CHAPTER – 7

Summary and Conclusions
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7.1 SUMMARY

The present project was executed in an attempt to evaluate the antihyperlipidemic potential of selected antihyperlipidemic plants. Leaves of *Hibiscus rosa sinensis*, fruits of *Trichosanthes anguina*, *amorphophallus campanulatus*, *Luffa cylindrical* and seeds of *Foeniculum vulgare* were screened and the selected plants *H. rosa sinensis* and *T. anguina* were studied for their antiobesity potential in High fat diet (HFD) induced obese rats. The study was extended to assess the mode of action of extracts on lipid metabolizing enzymes *in vitro*.

Keeping in view the aim and objectives of the study, initially an extensive review of literature was undertaken. Based on the abundance of phytochemicals of therapeutic importance present in the plants, five medicinal plants parts were selected for the study. They were locally collected, dried, pulverised in a mechanical grinder and stored in dry place. In order to prepare the extracts, the dried powders of the above mentioned plants were subjected to hot solvent extraction using methanol. The crude extracts of five plants were screened for their antihyperlipidemic effect on Triton WR-1339 induce hyperlipidemic mice model. It was observed that among the five plant extracts, methanolic extracts of *H. rosa sinensis* (MEHR) and *T. anuigna* (META) demonstrated maximum reduction in total cholesterol levels when compared to the Triton control group. Hence, MEHR and META were selected for the further study.

MEHR and META were screened for the determination of dose dependent activity in Triton WR-1339 induced hyperlipidemic rat model. The finding revealed that both the plant extracts at a dose of 500 mg/kg body weight exhibited significant reduction in serum lipid profile. Hence, 500 mg/kg body weight dose was taken for the evaluation of antiobesity and antihyperlipidemic activities.

In order to ascertain the phytochemicals possibly playing a role in the observed antihyperlipidemic activity, MEHR and META were phytochemically evaluated. This includes extractive values, ash value, physicochemical parameters, fluorescence analysis, preliminary phytochemical studies, TLC and HPTLC fingerprint analysis. The present finding showed the presence of flavonoids, steroids and saponins in the extracts. HPTLC chromatograms confirmed the presence of β-sitosterol and quercetin in both the plant extracts.
The antiobesity and antihyperlipidemic activities of MEHR and META were assessed in HFD induced obese rats. HFD induced obese rats showed significant increase in the levels of body weights, abdominal circumferences, food intake, serum cholesterol levels, blood glucose and increase in organ weight of liver, peritoneal and perirenal fat tissues. However, administration of MEHR and META in obese rats at a dose of 500 mg/kg body weight for 10 days significantly decreased the body weights and serum lipid levels along with abdominal circumferences. Also, significant decrease in the liver, peritoneal and perirenal fat tissue weights were observed after MEHR and META administration. Food intake tendency was also decreased considerably in the META treated group alone. Quantification of liver and fecal lipid content was also done and it was observed that fecal excretions of total lipids were significantly higher in META and MEHR treated groups than those in HFD control group. Conversely, hepatic lipid content was considerably reduced in META and MEHR treated rat livers. This indicates that hepatic lipid-lowering effect of MEHR and META is probably related to a lower intestinal lipid absorption, resulting in an increase of hepatic excretion of fats through bile acids and finally leading to the decreased hepatic lipid accumulations. Treatment with MEHR and META caused significant decrease in total cholesterol, triglycerides, low density lipoprotein cholesterol and very low density lipoprotein cholesterol levels. This could be due to presence of phytosterols in the extracts, as they possess more affinity for micelles than cholesterol and reduce incorporation of cholesterol in micelles in the intestine (Ikeda and Sugano, 1998). MEHR and META treatment, also significantly lowered serum blood glucose levels. However, significant elevation in serum AST and ALT levels were observed in META treated group. This could be due to presence of tannins or alkaloids in the extracts that are reported to be toxic when present in high amounts in herbal products. Hence, the above results show that MEHR and META possess antihyperlipidemic and antiobesity activities.

MEHR and META were additionally evaluated for their possible mode of action on lipid metabolic enzymes i.e., pancreatic lipase (PL) and HMG CoA reductase (in vitro and ex vivo). Inhibitory effects of plant extracts MEHR and META were checked against porcine PL enzyme, using \( p \)-nitrophenyl butyrate as substrate. Both the plant extracts showed significant inhibitory activity against PL. Effect on HMG CoA reductase activity in liver microsomal preparations was
measured *in vitro* as oxidation of NADPH at different concentrations of MEHR and META. Similarly, the *ex vivo* enzyme activity was analyzed in liver samples obtained from obese rats treated with the plant extracts. *In vitro* studies showed that MEHR significantly inhibited the HMG CoA reductase activity whereas META showed significant inhibition only at very high concentrations with an IC\textsubscript{50} value of more than 500 μg/ml. *Ex vivo* studies also showed similar results wherein MEHR treated rats showed significant reduction in HMG CoA reductase activity in liver samples whereas no significant effect was observed with META. Therefore, the results indicate that META exhibited antihyperlipidemic activity through a process independent of inhibition of HMG CoA reductase. Also, the results suggest that the possible mechanism of action of MEHR and META towards control of obesity could be through inhibition of pancreatic lipase.

*In vivo* acute toxicity of MEHR and META as per the OCED guidelines 423 were evaluated. The results demonstrated that both the extracts did not cause any mortality and toxicity and hence were considered safe for use. The repeated dose oral toxicity test for META was carried out as per OECD guideline 407. The study was conducted as increased levels of AST and ALT were observed in rats treated with META. The extent of damage as well as its reversibility were assessed by this test. Results showed significant elevation in serum AST, ALT and ALP levels after treatment with META extract for 28 days. However, after 14 days of recovery period the results showed reversal of the damage caused by intake of META. Serum AST, ALT and ALP levels were significantly restored.

### 7.2 CONCLUSIONS

- The results obtained in the present study indicate that amongst the five medicinal plants tested for antihyperlipidemic activity, *H. rosa sinensis* and *T. anguina* were most effective in reducing the total cholesterol levels in Triton WR-1339 induced hyperlipidemic animals model.

- MEHR showed antihyperlipidemic activity through inhibition of HMG CoA reductase enzyme whereas META exhibited antihyperlipidemic activity through a process independent of inhibition of HMG CoA reductase.
Presence of flavonoids, steroids and saponins in both the plants may be responsible for the observed bioactivities. Additionally, concentration of quercetin and β-sitosterol were quantified in both the extracts.

MEHR and META also possess significant antihyperlipidemic and anti-obesity activities in HFD induced obese rats. The observed anti-obesity and anti-hyperlipidemic activity of the crude extracts of the plants against the wide spectrum of parameters tested could be due to either the combined effect of two or more compounds/classes of compounds or their individual effects on different parameters.

Both extracts showed antiobesity activity by modulating the fat metabolism through inhibition of pancreatic lipase enzyme.

Treatment related toxicity shown by META was reverted after a recovery period of 14 days.

The data gathered in this study may assist in the formulation of these extracts as anti-obesity agents that may be used as adjuvants with the currently used synthetic drugs and thereby aid in circumventing the side effects of the same.

### 7.3 FUTURE PROSPECTS

- To isolate and identify the specific phytoconstituents responsible for the antihyperlipidemic, antihyperglycemic and preventive activity.
- It may be a novel approach to inhibit excess adipose tissue accumulation. The plant extracts can be evaluated for this effect. Future studies are required to show whether this approach improves the metabolic status or on the contrary leads to adverse effects through ectopic lipid accumulation.