CHAPTER 7 - SUMMARY

The present study was carried out to investigate the possible beneficial effects of Alpinia galanga rhizomes (AG) and Argyreia speciosa roots (AS) in cafeteria diet (CD) and atherogenic diet (AD) induced obesity in rats. The pet. ether (40-60°C), chloroform, ethanol and aqueous extracts of the selected plant materials were prepared and subjected to preliminary qualitative tests, which showed the presence of various constituents mainly phenolic compounds, flavonoids, phytosterols, and tannins. The extracts were found to be safe even at the highest dose of 2000 mg/kg in albino rats. Hence, 1/4th of maximum tested dose i.e. 500 mg/kg was selected as experimental dose to screen the anti-obesity effects in rats.

The CD or AD was administered along with normal diet to experimental rats everyday for 6 weeks to develop obesity like condition. The body weight and food intake was measured on day 1 and then repeated at weekly intervals. On day 42, serum levels of glucose, lipids, leptin, GOT and GPT were estimated. Animals were sacrificed; liver and parametrial adipose tissues were removed and weighed. The liver tissue was subjected to estimation of triglycerides, lipid peroxidation and antioxidant enzymes and finally histopathological study of liver tissue was also carried out by using Haematoxylin-Eosin dye.

Administration of a CD or AD along with normal diet for 6 weeks in rats produced obesity-like conditions, with increase in body weight, parametrial adipose tissue weight, and serum lipid levels. Furthermore, it also induced a fatty liver with the accumulation of hepatic triglycerides.

Treatment with extracts at the dose of 500 mg/kg/day, significantly reduced the increase in body weight induced by a CD/AD - a clear sign of an anti-obesity effect. The
food intake measured once in every week was significantly reduced as compared to CD/AD control rats. This result suggests that the body weight reducing effect of extracts in CD/AD fed rats may be produced due to the hypophagic property.

Treatment with extracts caused significant changes in the blood parameters, including decreased levels of TC, LDL-C, and TG, but increased HDL-C. These results indicate a significant improvement in lipid profile by the treatment with extracts. AIP correlates with the size of the pro- and anti-atherogenic lipoprotein particles and is known to predict a cardiovascular risk. An AIP value of less than 0.10 predicts a low cardiovascular risk, which was observed in animals treated with extracts and sibutramine.

Treatment with various extracts of AG and AS markedly reduced the SGPT and SGOT concentrations which were increased by high fat diets. However, effect produced by ethanol extracts was highly significant. These results suggest that liver function was significantly changed due to high fat diet, which was effectively reversed by treatment with ethanol extracts.

It is reported that consumption of a high-fat diet results in the development of leptin resistance in rodents, marked by an increased circulating leptin level, and is measured as a failure of leptin either to inhibit food intake or to induce weight loss. In the present study, the serum leptin was raised in the CD/AD control group, indicating that obese rats accompanied with leptin resistance; however, treatment with extracts resulted in a significant reduction in serum leptin levels when compared to the CD/AD control group, suggesting that, the extracts could improve leptin resistance induced by obesity.

The extracts produced a significant decrease in the liver and parametrial adipose tissue weight and the accumulation of liver triglycerides in comparison with the CD/AD
control group. The rate of reduction of body weight corresponded with that in the parametrial adipose tissue weight.

In order to investigate if oxidative stress was increased in high fat diet fed rats, we measured lipid peroxidation in liver tissue by estimating the malondialdehyde content using TBARS essay. The high concentration of liver tissue malondialdehyde observed in CD and AD control rats was an indication of increased oxidative stress in high fat diet rats. However, the extract supplemented animals showed significant decrease in malondialdehyde concentration, thus reducing lipid peroxidation. The ability of the ethanol extracts to significantly suppress lipid peroxidation could be due to the anti-free radical activities of its phenolic components, known to act as free radical scavengers and to increase in the activity of antioxidant enzymes regardless of the available lipids.

Obesity has been shown to be one of the conditions that decrease antioxidant capacity of the body. Obesity seems to decrease antioxidant defense by lowering the levels of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. Accordingly, in our study also, there was significant decrease in concentration of these antioxidant enzymes in liver tissue of CD and AD control rats. However, the animals treated with extracts showed an elevation in their antioxidant enzymes concentration. The ethanol extracts were highly significant to improve antioxidant defense system by enhancing in vivo antioxidant enzymes activities.

In histopathological studies, rats fed with high fat CD or AD showed mild fatty change surrounding central vein, and congestion in hepatic vein and dilated central vein with kupffer cells hyperplasia and there was vacuolation seen. However, rats supplemented with sibutramine or ethanol and pet. ether extracts of AG and AS significantly reversed these pathological changes and exhibited almost normal architecture.
Since, the ethanol extracts of AG and AS significantly increased in vivo antioxidant defensive mechanisms, the in vitro antioxidant potential of the these extracts was also determined by estimating free radical scavenging abilities against DPPH, NO and OH free radicals. The ethanol extracts produced marked scavenging of these free radicals.

The ethanol extracts were analyzed for their total phenolic compounds and total flavonoids. Both the extracts contained approximately the same amount of phenolic compounds and the total flavonoid content was relatively less in AS extract compared to AG extract. Thus, the in vitro and in vivo antioxidant activities of the ethanol extracts of AG and AS could be related to their high flavonoid and phenolic contents.

The results of the anti-obesity and antioxidant study suggests that, ethanol extract of AG and AS produces significant beneficial effects in treatment of high fat diet induced obesity in experimental rats, which was comparable with standard anti-obese drug, sibutramine.

The ethanol extracts, which exhibited highly significant anti-obesity activity, were subjected for isolation and characterization of phytoconstituents by chromatographic methods and spectral studies. The ethanol extracts were fractionated with pet. ether (40-60°C), chloroform, ethyl acetate and water and the obtained fractions were screened for in vitro antioxidant activity by using DPPH radical scavenging assay. Among them, the ethyl acetate fraction of AG and pet. ether and aqueous fractions of AS showed most potent DPPH scavenging activity. Hence, these fractions were further isolated by using column chromatography to yield two compounds from each plant. The isolated compounds were further characterized by using TLC and spectroscopic analysis. The compounds from AG were characterized as flavonoids i.e. Galangin and
Kaempferide; and the compounds from AS were characterized as Gallic acid and β-Sitosterol by comparing with standard spectral data.

Pancreatic lipase inhibition is one of the most widely studied mechanisms for the determination of the potential efficacy of natural products as anti-obesity agents. All the isolated compounds markedly inhibited the action of the in vitro pancreatic lipase enzyme, except β-Sitosterol (12.32%). The maximum inhibition of enzyme activity was observed with galangin (51.86%). This was further confirmed by reduction in plasma triacylglycerol content after oral lipid emulsion load in rats. Thus, the anti-obesity and antioxidant effects of the ethanol extracts of AG rhizomes and AS roots could be mediated by the inhibition of pancreatic lipase enzyme leading to decrease in digestion and absorption of dietary fat.

Thus, the present study demonstrates that, ethanol extracts of *Alpinia galanga* rhizomes and *Argyreia speciosa* roots significantly modulates obesity and obesity derived complications by reducing the excess accumulation of body fat due to inhibition of pancreatic lipase activity, altering the lipid profile, decreasing the calorie intake, overcoming the leptin resistance, and enhancing the antioxidant defensive mechanisms of the body. The active components responsible for the activity were identified as flavonoids such as galangin and kaempferide from *Alpinia galanga* extract, and a phenolic compound (gallic acid) and phytosterol (β-Sitosterol) from *Argyreia speciosa* extracts.