Chapter VIII

SUMMARY

AND

CONCLUSION
Obesity can be described as the "New World Syndrome" (Nammi et al., 2004). Its prevalence is increasing in both developed and developing countries throughout the world. The International Obesity Task Force estimates that more than 300 million individuals worldwide are obese body mass index (BMI ≥ 30 kg/m²) and 800 million are overweight (BMI between 25 and 29.9 kg/m²) (Haslam and James, 2005).

Obesity is associated with increased risk for mortality and numerous other medical and health hazards including hypertension, dyslipidemia, cardiovascular disease, stroke, type 2 diabetes mellitus, gallbladder disease, osteoarthritis, cancers of the breast, endometrium, prostate, and colon, respiratory problems, including asthma and sleep apnea, and perhaps depression (Racette et al., 2003).

Human studies have shown that increased fat intake is associated with body weight gain which can lead to obesity and other related metabolic diseases. Animal rodent models are therefore useful tools for studying obesity as they will readily gain weight when fed high-fat diets (Buettner et al., 2007).

The present research study was entitled "Evaluation of Gymnema sylvestre extract (Gurmar) for antiobesity activity in high fat diet-induced obesity in normal and diabetic albino rats". High fat diet used in the present study to induce obesity was designed and standardized by National Centre for Laboratory Sciences (NCLAS), National Institute of Nutrition (NIN), Hyderabad, Andhra Pradesh, India.

Gymnema sylvestre R. Br. (family: Asclepiadaceae) is a native plant in the south west of India, Australia and tropical Africa. From ancient times, G. sylvestre has been used in Indian traditional medicine ("Ayurvedic medicine") and is considered to be effective in improving urination, stomach stimulation, and diabetes (Nadkarni, 1982). Bishayee and Chatterjee (1994) reported the hypolipidemic and antiatherosclerotic effect of oral Gymnema sylvestre leaf extract in albino rats fed on high fat diet. The ethanolic extract of Gymnema sylvestre leaves showed an antimicrobial activity (Satdive et al., 2003). Standardized G. sylvestre extract in combination with niacin-
bound chromium (NBC) and hydroxycitric acid (HCA-SX) has been evaluated for antiobesity activity in moderately obese subjects (Preuss et al., 2004). Gymnema sylvestre leaves extract has anti-inflammatory activity in rats (Malik et al., 2008). Rachh et al. (2010) reported that Gymnema sylvestre R. Br. leaves extract has antihyperlipidemic activity in rats fed with high cholesterol diet.

First part of the research work dealt with extraction of air dried leaves of Gymnema sylvestre with 70% ethanol in Soxhlet's apparatus and ethanolic Gymnema sylvestre extract was standardized according to the WHO Guidelines. The ethanolic Gymnema sylvestre extract was sub fractionated into water-soluble (W-S) fraction and water-insoluble (W-INS) fractions according to method reported by (Alam et al., 2005).

The present study reported that oral administration of high fat diet (20 g/d/rat) to the Wistar albino rats for a period of 28 days produced significant obesity in rats as evidenced by increased in body mass index and body weight gain which is supported by Altunkaynak (2005) who reported that BMI was significantly increased in rats with high fat diet fed for 8 weeks as compared to the control group i.e. (from 3.2 ± 0.3 kg/m^2 to 5.6 ± 0.5 kg/m^2), and Matsuo et al. (2002) reported that body weight gain was greater in beef tallow diet group than in the other dietary groups. Water soluble fraction of G. sylvestre ethanolic extract and standard drug (i.e. rimonabant) significantly reduced the body mass index and body weight gain while water insoluble fraction of G. sylvestre ethanolic extract (80 mg/kg/p,o.) did not produce significant changes in the BMI and body weight gain. This may be due to decrease in food intake and that leads to decrease in calorie intake (Nakamura et al., 1999).

Furthermore, systolic BP, diastolic BP, mean arterial BP, and heart rate were significantly increased in high fat diet induced-obesity in Wistar rats (i.e. group II) as compared to normal healthy control rats (i.e. group I). Kaufman et al. (1999) investigated the effect of HFD on BP and sympathetic nervous activity (SNA) and reported that BP and urinary norepinephrine (NE) excretion were higher in HFD-fed rats than in low-fat diet-fed rats. Water soluble fraction of G. sylvestre ethanolic extract
and standard drug (i.e. rimonabant) significantly reduced the hemodynamic changes (i.e. systolic, diastolic, mean arterial BP and heart rate) as compared to the group II. While water insoluble fraction of ethanolic *G. sylvestre* extract (80 mg/kg/p.o.) decreased the hemodynamic changes but non-significantly.

Serum leptin levels in the HFD group (i.e. group II) were significantly (P<0.05) increased as compared with those in the normal healthy control group (i.e. group I). The administration of HFD for 10 weeks, significantly increased serum leptin levels in the HFD fed group as compared to the normal healthy control group (Hsu and Yen, 2007). Plasma leptin concentrations were directly correlated with the degree of adiposity (Woods *et al.*, 2003). Serum leptin levels in the HFD + WS of GSE groups were significantly decreased as compared with those in the HFD group. The serum leptin levels were not significantly decreased by water insoluble fraction of ethanolic *G. sylvestre* extract (80 mg/kg/p.o.) treatment.

The levels of serum insulin were increased in the high fat diet fed rats (i.e. group II) as compared to the normal healthy control rats (i.e. group I). Mehta *et al.* (2002) reported that HFD leads to insulin resistance through oxidative stress. Serum insulin levels in the HFD + WS of GSE groups were significantly decreased as compared with those in the HFD group. The levels of serum insulin were non-significantly changed by water insoluble fraction of ethanolic *G. sylvestre* extract (80 mg/kg/p.o.) treatment.

Serum glucose levels were significantly increased in the HFD group (i.e. group II) as compared with those in the normal healthy control group (i.e. group I). Diet-induced obesity dysregulated glucose homeostasis and causes hyperglycemia (Chang *et al.*, 1990). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o.) significantly (p<0.01) decreased the levels of glucose as compared to the group II. The leaf extract of *Gymnema sylvestre* contains four triterpinoid saponins, designated gymnensians A–D (1–4), besides the known four isomeric gymnemic acids.
These compounds are assumed to be either wholly or partly responsible for the observed antihyperglycemic effect (Chattopadhyay, 1998).

There was significant increase in the lipids (TC, TGs, LDL-C, and VLDL-C) levels in the HFD group as compared to the normal healthy control group. Lavie and Milani (2003) indicated that obesity adversely affects plasma lipids, especially by increasing TC, LDL- C, VLDL-C, TGs and decreasing the level of HDL-cholesterol. The HFD might lead to an increase in the synthesis of phospholipids and cholesterol esters in rats (Jayakumar et al., 1991). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o.) significantly (p<0.01) decreased the levels of TGs, TC, LDL-C, VLDL-C and atherogenic index. While water insoluble fraction of ethanolic Gymnema sylvestre extract produced non-significant changes in the lipid levels. Antihyperlipidemic activity may be due to presence of flavonoids, phenols, tannins (phenolic compounds) and triterpenoids found in the Gymnema sylvestre ethanolic extract (Rachh et al., 2010).

Serum apolipoprotein A1 and HDL-C levels were significantly decreased in the HFD group (i.e. group II) as compared to the normal healthy control group. While water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o.) significantly (p<0.01) increased the levels of apolipoprotein A1 and HDL-C levels as compared to the group II.

Hepatic cholesterol levels, organs (heart, liver and kidneys) and visceral fat pad (mesenteric, perirenal and epididymal) weights were significantly increased in the HFD group (i.e. Group II) as compared to the normal healthy control group (i.e. group I). The visceral fat (mesenteric, perirenal, epididymal) pads of HFD rats weighed 50% more than those of normal healthy control rats as feeding of high fat diet in rats increases body weight, adiposity and visceral fat deposition (Corbett et al., 1986). The liver is the central organ for cholesterol, phospholipid, and lipoprotein metabolism. Liver cholesterol levels were higher in the HFD group (Hsu and Yen, 2007). Water soluble fraction of ethanolic Gymnema sylvestre extract (120 mg/kg/p.o.) significantly
(p<0.01) decreased these levels as compared to the group II. These levels were non-significantly changed by water insoluble fraction of ethanolic Gymnema sylvestre extract (80 mg/kg/p.o.) treatment. Gymnema sylvestre would safely suppress the accumulation of liver lipids and visceral fat and improve obesity as a whole when administered for a long time under a high fat diet (Shigematsu et al., 2001a).

Obesity is associated with reduction of tissue Na\(^+\) K\(^+\)-ATPase, linked to hyperinsulinemia, which may repress or inactivate the enzyme, influencing thermogenesis and energy balance (Iannello et al., 2006). In the present study, HFD (i.e. group II) damage cell membrane as evident from significant decrease in hepatic levels of membrane bound enzymes like Na\(^+\)/K\(^+\) ATPase as compared to normal healthy control group while water soluble fraction of ethanolic Gymnema sylvestre extract significantly increased the Na\(^+\)/K\(^+\) ATPase levels.

High-fat diet feeding for a period of 4 weeks, triggered myocardial apoptosis, as assessed by the elevated caspase-3 activity assays. Cardiac caspase-3 levels, and DNA laddering was significantly increased in the HFD group (i.e. group II) as compared to the normal healthy control group, while cardiac Na\(^+\) K\(^+\) ATPase activity was significantly decreased in the HFD group (i.e. group II) as compared to the normal healthy control group. Caspase activities were greater in high-fat obese but not in weight control mouse hearts (Fang et al., 2008). Water soluble fraction of ethanolic Gymnema sylvestre extract and standard drug (i.e. rimonabant) significantly reduced the cardiac caspase-3 levels while DNA band was preserved in these groups as compared to the group II. Wang et al. (2008) reported that the apoptotic hepatocytes were significantly greater in livers of rats fed HFD than in those fed control diet, and these were associated with a higher levels of cleaved caspase-3.

The levels of antioxidant enzymes (catalase, SOD, GPx, GR, and GST) were significantly decreased in the HFD group (i.e. group II) as compared to the normal healthy control group (i.e. group I) while levels of lipid peroxidation were significantly increased in the HFD group (i.e. group II) as compared to the normal healthy control group.
healthy control group (i.e. group I). Obesity, one of the conditions that decrease antioxidant capacity. Obesity decreases the antioxidant defense by lowering the levels of antioxidant enzymes [catalase, glutathione peroxidase (GPx) and glutathione reductase (GRd)] (Asayama et al., 2001). It has been reported that high levels of fat increase fat-mediated oxidative stress and decrease antioxidative enzyme activity (Slim et al., 1996). Water soluble fraction of ethanolic Gymnema sylvestre extract and standard drug (i.e. rimonabant) significantly elevated the levels of antioxidant enzymes as compared to the group II. The increase of antioxidant enzymes levels and decrease of lipid peroxidation in terms of malondialdehyde (MDA) by Gymnema sylvestre extract due to the presence of gymnemic acid (Bhatia et al., 2008).

Histopathological findings revealed that heart tissue of high fat diet group showed deposition of fat globules in the myocardial cells as compared to normal healthy control rat’s heart tissue which showed normal architecture with regular morphology of myocardial cell membrane and well preserved cytoplasm while water soluble fraction of ethanolic G. sylvestre extract group showed no pathological changes in the myocardial cells. Water insoluble fraction of Gymnema sylvestre ethanolic extract showed microvascular fat deposition in myocardial cells.

Histopathological examination of hepatocyte in the HFD-fed group showed marked accumulation of fat droplets in liver cells as compared to normal healthy control group. HFD group showed more profound steatosis with macrovesicular fat accumulation. Staining with haematoxylin–eosin confirmed the presence of lipid droplets within hepatocytes of the rats fed on HFD (Hsu and Yen, 2007). In obesity, the liver is the receiver of large amounts of fatty acids, which cause its steatosis (Festi et al., 2004). The number of lipid droplets was significantly reduced by the water soluble fraction of ethanolic G. sylvestre extract and standard drug groups as compared with that of the HFD group. WINS fraction of GSE (80 mg/kg/p.o.) group showed ballooning degeneration and congestion in the liver cells.
The results of the present research work states that water soluble fraction of ethanolic *G. sylvestre* extract showed good antiobesity effect in HFD-induced obesity in Wistar albino rats.

Hossain *et al.* (2007) reported that the increase in the prevalence of type 2 diabetes is closely linked to the upsurge in obesity. About 90% of type 2 diabetes is attributable to excess weight. Furthermore, approximately 197 million people worldwide have impaired glucose tolerance, most commonly because of obesity and the associated metabolic syndrome. This number is expected to increase to 420 million by 2025.

There is an increasing amount of data showing that being overweight during childhood and adolescence is significantly associated with insulin resistance, dyslipidemia, and elevated blood pressure in young adulthood. Type 2 diabetes is strongly associated with obesity and cardiovascular risk.

In Model II, we developed Model II i.e. high-fat diet-fed and STZ-injected rat as a model for study of antiobesity activity in diabetic rats. The present study was planned to evaluate the antiobesity effect of water soluble fraction of *Gymnema sylvestre* ethanolic extract in HFD-induced obesity in diabetic rats. Since water insoluble fraction showed no significant changes hence, we used WS fraction of GSE only. Animals were treated with streptozotocin (45 mg/kg, i.v. single dose), after 72 hrs fasting blood glucose (FBG) level was measured. Those animals having FBG $\geq 200$ mg/dl were selected and obesity was induced in these rats by feeding of high fat diet for a period of 4 weeks. This rat model showed type 2 diabetic syndrome such as hyperglycemia, dyslipidemia, impaired glucose tolerance, and insulin resistance. Diabetic rats fed with high sucrose-fat diet, showed obvious obesity and impaired glucose tolerance after 8 weeks. Fasting blood glucose levels increased moderately, and were accompanied by hyperleptinemia, hyperinsulinemia, dyslipidemia, with mild $\beta$-cell dysfunction (Ding *et al.*, 2005).
As carbohydrate and lipid metabolisms are closely linked processes, derangement in the carbohydrate metabolism produces dyslipidemia, hence, STZ + HFD model is one of the ideal model for screening of antiobesity activity in diabetic rats. This is supported by the findings of Ding et al. (2005) who reported that Wistar rats injected intraperitoneally with low dose of STZ (30 mg/kg) and fed with a high sucrose-fat diet for 8 weeks, develop significant insulin resistance and obesity. Similarly, Srinivasan et al. (2005) reported that high fat diet –fed and low dose of STZ (35 mg/kg, i.p.) treated rats simulate natural disease progression and metabolic characteristics typical of individuals at increased risk of developing type 2 diabetes because of insulin resistance and obesity.

Since water insoluble fraction showed non significant (P>0.05) decrease in body mass index, body weight gain, serum leptin, lipid levels, visceral fat pad weights, cardiac caspase-3 levels and lipid peroxide levels hence, we used water soluble fraction of Gymnema sylvestre ethanolic extract in this Model II, which showed significant effect on obesity biomarkers viz. body mass index, body weight gain, food and water intake, hemodynamic parameters, serum leptin, insulin, glucose, lipids, apolipoproteins, visceral fat pad and organs weights, cardiac caspase-3, antioxidant enzymes levels and histopathological changes.

In the present study, we injected STZ at low doses (45 mg/kg) to Wistar rats to induce light damage of islet cells. On this basis, a high fat diet was followed to induce obesity which was characterized by significant hyperleptinemia, dyslipidemia, insulin resistance as well as hyperglycemia and increased cardiovascular risk, in rats as evidenced by increased body mass index, blood pressures and heart rate, serum leptin, insulin, glucose, glycohemoglobin, serum total cholesterol, low density lipoprotein-cholesterol, triglycerides, apolipoprotein-B, LDH levels and tissue lipid peroxides, cardiac caspase-3 levels and decreased levels of serum high density lipoprotein-cholesterol and tissue glutathione, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, superoxide dismutase and catalase as compared to normal healthy control rats.
The administration of STZ (45 mg/kg i.v. single dose), to the Wistar albino rats produced significant diabetes as well as hyperlipidemia and increased cardiovascular risk, in rats as evidenced by increased food intake, water intake, serum glucose, glycohemoglobin, and increased blood pressures and heart rate, serum total cholesterol, LDL-C, VLDL-C, TGs, apolipoprotein-B, LDH levels and tissue lipid peroxides, cardiac caspase-3 levels and decreased levels of serum leptin, insulin, HDL-C and tissue sodium potassium ATPase activity, glutathione, GPx, GST, GR, superoxide dismutase and catalase levels as compared to normal healthy control rats. Oral administration of water soluble fraction of *G. sylvstre* extract (120 mg/kg) and standard drug (i.e. pioglitazone) significantly reduced the blood pressures, serum glucose, lipids, apolipoprotein B and LDH, glycated hemoglobin levels and cardiac caspase-3 levels where as significant increase the levels of antioxidant enzymes and serum leptin, insulin, HDL-C levels.

Histopathological observation of rat’s heart tissue of STZ treated group showed few calcified lesions with fatty particles in the myocardial cells as compared to normal healthy control rat’s heart tissue which showed normal architecture with regular morphology of myocardial cell membrane and well preserved cytoplasm. Histopathological observation of rat’s liver tissue of STZ treated group showed marked hypertrophy of hepatocytes with dense cytoplasm and fatty changes as compared to normal healthy control rat’s liver tissue which showed normal fat deposition in myocardial cells i.e. within normal limit. Water soluble fraction of *G. sylvstre* extract and standard drug groups showed normal morphology of heart and liver tissues as compared to the STZ treated group.

In diabetic rats fed with high fat diet increased the body mass index significantly (P<0.01) which was decreased by water soluble fraction of *Gymnema sylvestre* ethanolic extract treatment.
Hemodynamic parameters (systolic, diastolic, mean arterial BP and heart rate) were significantly (P<0.01) increased in the diabetic rats fed with high fat diet which were decreased water soluble fraction of Gymnema sylvestre ethanolic extract treatment.

The diabetic rats fed with high fat diet showed significant elevation in circulating TG, TC, LDL-C, VLDL-C and hepatic cholesterol levels. The hypertriglyceridemia observed in these fat-fed/STZ rats may be due to increased absorption and formation of triglycerides in the form of chylomicrons following exogenous consumption of diet rich in fat or through increased endogenous production of TG-enriched hepatic very low density lipoprotein (VLDL) and decreased TG uptake in peripheral tissues. Hypercholesterolemia may be attributed to increased dietary cholesterol absorption from the small intestine following the intake of HFD in a diabetic condition (Colca et al., 1991; Shafir, 2003). Water soluble fraction of G. sylvestre ethanolic extract significantly reduced these levels.

Serum glucose, insulin and leptin concentrations were significantly increased in the diabetic rats fed with high fat diet. High sucrose-fat diet elevated the serum levels of glucose and insulin compared with control rats (Ding et al., 2005). Water soluble fraction of G. sylvestre ethanolic extract and standard drug (i.e. pioglitazone) significantly reduced the blood glucose, glycohemoglobin, serum, leptin, insulin, and LDH levels.

Cardiac caspase-3 levels and DNA laddering significantly increased in the diabetic rats fed with high fat diet while water soluble fraction of G. sylvestre ethanolic extract and standard drug (i.e. pioglitazone) treatment significantly decreased the cardiac caspase-3 levels and DNA laddering. Cardiac Na\(^+\) K\(^+\) ATPase levels significantly decreased in diabetic group fed with high fat diet while these levels were significantly increased in water soluble fraction of G. sylvestre ethanolic extract treatment group.

Oxidative stress was significantly increased in the diabetic rats fed with high fat diet as evidenced by increased lipid peroxidation and decreased antioxidant enzymes.
(GPx, GR, GST, SOD and catalase) levels in heart and liver tissues. Water soluble fraction of *G. sylvestre* ethanolic extract and pioglitazone treatment decreased the oxidative stress in heart and liver tissues by decreasing the levels of lipid peroxides and increased the tissue glutathione, GPx, GR, GST, SOD and catalase levels.

Histopathological observation of rat’s heart tissue of STZ+HFD treated group showed dense focal fatty infiltration in the myocardial cells as compared to normal healthy control rat’s heart tissue which showed normal architecture with regular morphology of myocardial cell membrane and well preserved cytoplasm. Water soluble fraction of *G. sylvestre* ethanolic extract and standard drug groups showed normal morphology of heart tissues as compared to the STZ+HFD treated group.

Histopathological observation of rat’s liver tissue of STZ+HFD treated group showed few fatty particles were seen in the hepatocytes and no other pathological changes observed as compared to normal healthy control rat’s liver tissue which showed normal fat deposition in myocardial cells i.e. within normal limit. Standard drug groups showed normal morphology of liver tissues as compared to the STZ+HFD treated group, while water soluble fraction of *G. sylvestre* ethanolic extract group showed microvesicular fat accumulation in the hepatocytes.

The results of this study reveal that water soluble fraction of *G. sylvestre* ethanolic extract effectively alleviates the deleterious effects produced by HFD in diabetic rats. Further, the *G. sylvestre* ethanolic extract offers cardiac protection by decreasing serum leptin, insulin, lipids, cardiac caspase-3 levels, DNA laddering, oxidative stress, and maintaining normal architecture of myocardium. The present study supports the potential of *G. sylvestre* in diaobesity disorder.

Our study demonstrates that a combination of HFD (20 g/d/rat) and low dose of STZ (45 mg/kg) treatment can be effectively used to generate a rat model that mimic the natural history and metabolic characteristics of the common type 2 diabetes in obese humans. It is easy to develop and most suited for studying the pathophysiology of
type 2 diabetes and is also useful in evaluating the therapeutic compounds for the treatment of diabesity disorder.

In conclusion, the results of present study reveal for the first time that the water soluble fraction of *Gymnema sylvestre* ethanolic extract along with the HFD decreased body mass index, body weight gain, the weights of liver, heart, kidneys and visceral fat, serum parameters (TC, LDL-C, VLDL-C, TGs, apolipoprotein B, insulin and leptin) and hepatic steatosis. Water soluble fraction of *Gymnema sylvestre* ethanolic extract reduced oxidative stress (enhanced GSH, GPx, GRd and GST) in rats with obesity induced by a HFD. Our study also reported for the first time a marked state of insulin resistance and obesity in diabetic rats that is associated with various defects in glucose and lipid metabolism, including the oxidative stress.

Despite the persistence of obesity in these animals, water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o.) treatment led to an apparent improvement in overall insulin resistance and obesity biomarkers by ameliorating hyperleptinemia, dyslipidemia, hyperinsulinemia, and hyperglycemia, as well as affecting oxidative stress. The reduced serum leptin, insulin levels, decreased appetite, reduced food intake, and increased fat oxidation may be, at least in part, responsible for this positive outcome and decrease the risk factors for obesity related degenerative diseases and mortality.

These results provide initial evidence that water soluble fraction of *Gymnema sylvestre* ethanolic extract raises new horizon in the treatment of obesity and its related disorders. Further, experimental and clinical trials are needed to evaluate the antiobesity potential of *Gymnema sylvestre* before being recommended to treatment of obesity, diabetes and hyperlipidemia.