CHAPTER VIII
SUMMARY AND CONCLUSION

Elephantopus scaber Linn, a traditional medicinal plant, was analyzed for its phytochemicals as well as its pharmacological effects with special emphasis on hepatoprotection. This study revealed that the whole plant of *E. scaber* especially the root possessed antihepatotoxic and antioxidant properties in different models of hepatopathy.

Preliminary studies started with the extraction of plant powder in petroleum ether, chloroform, ethanol and methanol. Preliminary phytochemical screening indicated the presence of flavonoids, phenols, tannins, carbohydrates, proteins, oils and terpenoids in the different extracts.

In *vitro* antioxidant effect of the different extracts of *Elephantopus scaber* was conducted. The different extracts utilized were petroleum ether, chloroform, ethanol and methanol. Methanolic extract exhibited better efficacy and was selected for further *in vivo* studies. The IC$_{50}$ of methanolic extract of *Elephantopus scaber* was found out. Curcumin was used as the standard in all these experiments.

Pharmacological and toxicological evaluation of methanolic extract of *Elephantopus scaber* was done in male wistar rats. CCl$_4$ was used to induce liver damage in rats. Administration of methanolic extract of *Elephantopus scaber* root at a dose of 150mg/kg body weight evoked the maximum hepatoprotective activity as indicated by the restoration of altered values of different parameters in hepatotoxic rats to near normalcy.
The different parameters considered were the activities of AST, ALT, ALP, GGT, total protein and albumin.

Methanolic extract of *E. scaber* elicited antioxidant activity in CCl$_4$ treated rats as manifested by the attainment of normalcy in the altered values of TBARS, CD, SOD, Catalase, and GSH levels in the liver of drug administered rats. Histopathological alterations caused by CCl$_4$ were returned to normal by the administration of the methanolic extract of *E. scaber*.

The protective effect of methanolic extract of *Elephantopus scaber* root against paracetamol induced liver toxicity was also studied in male wistar rats. Pre-treatment with methanolic extract at doses of 75mg and 150mg/kg body weight significantly prevented the increase in serum AST, ALT, ALP and GGT activity. It also prevented lipid peroxidation and the depletion of hepatic glutathione (GSH) in liver. Methanolic extract of *Elephantopus scaber* increased the levels of total protein and albumin. The levels of TBARS and CD were decreased. The levels of SOD and catalase were increased by administration of the plant extract. Histological changes in the liver sections induced by paracetamol include centrilobular necrosis, dilated sinusoidal spaces and diffuse hyaline necrosis with blood pooling in sinusoidal spaces and central venule. These changes were reduced by the treatment of methanolic extract of *Elephantopus scaber* root. Standard drug silymarin also produced a similar effect.

Anti-inflammatory activity of *Elephantopus scaber* on rat paw edema model was also investigated. In this study, both carrageenan and formalin were used for inducing paw edema. Carrageenan induced paw edema was used for determining the acute phase of inflammation. Rat paw
volumes at different intervals were measured. *Elephantopus scaber* root methanolic extract at doses of 100mg/kg and 200mg/kg body weight showed considerable anti-inflammatory activity. This was evident by the decrease in rat paw volume. Formalin induced rat paw edema model was used as the chronic model for measuring inflammation. Rat paw volumes at different intervals were significantly reduced by the administration of methanolic extract of *Elephantopus scaber* root at doses of 100mg/kg and 200mg/kg body weight. Diclofenac sodium was used as the standard reference drug in both acute and chronic models.

Toxicity study was performed to assess the safety of the plant extract used for *in vivo* experiments. In acute toxicity study, rats were given a single dose of methanolic extract of *Elephantopus scaber* at 5gm/kg body weight. The animals were sacrificed on the fifteenth day. No significant variation in glucose, cholesterol, triglycerides, urea, creatinine, AST and ALT levels between the control and treatment groups were observed. Subacute toxicity study on rats at 1gm/kg body weight daily was done for a period of thirty days. The levels of glucose, cholesterol, triglycerides, urea, creatinine, AST and ALT were measured. No significant variation in the levels of biochemical parameters between the control and treatment groups provides support to the nontoxic effect of methanolic extract of *Elephantopus scaber* on multiple organs of the body including heart, kidney, liver, pancreas etc. So *Elephantopus scaber* can be safely used for drug development.

The antifibrotic effect of *E.scaber* methanolic extract was also studied in male wistar rats. It was effective in preventing and curing hepatic fibrosis as evidenced by biochemical parameters. It effectively reduced the hydroxyproline content of the liver. Histopathological observations of the
tissues of various experimental groups further corroborated the biochemical findings. Immunohistochemical analysis of liver sections specifically stained for Collagen-III showed that Collagen-III was predominantly expressed along fibrous septa in CCl₄ treated rats. Weak immunostaining of localized Collagen - III was noticed in Elephantopus scaber extract treated rats, at doses 100 mg/kg and 200 mg/kg body weight co-exposed with CCl₄. Weak immunostaining of localized Collagen III was observed in the standard drug silymarin treated rats.

Purification and characterization of the active fraction was carried out. In this study, the methanolic extract of the plant was partitioned between petroleum ether (60-80°C) and 10% aqueous methanol. The 10% aqueous methanolic extract was subjected to silica gel column chromatography and was eluted with different concentrations of hexane:ethyl acetate mixture. The eluted fractions were collected and allowed to evaporate. One of the fractions on recrystallisation from chloroform gave yellow needle shaped crystals. LCMS analysis of the purified sample was conducted. The presence of scabertopin, dihydroelephantopin, elascaberin, luteolin and methoxy cinnamaldehyde were detected. The purified fraction was tested for hepatoprotective activity in CCl₄ treated rats. The purified fraction was found to be a potent hepatoprotectant. The antioxidant and antifibrotic properties of Elephantopus scaber extract may be due to the combined effect of all these compounds.

In this study, Elephantopus scaber Linn, a traditional medicinal plant, used for the treatment of hepatic disorders was subjected to scientific evaluation with the help of modern medicinal chemistry and pharmacology. The plant was found to be highly effective in resisting hepatic fibrosis.
In total this study validates the traditional use of *E.scaber* for the treatment of hepatic disorders. Future studies may be earmarked towards improvised technique to synthesize the active principle from the plant preferably by plant tissue culture methods. This can be utilized to effectively combat the ever increasing incidence of hepatic disorders. Also, the protective mechanism of action of the active principle can be explored. The immunomodulatory properties of the active fraction can also be studied. Ample chances are awaiting researchers in this direction.