/Kg)</td>
<td>222 ± 26</td>
<td>95 ± 11</td>
<td>36 ± 13</td>
</tr>
<tr>
<td>2.</td>
<td>Paracetamol + Saline (640 mg/Kg + 10 mL/Kg)</td>
<td>331 ± 23&lt;sup&gt;★&lt;/sup&gt;</td>
<td>1430 ± 217&lt;sup&gt;★★&lt;/sup&gt;</td>
<td>483 ± 125&lt;sup&gt;★★★&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.</td>
<td>Paracetamol + Extract (640 mg/Kg + 500 mg/Kg)</td>
<td>247 ± 27&lt;sup&gt;★★&lt;/sup&gt;</td>
<td>70 ± 17&lt;sup&gt;★★★&lt;/sup&gt;</td>
<td>38 ± 08&lt;sup&gt;★★★&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received three doses of plant fruits extract (500 mg/Kg) at 6 hrs intervals, started 6 hrs after paracetamol administration.

★ P < 0.01, ★★ P < 0.001; Compared to group 1.

★★ P < 0.05, ★★★ P < 0.01, ★★★★ P < 0.001; Compared to group 2.
Appendix 23.
Curative effect of berberine on paracetamol-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle</td>
<td>198 ± 13</td>
<td>93 ± 16</td>
<td>48 ± 10</td>
</tr>
<tr>
<td></td>
<td>(1 mL/Kg + 13 mL/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Saline + Paracetamol</td>
<td>261 ± 19'</td>
<td>996 ± 228''</td>
<td>495 ± 133''</td>
</tr>
<tr>
<td></td>
<td>(1 mL/Kg + 640 mg/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Berberine + Paracetamol</td>
<td>210 ± 14''</td>
<td>138 ± 29'''</td>
<td>52 ± 15'''</td>
</tr>
<tr>
<td></td>
<td>(4 mg/Kg + 640 mg/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as 1U/L. Group 3 received three doses berberine (4 mg/Kg) at 6 hrs. intervals started 6 hrs. after paracetamol administration.

* P < 0.05; ** P < 0.01; Compared to group 1 (control).

* P < 0.01, ** P < 0.001; Compared to group 2.
Appendix 24.

Effect of *Berberis aristata* shoot extract post-treatment on CCl₄-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vehicle + Saline (7.5 mL/Kg + 10 mL/Kg)</td>
<td>215 ± 18</td>
<td>105 ± 18</td>
<td>42 ± 16</td>
</tr>
<tr>
<td>2.</td>
<td>CCl₄ + Saline (1.5 mL/Kg + 10 mL/Kg)</td>
<td>327 ± 28&quot;</td>
<td>572 ± 192'</td>
<td>343 ± 121'</td>
</tr>
<tr>
<td>3.</td>
<td>CCl₄ + Extract (1.5 mL/Kg + 500 mg/Kg)</td>
<td>297 ± 32'</td>
<td>394 ± 131'</td>
<td>278 ± 38'</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received three doses of plant shoots extract (500 mg/Kg) at 6 hrs intervals started 6 hrs after CCl₄ administration.

* P < 0.05, ** P < 0.01; Compared to group 1.

* P > 0.05; Compared to group 2.
Appendix 25.
Effect of *Berberis aristata* fruit extract post-treatment on CCl₄-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>AJP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vehicle + Saline (7.8 mL/Kg + 10 mL/Kg)</td>
<td>193 ± 16</td>
<td>91 ± 08</td>
<td>35 ± 14</td>
</tr>
<tr>
<td>2.</td>
<td>CCl₄ + Saline (1.5 mL/Kg + 10 mL/Kg)</td>
<td>297 ± 13*</td>
<td>602 ± 212*</td>
<td>273 ± 96*</td>
</tr>
<tr>
<td>3.</td>
<td>CCl₄ + Extract (1.5 mL/Kg + 500 mg/Kg)</td>
<td>321 ± 40*</td>
<td>185 ± 14*</td>
<td>118 ± 33*</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received three doses of plant fruits extract (500 mg/Kg) at 6 hrs intervals started 6 hrs after CCl₄ administration.

* P < 0.05, ** P < 0.001; Compared to group 1.

* P > 0.05; Compared to group 2.
Appendix 26.

Effect of berberine post-treatment on CCl₄-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle</td>
<td>201 ± 14</td>
<td>99 ± 17</td>
<td>46 ± 12</td>
</tr>
<tr>
<td></td>
<td>(1 mL/Kg + 7.5 mL/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Saline + CCl₄</td>
<td>268 ± 26'</td>
<td>911 ± 237''</td>
<td>375 ± 92''</td>
</tr>
<tr>
<td></td>
<td>(1 mL/Kg + 1.5 mL/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Berberine + CCl₄</td>
<td>239 ± 23'</td>
<td>644 ± 198'</td>
<td>262 ± 73'</td>
</tr>
<tr>
<td></td>
<td>(1 mg/Kg + 1.5 mL/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received three doses of berberine (4 mg/Kg) at 6 hrs intervals started 6 hrs after CCl₄ administration.

* P < 0.05, ** P < 0.01; Compared to group 1.

* P > 0.05; Compared to group 2.
Appendix 27.

Effect of *Cichorium intybus* shoots extract on paracetamol-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
</table>
| 1.    | Saline + Vehicle  
(10 mL/Kg + 13 mL/Kg) | 231 ± 12  | 97 ± 10   | 26 ± 02   |
| 2.    | Saline + Paracetamol  
(10 mL/Kg + 640 mg/Kg) | 410 ± 24* | 1447 ± 182* | 899 ± 201* |
| 3.    | Extract + Paracetamol  
(600 mg/Kg + 640 mg/Kg) | 224 ± 21** | 487 ± 301* | 77 ± 13** |

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of plant extract (600 mg/Kg) at 12 hrs intervals before paracetamol administration.

* P < 0.001; Compared to group 1.

* P < 0.05 & ** P < 0.001; Compared to group 2.
Appendix 28.
Effect of *Cichorium intybus* seeds extract on paracetamol-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (10 mL/Kg + 13 mL/Kg)</td>
<td>198 ± 15</td>
<td>76 ± 07</td>
<td>39 ± 09</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + Paracetamol (10 mL/Kg + 640 mg/Kg)</td>
<td>393 ± 28**</td>
<td>767 ± 215*</td>
<td>692 ± 191*</td>
</tr>
<tr>
<td>3.</td>
<td>Extract + Paracetamol (500 mg/Kg + 640 mg/Kg)</td>
<td>228 ± 16**</td>
<td>68 ± 10*</td>
<td>41 ± 08*</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of seeds extract (500 mg/Kg) at 12 hrs intervals before paracetamol administration.

* P<0.01, ** P<0.001; Compared to group 1 (control).

* P<0.01, ** P<0.001; Compared to group 2.
Appendix 29.
Effect of esculetin on paracetamol-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (3 mL/Kg + 13 mL/Kg)</td>
<td>219 ± 10</td>
<td>84 ± 12</td>
<td>33 ± 07</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + Paracetamol (3 mL/Kg + 640 mg/Kg)</td>
<td>262 ± 14*</td>
<td>1210 ± 242***</td>
<td>735 ± 223**</td>
</tr>
<tr>
<td>3.</td>
<td>Esculetin + Paracetamol (6 mg/Kg + 640 mg/Kg)</td>
<td>204 ± 18**</td>
<td>137 ± 41***</td>
<td>71 ± 34**</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of esculetin (6 mg/Kg) at 12 hrs intervals before paracetamol administration.

* P < 0.05, ** P < 0.01, *** P < 0.001; Compared to group 1.

* P < 0.05, ** P < 0.01, *** P < 0.001; Compared to group 2.
Appendix 30.
Effect of *Cichorium intybus* shoots extract on CCl₄-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (10 mL/Kg + 7.5 mL/Kg)</td>
<td>176 ± 05</td>
<td>95 ± 11</td>
<td>42 ± 13</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + CCl₄ (10 mL/Kg + 1.5 mL/Kg)</td>
<td>276 ± 13**</td>
<td>532 ± 89**</td>
<td>263 ± 74*</td>
</tr>
<tr>
<td>3.</td>
<td>Extract + CCl₄ (600 mg/Kg + 1.5 mL/Kg)</td>
<td>173 ± 11**</td>
<td>127 ± 29**</td>
<td>63 ± 15*</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of shoots extract (600 mg/Kg) at 12 hrs intervals before CCl₄ administration.

* P < 0.01, ** P < 0.001; Compared to group 1.

* P < 0.05, ** P < 0.001; Compared to group 2.
Appendix 31.
Effect of *Cichorium intybus* seeds extract on CCl₄-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle</td>
<td>215 ± 16</td>
<td>79 ± 18</td>
<td>49 ± 10</td>
</tr>
<tr>
<td></td>
<td>(10 mL/Kg + 7.5 mL/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Saline + CCl₄</td>
<td>312 ± 20''</td>
<td>503 ± 98''</td>
<td>407 ± 109''</td>
</tr>
<tr>
<td></td>
<td>(10 mL/Kg + 1.5 mL/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Extract + CCl₄</td>
<td>222 ± 27''</td>
<td>114 ± 23''</td>
<td>68 ± 14''</td>
</tr>
<tr>
<td></td>
<td>(500 mg/Kg + 1.5 mL/Fg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.”M., of 10 determinations expressed as 1U/L. Group 3 received four doses of seeds extract (500 mg/Kg) at 12 hrs intervals before CCl₄ administration.

* P<0.01, ** P<0.001; Compared to group 1.

* P<0.05, ** P<0.01; Compared to group 2.
Appendix 32.

Effect of esculetin on CCl₄-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (3 mL/Kg + 7.5 mL/Kg)</td>
<td>198 ± 13</td>
<td>103 ± 15</td>
<td>48 ± 12</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + CCl₄ (3 mL/Kg + 1.5 mL/Kg)</td>
<td>267 ± 25*</td>
<td>868 ± 249**</td>
<td>576 ± 159**</td>
</tr>
<tr>
<td>3.</td>
<td>Esculetin + CCl₄ (6 mg/Kg + 1.5 mL/Kg)</td>
<td>207 ± 12*</td>
<td>133 ± 23**</td>
<td>85 ± 27**</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of esculetin (6 mg/Kg) at 12 hrs intervals before CCl₄ administration.

* P < 0.05, ** P < 0.01; Compared to group 1 (control).

* P < 0.05, ** P < 0.01; Compared to group 2.
Appendix 33.

Effect of *Cyperus scariosus* rhizomes extract on paracetamol-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline + Vehicle</td>
<td>202 ± 36</td>
<td>59 ± 14</td>
<td>38 ± 07</td>
</tr>
<tr>
<td></td>
<td>(10 mL/Kg + 13 mL/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Saline + Paracetamol</td>
<td>430 ± 68&quot;</td>
<td>867 ± 305'</td>
<td>732 ± 212&quot;</td>
</tr>
<tr>
<td></td>
<td>(10 mL/Kg + 640 mg/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Extract + Paracetamol</td>
<td>192 ± 31&quot;</td>
<td>63 ± 09'</td>
<td>35 ± 08&quot;</td>
</tr>
<tr>
<td></td>
<td>(500 mg/Kg + 640 mg/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of plant extract (500 mg/Kg) at 12 hrs intervals before paracetamol administration.

* P < 0.05 & " P < 0.01; Compared to group 1.

** P < 0.05 & "" P < 0.001; Compared to group 2.
Appendix 34.
Effect of *Cyperus scariosus* rhizomes extract on CCl₄-induced elevation of serum enzymes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (10 mL/Kg + 7.5 mL/Kg)</td>
<td>177 ± 21</td>
<td>106 ± 15</td>
<td>47 ± 12</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + CCl₄ (10 mL/Kg + 1.5 mL/Kg)</td>
<td>328 ± 30**</td>
<td>493 ± 102**</td>
<td>357 ± 109***</td>
</tr>
<tr>
<td>3.</td>
<td>Extract + CCl₄ (500 mg/Kg + 1.5 mL/Kg)</td>
<td>220 ± 30*</td>
<td>207 ± 90*</td>
<td>75 ± 38*</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of plant extract (600 mg/Kg) at 12 hrs intervals before CCl₄ administration.

* P < 0.05, ** P < 0.01; *** P < 0.001; Compared to group 1.

* P < 0.05; Compared to group 2.
Appendix 35.

*Effect of *Rubia cordifolia* roots extract on paracetamol-induced elevation of serum transaminase levels in rats.*

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (10 mL/Kg + 13 mL/Kg)</td>
<td>97 ± 10</td>
<td>36 ± 11</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + Paracetamol (10 mL/Kg + 640 mg/Kg)</td>
<td>1447 ± 182'</td>
<td>899 ± 201'</td>
</tr>
<tr>
<td>3.</td>
<td>Extract + Paracetamol (500 mg/Kg + 640 mg/Kg)</td>
<td>161 ± 48'</td>
<td>73 ± 25'</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of extract (500 mg/Kg) at 12 hrs intervals before paracetamol administration.

* P < 0.01; Compared to group 1.

" P < 0.01; Compared to group 2.
Appendix 36.

Effect of *Rubia cordifolia* root extract on CCl₄-induced elevation of serum transaminase levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (10 mL/Kg + 7.5 mL/Kg)</td>
<td>99 ± 15</td>
<td>29 ± 08</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + CCl₄ (10 mL/Kg + 1.5 mL/Kg)</td>
<td>422 ± 102*</td>
<td>354 ± 74**</td>
</tr>
<tr>
<td>3.</td>
<td>Extract + CCl₄ (500 mg/Kg + 1.5 mL/Kg)</td>
<td>95 ± 09**</td>
<td>33 ± 07*</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. of 10 determinations expressed as IU/L. Group 3 received four doses of plant extract (500 mg/Kg) at 12 hrs intervals before CCl₄ administration.

* P < 0.01, ** P < 0.001; Compared to group 1.

* P < 0.05, ** P < 0.01; Compared to group 2.
12. PUBLICATIONS & COMMUNICATIONS
LIST OF PUBLICATIONS & COMMUNICATIONS

Papers:


General Pharmacology 26, 619-623.


Abstract/Communications:


301
14-16, Karachi, Pakistan.

