

7. Conclusion

In present study a number of chemical constituents were investigated for antioxidant activities using different methods. As a result, several chemical constituents were found to possess radical scavenging and xanthine oxidase inhibition activities. Some of these were found to possess both antiradical and xanthine oxidase inhibition potential, whereas some were found to be xanthine oxidase inhibitors. The natural compounds that showed radical scavenging and xanthine oxidase inhibition potential were cinnamic acid, coumarins and aurone derivatives whereas the synthetic compounds belonged to oxadiazole thione, thiadiazole and coumarin classes. Most of these compounds contained phenolic groups in their structure. It is concluded that this functional group contributes to their radical scavenging and enzyme inhibition properties.

Moreover three phytochemical constituents belonging to the coumarin and stilbene classes of compounds and four synthetic coumarin derivatives were found to inhibit xanthine oxidase specifically but were not found to be radical scavengers. Most of the compounds were found to be radical scavengers but did not show xanthine oxidase inhibition activity.

Some xanthine oxidase inhibitors were further investigated for their mechanistic action with xanthine oxidase and were found to be mixed type and uncompetitive inhibitors.

The selected compounds, possessing radical scavenging activities in *in vitro* assay models, were further investigated for their hepatoprotective effect in CCl₄-induced hepatotoxicity assay model in rats. Most of these compounds exhibited

hepatoprotective activity. The results of this *in vivo* study indicated that the levels of serum biochemical parameters, sAST, sALT, total, direct, and indirect bilirubins were largely maintained ($p < 0.05$) after pretreatment with triterpene (2), cinnamic acid ester derivatives (8 and 11), aurone derivatives (33 and 34), acyl hydrazide derivatives (45, 49, 51-56 and 58), oxadiazole thione derivatives (66 and 68) and benzothiazepine derivative (102). Among the natural compounds, the most hepatoprotective were the triterpene (2) and the cinnamic acid ester derivatives (8 and 11). In the case of synthetic compounds, the acyl hydrazides 45, 49, 51-56, 58 were found to be the most effective against the hepatotoxic action of CCl_4 as compared to other classes. The mechanism of CCl_4 -induced hepatotoxicity, as suggested by several researchers, is that CCl_4 is converted into the trichloromethyl free radicals ($\cdot\text{CCl}_3$) by the NADPH-cytochrome P-450 system, transferring an electron from NADPH to CCl_4 . The resulting radical species ($\cdot\text{CCl}_3$) reacts with oxygen to form (trichloromethyl peroxy radical) $\text{CCl}_3\text{OO}\cdot$, which is capable of initiating lipid peroxidation of cellular and organelle membranes, leading to the release of large quantities of cytosolic enzymes into the blood stream. Therefore, it can be suggested that the compounds which have been found to be hepatoprotective in this study, may provide hepatoprotection by a radical scavenging mechanism as these have already been found to possess antiradical activity in *in vitro* assay models.

Moreover, all the compounds which were found to be hepatoprotective were evaluated for their toxicity on freshly isolated human neutrophils. Most of these compounds were found to be non-toxic upto concentrations of 200 $\mu\text{g}/\text{mL}$ but some

of these compounds i.e. cinnamic acid ester derivative (11), acyl hydrazide derivatives (54 and 55) and oxadiazole thione derivative (61) were found to be toxic at even low concentration i.e. 12.5 µg/mL.

Interestingly, the phytochemicals triterpene (2), cinnamic acid ester derivative (8) and aurone derivatives (33 and 34) isolated for the first time showed significant radical scavenging activities in *in vitro* assay models as well as in *in vivo* system. The known constituent, cinnamic acid ester derivative (11), which was evaluated for the first time as antiradical in this study, was also found to be an effective radical scavenger in *in vitro* and *in vivo* models. The interesting action of cinnamic acid ester derivative (11) was observed during cytotoxicity studies on human neutrophils where this compound was found to be most toxic at lower concentrations (12.5 µg/mL). By comparison, the synthetic acyl hydrazide derivatives (45, 49, 51-56 and 58), oxadiazole thione derivatives (66 and 68) and benzothiazepine derivative (102), evaluated for the first time as antioxidants, were found to be active both in *in vitro* and *in vivo* assay models. Again some of these compounds, two acyl hydrazide derivatives (54 and 55) and an oxadiazole thione derivative (61), were found to be most toxic for neutrophils. Therefore, it is concluded that although these compounds provide hepatoprotection, they are not necessarily non-toxic for neutrophils.

“Antioxidants” act by a number of mechanisms include free radical scavenging activities, metal chelating properties and enzyme inhibiting potential, etc. All these mechanisms help stop the oxidation processes in any particular condition. The work presented in this thesis evaluates the compounds for their antioxidant activities, i.e.

having either free radical scavenging activity or xanthine oxidase enzyme inhibiting potential or both.

On the basis of the results presented here additional studies are required to investigate these compounds as potential drug agents. Further studies need to be conducted on the effects of these active chemical constituents on termination of the lipid peroxidation process in different systems by various methods. Further, these compounds may also be investigated for metal chelating potential. The resulting data would be helpful to pick out the most effective and suitable compounds for further research in the area of drug development.