Obesity can be described as the "New World Syndrome" (Nammi et al., 2004). Its prevalence is on continuous rise in all age groups of many of the developed countries in the world. Statistical data reveals that the problem of obesity has increased from 12–20% in men and from 16–25% in women over the last ten years (Flegal et al., 1998). Recent studies suggest that nearly 15–20% of the middle aged European population are obese (Björntorp, 1997) and that in USA alone it is responsible for as many as 3,00,000 premature deaths each year (US Department of Health and Human Services, 2001). Obese patients have been associated with increased risk of morbidity and mortality relative to those with ideal body weight (Manson et al., 1995). Prevalence of obesity in Indian population is 20% in adults and 10% in children. In Northern India, obesity was most prevalent in urban populations (male = 5.5%, female = 12.6%), followed by the urban slums (male = 1.9%, female = 7.2%). Obesity rates were the lowest in rural populations (male = 1.6%, female = 3.8%) (Yadav and Krishnan, 2008).

Obesity results from an imbalance between food intake and energy expenditure, culminating in excessive accumulation of fat in adipose tissue, liver, muscle, pancreatic islets, and other organs involved in metabolism. Obesity increases the risk of diabetes, coronary artery disease, fatty liver, gall stones, sleep apnea, arthritis, and cancer and may shorten the lifespan (Ogden et al., 2007).

Obesity and its associated conditions such as insulin resistance, type 2 diabetes, dyslipidemia, and steatosis hepatitis termed as the 'metabolic syndrome', represent major challenges for basic science and clinical research. It is obvious that appropriate animals models are crucial for studies on the pathogenesis and therapy of this complex metabolic disorder (Buettner et al., 2007).

Dietary fat is one of the most important environmental factors associated with the incidence of cardiovascular diseases (CVD); high cholesterol and saturated fat diets have been shown to promote atherosclerosis (Hsu and Yen, 2007). Fat-enriched diets have been used for decades as a model of obesity, dyslipidemia and insulin intolerance in rodents (Almind and Kahn 2004; Buettner et al., 2006). It has been
observed that the disorders achieved by high-fat feeding resemble the human metabolic syndrome as was characterized by the increased body weight (obesity), mild hyperglycemia, hypertriglyceridemia, hypercholesterolemia and compensatory hyperinsulinemia together with reduced glucose disappearance rate, and this also may extend to the cardiovascular complications (Woods et al., 2003; Srinivasan et al., 2005).

In the present study, well controlled and standardized experimental animal models i.e. high fat died-induced obesity in normal albino rats (Model I) and high fat diet- induced obesity in diabetic albino rats (Model II) were selected as obesity models.

Many epidemiological studies reported that feeding of high fat diet containing 40% fat content caused obesity (Black et al., 1998; Han et al., 1999) but the high fat diet containing 25% fat content slightly increases the body weight (Anai et al., 1999). Further, it has been reported that diet-induced obesity (DIO) rats overeat and become obese over 3–4 weeks on a 31% fat diet (Levin and Dunn-Meynell, 2002; Levin et al., 2003). A model of high fat diet (HFD) - induced obesity in rats is well controlled and shares many features with human obesity. A rodent model of obesity based on the intake of HFD is advantageous in studying obesity-related cardiovascular abnormalities (Carroll et al., 2006). The feeding of HFD for 4 weeks produced a significant increase in body weight, total fat pad weight, basal/fasting plasma glucose, insulin, basal triglyceride (TG) and total cholesterol (TC) levels in male rats (Srinivasan et al., 2004).

In the present study, we used high fat diet to induce obesity in Wistar rats which was designed and standardized by National Centre for Laboratory Animal Sciences (NCLAS), National Institute of Nutrition (NIN), Hyderabad, Andhra Pradesh, India. It consisted of [(g/kg): Casein-342g; Cystine-3g; Starch-172g; Sucrose-172; Cellulose-50g; G. N. oil-25g; Tallow-90g; Mineral oil-37g; Vitamin Mix-10g]. The HFD was mixed with distilled water to make dough, and then pellets
were made 10 gm of weight each. These pellets were air dried and were fed orally in a
dose of 20 gm/rat/day was fed orally to each animal.

Oral treatment with hydroalcoholic extract of Gymnema sylvestre leaves (200
mg/kg) significantly (P<0.01) decreased TC, TGs, LDL-C, VLDL-C and increased
the HDL-C in hyperlipidemic rats (Rachh et al., 2010). Oral administration of
hydroalcoholic extract of Gymnema sylvestre leaves to rats fed a high fat diet for a
period of 3 weeks suppressed body weight gain, accumulation of liver lipids, and
intraperitoneal fat (Shigematsu et al., 2001a). Till date, no animal study has been
reported to study the antiobesity effect of Gymnema sylvestre ethanolic extract on
HFD – induced obesity in normal as well as diabetic Wistar albino rats. Therefore,
present research work was planned to investigate the role of Gymnema sylvestre
ethanolic extract treatment on HFD-induced obesity in normal and diabetic murine
models.

The problem posed for the present study was entitled “Evaluation of
Gymnema sylvestre extract (Gurmar) for antiobesity activity in high fat diet-
induced obesity in normal and diabetic albino rats”. We have used Rimonabant as
a standard antiobesity drug in the present Model (i.e. Model I). It has been reported
that rimonabant reduces food intake and increases energy expenditure (Singh and
Budhiraja, 2006). Chronically administered rimonabant reduced body weight in obese
rodents via central nervous system-independent effects that might involve increased
lipolysis from the adipose tissue and enhanced energy expenditure (Herling et al.,
2008).

The ethanolic extract of air dried Gymnema sylvestre leaves obtained by
Soxhlet’s extraction and was standardized according to WHO guidelines (WHO,
1998). The ethanolic extract of Gymnema sylvestre was further subfractionated into
water-soluble (W-S) fraction (60%) and water-insoluble (W-INS) fraction (40%)
respectively to pinpoint the subfraction which is responsible for antiobesity activity.
The fractions were made according to method reported by Alam et al. (2005).
In the HFD-induced obesity in normal Wistar rats (i.e. Model I), the effects of water soluble fraction and water insoluble fraction of ethanolic extract of *G. sylvestre* leaves (GSE) were investigated in high fat diet fed rats for a period of 4 weeks and the effects of HFD on various biomarkers of obesity were investigated. The results were compared with the standard antiobesity drug, rimonabant.

In present study, there was significant increase in body mass index (BMI) (from 4.26±0.04 kg/m\(^2\) to 5.87±0.18 kg/m\(^2\)) and body weight gain (from 75.13±5.02 gm to 126.25±13.90 gm) in rats fed with high fat diet as compared to the normal healthy control rats (i.e. Group I). BMI is a simple index of weight-for-height that is commonly used to classify underweight, overweight and obesity in adults (WHO, 1995). Our study corroborate the findings of 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\(^2\)). Further, Matsuo et al. (2002) reported that body weight gain was greater in beef tallow diet group than in the other dietary groups. The increased body weight found in HFD rats might be due to the consumption of a diet rich in energy in the form of saturated fats (lard) and its deposition in various body fat pads and decreased energy expenditure (Srinivasan et al., 2004). WS fraction of GSE (120 mg/kg/p.o.) and rimonabant treatment significantly (p<0.01) decreased the BMI and body weight gain as compared to the HFD treated group while water insoluble fraction of ethanolic *Gymnema sylvestre* extract did not produce significant changes in the body mass index 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).

Further, rats fed with HFD for a period of 4 weeks consumed considerably more food and water than the control rats throughout the experiment. Hence, their caloric intake was increased and they showed a large increase in perirenal visceral adipose tissue mass, suggesting that the excess energy led to the buildup of adiposity. Rat consuming the high fat diet actually received about 27% more kilocalories, more weight, and had larger fat pads than rats fed only chow (Amin and Nagy, 2009). Further, our results showed a significant (p<0.05) decrease in food intake and water...
intake by oral administration of WS fraction of GSE (120 mg/kg) i.e. group III rats and these results were comparable to rimonabant treatment i.e. group V rats, while there was no significant changes in the food intake and water intake by the water insoluble fraction of Gymnema sylvestre ethanolic extract.

After 4 weeks feeding with HFD, hemodynamic parameters were significantly (p<0.01) elevated in HFD fed rats as compared to normal healthy control rats. Activation of the sympathetic nervous system contributes to blood pressure (BP) elevation in high-fat diet-induced obesity (Iwashita et al., 2002). A high-fat diet (HFD), which frequently induces BP elevation, could derange the neurohumoral control of the kidney (Hall et al., 1993). 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. These data suggest that activation of the sympathetic nervous system has an important role in HFD-related BP elevation. This may be also due to HFD intake, increases Ca\(^{2+}\) channel numbers or alter channel regulation, leading to increased transmembrane Ca\(^{2+}\) influx. Elevation in Ca\(^{2+}\) current density is associated with significantly elevated blood pressure (Wilde et al., 2000). The results of significant (p<0.01) increase in systolic BP (from 127.83±6.96 mm Hg to 165.83±10.33 mm Hg) and diastolic BP (from 96±4.65 mm Hg to 116.66±7.1 mm Hg) in the present study corroborates with the study of Aubin et al. (2008) who also reported that in rats fed a HFD, systolic BP (171 ± 7 mm Hg) and diastolic BP (109 ± 3 mmHg) were increased significantly as compared to a standard diet, fed rats (systolic BP, 134 ± 8 mm Hg; diastolic BP, 96 ± 5 mm Hg). When HFD fed rats were treated along with water soluble fraction of GSE (120 mg/kg/p.o.), there were significant (p<0.01) decrease in hemodynamic changes and results were comparable to rimonabant, a standard antiobesity drug.

The results of present study indicated that serum leptin levels in the HFD treated rats i.e. Group II were significantly (p<0.05) increased as compared with those in the normal control group i.e. Group I. Fried et al. (2000) indicated that basal levels of leptin are known to be strongly positively correlated with body fat on a HFD. Their
report indicated that leptin might contribute to hepatic steatosis by promoting insulin resistance and also, by altering insulin signaling in hepatocytes, so as to promote increased intracellular fatty acids (Uygun et al., 2000). Therefore, WS fraction of GSE (120 mg/kg/p.o.) prevents the increase of leptin levels due to its decrease of the body fat content of rats fed with HFD while WINS fraction of GSE (80 mg/kg/p.o.) has no effect on serum leptin levels.

The obese rats are both hyperleptinemic and hyperinsulinemic, and as occurs in humans, both plasma insulin and leptin concentrations were directly correlated with the degree of adiposity (Woods et al., 2003). In all likelihood, the hyperinsulinemia in the HFD fed rats was a result of insulin resistance, a common feature of human obesity and one that is central to the development of diabetes and cardiovascular disease. In the present research work, serum insulin levels were significantly (p<0.01) increased in HFD group as compared to the normal control healthy group. This may be due to insulin resistance caused by HFD. Mehta et al. (2002) indicated that HFD lead to insulin resistance through oxidative stress. In another study, serum insulin levels were higher in the HFD group as compared to the normal control healthy rats (Hsu and Yen, 2007). Serum insulin levels in the rats treated with HFD + WS fraction of GSE (120 mg/kg/p.o.) i.e. Group III, and as well as in standard drug, rimonabant treated rats i.e. Group V were significantly decreased as compared with those in the HFD group i.e. Group II. WINS fraction of GSE (80 mg/kg/p.o.) has no significant effect on serum insulin levels.

In the present study, serum glucose levels were increased in the HFD group as compared to the normal control group, while glucose levels were significantly decreased by WS fraction of GSE (120 mg/kg/p.o.) but not WINS fraction of GSE (80 mg/kg/p.o.) and rimonabant (10 mg/kg/p.o.). Diet-induced obesity dysregulated glucose homeostasis and causes hyperglycemia (Chang et al., 1990). This is consistent with previous study that feeding of HFD for a period of 4 weeks produced a significant increase in plasma glucose levels (Srinivasan et al., 2004). Other studies have also reported that Gymnema sylvestre controls the blood glucose levels in both
non-insulin-dependent diabetes mellitus and insulin-dependent diabetes mellitus patients (Baskaran et al., 1990; Shanmugasundaram et al., 1990).

A fat-enriched diet is regarded as an important factor in the development of cardiac diseases because it leads to the development of hyperlipidemia, atherosclerosis, and abnormal lipid metabolism (Onody et al., 2003). Our data clearly showed that feeding of the HFD (20 g/day/rat) for a period of 4 weeks increased the concentrations of serum TC, LDL-C, VLDL-C, TGs in experimental rats. 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). The levels of TGs, TC, LDL-C, VLDL-C and atherogenic index were significantly (p<0.01) decreased by water soluble fraction of ethanolic Gymnema sylvestre extract (120 mg/kg/p.o.) and rimonabant (10 mg/kg/p.o.) treatment groups when rats fed with high fat diet while these levels were not significantly decreased by water insoluble fraction of ethanolic Gymnema sylvestre extract (80 mg/kg/p.o.) treatment group (i.e. group IV). Rachh et al. (2010) reported that oral administration of hydroalcoholic extract of Gymnema sylvestre (200 mg/kg) along with the high cholesterol diet for 7 days, significantly decreased TC, TGs, LDL-C, VLDL-C and increased the HDL-C levels. The results of present study are consistent with the findings of Rachh et al. (2010).

Measurement of the levels of the important lipoproteins such as apo-A1 and apo-B can throw light on lipid metabolism and the effect of various drugs on the same. Additionally, the apolipoprotein-A class of lipoprotein, besides being associated with HDL particle, is known to play important antioxidant and anti-inflammatory roles in atherosclerosis (Garner et al., 1998). Apolipoprotein A1 (apo-A1) is associated with HDL, having several antiatherogenic properties. Apolipoprotein B (apo-B) is associated with low density lipoprotein, intermediate-density lipoprotein (IDL), very low-density lipoprotein (VLDL) and chylomicrons (D’Souza et al., 2007). In the present study, serum apo-B levels were significantly (p<0.01) increased in HFD rats as compared to the normal healthy control rats where
the levels of serum apo-A1 were significantly (p<0.01) decreased in HFD rats i.e. Group II as compared to the normal healthy control rats. Water soluble fraction of GSE (120 mg/kg/p.o.) and rimonabant significantly (p<0.01) decreased apo-B levels as compared to the group II but water insoluble fraction of Gymnema sylvestre ethanolic extract (80 mg/kg/p.o.) treatment group showed less significant (p<0.05) decrease in apo-B levels. Apo-B secretion by the liver is regulated by factors such as rate of cholesterol biosynthesis, availability of triglycerides and cholesterol esters (Dixon et al., 1991).

In the present study, hepatic cholesterol, visceral fat pad (mesenteric, perirenal, epididymal) and organs (heart, liver and kidney) weights were significantly affected by high fat diet in HFD-fed group i.e. Group II rats. WS fraction of Gymnema sylvestre ethanolic extract significantly (p<0.01) decreased the hepatic cholesterol levels as compared to HFD fed group but WINS fraction of Gymnema sylvestre ethanolic extract produced no significant changes in the hepatic cholesterol levels. It has been reported by Shigematsu et al. (2001a) that the decrease of cholesterol in liver may result from (1) the effects of transfer acceleration of cholesterol from liver to blood, (2) the inhibition of cholesterol synthesis in liver, (3) increased of cholesterol to bile acid, (4) the hydrolysis improvement of cholesterol ester in liver, and (5) suppression of absorption of cholesterol from intestine.

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). WS fraction of GSE (120 mg/kg/p.o.) significantly (p<0.01) decreased the weight of visceral fat and organs as compared to the HFD group while WINS fraction of GSE (80 mg/kg/p.o.) treatment did not show significant changes in the weights of visceral fat and organs. The results were comparable to rimonabant treatment group.

HFD-induced obesity leads to cardiac abnormalities such as cardiac oxidative stress assessed by lactate dehydrogenase (LDH) enzyme (Diniz et al., 2008). The measurement of cytoplasmic LDH activity is a well-accepted assay to quantify viable
cell numbers and monitor cell proliferation (Mosmann, 1983). On the other hand, the leakage of cytoplasmic LDH caused by the damage of cell membrane integrity is also a good indicator of cell death and is used to estimate cytotoxicity (Arechabala et al., 1999). In the present study, serum LDH levels were significantly (p<0.01) increased in the HFD group, as compared to the normal control animals i.e. Group I. The levels of LDH were significantly (p<0.01) decreased by WS fraction of GSE (120 mg/kg/p.o.) and rimonabant but not in WINS fraction of GSE (80 mg/kg/p.o.) treatment group.

Another detectable parameter in the present study was lipid peroxidation. Free radicals are known to be involved in a variety of human pathologies, including atherosclerosis (Steinberg, 1997), and obesity (Van Gaal et al., 1995). Lipid peroxidation levels were significantly increased in the HFD group i.e. Group II as compared to the levels in Group I rats, while these levels were significantly decreased by WS fraction of GSE (120 mg/kg/p.o.) and rimonabant but not by WINS fraction of GSE. A potential mechanism for the generation of free radicals may be the activation of β-adrenergic receptors reported for obesity prone rats (Levin et al., 1983). This could increase lipolysis to yield free fatty acids that are able to uncouple the mitochondrial phosphorylation and further, generate free radicals (Turrens, 1997).

Liou et al. (1993) have shown that hyperlipidaemia reduces the strength of the antioxidative defence system. Mehta et al. (2002) reported that HFD leads to liver injury and insulin resistance through oxidative stress. The reports indicate that antioxidants can modify cholesterol absorption and increase antioxidant status (Ko et al., 2005). Fardet et al. (2008) suggested that the diet-induced obesity in rat models showed an increase in the levels of oxidative stress in their liver and that oxidative stress can result from the excessive production of reactive oxygen species and/or deficient anti-oxidant capacity.

In animal and human studies, obesity is associated with a decrease in tissue or plasma antioxidant capacity (Ozata et al., 2002). GSH constitutes the first line of defence against free radicals and is also responsible for the maintenance of protein
thiols and acts as a substrate for GPx and GST (Prakash et al., 2001). The present data indicate that GSH content was depleted in the rats with obesity induced by a HFD. Enzymatic antioxidants, such as superoxide dismutase, catalase or GPx, GR and GST can scavenge reactive oxygen species and free radicals or prevent their formation (Husain et al., 2005). The present results showed that antioxidant enzyme activities (GPx, GR and GST) in the HFD group i.e. Group II were significantly (p<0.01) decreased, while HFD + WS fraction of GSE (120 mg/kg/p.o.) and rimonabant groups had significantly increased activities of antioxidant enzymes in the liver and heart but not in WINS fraction of GSE (80 mg/kg/p.o.) treatment group. This is inconsistent with previous reports that high-fat diet induces critical oxidative damage in the liver (Schrauwen, 2007).

Na\(^+\) K\(^+\) ATPase (liver and kidney) is a membrane enzyme that energizes the Na-pump, hydrolyzing ATP and wasting energy as heat so playing a role in thermogenesis, energy balance, and obesity development (Iannello et al., 2006). 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 (p<0.01) decrease in hepatic levels of membrane bound enzymes like Na\(^+\)/K\(^+\) ATPase as compared to normal healthy control group. Our results are in agreement with the findings of Takeuchi et al. (1995) who reported the activity of Na\(^+\) K\(^+\) ATPase in the liver and skeletal muscle was lower in rats fed with the lard diet. The decrease of ATPases could be due to enhanced lipid peroxidation by free radicals. Since this membrane bound enzymes are 'SH' group containing enzymes, so are lipid dependant. In the present study, Na\(^+\) K\(^+\) ATPase activity was significantly increased in the WS fraction of GSE (120 mg/kg/p.o.) treatment group as compared to the HFD fed group. These results were comparable with the results of rimonabant treatment group but not in water soluble fraction of ethanolic Gymnema sylvestre (80 mg/kg/p.o.) extract treatment group. Chueh et al. (2001) reported that blocking the Na\(^+\) K\(^+\) pump can induce apoptosis. A study showed that apoptotic thymocytes had decreased protein levels of Na\(^+\) K\(^+\) ATPase (Mann et al., 2001). Previous investigations have suggested
that sodium-potassium-ATPase activity is lower in severely obese patients than in normal controls (Beutler et al., 1983). This is a significant finding of the study and has not been reported earlier.

Obesity-related cardiomyopathy typically occurs in persons with severe and long-standing obesity, which may progressively develop various cardiac abnormalities, such as dilated heart, congestive heart failure, and sudden cardiac death (Alpert, 2001). Wang et al. (2008) have 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.

Apoptosis results from the activation of caspases that cleave various subcellular cytoplasmic proteins and fragment nuclear DNA (Trivedi and Barouch, 2008). In the present study, the caspase dependent apoptotic pathway was significantly increased in cardiac tissues of HFD-induced obese rats, as evidenced by increase in cardiac activated caspase-3 levels in obese rats’ hearts. Caspase-3 is a key player involved in the caspase-dependant apoptotic pathway (Yong-hao et al., 2008). WS fraction of GSE (120 mg/kg/p.o.) significantly (p<0.01) reduced the caspase-3 levels but not by WINS fraction of GSE (80 mg/kg/p.o.) treatment as compared to the HFD fed group. The results were comparable with the standard rimonabant treatment. This is the first reported study on Gymnema sylvestre extract.

DNA laddering is an index of myocyte apoptosis (Trivedi and Barouch, 2008). In the present study, apoptotic death of myocardial cells was demonstrated in HFD-induced obesity in Wistar rats by the DNA agarose-gel electrophoresis. DNA electrophoresis demonstrates the presence of small DNA fragments in the form of a DNA ladder in HFD fed group. The finding is in accordance with that of Li et al. (2005) who reported that HFD feeding significantly elevated cytoplasmic DNA fragmentation in heart and liver samples of high-fat diet fed rats compared with those from low-fat diet fed group. WS fraction of GSE treatment (120 mg/kg/p.o.) group, DNA laddering was preserved while in the WINS fraction of GSE treatment (80
mg/kg/p.o.) group little DNA laddering was present. This is a significant finding of the study and has not been reported earlier.

In the histology study, haematoxylin–eosin stained heart tissue of HFD fed rats (i.e. Group II rats) showed deposition of fat globules in myocardial cells with marked fatty changes in portal region as compared to normal healthy control rat’s heart tissue (i.e. Group I) which showed normal architecture with regular morphology of myocardial cell. The fatty changes were reduced by WS fraction of GSE (120 mg/kg/p.o.), induced by HFD in the heart however, WINS fraction of GSE (80 mg/kg/p.o.) showed microvascular fat deposition in myocardial cells. The results signify that water soluble fraction of ethanolic Gymnema sylvestre extract reduced the fatty changes in the myocardial tissue.

Further, haematoxylin–eosin stained liver tissue showed a large number of lipid droplets in the HFD group i.e. Group II, while the number of lipid droplets was significantly reduced by the water soluble fraction of ethanolic G. sylvestre extract and standard drug groups as compared to the HFD group. WINS fraction of GSE (80 mg/kg/p.o.) group showed ballooning degeneration and congestion in the liver cells. The liver is the central organ for cholesterol, phospholipid, triacylglycerol and lipoprotein metabolism. In obesity, the liver is the receiver of large amounts of fatty acids, which cause its steatosis (Festi et al., 2004).

The results of the present study indicated that administration of high fat diet for a period of resulted in significant obesity in Wistar rats as indicated by increased body mass index, body weight gain, blood pressure, heart rate, and an increase in levels of serum leptin, insulin, lipids, and apo-B levels and decrease in serum apo-A1 and HDL- C levels, Na<sup>+</sup>/K<sup>+</sup> ATPase activity and antioxidant enzymes levels in cardiac and liver tissues which were further supported by histopathological findings. The oral feeding of water soluble fraction of ethanolic Gymnema sylvestre extract produced significant reduction in body mass index, body weight gain, blood pressure, heart rate, and an increase in levels of serum leptin, insulin, lipids, and apo-B levels and elevation in serum apo-A1 and HDL- C levels, Na<sup>+</sup>/K<sup>+</sup> ATPase activity and
antioxidant enzymes in cardiac and liver tissues which were further supported by histopathological findings. However, water insoluble fraction of ethanolic *Gymnema sylvestre* extract could not produce significant protection in obese Wistar rats. The results in the first in-depth study revealing significant antiobesity potential of water soluble fraction of *Gymnema sylvestre* ethanolic extract. Hence, in the Model II, the role of water soluble fraction of *Gymnema sylvestre* ethanolic extract was investigated in diabesity in rats.

In Model II i.e. high fat diet-induced obesity in streptozotocin-induced diabetes in rats, we had 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 Model II study was planned to evaluate the antiobesity effect of water soluble fraction of *Gymnema sylvestre* ethanolic extract in HFD-induced obesity in diabetic rats. We used Pioglitazone as a standard drug in this Model (i.e. Model II). Ding et al. (2005) have reported that treatment with pioglitazone (20 mg/kg/p.o.) improves insulin sensitivity in low-dose STZ and high sucrose-fat diet induced obese rats. Hence, we have used pioglitazone as a standard drug in the HFD-induced obesity in diabetic rats (i.e. Model II).

Diabetes mellitus was induced by single injection of streptozotocin (45 mg/kg, i.v. in citrate buffer, pH-4.5). Streptozotocin (STZ) is a naturally occurring diabetogen that is particularly toxic to the insulin-producing beta cells of the pancreas in mammals. The STZ was discovered in a strain of the soil microbe *Streptomyces achromogenes* and used to induce diabetes mellitus (NIDDM) in experimental models (Nakhaee et al., 2009).

In the present Model (i.e. Model II) single injection of streptozotocin (45 mg/kg, i.v. in citrate buffer, pH-4.5), produced significant increase (p<0.01) in blood glucose levels after 72 hrs. Those animals having FBG≥ 200 mg/dl were selected in the present study. These rats were further subjected for induction of obesity. Obesity was induced in these rats by feeding of high fat diet (HFD) 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 β-cell dysfunction (Ding et al., 2005).

Diabetic rats when fed with high fat diet produce a metabolic syndrome characterized by insulin resistance, dyslipidemia, type-2 diabetes and central obesity, which is similar with the metabolic syndrome caused by obesity (Steinberger and Daniels, 2003). 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 when injected intraperitoneally with low dose of STZ (30 mg/kg) and fed with a high sucrose-fat diet for 8 weeks, developed 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. Further, Zhang et al. (2008) demonstrated that a combination of HFD and a low dose of STZ (45 mg/kg) injection effectively used to generate a rat model that mimics the natural history and metabolic characteristics of type 2 diabetes in humans. Hence, in the present study selected a dose of 45 mg/kg of STZ to induce diabetes in Wistar rats.

Previous reports suggest that diabetic animals maintained on a HFD were hyperphagic relative to diabetic rats maintained on a normal diet, consuming 40% more calories on a daily basis (Friedman, 1978). In the present model II, HFD induced obesity in diabetic rats, food intake in rats was not significantly changed in HFD fed diabetic group as compared to the other groups. The body weight gain in the STZ/HFD- fed rats i.e. Group XII rats was significantly reduced as compared to the normal healthy control rats i.e. Group XI rats. Water soluble fraction of ethanolic Gymnema sylvestre extract (120 mg/kg/p.o.) showed no significant difference in body
weight gain as compared to the Group XII rats. Whereas in Model I i.e. HFD only, there was significant increase in body weight in Group II as compared to Group I.

In the present study, blood pressures and heart rate were significantly (P<0.01) increased in STZ/HFD fed rats as compared to the normal healthy control rats, while these levels decreased significantly by WS fraction of GSE i.e. Group XIII. HFD induces BP elevation, could derange the neurohumoral control of the kidney (Hall et al., 1993). At the same time, diabetes is also associated with alterations in resting heart rate and blood pressure (Hicks et al., 1998). Teuscher et al. (1989) reported that the prevalence of hypertension in diabetic individuals appears to be approximately two folds that in the nondiabetic population.

STZ/ HFD- fed rats showed an increased in the lipid levels (TC, LDL-C, VLDL-C, TGs and hepatic-cholesterol ), while the lipid levels were significantly decreased in WS of GSE treated group. These results are comparable with the standard pioglitazone treatment. STZ/ HFD-fed animals showed abnormalities in lipid metabolism as evidenced from increased serum TC, LDL-C, VLDL-C and TGs levels, as in case of human type 2 diabetic patients which might contribute to various cardiovascular complications. 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 (Sahu et al., 1990). Hypercholesterolemia may be attributed to increased dietary cholesterol absorption from the small intestine following the intake of HFD in a diabetic condition (Strack et al., 1995).

Serum apolipoprotein B levels were increased in the diabetic group maintained on HFD as compared to the normal control group as apolipoprotein B is associated with low density lipoprotein, intermediate-density lipoprotein (IDL), very low-density lipoprotein (VLDL) and chylomicrons (D'Souza et al., 2007).
In the present study serum leptin levels were significantly increased in HFD fed diabetic rats. Diabetic rats fed the high fat diet had significantly elevated plasma leptin levels relative to diabetic rats fed the high cholesterol diet (Chavez et al., 1998). In fat tissue, leptin mRNA expression levels increased almost 2 to 3 folds as compared with those of control group (Wang et al., 2007). The increased leptin levels were significantly decreased by WS fraction of GSE (120 mg/kg/p.o.) treatment. Many studies have reported that the high-fat diet (HFD) feeding rats; develop insulin resistance (Zhao et al., 2008). At the same time, low-dose STZ has been known to induce a mild impairment of insulin secretion which is similar to the feature of the later stage of type 2 diabetes characterized by a progressive decline in insulin action (insulin resistance), followed by hyperinsulinemia (Srinivasan et al., 2005). Serum insulin concentrations were more than 2-fold greater in fat-fed/STZ rats than in chow-fed/STZ rats (Reed et al., 2000). STZ/HFD fed rats showed hyperinsulinemia which was significantly decreased by WS fraction of GSE (120 mg/kg/p.o.) treatment.

Serum glucose concentrations were significantly elevated in the diabetic rats maintained on the HFD relative to the normal control group, these concentrations were significantly decreased by WS fraction of GSE (120 mg/kg/p.o.) treated group. Wang et al. (2007) reported that serum glucose was significantly increased in the low dose of STZ/ HFD fed rats. Fat-fed rats had an almost 3-folds higher proportion of severe hyperglycemia after STZ-injection than did chow-fed animals (Reed et al., 2000).

Diabetes is characterized by the development of a cardiac myopathy that is independent from intercurrent coronary pathologic conditions (Bell, 1995). The increase in glucose level after STZ administration was coupled with the greatest degree of cell death. A 30% reduction of myocytes was measured in the diabetic left ventricle at 4 weeks after STZ administration (Fiordaliso et al., 2000). Moraes et al. (2009) showed that neuronal apoptosis is induced by the fat-rich diet.

The treatment with WS of GSE for 3 weeks suppressed the increase in caspase-3 levels, Na⁺ K⁺ ATPase activity, and DNA laddering as compared to the
STZ/HFD fed group. DNA electrophoresis demonstrates the presence of small DNA fragments in the form of a DNA ladder in HFD fed group, while in the WS fraction of GSE (120 mg/kg/p.o.) treatment group, DNA laddering was preserved. The elevated myocardial DNA fragmentation levels (a hallmark of apoptosis) in overweight subjects that were further increased in patients with concomitant type 2 diabetes. These data indicate that obesity and diabetes have additive effect on cardiac apoptosis in humans (Baranowski et al., 2009).

Organ’s weight (liver, heart, kidney and pancreas) and visceral fat pad weights (perirenal, mesentric fat and epididymal fat) were significantly increased in diabetic rats fed with HFD. HFD produces adiposity i.e. deposition of fat on the adipose tissues. But WS fraction of GSE (120 mg/kg/p.o.) showed no significant difference in the organs weight as compared to the STZ/ HFD fed rats. Visceral fats were significantly decreased by WS fraction of GSE (120 mg/kg/p.o.). These results were comparable with the standard pioglitazone treatment.

In the present study, antioxidant enzymes (GPx, GR, GST, SOD and catalase) levels were significantly increased by WS fraction of GSE (120 mg/kg/p.o.) treated group as compared to the STZ/ HFD treated group. This may be due to the high level of fat increases fat-mediated oxidative stress and decrease antioxidative enzyme activity (Slim et al., 1996), as well as hyperglycemia was found to increase the production of free radicals that is associated with increased production of reactive oxygen species (ROS), resulting in tissue damage that is assessed by the measurement of lipid peroxides (Nakhaee et al., 2009). The increase in lipid peroxidation indicates an increased oxidative stress as a result of excessive generation of free radicals.

There was dense focal fatty infiltration in hematoxylin-eosin stained myocardial cells of STZ/HFD fed rats as compared to the normal control group 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. sylvstre ethanolic extract (120 mg/kg/p.o.) (i.e. group XIII) and standard drug i.e.
pioglitazone groups (20 mg/kg/p.o., i.e. group XIV) showed normal morphology of heart showed no pathological changes with regular architecture of myocardium.

Very few fatty changes were observed in the hematoxylin-eosin stained hepatocytes of the STZ/HFD fed group and no other pathological changes observed as compared to normal healthy control rat’s liver tissue which showed normal fat deposition in hepatocytes i.e. within normal limit. Water soluble fraction of G. sylvestre ethanolic extract group (120 mg/kg/p.o.) (i.e. group XIII) showed microvesicular fat accumulation in the hepatocytes while standard drug i.e. pioglitazone group (20 mg/kg/p.o., i.e. group XIV) showed normal morphology of liver tissues as compared to the STZ+HFD treated group. Liver steatosis is a well-known pathology in severely obese patients and is especially associated with visceral adiposity and diabetes (Adams et al., 2005). These results were comparable with the pioglitazone treatment.

The results of this study reveal for the first time that water soluble fraction of ethanolic Gymnema sylvestre extract effectively alleviates the deleterious effects such as insulin resistance, hyperlipidemia, hypertension and oxidative stress produced by HFD in diabetic rats. Further, the water soluble fraction of ethanolic Gymnema sylvestre extract offers cardiac protection by preventing cellular obesity changes and cardiac organ damage as revealed by decreasing cardiac caspase-3 levels, Na⁺K⁺ ATPase activity, DNA laddering, oxidative stress, and maintaining normal architecture of myocardium. The present study supports the potential of water soluble fraction of ethanolic Gymnema sylvestre extract in diabesity disorder.