CHAPTER 7

SUMMARY AND CONCLUSION

Obesity is a condition characterised by accumulated fat that might have a negative effect on one’s health. Surveillance system on obesity reports growing numbers of obese persons since the last decade which has reached epidemic proportions worldwide. According to WHO (2014), there were more than 600 million (13 %) who were obese. This alarming scenario of obesity has economically burdened many countries across the world. According to the latest reports, many countries incur billions of dollars on obesity related medical expenses.

It has become imperative to find a more economical, effective and natural alternative solution to reduce the burden on health care costs. Managing obesity by natural therapy offers a perfect solution to stem the prevailing trend of obesity.

Our work is directed on the following objectives such as development of a Poly Herbal Formulation (PHF), its validation by different in vitro antioxidant models and in vivo animal models.

A review on antiobesity plants was done, their availability, affordability, presence of important phytoconstituents showing specific antiobesity actions. Based on the above mentioned criteria we selected four plants for our study.
The four herbal drugs *Phyllanthus emblica. L: Curcuma longa. L: Macrotyloma uniflorum. L: Plumbago zeylanica. L* were taken in the ratio of 6:3:12:1 (w/w). Chemical and physical evaluation of the herbal drugs and the developed PHF were carried out, that included ash values- total ash, acid insoluble ash, water soluble ash and sulphated ash, extractive values (water soluble extractives, alcohol insoluble extractives, ether soluble extractives, pH and moisture content, bulk density and tapped density were carried out and microbial limit tests were also conducted according to the World Health Organisation, (2011).

Qualitative analysis was done by phytochemical analysis for the different solvent extracts of the individual herbals and the PHF. Screening and detection of polyphenols (quercetin, rutin, apigenin, curcumin and plumbagin) using HPTLC was carried out. Furthermore, quantification of biomarkers by HPTLC fingerprinting in the optimized solvent front was also carried out.

Quantitative analysis for the estimation of total phenols, flavonoids and tannins were done. Nutritional analysis of the developed PHF with respect to crude fibre content, total carbohydrates, proteins, lipids and macro/micronutrients were estimated according to the well established protocols. Detection of heavy metals as a procedure for authentication of the herbal drugs and the developed PHF was done. The *in vitro* antioxidant potential of the Poly Herbal Formulation and its individual herbals were carried out by standard methods such as DPPH, ABTS, Superoxide, Nitric oxide free radical scavenging assays and comparisons were made with respective standards.

Pharmacological evaluation for antiobesity properties of the PHF was carried out using Wistar rats. A total of 30 Wistar rats each of female and male sexes were randomly assigned for evaluation. Obesity was induced for 6 weeks
by feeding our High Fat Diet (HFD) and thereafter treatment period was followed for 4 weeks.

Changes in indices of obesity such as food intake, body weight, Body Mass Index (BMI), Abdominal Circumference (AC) during the period of induction and treatment were observed. The body temperature was recorded using rectal thermometer before and after drug administration. Locomotor activity in different groups was also recorded using open field behavior test apparatus.

The animals were sacrificed by cervical dislocation and different organs (liver, kidney and heart) and fat pads (mesenteric, kidney, epididymal and uterine fat pads) were removed and weighed immediately. Biochemical changes during the pre-treatment and post-treatment periods were measured in serum samples for glucose, Total Cholesterol (TC), Total Triglycerides (TG), Very Low Density Lipoproteins (VLDL), and High Density Lipoproteins (HDL) by enzymatic colorimetric methods using biochemical kits (M/s. Span Diagnostics, India). The TC and TG levels in liver tissues were also carried out. Serum ALT, AST activities, urea, uric acid and creatinine levels, CK-MB and LDH activity were measured colorimetrically using kits (M/s. Span Diagnostics, India). The levels of Reactive Oxygen Species (ROS) that are controlled by antioxidant enzymes such as Super Oxide Dismutase, Catalase, Glutathione Peroxidase, Glutathione Reductase, and Glutathione-S-Transferase and non-enzymatic scavengers: reduced glutathione (GSH), Vitamin C and Vitamin E were measured in serum and liver tissue samples. Lipid peroxidation levels were measured according to the well established methods.

The evaluation of cardioprotective potential of the PHF was also studied. A pre-treatment approach of the male Wistar rats with the experimental drugs
was carried out for 14 days for atorvastatin and 15 days for quercetin and PHF respectively.

It was observed that the extractive and ash values of the herbal drugs were within the specified pharmacopoeia limits. The formulation showed values matching with the average of the individual drugs added. The observed pH values of 1% and 10% suspensions of the PHF indicated its suitability for human use. The tapped and bulk density values indicated that the PHF was less bulky. Values of total viable count and fungal count of the PHF were within the standard limits while *Escherichia coli, Salmonella typhi, Psuedomonas aeruginosa* and *Staphylococcus aureus* were completely absent.

Phytochemical investigations for the aqueous and ethanolic extracts of individual herbals (*P. emblica, P. zeylanica, M. uniflorum, and C. longa*) and different extracts of PHF revealed the strong presence of carbohydrates, amino acids and secondary metabolites such as alkaloids, phenols, flavonoids, tannins,. Sterols and saponins were absent. However, the percentage yield calculated for each of the solvent extracts showed an order as follows: Ethanol > Water > Chloroform > Petroleum ether. Quantitative analysis revealed a higher percentage of phenolic, flavonoid and tannin contents for the ethanolic extracts. Heavy metals (Arsenic, Lead, Cadmium and Mercury) detected were found to be below 1 ppm which complied with the WHO limits. HPTLC chromatogram of the sample showed peaks with $R_f$ values matching with biomarkers: quercetin, apigenin, rutin, gallic acid, plumbagin and curcumin.

In the *in vitro* antioxidant assays, ethanolic extract of PHF showed greater antioxidant activity compared to the aqueous extract. Regression analysis showed significant correlation between antioxidant properties and polyphenol/ flavonoid contents, latter known for their strong antioxidant potentials.
In animal models, induction of obesity by a modified HFD promoted an increase in food and calorie intake, resulting in body weight gain, BMI, increased adipose tissue and alteration of serum/tissue lipids (dyslipidemia, hypercholesterolemia and hypertriglyceridemia). Therefore, we can conclude that adiposity was directly related to the proportion of fat in the diet which was induced for 6 weeks by HFD. The weight reducing effect of the PHF can be attributed to its potential to inhibit lipogenesis and enhanced thermogenesis, similar to the effect of L-Carnitine. HFD induced obesity in both the sexes of rats showed a positive correlation between obesity and altered lipid content. This alteration was shown to be accompanied by an increase in Lipid Peroxidation (LPO) in turn inactivating the antioxidant enzymes such as SOD, CAT, GST, GPx, and GSH, causing hepatic oxidative stress. Obesity induced structural changes in liver, heart, adipose tissue etc were restored by PHF to normal architecture.

Our studies on cardioprotective activity revealed that administration of PHF showed positive effect in reducing the extent of myocardial damage and significantly counteracted the oxidative stress during isoproterenol-induced myocardial infarction in rats. In conclusion, we have developed a model for High Fat Diet (HFD) induced obesity having several features that make it useful for investigating the mechanisms by which dietary composition contributes to body weight regulation.

Preclinical studies of the Poly Herbal Formulation (PHF) provided a scientific justification for their effective use for obesity control and proved that they are safe and ecofriendly. A cost effective PHF was developed which is comparatively cheaper than some of the existing commercially available natural products such as Ayur Slim, Biolean, Erboslim, Herbalife, etc. Our cost effective PHF is nutritionally rich in protein, fibre and macro and micronutrients and
contains active phytochemical constituents such as polyphenols, flavonoids, tannins, etc.

In summary our PHF shows the following mechanisms of action for antiobesity effects such as reduced body weight, fat mass, triglycerides through enhanced thermogenesis, suppression of appetite, decreased absorption of lipids.

Previous review of literature on the four herbals that were chosen for the development of our PHF provide substantial evidence of multitudinous medicinal properties such as anti-inflammatory, anticancer, antimicrobial, antihyperlipidemic, antidiabetic, immunomodulatory, anti-atherosclerotic, memory enhancers etc. This work demonstrated that the newly developed PHF has antiobesity, hypolipidemic, hepatoprotective, antioxidant and cardioprotective effects. In addition, our PHF can be used also be recommended as a general health promoter which will help in overall well-being. Further investigations by clinical trials on human volunteers have to be done for a commercially successful patentable product accessible to the different sections of the society.