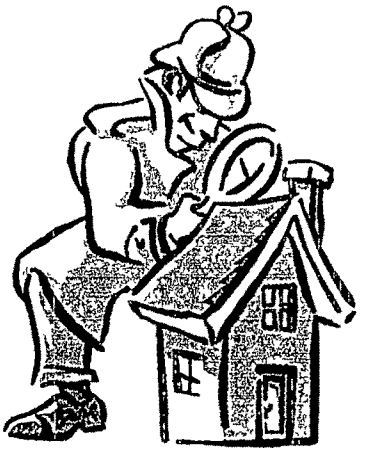
CHAPTER 6



SUMMARY AND CONCLUSIONS

The occurrence of reactive oxygen species, known as pro-oxidants, is an attribute of normal aerobic life. "Oxidative stress" results from imbalance in this pro-oxidant-antioxidant equilibrium in favor of the pro-oxidants. A number of diseases are associated with oxidative stress, being the basis of antioxidant therapy (Sies, 1991).

Free radical reactions have been implicated in the pathology of many human diseases including atherosclerosis, ischaemic heart disease, ageing process, inflammation, diabetes, immunodepression, neurodegenerative condition and other disease conditions (Maxwell, 1995). Antioxidants (Free radical scavengers) fight free radicals, and therefore may be able to help prevent the diseases that free radicals promote.

Antioxidant supplements were once thought to be harmless but increasingly we are becoming aware of potential interactions and potential toxicity. As an example, in normal concentrations found in humans, vitamin C and beta-carotene are antioxidants; but at higher concentrations they are pro-oxidants and can be harmful. It is also possible that unforeseen metabolic disturbances may occur after prolonged use of highly bioavailable pure compounds; such effects may not be apparent when antioxidants are obtained from natural foods. Thus, supplementation of antioxidants using natural sources seems to be a safe approach (Pillai and Pillai, 2002). As plants produce a lot of antioxidants to control the oxidative stress, they can represent a source of new compounds with antioxidant activity. Crude drugs or natural diet food which possess antioxidant or free radical scavenging activity has become a central focus for research designed to prevent or ameliorate tissue injury and may have a significant role in maintaining health (Jer-Min et al., 1995).

The present study on the *in vitro* and *in vivo* testing of herbal/herbomineral formulations DHC-1, Activit, Pepticare, Normacid and Drug-X showed that these formulations possessed weak scavenging activity of free radicals generated in *in vitro* systems. Study on *in vivo* models such as pylorus-ligation and ethanol-induced ulcers,

isoproterenol-induced myocardial infarction, cisplatin-induced nephrotoxicity and carbon tetrachloride-induced hepatotoxicity showed that these formulations possess good antioxidant activity *in vivo*. The study also proved that these formulations can ameliorate the tissue injuries inflicted by the release of free radicals as a result of the disease process or toxic actions of chemicals. Quantitative data obtained from this study will also be useful to carry out clinical studies and then launch these products in the market to make it available for the benefit of mankind.

The following studies were conducted to substantiate these findings.

- Preliminary radical scavenging activity of the formulations DHC-1, Activit, Pepticare and Normacid was tested by their ability to bleach the stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH). The study showed that the methanolic extracts of the formulations DHC-1, Activit and Pepticare possessed moderate, while Normacid had a mild DPPH quenching capacity.

- The methanolic extracts of the formulations DHC-1 and Activit were found to possess moderate, while Pepticare and Normacid were found to be weak scavengers of superoxide radical generated in riboflavin-NBT-light system *in vitro*.

- Administration of the formulations DHC-1, Activit, Pepticare and Normacid, produced a significant reduction in ulcer index in pylorus-ligation ulcer model. They also increased the pH and decreased the acid volume and total acidity of gastric fluid in pylorus-ligated rats, thus proving their anti-ulcer activity. Pylorus-ligation increased lipid peroxidation and decreased SOD, catalase, reduced glutathione and membrane bound ATPases thus leading to oxidative stress. Administration of these formulations resulted in a significant reduction in lipid peroxidation and enhancement in levels of SOD, catalase, reduced glutathione and membrane bound ATPases, which suggests

their efficacy in preventing free radical induced damage in stomach. Thus it can be concluded that the anti-ulcer activity shown by DHC-1, Activit, Pepticare and Normacid may be due to the modulation of defensive factors by improvement in gastric cytoprotection and partly due to antioxidant property and hence indirectly protect the gastric mucosa from oxidative stress

- Acute p.o. administration of ethanol to fasted rats produced extensive necrosis of gastric mucosa. Pretreatment with p.o. administration of DHC-1, Activit, Pepticare and Normacid could effectively and dose-dependently prevent such necrosis. These formulations also prevented the increase in lipid peroxidation and lowering of endogenous antioxidants and membrane bound ATPases produced by ethanol administration. Thus they protected the gastric mucosa from the damaging oxidative effects of ethanol.

- In isoproterenol-induced myocardial infarction model, isoproterenol increased the serum levels of creatine kinase, lactate dehydrogenase GOT and uric acid. The administration of formulations DHC-1, Activit, Pepticare and Normacid resulted in significant decrease in the activities of serum enzymes and uric acid; and increase in the levels of endogenous antioxidants (SOD, catalase and reduced glutathione), reduction in lipid peroxidation and prevention of associated histopathological changes induced by isoproterenol myocardial injury. Thus, the results obtained from this study indicate that pretreatment with DHC-1, Activit, Pepticare or Normacid offer significant protection to heart (cardioprotective effect) and thus reduces the risk of isoproterenol-induced cardiac damage by inhibiting lipid peroxidation and activating antioxidant defense enzymes in the organ.

- Cisplatin increased the serum levels of creatinine, urea, uric acid and BUN. It inhibited the activities of antioxidant enzymes (SOD and catalase), increased lipid peroxidation and depleted reduced

glutathione in rat kidneys suggesting that cisplatin nephrotoxicity results from generation of reactive oxygen species. Pretreatment with the formulations DHC-1, Activit, Pepticare or Normacid significantly reduced lipid peroxidation and increased the levels of glutathione, catalase and SOD in the chronic model, which suggests their efficacy in preventing free-radical induced damage. The reduction in serum levels of creatinine, urea, uric acid and BUN by the formulations, in the chronic model, may be due to their protective effect on the kidneys. The formulations DHC-1, Activit, Pepticare and Normacid also significantly increased the activity of ATPases in the chronic model and also completely protected the animals against cisplatin-induced decrease in body weight. Thus, the results obtained in the chronic study indicate that the formulations offer significant protection to kidney (nephroprotective effect) and reduce the risk of cisplatin-induced nephrotoxicity by their antioxidant mechanism of action.

- The formulations DHC-1, Activit, Pepticare and Normacid did not offer complete protection to the kidneys in the acute model.

- Administration of CCl_4 resulted in a significant elevation in SGPT, SGOT, alkaline phosphatase and total bilirubin levels. Depletion of elevated bilirubin level together with the suppression of the activity of SGPT, SGOT and ALP in the serum of rats treated with DHC-1 suggests the possibility of the herbal product being able to stabilize biliary dysfunction of rat liver during chronic injury with CCl_4. Reduction in lipid peroxidation and enhancement in the levels of endogenous antioxidants (glutathione, catalase and SOD) proves the efficacy of the drug, DHC-1 in preventing free-radical induced damage in liver by CCl_4. Thus the results prove that DHC-1 protects the liver from the damaging effects of CCl_4 by its antioxidant mechanism of action and can thus be used as hepatoprotectant against such chemical insults and other pathological conditions. Histopathological studies also correlate with the above results. The other three formulations, Activit,

Pepticare and Normacid did not completely protect the organ from the damaging effects of carbon tetrachloride.

- Administration of *M.oleifera* (200 mg/kg/day, p.o.) to rabbits fed a standard laboratory diet or hypercholesterolemic diet for a period of 120 days decreased the serum total cholesterol, phospholipid, triglycerides, LDL, VLDL, total lipids as compared to the corresponding control groups. Treatment of hypercholesterolemic rabbits with *M.oleifera* showed a significant increase in HDL levels. *M.oleifera* treated hypercholesterolemic rabbits also showed decrease in the lipid profile of liver and heart as compared to the corresponding control groups. The present study suggests that *M.oleifera* has hypolipidaemic action, but showed no significant enhancement in endogenous antioxidants nor reduction in lipid peroxidation in the organs, liver and heart. Thus the study showed that Drug-X exerts a hypolipidaemic effect, which is not due to the antioxidant effect but due to some other mechanism.

With all these findings it can be concluded that the formulations DHC-1, Activit, Pepticare and Normacid though not very effective free radical scavengers *in vitro* proved to be good antioxidants *in vivo*.

The present investigations, using a variety of experimental models of oxidative stress in several tissues, indicates that DHC-1, Activit, Pepticare and Normacid, have potent and non-tissue specific antioxidant action. It is therefore likely that DHC-1, Activit, Pepticare and Normacid may prove to be beneficial in disorders induced by oxidative stress.

Thus, the present investigation provides conclusive evidence for the antioxidant action of DHC-1, Activit, Pepticare and Normacid against several models of oxidative stress with diverse etiologies. However, clinical investigations are required to confirm these observations. Such investigations will help in rationalizing the uses of these formulations, particularly in conditions where oxidative stress is likely to be involved as part of the disease etiopathogenesis.