CHAPTER 5

DISCUSSION

The antioxidant activity of the formulations DHC-1, Activit, Pepticare and Normacid, was studied using *in vitro* models and in several experimentally induced disease conditions as *in vivo* models.

The *in vitro* free radical scavenging activity of these formulations was tested by their ability to bleach the stable radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH). It is one of the free radicals generally used for testing preliminary radical scavenging activity of a compound or a plant extract. This assay provides information on the reactivity of test compounds with a stable free radical, independently of any enzymatic activity. Because of its odd electron DPPH gives a strong absorption band at 516nm in visible spectroscopy (deep violet colour). As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes, and the resulting decolorization is stoichiometric with respect to the number of electrons taken up (Russo et al., 2002). 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 as compared to the standard, pyrogallol.

Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxygen species (Halliwell and Gutteridge, 1985). The NBT (Nitro blue tetrazolium) reduction method showed that the methanolic extracts of the formulations DHC-1 and Activit possessed moderate, while Pepticare and Normacid were weak scavengers of superoxide radical generated in riboflavin-NBT-light system *in vitro* as compared to the standard, ascorbic acid.

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Although in most of the cases the etiology of ulcer is unknown, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism (Piper and Stiel, 1986). To regain the balance, different therapeutic agents including herbal preparations are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production. Traditional medicine uses plants to treat gastrointestinal disorders, including peptic ulcers.

Effect of various doses (125, 250, 500 and 1000 mg/kg, p.o.) of DHC-1, Activit, Pepticare and Normacid was tested against gastric ulcers induced by pylorus-ligation and ethanol, the experimental models related to lesion pathogenesis with production of reactive species.

It is well known that pylorus-ligation causes gastric hypersecretion [Shay et al., 1945; Brodie et al., 1962; Ishii, 1969] due to poorly understood mechanisms. The activation of the vagus-vagal reflex by stimulation of pressure receptors in the antral gastric mucosa in the hypersecretion model of pylorus ligature is believed to increase gastric tonus and secretion. The stomach digestive effect of accumulated gastric juice in the induction of gastric ulcers is well documented in the pylorus-ligation model [Brodie, 1966]. The gastric distension produced by accumulated secretion seems to influence the secretion of gastric acid in this model, possibly by increasing the release of gastrin hormone, and consequently further increasing acid secretion [Nagy et al., 1968]. Administration of DHC-1, Activit, Pepticare and Normacid, produced a significant reduction in ulcer index. They also increased the pH and decreased the acid volume and total acidity of gastric fluid in pylorus-ligation model, thus proving their anti-ulcer activity. These effects of drug treatment on the parameters that influence the initiation and induction of ulceration may be considered as highly desirable property of anti-ulcerogenic agent.

Ethanol is a necrotizing agent that produces gastric ulceration by causing direct damage to the mucosa independent of gastric acid secretion [Takase et al., 1994]. Studies focusing on the pathogenesis of ethanol-induced injury have suggested that several factors are implicated in such processes: products of arachidonate metabolism (e.g.leukotriene) [Peskar et al., 1986], mast cell secretory products [Oates and Hakkinen, 1988] and reactive oxygen species [Pihan et al, 1987; Mizui et al., 1987]. Acute oral administration of ethanol to fasted rats produced extensive necrosis of gastric mucosa. Pretreatment with oral administration of DHC-1, Activit, Pepticare and Normacid could effectively and dose-dependently prevent such necrosis. This protective effect is called "cytoprotection". A gross

examination of the gastric mucosa showed a marked improvement in groups receiving DHC-1, Activit, Pepticare and Normacid.

Some of the ingredients of DHC-1 are known for their anti-ulcer properties. The ulcer protective potential of methanolic extract of *Emblica officinalis* Gaertn. was assessed by Al-Rehaily et al. (2002) in different acute gastric ulcer models in rats induced by aspirin, ethanol, cold restraint stress and pyloric ligation and healing effect in chronic gastric ulcers induced by acetic acid in rats. It showed dose-dependent ulcer protective effects in all the above acute ulcer models. Further study on gastric mucosal factors showed that it significantly decreased the offensive factors like acid and pepsin and increased the defensive factors like mucin secretion, cellular mucus and life span of mucosal cells. It also showed significant antioxidant effect in these stressed animals. Rajeshkumar et al. (2001) found that oral administration of *E. officinalis* juice (50mg/kg) produced a dose-dependent protective effect against gastric damage induced by ethanol, indomethacin and histamine. The protection afforded by *E. officinalis* fruits was found to be better than that of ranitidine (50mg/kg). The results of the study suggested the novel cytoprotective activity of *E. officinalis* fruits on gastric mucosal cells.

The antiulcer activity of another ingredient of DHC-1, *Glycyrrhiza glabra* (licorice) has been demonstrated both experimentally and clinically. Intraperitoneal or oral administration of its aqueous or alcoholic extracts reduced gastric secretions in rats and inhibited the formation of gastric ulcers induced by pyloric ligation, aspirin, and ibuprofen. Glycyrrhizin and its aglycone (glycyrrhetic acid, enoxolone), two of its active constituents, both have antiphlogistic activity and increase the rate of mucus secretion by the gastric mucosa. The mechanism of antiulcer activity involves acceleration of mucin excretion through increasing the synthesis of glycoprotein at the gastric mucosa, prolonging the life of the epithelial cells, and antipepsin activity (De et al., 1997). The mechanism underlying the anti-ulcer effects of licorice appears to involve the ability of GL and GA (glycyrrhetinic acid - a partially hydrolyzed form of glycyrrhizin) to inhibit the enzymes 15-hydroxyprostaglandin dehydrogenase and D-13-

prostaglandin reductase, which inactivates the protective prostaglandins (PG) in the gastric mucosa. Chaudhary, R.D. (1996) has also mentioned the anti-ulcer property of *Syzygium aromaticum.* Thus, the anti-ulcer activity of DHC-1 may be due to the gastroprotective effect of its ingredients.

The ingredients of Activit, *Asparagus racemosus, Centella asiatica* and *Piper longum* also possess anti-ulcer properties. The methanolic extract of fresh roots of *A.racemosus* showed significant protection against acute gastric ulcers induced by cold restraint stress, pyloric ligation, aspirin plus pyloric ligation, and duodenal ulcers induced by cysteamine. It also significantly healed chronic gastric ulcers induced by acetic acid; however, was ineffective against aspirin- and ethanol-induced gastric ulcers (Sairam, 2003). Further, gastric juice and mucosal studies showed that it significantly increased the mucosal defensive factors and also possessed significant anti-oxidant effect, but had little or no effect on offensive factors like acid and pepsin (Sairam, 2003). The extract of the fresh plant of *Centella asiatica* prevented ethanol-induced gastric mucosal lesions by strengthening the mucosal barrier and reducing the damaging effects of free radicals (Cheng and Woo, 2000). It was also found to provide significant protection against aspirin and pylorus-ligation induced gastric ulcers in rats (Sairam et al., 2001). *P.longum* showed significant protection against gastric ulcers induced by 2 h cold restraint stress, aspirin (200 mg/kg, 4 h) and 4 h pylorus-ligation. The anti-ulcerogenic effect seemed to be due to the augmentation of mucin secretion and decreased cell shedding rather than offensive acid and pepsin secretion, which however were increased by them (Agrawal et al., 2000).

Apart from *Emblica officinalis* and *Glycyrrhiza glabra,* the anti-ulcer reports of which have been mentioned in DHC-1, Pepticare also contains Sutashekhar ras, a herbomineral ayurvedic preparation and Kapardi bhasma. Clinical trial of Sutashekhar ras has proved its efficacy in the management of duodenal ulcers (Dash et al., 1987). Kapardi bhasma is also well known for its antacid property and used in acid peptic disorders

(Anonymous, 1978). It can thus be said that Pepticare has an anti-ulcer effect due to the potentiated synergistic effects of the herbal ingredients alongwith the minerals present in the formulation.

The anti-ulcer property of *Solanum nigrum* and Shankh bhasma, components of Normacid has been mentioned. Antiulcerogenic activity of *Solanum nigrum* was studied against aspirin-induced gastric ulcers in rats. In addition, its effect on output of gastric acid and pepsin and hexosamine concentrations in gastric fluid was recorded in ulcerated and non-ulcerated rats. *Solanum nigrum* (aerial parts) powder and its methanolic extract decreased the ulcer index significantly. The activity may be due to inhibition of acid and pepsin secretions and/or their *in vitro* ability to bind these (Akhtar and Munir, 1989). Pandit et al. (2000) studied the anti-ulcer activity of Shankh bhasma, another ingredient of Normacid, in rats and found that it offered significant protection in indomethacin and cold restraint stress induced gastric ulcers. The study also exhibited a potent anti-peroxidative effect, suggesting that Shankh bhasma may act as gastric cytoprotective agent by modulating scavenging of free radicals. The other ingredients of Normacid, Bhunimbadi kwath, Mouktika bhasma and Kapardi bhasma are also well known for their antacid property and used in acid peptic disorders (Anonymous, 1978). Thus, it is apparent that the anti-ulcer activity of Normacid is due to the presence of above components in the formulation.

Reactive oxygen species are involved in the pathogenesis of pylorus ligation-induced (Rastogi et al, 1998) and ethanol-induced (Pihan et al, 1987) gastric mucosal injury *in vivo*. As compared to normal rats, pylorus-ligation and ethanol administration increased lipid peroxidation and decreased SOD, catalase and reduced glutathione in the control groups, thus leading to oxidative stress. Thus, results in the present study also indicate similar alterations in the antioxidant status in rats after pylorus ligation and ethanol induced ulcers.

Preventive antioxidants, such as superoxide dismutase (SOD) and catalase (CAT) enzymes are the first line of defense against reactive oxygen

species. Reduced glutathione (GSH) is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation (Halliwell, B., 1995).

Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease states. It involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids which eventually results in destruction of membrane lipids. Biological membranes are often rich in unsaturated fatty acids and bathed in oxygen-rich metal containing fluid. Therefore it is not surprising that membrane lipids are susceptible to peroxidative attack (Cheesman, 1993).

Administration of DHC-1, Activit, Pepticare and Normacid resulted in a significant decrease in lipid peroxidation and increase in the SOD, catalase and reduced glutathione levels as compared to the control animals, which suggests their efficacy in preventing free radical induced damage. Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase are membrane bound enzymes. The inactivation or decrease of ATPases on pylorus-ligation and ethanol administration could be due to enhanced lipid peroxidation by free radicals (Gubdjarson et al., 1983). Lipid peroxidation impairs the function of membrane ion motive ATPases and glucose and glutamate transporters by a mechanism involving covalent modification of the transport proteins by the aldehyde product of lipid peroxidation, 4-hydroxynonenal (HNE). An enhancement in the membrane bound ATPases in both the ulcer models was observed in DHC-1, Activit, Pepticare and Normacid treated groups.

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

Isoproterenol-induced myocardial infarction serves as a well-standardized model to study the beneficial effects of many drugs. Isoproterenol, a non-selective β- adrenergic agonist, has been reported to cause oxidative stress in the myocardium resulting in infarct like necrosis of the heart muscle (Wexler and Greenberg, 1978). Isoproterenol

administration produces free radicals and via β- adrenoceptor mechanism, affects the cell metabolism to such a degree that cytotoxic free radicals are formed, producing myocardial cell necrosis (Noronha-Dutra et al., 1984). The cytotoxic free radicals cause the loss of membrane integrity with disintegration of polyunsaturated fatty acids in the membrane bilayer and exert unfavourable effects on the heart structure and function. Isoproterenol has been reported to increase lipid peroxidation in the heart tissue through free radical formation (Sushmakumari et al., 1989). Activated lipid peroxidation is an important pathogenic element in myocardial infarction, with lipid peroxide levels reflecting the major stages of disease and its complications (Golikov et al., 1989). Increased levels of lipid peroxides indicate the excessive formation of free radicals and activation of free radicals resulting in irreversible damage to the heart in animals subjected to isoproterenol stress. During myocardial infarction, superoxide radicals generated at the site of damage modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide ions, which damage the myocardium (Manjula et. al., 1994).

Isoproterenol increased lipid peroxidation in the heart tissue and decreased SOD and catalase activities (Sushmakumari et al., 1989; Manjula et al., 1994). The administration of formulations DHC-1, Activit, Pepticare and Normacid resulted in significant reduction in lipid peroxidation in the heart and an increase in the levels of endogenous antioxidants (SOD, catalase and reduced glutathione), which suggests their efficacy in preventing free radical induced damage. It has been reported that administration of isoproterenol (Chernysheva *et al.*, 1980; Manjula and Devi, 1993) alone resulted in decrease in the activities of membrane bound ATPases (Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase). The results obtained in this study also correlate with the above reports. The formulations DHC-1, Activit, Pepticare and Normacid significantly increased the activity of ATPases in the heart.

Serum levels of creatine kinase, lactate dehydrogenase and GOT are the diagnostic indicators of myocardial infarction. The increased levels of serum enzymes in myocardial ischemia are due to the leakage of enzymes from the damaged heart cells into blood as a result of necrosis induced by

isoproterenol (Kaul and Kapoor, 1991). Increase in serum uric acid could be due to excessive degradation of purine nucleotides and proteolysis (Iriama, 1987). Treatment with DHC-1, Activit, Pepticare and Normacid significantly decreased the serum enzyme levels by preventing the release of lysosomal enzymes, which may be due to its membrane stabilizing activity. The drugs also decreased the levels of uric acid and prevented the 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 offers significant protection to heart (cardioprotective effect) and thus reduce the risk of isoproterenol-induced cardiac damage by inhibiting lipid peroxidation and activating antioxidant defense mechanism in the organ.

Cisplatin [cis-diamminedichloroplatinum (II): CDDP] is a widely used cancer chemotherapeutic agent whose clinical use is limited by its renal toxicity (Goldstein and Mayor, 1983). Previous reports suggest that cisplatin-induced nephrotoxicity is by the increase in lipid peroxidation (Hanneman and Baumann, 1991) and depletion of cellular thiols (Levi et al., 1980) in kidney tissues following cisplatin treatment. Cisplatin inhibits activities of antioxidant enzymes (SOD and catalase) in rat kidneys suggesting that cisplatin nephrotoxicity results from generation of reactive oxygen species (Sdzuka et al., 1992). It has been reported that administration of cisplatin (Devi Priya and Shyamala Devi, 1999) alone resulted in decrease in the activities of membrane bound ATPases (Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase).

Acute tubular necrosis is a prominent feature of cisplatin nephrotoxicity and is clinically manifested by elevations in blood urea nitrogen (BUN), serum creatinine, urea and uric acid due to decreased clearance by kidney (Goldstein and Mayor, 1983).

Cisplatin administration significantly decreased the levels of endogenous antioxidants, SOD, catalase and reduced glutathione and increased lipid peroxidation (MDA content) in both acute and chronic model. Thus, the results obtained in the cisplatin control group correlates

with the previous reports. Pretreatment with the formulations DHC-1, Activit, Pepticare or Normacid did not significantly affect the serum levels of creatinine, uric acid, urea and BUN, indicators of kidney damage and the various antioxidant parameters in the acute model. The formulations also could not completely protect the animals against cisplatin-induced decrease in body weight. However, the formulations significantly reduced the serum levels of creatinine, uric acid, urea and BUN in the chronic model. They also significantly reduced the lipid peroxidation and increased the levels of glutathione, catalase and SOD of kidneys in the chronic model, which suggests their efficacy in preventing free-radical induced damage. 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.

The reduction in serum levels of creatinine, uric acid, urea and BUN, the indices of renal functional impairment, in the chronic model, may be due to the protective effect of the formulations on the kidneys. Histopathological studies also confirm the above findings. **Thus, the results obtained in the chronic study indicate that the formulations, DHC-1, Activit, Pepticare and Normacid offer significant protection to kidney (nephroprotective effect) and reduce the risk of cisplatin-induced nephrotoxicity by their antioxidant mechanism of action. The results also suggest the possibility that multiple doses of the formulations DHC-1, Activit, Pepticare and Normacid are more effective in protecting the kidneys from the toxic effects of cisplatin rather than a single dose.**

CCl_4-induced hepatotoxicity in rats is a commonly used experimental model for investigating lipid peroxidation-related tissue injury. Ko et al. (1993) have examined the impairment in hepatic antioxidant status during the development of CCl_4-induced hepatotoxicity in rats and the protection of such tissue injury by pretreatment with vitamin E, herbal extracts or herbal preparations known to possess antioxidant activities. Twenty-four hours following oral administration of CCl_4, a decrease in hepatic GSH content and activities of antioxidant

enzymes was observed. This generalized impairment in hepatic antioxidant defense mechanism was paralleled by an elevation in SGPT and SGOT activity, an indication of hepatocellular damage (Naziroglu et al., 1999). The leakage of cytosolic enzymes from the liver may be a consequence of the membrane destabilizing effect of CCl_4 on hepatocytes and/or the membrane damage caused by free radicals arising from CCl_4 metabolism. In this regard, reactive free radicals or their reaction products generated from CCl_4 metabolism can diffuse across the cytoplasm or via membrane contiguous with the plasma membrane, leading to the peroxidation of plasma membrane lipids. The activity of serum alkaline phosphatase was also elevated during CCl_4 administration. Alkaline phosphatase is excreted normally via bile by the liver. In liver injury due to hepatotoxin, there is a defective excretion of bile by the liver, which is reflected in their increased levels in serum (Rao, 1973). Hyperbilirubinaemia is a very sensitive test to substantiate the functional integrity of the liver and severity of necrosis which increases the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocyte degeneration rate (Singh et al., 1998).

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 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 DHC-1 in preventing free-radical induced damage in liver by CCl_4. The administration of Activit or Normacid decreased the levels of SGOT and alkaline phosphatase, but did not affect the levels of SGPT and total bilirubin. Pepticare significantly reduced the levels of total bilirubin and alkaline phosphatase but did not affect the levels of SGPT and SGOT. **Thus, only DHC-1 could completely protect the liver from hepatotoxic effects of CCl_4; whereas the other three formulations, Activit, Pepticare and Normacid did not completely protect the organ from the damaging effects of carbon tetrachloride.**

Results of histopathological study correlated with the changes of serum enzymatic alternation.

Pretreatment with DHC-1 significantly reduced lipid peroxidation and increased the levels of endogenous antioxidants, which suggests its efficacy in preventing free-radical induced damage. However, Activit, Pepticare and Normacid did not affect the levels of lipid peroxidation, SOD and catalase but significantly increased the GSH levels of liver.

Thus the results prove that DHC-1 protected 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.

The hepatoprotective effect of DHC-1 can be due to the ingredients, *Emblica officinalis* (Jose and Kuttan, 2000) and *Glycyrrhiza glabra* (Wang and Han, 1993), which have individually been shown to protect the liver from the toxic effects of carbontetrachloride.

Regarding the antioxidant effects of the drugs Activit, Pepticare and Normacid in carbon tetrachloride toxicity without complete amelioration of its hepatotoxic effects, it is known that any effect on glutathione based antioxidant defense is a secondary effect in this toxicity model. There is no evidence that GSH based antioxidant status is critical for carbon tetrachloride toxicity. Therefore, it is not surprising that these antioxidant drugs are without much positive effect in ameliorating hepatotoxic effects of carbon tetrachloride.

The partial protection afforded to the kidney by Activit, Pepticare and Normacid may be due to the presence of liver protecting ingredients like *Tinospora cordifolia* (Bishayi et al., 2002), *Piper longum* (Koul and Kapil, 1993), *Emblica officinalis* (Jose and Kuttan, 2000), *Glycyrrhiza glabra* (Wang and Han, 1993), *Centella asiatica* Darnis et al. (1979), *Solanum nigrum* (Sarwat et al., 1995) and *Tribulus terrestris* (Li et al., 1998).

The sections above dealt with the results pertaining to the study of the formulations DHC-1, Activit, Pepticare and Normacid in *in vitro* and *in vivo* models for the amelioration of experimentally induced tissue damage. The discussion given below deals with the antioxidant mechanism of DHC-

1, Activit, Pepticare and Normacid in rectifying the disease induced by oxidative stress.

The *in vitro* antioxidant activity of *B. monniera,* one of the ingredients of DHC-1 was evaluated earlier by $FeSO_4$ induced lipid peroxidation in rat brain homogenate and the mechanism of action was thought to be through metal chelation at the initiation level and also as a chain breaker (Tripathi et al., 1996). The active tannoid principles of *Emblica officinalis* (amla), another ingredient of DHC-1, were found to induce an increase in both frontal cortical and striatal concentrations of the oxidative free radical scavenging enzymes, SOD, catalase and glutathione peroxidase and concomitant decrease in lipid peroxidation in these brain areas (Bhattacharya et al., 1999). Wang and Han (1993) suggested that the anti-lipid peroxidation effect of Glycyrrhiza flavonoids contributed to its protective action against carbon tetrachloride-induced hepatotoxicity. Haraguchi et al. (2000) also studied the protection of mitochondrial functions against oxidative stresses by isoflavans from *Glycyrrhiza glabra.* The extract of *Mangifera indica* reduces ischemia-induced neuronal loss and oxidative damage in the gerbil brain most probably due to the antioxidant activity of the extract (Martinez Sanchez et al., 2001). The antioxidant activity of the extract was also studied on hydroxyl-mediated oxidation of bovine serum albumin (BSA) and in a hepatic microsome system and was found to reduce the oxidation of BSA and inhibited lipid peroxidation, which was, initiated enzymatically by NADPH. The results suggested that the extract has an antioxidant activity probably due to its ability to scavenge free radicals involved in microsome lipid peroxidation. In addition, the extract's antioxidant profile *in vitro* was probably similar to its principal polyphenolic component, mangiferin, a glycosylated xanthone (Martinez et al., 2001). In another study, Vimang, an aqueous extract of *M.indica* was found to provide significant protection against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced oxidative damage in serum, liver, brain as well as against hyperproduction of ROS by peritoneal macrophages. Thus Vimang could be useful to prevent the production of ROS and the oxidative tissue damages *in vivo* (Sanchez et al., 2000). The aroma extracts and aroma components isolated from

Syzygium aromaticum (clove) were found to inhibit malondialdehyde formation from blood plasma oxidised with Fenton's reagent (Lee and Shibamoto, 2001).

Activit contains extracts derived from *Anacyclus pyrethrum, Argyreia speciosa, Asparagus racemosus, Centella asiatica, Mucuna pruriens, Nux vomica, Piper longum, Tinospora cordifolia, Tribulus terrestris, Withania somnifera* and shring bhasma. The methanolic extract of fresh roots of *Asparagus racemosus* showed significant protection against acute gastric ulcers induced by cold restraint stress, pyloric ligation, aspirin plus pyloric ligation, and duodenal ulcers induced by cysteamine and was also found to possess significant anti-oxidant effect (Sairam, 2003). *A.racemosus* has also been shown to possess potent antioxidant properties against damage induced by gamma radiation in mitochondrial membranes of rat liver (Kamat et al., 2000). The extract of the fresh plant of *Centella asiatica* prevented ethanol-induced gastric mucosal lesions by strengthening the mucosal barrier and reducing the damaging effects of free radicals (Cheng and Woo, 2000). Other studies have indicated an antioxidant effect of asiaticoside. When applied topically, asiaticosides derived from Centella was found to enhance induction of antioxidant (SOD, catalase, glutathione peroxidase, vitamin E and ascorbic acid) levels at an initial stage of healing wounds, which may be an important contributory factor in the healing properties of this substance (Shukla et al., 1999). Studies also indicated that *C. asiatica* has cognitive enhancing effect and an antioxidant mechanism is involved (Veerendra Kumar and Gupta, 2002). The alcohol extract of seeds of *Mucuna pruriens* was found to have an antilipid peroxidation property, which is mediated through the removal of superoxides and hydroxyl radicals (Tripathi and Upadhyay, 2002). The active principle of *Piper longum*, Piperine exerted significant protection against tert-butyl hydroperoxide and carbon tetrachloride hepatotoxicity by reducing both *in vitro* and *in vivo* lipid peroxidation, enzymatic leakage of GPT and alkaline phosphatase, and by preventing the depletion of GSH and total thiols in the intoxicated mice (Koul and Kapil, 1993). Strychnine, the major active principle in the alcoholic extract of the seeds of *Strychnos*

nux-vomica, is responsible for its antilipid peroxidative property. The mechanism of action of this drug is through the chelation of the free iron in the system. It has also been observed that strychnine does not have any pro-oxidant property, because it does not convert Fe^{3+} to Fe^{2+} and vice versa in the reaction system, as has been observed with several other antioxidants (Tripathi and Chaurasia, 2000). *Tinospora cordifolia* extract quenched superoxide radicals and hydroxyl radicals in *in vitro* assays. The extract also inhibited lipid peroxidation in isolated liver microsomal fractions. The antioxidant activity was further studied in cell cultures. When incubated with activated macrophages, the extract inhibited the production of superoxide generated by the oxidative burst of these immune cells. The antioxidant activity of the extract was also found to be useful in the amelioration of cyclophosphamide-induced toxicity (Mathew and Kuttan, 1997). The antioxidant activity of an arabinogalactan polysaccharide (TSP) isolated from *Tinospora cordifolia* was studied by Subramanian et al. (2002). The polysaccharide showed good protection against iron-mediated lipid peroxidation of rat brain homogenate as revealed by the thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxide (LOOH) assays. TSP also provided significant protection to protein against gamma ray induced damage. The protective action can possibly be explained by its very high reactivity towards DPPH, superoxide radicals and the most damaging of radicals, the hydroxyl radical. Aqueous extract of *T.cordifolia* inhibited Fenton ($FeSO_4$) reaction and radiation mediated 2-deoxyribose degradation in a dose dependent fashion. The results revealed that the direct and indirect antioxidant actions of T cordifolia probably act in corroboration to manifest the overall radioprotective effects (Goel et al. 2002). Experiments suggest that its ability to scavenge free radicals and to block free radicals and to inhibit radical-induced membrane damage may be the underlying feature of many of the biological activities of this herb. *Withania somnifera* (Ashwagandha) roots are known to possess restorative and adaptogenic properties. The free radical scavenging activity of *W.somnifera* and its protective effect on H_2O_2-induced cytotoxicity and DNA damage was studied by Russo et al. (2001). The antioxidant activity of *W.somnifera* glycowithanolides was also

assessed in chronic footshock stress induced changes in rat brain frontal cortex and striatum, lending support to the clinical use of the plant as an anti-stress adaptogen (Bhattacharya et al., 2001). Studies have also suggested that the ameliorating role of root extract of ashwagandha in the lead intoxicated mice could be the result of its antiperoxidative action (Chaurasia et al., 2000).

Pepticare consists of *Emblica officinalis*, *Glycyrrhiza glabra* and *Tinospora cordifolia*, alongwith Sootshekhar ras, Praval bhasma, Swarna bhasma, Kapardi bhasma and Shodhit gairik. The antioxidant properties of *Emblica officinalis* and *Glycyrrhiza glabra* have already been discussed in DHC-1. *Emblica officinalis* and *Tinospora cordifolia* are categorized as 'rasayanas' (rejuvenatives). Rasayanas are non-toxic Ayurvedic complex herbal preparations or individual herbs used to rejuvenate or attain the complete potential of an individual in order to prevent diseases and degenerative changes that leads to disease. Various activities of rasayanas have been reviewed by Vayalil et al. (2002) to support the above concept, its role as a prophylactic medication and significance in the prevention of diseases in both healthy as well as diseased individuals. The emerging data suggest that the possible mechanisms may be by immunostimulation, quenching free radicals, enhancing cellular detoxification mechanisms; repair damaged non-proliferating cells, inducing cell proliferation and self-renewal of damaged proliferating tissues, and replenishing them by eliminating damaged or mutated cells with fresh cells. The clinical efficacy of the fruits of *E.officinalis* is held in high esteem in Ayurveda and amla is referred to as a maharasayana. By virtue of their properties and clinical use in Ayurveda, the rasayanas may provide potential therapeutic intervention against oxidative threats, both in health and disease. Another constituent of Pepticare, Swarnabhasma, has been used since ancient times in several clinical manifestations including loss of memory, defective eyesight, infertility, overall body weakness and incidence of early aging. According to modern concept, the scientific basis for its application in degenerative diseases may arise, atleast in part, through enhancement in free radical concentrations. It was

found to induce enhanced activity of SOD and catalase. Swarnabhasma was found to produce no signs of toxicity indicating that the drug can be used safely in oral route for atleast a few months (Mitra et al., 2002). Shah and Vohora (2002) have also reported the antioxidant effects of calcined gold preparations used in Indian systems of medicine against global and focal models of ischemia.

The formulation Normacid contains Bhunimbadi kwath, *Solanum nigrum*, Mouktika bhasma, Shuddha gairika bhasma, Kapardi bhasma, Swarna bhasma, Praval bhasma and Shankh bhasma. *Solanum nigrum* has been reported to possess hepatoprotective effect due to its ability to suppress the oxidative degradation of DNA in the tissue debris (Sarwat et al., 1995). Pandit et al. (2000) have suggested that Shankh bhasma may act as gastric cytoprotective agent by modulating scavenging of free radicals. The enhanced activity of SOD and catalase by Swarna bhasma has been mentioned above.

Experimental hyperlipidaemia in rabbits was associated with an increase in serum lipid parameters. Administration of Drug X consisting 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 normal rabbits with Drug X decreased the HDL levels as compared to the normal control group. However, treatment of hypercholesterolemic rabbits with Drug X showed a significant increase in HDL levels. The change in the lipid profile with Drug X treatment may contribute to the decreased incidence of atherosclerosis and coronary heart disease. Drug X treated hypercholesterolemic rabbits also showed decrease in the lipid profile of liver and heart as compared to the corresponding control group while similar treatment of normal animals did not produce significant reduction in the heart. The reduction in liver cholesterol, triglyceride and phospholipid levels may be because of partial inhibition of cholesterol synthesis *de novo* or by inhibition of cholesterol absorption, thereby

depleting critical intracellular pool of sterols in the liver. The reduction of lipid content of heart may serve as a useful index of the severity of atherosclerosis.

Plant sterols inhibit the absorption of dietary cholesterol, but the resulting decrease in serum cholesterol has been slight (Lees et al., 1977; Grundy et al., 1969). A more recent study (Tatu et al., 1995) has found a 10.2% reduction in serum cholesterol. Although *M.oleifera* has been shown to contain β-sitosterol, the amount contained is unknown. The cholesterol lowering effect may be due to this inhibition in reabsorption of cholesterol from endogenous sources. The present study suggested that Drug X (*M.oleifera*) has hypolipidaemic action.

It is well documented that elevated cholesterol and LDL levels promote atherosclerosis (Diaz et al., 1997). Oxidative modification of LDL appears to have an important role in coronary artery diseases and atherogenesis (Camejo et al., 1976). The agents, which can scavenge the free radicals or inhibit their production and protect membranes from peroxidation, have gained wide therapeutic value. Many plant products are increasingly recognized as having protective role in coronary artery diseases, stroke through several mechanisms including antioxidant and hypocholesterolemic properties (Chander et al., 1996). The search for hypolipidaemic drugs follows rationale that high levels of plasma cholesterol are associated with an increased incidence of coronary heart diseases. Reduction in LDL cholesterol and increase in HDL cholesterol are significantly related to lipid lowering therapy (Miller, 1995).

The pathogenicity of atheromatous lesion is known to be closely related to the toxic effects of lipid peroxides (Glavind et al., 1952) and higher rate of lipid peroxidation has also been demonstrated in experimental hypercholesterolemia (Tsai 1975). Also decrease in the content of reduced glutathione (GSH) was demonstrated by Tikekar and Chakrabarti (1973) in prolonged cholesterol fed rats. Our present finding of enhancement in hepatic and cardiac lipid peroxidation and reduction in reduced glutathione in hypercholesterolemic (HCD) control group is quite in agreement with that of the earlier workers. Treatment with Drug-X neither showed significant enhancement in endogenous antioxidants

(SOD, catalase and GSH) nor reduction in lipid peroxidation in liver and heart, which suggests that this formulation does not afford an *in vivo* resistance to lipoperoxide formation. **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. Thus, the results demonstrated a hypolipidaemic effect of Drug-X independent of any antioxidant effect of the drug.** As Drug-X was not found to possess antioxidant activity, it was not studied further for this effect in the earlier mentioned *in vivo* and *in vitro* methods.

The results thus prove that the formulations DHC-1, Activit, Pepticare and Normacid though are not very good free radical scavengers *in vitro* but possess good antioxidant activity *in vivo*. The study proved that these formulations can protect the vital organs of our body from oxidative stress and the diseases involving the same. The study thus provides a basis for the clinical use of DHC-1, Activit, Pepticare and Normacid in several diseases like ulcers; cardiac, renal and hepatic dysfunction caused either due to pathological or toxicological conditions.