Increased rates of obesity due to low levels of physical activity and high-energy diets are driving the global epidemic of type 2 diabetes (Zimmet et al., 2001). Obesity is set to be the world’s major cause of morbidity and mortality in the 21st Century. According to World Health Organization (WHO), more than 1.4 billion adults were overweight in the world, and at least 200 million men and nearly 300 million women from them were obese (WHO, 2012). The incidence of diabetes mellitus has increased dramatically in recent decades, predominantly because of changes in life style, an increase in the prevalence of obesity and longevity (Heydari et al., 2010). Current projections estimate that there are about 366 million diabetic people around the world and the number is expected to reach 552 million cases by the year 2030 (IDF, 2011). India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the ‘diabetes capital of the world’ (Mohan et al., 2007).

The rising concern over obesity as a global health hazard is relatively recent and it has outpaced the pharmaceutical industry’s ability to develop new and safe drugs. Despite the increasing need for new anti-obesity therapeutics, the food and drug administration (FDA) has not approved a new anti-obesity drug since orlistat in 1999. In past years, numerous drugs have been approved for the treatment of obesity; however, most of them have been withdrawn from the market because of their adverse effects. This, in part, reflects the lack of new approaches to drug development in the past years and the efficacy and safety requirements set by the FDA. In order to win approval, a drug must induce a statistically significant weight loss of at least 5% at one year when compared to placebo, and at least 35% of patients must achieve at least a 5% weight loss and show improvement in metabolic biomarkers (FDA, 2007). Several clinical trials have demonstrated that progression to diabetes in obese patients with impaired glucose tolerance can be prevented through weight reduction and increased levels of physical activity (Tuomilehto et al., 2001; DPP, 2002).

For patients who have developed type 2 diabetes, intentional weight loss has many potential benefits, including improved metabolic control and a reduced need for antidiabetic medications. One of the challenges of using antidiabetic therapy in obese or overweight patients is the prospect of substantial iatrogenic weight gain with many widely used drug classes. Since lifestyle interventions, including weight loss, are usually very difficult to achieve and/or sustain, the practitioner frequently is left with few options when trying to
Avoid adverse effects of antidiabetic therapy that are counterproductive to efforts directed at weight loss (UKPDS, 1995; Scheen, 2003).

The recent consensus statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) recommends initial treatment with lifestyle intervention and metformin were safe and tolerated for all patients with type 2 diabetes, whether obese or not. The statement also emphasises the need for rapid addition of complementary medications or changes in regimens to promptly achieve and maintain glycemia at levels as close to normal as possible (Krentz, 2007). Discoveries of new molecular targets that control appetite, lipogenesis, and metabolism are anticipated to lead to a new wave of targeted pharmacotherapies that feature higher efficacies and improved safety profiles for the prevention and treatment of obesity and type 2 diabetes. An array of potential new drugs, either alone or in combination, may provide the millions of obese diabetic patients a tool to help reach a healthy weight (Colon-Gonzalez et al., 2013).

The selected plan for the present study was entitled ‘Evaluation of ethanolic extract of *Embelia ribes* in high fat diet plus low dose streptozotocin induced type-2 diabetes in Wistar rats’.

Herbal traditional medicines have gained considerable momentum worldwide during the past decade and play a paramount role in health care programs due to their long historical clinical practice and less side effects. However, a key obstacle, which has hindered the acceptance of the traditional medicines is the lack of standardization techniques. Hence, quality control for the efficacy and safety of herbal products is essential. Hence, in the present study an attempt was made to standardize the fruits of *E.ribes* on the basis of physio-chemical and phytochemical characteristics. Chemical characterization of the main biologically active constituent of *E.ribes* extract was achieved by HPTLC and HPLC, showing the presence of embelin, a naturally occurring alkyl substituted hydroxy benzoquinone. The study also included isolation of pure embelin from dry fruits of *E.ribes* and characterization of isolated embelin by UV spectroscopy, HPLC, and FT-IR spectroscopy. Madhavan et al. (2011) reported the HPLC method for the estimation of embelin as marker in *E.ribes* and its polyherbal formulation.
As type 2 diabetes in humans is thought to be a consequence of polygenic interactions with the environment and as most patients have a history of foregoing obesity, the diet-induced animal strains are appropriate models to study those clinical features (Reuter, 2007). Hence, in the present study, High fat diet (HFD) was used to induced obesity, and HFD plus low dose of streptozotocin (STZ, 35 mg/kg bw, i.p., single injection) was used to induced type 2 diabetes in Wistar rats (Srinivasan et al., 2005).

In study-I, ‘antiobesity potential of ethanolic extract of E.ribes in HFD-induced obesity in wistar rats’ was investigated. First HFD-induced obesity model was validated by investigating the effect of feeding HFD for the period of 1 week on baseline characteristics of obesity in Wistar rats. The feeding of HFD for 1 week resulted in significant \( p < 0.05 \) increase in body weight, BMI as well as blood glucose, serum insulin, total cholesterol, triglycerides levels as compared to NPD-fed rats i.e. group I (Table 16), indicating the development of obesity characteristic. Ansari et al. (2012) characterized the obesity model by feeding HFD for the period of 1 week in Wistar rats. Results of the present study corroborate the findings of Ansari et al. (2012).

After characterization, antiobesity potential of ethanolic extract of E.ribes (100 mg/kg bw) was investigated by feeding HFD for the period of 4 weeks to induce obesity in Wistar rats. Bhandari et al., (2007, 2008) reported the significant reduction in the levels of blood glucose, hemodynamic parameters, lipid peroxidation in pancreatic tissue following oral administration of ethanolic extract of E.ribes in a dose of 100 mg/kg bw in STZ-induced diabetes in rats. Further, E.ribes extract significantly enhanced the antioxidant defense against reactive oxygen species produced under hyperglycaemic condition at 100 mg/kg bw dose level in rats. Furthermore, insulin resistance, dyslipidemia, hypertension and oxidative-stress are linked to obesity and related co-morbidities (Alegria Ezquerra et al., 2008). Therefore, dose of 100 mg/kg bw dose of ethanolic extract of E.ribes extract was selected and the study was carried out to test the hypothesis that ethanol extract of E.ribes have anti-obesity potential in HFD-induced obesity in Wistar rats.

Excessive growth of adipose tissue results in obesity which involves two growth mechanisms: hyperplasia (cell number increase) and hypertrophy (cell size increase; Mancini et al., 2001). The development of hyperplastic adipose tissue is associated with the
most severe forms of obesity and has the poorest prognosis for treatment and reduction in body fat content reduces the risk for obesity and decreases morbidity and mortality (Hausman et al., 2001). In the present study, significant reduction (p < 0.01) in body weight gain of HFD-fed rats was accompanied by a reduction of organ’s weight (heart, liver and kidney; Table 19) on treatment with ethanolic extract of *E.ribes* (100 mg/kg bw). Further, *E.ribes* treatment also produced significant decrease in the visceral fat pad weights (perirenal, mesenteric, epididymal; Figure 19) as compared to HFD-only fed rats i.e. group II. These results are in line with the findings reported by Kim et al., (2009) who observed the antiobesity potential of corn gluten by reduction in visceral fat pad weight and lipid regulating enzymes in HFD-induced obesity in rats.

In epidemiological studies, BMI is widely used as a measure of fatness because it is highly correlated with body fat and is nearly independent of height (Yalcin et al., 2005). In the present study, significant reduction in BMI of ethanolic extract of *E.ribes* treated group was observed. These results indicate that ethanolic extract of *E.ribes* (100 mg/kg bw, p.o) suppresses the HFD-induced increase in fat mass and body weight gain.

Though there was a significant difference in the body weights between the HFD-fed and normal control groups, no significant difference in the daily food intake and water intake between groups were observed (Table 17). This observation provides the fact that dietary caloric content was independent of the amount of food consumed by the animals. The HFD-fed rats continuously consumed similar quantities of food, regardless of the higher calories content in the diet. As a result, the caloric intake was raised significantly in the HFD-fed rats as compared to the normal control rats. The unchanged food intake in spite of the higher caloric content in the diet was proportional to the increment of the body weight, hence, resulting in the obese state in the present study.

In addition to its weight reducing effect, ethanolic extract of *E.ribes* (100 mg/kg bw, p.o) was also able to improve lipid metabolites including TC, TG, HDL-C, LDL-C and VLDL-C levels in obese rats (Figure 20). It is reported that obesity, especially abdominal obesity, is associated with dyslipidemia, characterized by elevated TG and reduced HDL-C concentrations. TGs are involved in the ectopic accumulation of lipid stores in the liver and are associated with a number of diseases such as metabolic syndrome. VLDL-C transports...
cholesterol and TG to the tissues, while high HDL-C is helpful in transporting excess cholesterol to the liver for excretion in the bile. High TC and LDL-C levels, and lower HDL-C level are risk factors for coronary heart disease (Chang et al., 2011). The ratio of total cholesterol to HDL-C (atherogenic index) and the ratio of LDL-C to HDL-C (coronary artery index) are strong and reliable indicators of whether or not cholesterol is deposited into tissues or metabolized and excreted (Hasty et al., 2007). The results of present study for the first time show that treatment with ethanolic extract of E.ribes (100 mg/kg bw, p.o) causes profound reductions in the atherogenic and coronary indices in experimental obese rats (Figure 21 and 22). In addition, ethanolic extract of E.ribes (100 mg/kg bw, p.o) treatment showed significant reduction in serum apolipoprotein-B levels as compared to HFD control group (Figure 23). Apolipoprotein-B is associated with LDL-C, intermediate density lipoprotein (IDL-C), VLDL-C and chylomicrons (D'Souza et al., 2007). Apolipoprotein-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). Thus, the present result suggested that ethanolic extract of E.ribes (100 mg/kg bw, p.o) would be helpful in the prevention of obesity complications by improving hyperlipidemia. Moreover, ethanolic extract of E.ribes (100 mg/kg bw, p.o) treatment showed improvements in the histological features (i.e. fatty degeneration) of heart and liver induced by HFD in rats (Figure 11, 12).

Obesity is associated with an impaired ability of tissue to respond to insulin and effectively store glucose. Further, in response to insulin resistance, glucose production is increased, leading to hyperinsulinemia, hyperglycemia and ultimately type 2 diabetes (Knight and Imig, 2007). In the present study, significant reduction in the blood glucose, serum insulin levels and HOMA-IR values (Figures 24,25,26) after the treatment with ethanolic extract of E.ribes (100 mg/kg bw, p.o) for 21 days in HFD-fed rats was observed.

Epidemiological studies have found a progressive increase in the prevalence of elevated blood pressure with increasing adipose tissue (Yalcin et al., 2005). Moreover, hypertension and hyperlipidemia often manifest concomitantly in the clinical context of obesity and insulin resistance. In consistent with improvement of lipid profile and insulin resistance by
ethanolic extract of *E.ribes* (100 mg/kg bw, p.o), treatment was also able to attenuate the development of hypertension in HFD-fed obese rats (Table 18).

Leptin is fat-derived key regulators of food intake and energy expenditure, where its concentration in the plasma is associated with general adiposity (Staiger and Haring, 2005). The reductions of leptin level reflect a decreased in the fat mass. Hypothalamus receives direct input from leptin, which cross the blood-brain barrier and provide information on the levels of peripheral adipose mass. The results from the present studies suggest that ethanolic extract of *E.ribes* (100 mg/kg bw, p.o) produced significant adipocytes loss, indicated by reduced serum leptin levels (Figure 27).

HFD-induced obesity leads to cardiac abnormalities such as cardiac oxidative stress assessed by LDH enzyme levels (Diniz *et al.*, 2008). In the present study, there was significant increase (p < 0.01) in serum LDH levels in HFD control rats as compared to normal control rats. Treatment with ethanolic extract of *E.ribes* (100 mg/kg bw, p.o) produced significant reduction (p < 0.01) in serum LDH levels as compared to HFD control rats (Figure 28).

Na⁺K⁺-ATPase 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. 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, hepatic and cardiac Na⁺K⁺-ATPase contents were significantly decreased in HFD control rats as compared to normal control rats. The hepatic and cardiac Na⁺K⁺-ATPase contents were significantly enhanced by treatment with ethanolic extract of *E.ribes* (100 mg/kg bw, p.o; Table 20).

Obesity and hyperlipidemia synergistically promote systemic oxidative stress-imbalance between tissue free radicals, reactive oxygen species (ROS) and antioxidants (Hasty *et al.*, 2007). ROS could react with polyunsaturated fatty acids which lead to lipid peroxidation (Memisogullar and Bakan, 2004). Malondialdehyde is a by-product of lipid peroxidation and reflect the degree of oxidation in the body (Ohkawa *et al.*, 1979). Possible mechanisms that generate oxidative stress in obesity include hyperglycemia, elevated lipid levels, inadequate antioxidant defenses and hyperleptinemia (Vincent *et al.*, 2007). In the present
study, cardiac and hepatic TBARS levels, determined by evaluating malondialdehyde content were significantly decreased (p < 0.01) after ethanolic extract of *E.ribes* (100 mg/kg, p.o) treatment in HFD-fed rats. Furthermore, in our study, the levels of GSH (a major endogenous antioxidant), GPx, GR, GST, SOD and CAT (scavenger of free radicals) decreased in HFD-fed rats as reported earlier (Ansari *et al.*, 2012). Treatment of HFD-fed rats with ethanolic extract of *E.ribes* (100 mg/kg bw, p.o) had reversed the levels of these enzymatic antioxidants towards normal. It is, therefore, reasonable to assume that ethanolic extract of *E.ribes* (100 mg/kg bw, p.o) treatment improves cardiac and hepatic oxidative balance in HFD-fed obese rats (Table 21, 22), because ethanolic extract of *E.ribes* (100 mg/kg bw, p.o) was able to reduce the level of TBARS and free radical generation.

In the present study, no significant difference (p > 0.05) was observed in body weight gain, fat pad weights, body mass index (BMI), mean arterial blood pressure, serum lipid profile, serum leptin, insulin, glucose and oxidative stress markers between *E.ribes* (100 mg/kg bw) *per se* and normal control group, suggesting that ethanolic extract of *E.ribes* has no effect *per se* in HFD-induced obesity in Wistar rats.

After evaluating the anti-obesity potential of ethanolic extract of *E.ribes* (100 mg/kg bw, p.o) in HFD-induced obesity in Wistar rats, embelin—the active principle of the *E.ribes* fruits was investigated for anti-obesity potential in HFD-induced obesity in Wistar rats (Plan-II).

Mahendran *et al.* (2011) showed the antihyperglycemic, lipid lowering and antioxidant activities of embelin at the doses of 25- and 50 mg/kg bw in alloxan induced diabetes in rats. They reported that among the two doses of embelin (25- and 50 mg/kg bw), 50 mg/kg bw dose produced significant antihyperglycemic, lipid lowering and antioxidant action. Thippeswamy *et al.* (2011) showed the protective effects of embelin at 25- and 50 mg/kg bw dose on ischemia/reperfusion-induced brain injury in rats. They reported that embelin pretreatment at 50 mg/kg bw dose exhibited better protection against ischemic damage than 25 mg/kg bw dose. Therefore, in the present study, a dose of 50 mg/kg bw dose of embelin was selected to evaluate the anti-obesity potential in HFD-induced obesity in Wistar rats.

In the present study, reduced body weight gain (Table 23) in rats was accompanied by a depletion of body fat stores (mesenteric, perirenal and epididymal; Figure 31) and organ’s
weight (heart, liver and kidney; Table 25), since treatment with embelin (50 mg/kg bw, p.o.) significantly reduced the weight of the visceral fat pads and organ’s compared with that of HFD-fed rats. Thus, this weight reducing effect of embelin might be contributory to the observed lower mean arterial blood pressure in HFD-fed obese rats as epidemiological studies have shown an association between BMI and blood pressure (Mertens and Van Gaal, 2000). However, this suppression on body weight gain did not depend on decreased food intake, as no significant differences were found in food intake among the different treatment groups (Table 23).

Similar to orlistat treatment, the oral administration of embelin significantly lowered serum TC, TG, LDL-C, and apolipoprotein-B levels in rats with HFD-induced obesity (Table 26, Figure 32). The effect of embelin treatment on the atherogenic and coronary artery risk indices was also notable. The results of this study show that treatment with embelin (50 mg/kg) produced profound reductions in the atherogenic and coronary indices in experimental obese rats (Table 26). Significant reduction in the blood glucose (Figure 33), insulin levels (Figure 34) and HOMA-IR values (Figure 35) after the treatment with embelin (50 mg/kg bw, p.o.) in HFD-fed rats were observed. In consistent with improvement of lipid profile and insulin resistance by embelin (50 mg/kg bw, p.o.), it is also able to attenuate the development of hypertension in HFD-fed obese rats (Table 24).

In the present study, the hepatic tissue of the HFD fed rats showed significant accumulations of fat. Embelin (50 mg/kg bw, p.o.) treatment showed improvements in the histological features of hepatic fatty degeneration induced by HFD in rats (Figure 37). Further, embelin (50 mg/kg bw, p.o.) treatment also improved the histological features of cardiac and kidney tissue fatty degeneration induced by HFD in rats (Figure 38, 39). These results seemed to correspond to the serum lipid profiles (Table 26) and liver, heart and kidney weights given in Table 25. In the present study, the serum leptin levels were decreased in the embelin (50 mg/kg bw, p.o.) treated rats compared with the HFD-fed rats (Figure 36). Therefore, the possible mechanism by which embelin reduced fat mass and lipid concentrations in HFD-fed obese rats may be by improvement in leptin sensitivity.

Hepatic and cardiac Na⁺K⁺-ATPase contents were significantly decreased in HFD control rats as compared to normal control rats. The hepatic and cardiac Na⁺K⁺-ATPase contents
were significantly enhanced by treatment with embelin (50 mg/kg bw, p.o; Table 27). In the present study, hepatic and cardiac TBARS levels, determined by evaluating malondialdehyde content were decreased after embelin (50 mg/kg bw, p.o.) treatment in HFD-fed rats. Furthermore, in the present study, the activities of GSH (a major endogenous antioxidant), GPx, GR, GST, SOD and CAT (scavenger of free radicals) decreased in HFD-fed rats as reported earlier (Table 28, 29; Ansari et al., 2012). Treatment of HFD-fed rats with embelin (50 mg/kg bw, p.o.) had reversed the activities of these enzymatic antioxidants. It is, therefore, reasonable to assume that embelin (50 mg/kg bw, p.o.) treatment improves hepatic oxidative balance in HFD-fed obese rats, because embelin was able to reduce the level of TBARS and free radical generation.

The results of the present study showed comparable effect of ethanolic extract of *E.ribes* and embelin on different parameters of obesity. The dose of the ethanolic extract *E.ribes* used in the present study was 100 mg/kg bw and that of embelin was 50 mg/kg bw, so, the dose of embelin was 0.5 times less than that of the extract.

The HPLC results of our study indicate that the 100 gm of extract of *E.ribes* contains 32803 mg of embelin [Embelin content in *E.cribes* extract = 32803 mg/100g). Possible explanation for therapeutic equivalence between embelin and *E.ribes* extract is Synergistic effects produced by constituents present in the *E.ribes* extract. *E. ribes* fruits contain a quinone derivative embelin, rapanone, embelinol, embeliaribylester, embeliol, sitosterol, daucosterol, an alkaloid christembine, a volatile oil vilangin, quercitol, fatty ingredients, a resinoid and tannins. Among them, embelin is considered one of the major bioactive constituents and marker compounds in *E. ribes* fruits. Embelin (3-undecyl 2,5-dihydroxy,1,4-benzoquinone) has a wide spectrum of biological activities (Madhavan et al., 2011). Further, many herbal drug extracts derived from traditional medicines showed therapeutic superiority as compared with single constituents thereof. Synergy effects of the mixture of bioactive constituents and their byproducts contained in plant extracts are claimed to be responsible for the improved effectiveness of many extracts. Synergistic effects can be produced, if the constituents of an extract affect different targets or interact with one another in order to
improve the solubility and thereby enhance the bioavailability of one or several substances of an extract (Wagner and Ulrich-Merzenich, 2009).

Hence, in Plan-III, ‘Antidiabetic potential of ethanolic extract of *E.ribes* in high fat diet-fed and low dose streptozotocin-induced type 2 diabetes in wistar rats’ was investigated.

Srinivasan *et al.* (2005) reported that the combination of HFD-fed and low-dose STZ (35 mg/kg bw, i.p)-treated rat serves as an alternative animal model for simulating type 2 diabetes in humans because of insulin resistance and obesity. Using methodology of Srinivasan *et al.* (2005), a rat model of type-2 diabetes was successfully developed in the present study, which showed metabolic changes such as hyperglycemia, hypertension, hyperlipidemia, impaired glucose tolerance, and decreased insulin sensitivity.

In the present study, HFD feeding for a period of 2 weeks in rats produced insulin resistance syndrome as was characterized by the increased body weight (obesity), mild hyperglycemia, hypertriglyceridemia, hypercholesterolemia and compensatory hyperinsulinemia (Table 15), a condition similar to prediabetic, insulin-resistant state in humans (Srinivasan *et al.*, 2005; Reaven, 1991). HFD has been shown to induce insulin resistance mainly through Randle or glucose-fatty acid cycle (Srinivasan *et al.*, 2005; Gaikwad *et al.*, 2007). The presence of high level of triglycerides due to excess fat intake could constitute a source of increased fatty acid availability and oxidation. The preferential use of increased fatty acids for oxidation blunts the insulin-mediated reduction of hepatic glucose output and reduces the glucose uptake or utilization in skeletal muscle leading to compensatory hyperinsulinemia, a common feature of insulin resistance. 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 (Srinivasan *et al.*, 2004) and decreased energy expenditure as compared to NPD-fed animals (Storlien *et al.*, 1986).

It is well known that injection of a high dose of STZ (>45 mg/kg) in rats produced insulin deficiency (mimicking type 1 diabetes) rather than the consequence of insulin resistance. On the other hand, 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 (Srinivasan *et al.*, 2005). The conversion of prediabetes to frank hyperglycemia in patients with type 2
diabetes is associated with decline in secretory capacity of pancreatic β-cells to compensate for the existing insulin resistance. But, there is only a relative insulin deficiency as the circulating day-long insulin concentrations in patients with type 2 diabetes are comparable in absolute terms to the values seen in non-diabetic individuals (Reed et al., 2000). In the present study, the evolution of disease pattern was achieved in insulin-resistant HFD rats upon injection with low dose of STZ (35 mg/kg bw, i.p.) which produced frank hyperglycemia in the presence of circulating insulin concentration almost comparable to normal rats (relative insulin deficiency) whereas the same dose did not significantly decrease the insulin secretory capacity enough to cause overt hyperglycemia in rodents fed with NPD (Table 31). It is interesting and noteworthy that the development of diabetes occurs only in insulin-resistant HFD-fed rats but not in NPD-fed normal rats following low dose of STZ (35 mg/kg bw, i.p.) in the present study. As such, they simulate natural disease progression and metabolic condition of individuals at increased risk of developing type 2 diabetes (because of insulin resistance and obesity). The reasons for the high degree of glycemic difference induced by STZ (35 mg/kg bw, i.p.) between these two groups might be that HFD-fed rats were already insulin resistant together with compensatory hyperinsulinemia to maintain glucose homeostasis, and hence, even the slight insult by low dose of STZ that could compromise the beta cell function might lead to drastic hyperglycemic effect as against the NPD-fed normal animals where in the effect could be compensated by normal defense homeostasis mechanisms. Apart from glucose, HFD-fed, insulin-resistant STZ animals also showed abnormalities in lipid metabolism as evidenced from increased serum triglycerides and total cholesterol levels in the present study (Table 31), as in case of human type 2 diabetic patients which might contribute to various cardiovascular complications. The hypertriglycerideremia observed in these HFD + STZ treated 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 (Srinivasan et al., 2004). 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; Shafrir, 2003).
In the present study, type 2 diabetic rats when treated with ethanolic extract of *E.ribes* at 100 and 200 mg/kg bw for 21 days produced a significant decline in body weight (Table 34), heart weight, heart index (heart weight/ body weight × 100), liver weight, liver index (liver weight/ body weight × 100), kidney weight and kidney index (kidney weight/ body weight × 100; Table 36), suggesting a weight reducing effect of ethanolic extract of *E.ribes* in type 2 diabetic rats. As diabetes generally causes increases of water and food intake, reductions of water and food intake after ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o) treatments are indicative of improvement of diabetic conditions (Table 33).

In order to clarify the antihyperglycemic effect of ethanolic extract of *E.ribes* in type 2 diabetic rats, fasting blood glucose levels were measured at weekly interval for the period of 3 weeks. It was found that ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o) treatment progressively reduced blood glucose levels in diabetic rats (Table 37). The observed antihyperglycemic effect of ethanolic extract of *E.ribes* is supported by previous report (Bhandari *et al.*, 2002). Further, HFD-fed and low dose STZ-treated diabetic rats had frank hyperglycemia in the presence of comparable amount of serum insulin concentrations, indicated the persistence of insulin resistance. The presence of insulin resistance is further indicated by higher values of HOMA-IR in diabetic control group. The HOMA-IR index was significantly decreased in ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o.) treated groups (i.e. Group III and IV) compared to diabetic control i.e. Group II (Table 39). Furthermore, insulin sensitizing capability of ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o.) was assessed by AUC of the glycemic response to an OGGT (Figure 40, 41). In the present study, severely impaired glucose tolerance in type 2 diabetic rats was observed. Ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o.) treatment alleviated the degree of impairment significantly, indicating the potential of *E.ribes* extract to reduce insulin resistance. Insulin sensitizing capability of ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o.) was further confirmed by serum insulin concentration-time curve for 120 min (AUC0-120 min) in OGGT (Figure 42, 43). As compared to diabetic control, ethanolic extract of *E.ribes* at 100- and 200 mg/kg bw treated group showed a clear increase in the early-phase serum insulin in Wistar rats (Figure 42). Serum insulin levels at all the time points and AUC0-120 min were were significantly (p< 0.05) higher in ethanolic extract of *E.ribes* treated group compared to diabetic control (DC) group (Figure 43), indicating the
insulin sensitizing potential of *E.ribes*. This finding corroborate the results of Yoshida *et al.*, (2010), wherein they reported that GPR119 agonist AS1535907 has the ability to stimulate the first phase of insulin release, which is important for preventing the development of postprandial hypoglycemia in db/db mice. Reports concerning the role of *E.ribes* extract in connection with insulin sensitivity are not available in the literature and the present study is the first to report that *E.ribes* boost insulin sensitivity.

Furthermore, the circulating serum insulin levels observed in the present study revealed significant effect of ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o.) on insulin secretion in HFD-fed and low dose STZ-induced diabetic rats during the 3 weeks of treatment period. These diabetic rats had significantly lower circulating serum insulin levels, whereas ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o.) treated diabetic rats had significantly (p< 0.01) increased serum insulin levels (Table 39). This indicates that *E.ribes* extract treatment might be able to regenerate β-cells and/or to recover β-cells functions in rats injected with low dose STZ (35 mg/kg bw, i.p)-induced diabetes under the current experimental conditions (Wang *et al.*, 2011). Further, insulin plays a homeostatic role in normal kidney function. However, insulin-resistance has been associated with alterations in glomerular hemodynamics and renal damage (Knight and Imig, 2007). Hence, insulin sensitizing drugs may have preventive effects on renal injury in obesity and insulin resistance. Thus, an important benefit of ethanolic extract of *E.ribes* in treating diabetic renal injury is to improve glycemic control by attenuating insulin resistance.

Blood pressure ≥ 135/85 mm Hg is an integral part of cardiometabolic syndrome. The underlying mechanisms for development of hypertension in the metabolic syndrome include obesity, insulin resistance and oxidative stress (Gaikwad *et al.*, 2007). Further, hypertension has been reported to aggravate diabetic nephropathy through its additive glomerular hypertension (Hostetter *et al.*, 1982). In consistent with improvement of insulin resistance by ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o.), it was also able to reduce the elevated blood pressure and heart rate in diabetic rats (Table 35). Thus, ethanolic extract of *E.ribes* may be beneficial in patients with diabetic nephropathy as optimal control and maintenance of normal sugar and blood pressure level are vital to prevent the progression of diabetic nephropathy (Balakumar *et al.*, 2009).
Adipokines particularly leptin, adiponectin and free fatty acid (FFA) play an important role in the pathogenesis of pancreatic β-cell dysfunction and type 2 diabetes (Yaturu, 2011). In the present study, diabetic rats had significantly (p< 0.01) decreased the serum leptin and adiponectin levels as compared to normal control rats. Ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o.) treatment was able to significantly increase the low circulating levels of leptin and adiponectin observed in diabetic rats (Table 39). In fact, the reversal of low circulating levels of leptin and adiponectin provides an explanation for the correction of glucose and lipid metabolism and insulin resistance by *E.ribes* extract treatment in the diabetic rats.

The role of dyslipidemia in the development of diabetes macrovascular complications has long been known (Haffner, 1998). Further, Studies have shown that diabetic nephropathy is resulted from elevated renal local lipid accumulation (Yokoyama et al., 2010). Regulation of serum or tissue lipid levels leads to decrease in the risk of lipid-induced glomerular injury (Dominguez et al., 2000). Ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o.) treatment was able to improve serum lipid profile in diabetic rats (Table 38). Results of the histopathological changes in heart, liver and kidney of HFD-fed and low dose STZ-induced type 2 diabetic rats, such as lipid accumulation and fatty degeneration are consistent with the results of serum lipid profile (Figures 46, 47, 48). Treatment of diabetic rats with ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o.) showed considerable reduction in cardiac, hepatic, and kidney lipid accumulation. Thus, this result suggested that *E.ribes* would be useful in the prevention of diabetic complications through improving dyslipidemia.

Lipases functions as a lipolytic enzyme that hydrolyzes TGs and phospholipids in circulating plasma lipoproteins. Among lipases, pancreatic lipase is the principal lipolytic enzyme synthesized and secreted by the pancreas. It is responsible for the hydrolysis of 50–70% of total dietary fats to monoglycerides and free fatty acids. Reduction of fat absorption by the inhibition of pancreatic lipase is known to be beneficial for the regulation of obesity and related metabolic disorders (Birari and Bhutani, 2007). Administration of ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o.) to diabetic rats produced considerable reduction in serum lipase activity (Table 39), suggesting its potential in prevention of lipid abnormalities and obesity.
Danda et al. (2005) showed that type 2 diabetic rats (induced by feeding HFD and low dose STZ) developed more pronounced kidney lesions than type 1 diabetic (induced by high dose STZ injection) rats. Hence, this is an appropriate experimental model to examine the effect of various therapeutic agents on diabetic nephrotoxicity. Diabetes is the leading cause of chronic kidney disease (CKD), which makes estimation of renal function crucial. To demonstrate an alteration of renal function in diabetic rats, LDH (lactate dehydrogenase), creatinine, ALP (alkaline phosphatase), albumin and total protein levels were measured in serum (Table 40). Increased levels of LDH are found in renal, hepatic, pulmonary and cardiovascular diseases. Currently, measuring urine albumin excretion and creatinine (for estimation of GFR) remains the most effective screening method for the early detection of diabetic nephropathy (Kramer, 2004). ALP levels are significantly increased in human patients suffering from renal insufficiency (Sanchez Navarro et al., 2002). Further, it has been shown that ALP-enzyme-regulating gene is expressed not only in the liver but also at high level in the kidneys too (Lindblom et al., 2007). The decrease in total protein and albumin levels in diabetic rats may be due to microproteinuria and albuminuria and/or may be due to increased protein catabolism. The present study showed that treatment with ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o.) significantly prevented enhanced levels of serum LDH, creatinine and ALP as well as alteration in serum albumin and total protein, valuable markers of renal disease (Table 40). The biochemical changes were correlated with a histopathological examination, which revealed that HFD- and low dose STZ caused a marked damage in renal structure showing congestion, proteinuria, haemorrhage, and tubular degeneration (Figure 48B). This finding is supported by previous pathological study on the pathological feature of nephrotoxicity (Danda et al., 2005). Treatment with ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o.) prevented such alterations and protected the histological aspects of kidney (Figure 48C, 48D).

Glycogen is the primary intracellular storable form of glucose and serves as a tissue reserve for the body's glucose needs. The conversion of glucose to glycogen in liver cells is dependent on the presence of insulin as insulin stimulates intracellular glycogen synthesis by stimulating glycogen synthase and inhibiting glycogen phosphorylase (Stalmans et al., 1991). In the present study, data obtained in diabetic rats demonstrated a statistically significant reduction (p< 0.01) in liver glycogen content from normal control values.
Diabetic rats treated with ethanolic extract of *E.ribes* (100- and 200 mg/kg bw, p.o.) brought back liver glycogen level to near normal (Table 41), which could be due to attenuation of insulin resistance. Similar observation was obtained in HFD-fed and STZ-treated diabetic rats that the liver glycogen content tended to be higher in the semi-purified fractions of *Averrhoa bilimbi* treated group (Tan *et al.*, 2005).

The hepatic Glc-6-Pase, an enzyme found mainly in the liver and the kidneys, plays a key role in the regulation of blood glucose homeostasis. Both gluconeogenesis and glycogenolysis result in the formation of glucose 6-phosphate (Glc-6-P), which is hydrolysed by Glc-6-Pase before being liberated as glucose into the circulation. Activities of liver microsomal Glc-6-Pase have been reported to be up-regulated in diabetic states (Pushparaj *et al.*, 2007). The data presented in this study showed that hepatic Glc-6-Pase activity in diabetic rats was significantly higher than that of normal control rats and *E.ribes* extract treatment (100- and 200 mg/kg bw/day) for 21 days significantly (p< 0.01) lowered its activity in diabetic rats (Figure 44). These results suggest that *E.ribes* extract might have corrected the defect of carbohydrate metabolism in diabetic rats, as evident by attenuation of hyperglycemia and insulin resistance, increase hepatic glycogen content and decrease in Glc-6-Pase activity.

Studies have shown that HFD feeding or obesity results in an increase in oxidative stress (Wang *et al.*, 2011), which is further increased with the development of diabetes (Wang *et al.*, 2011). Hyperglycemia can inactivate enzymatic antioxidants like SOD and CAT by glycating these proteins, thus, promotes free radical generation, which results in lipid peroxidation (Kennedy and Lyons, 1997). GSH (non-enzymatic antioxidant) plays a key role in protecting the cells from oxidative damage. GSH is one of the most prominent antioxidant in the liver and its level in liver reflects the detoxification potential of the liver. Results of the present study show that diabetes caused significant increase in lipid peroxides as indicated by a significant increase in TBARS concentration accompanied by a concomitant significant (p< 0.01) decrease in the activity of GSH, SOD and CAT in liver, heart, and kidney tissues of diabetic rats as compared with normal control rats (Table 42, 43, 44). These results are consistent with those of the previous studies which have shown an increase in the TBARS level and a decrease in the levels of the GSH, SOD and CAT levels (Wang *et
al., 2011; Zhang et al., 2010). Treatment of diabetic rats with *E.ribes* extract treatment (100- and 200 mg/kg bw/day) had reversed the activities of these antioxidants in liver, heart and kidney, which might be due to decreased oxidative stress as evidenced by decreased lipid peroxidation. In a nutshell, these findings suggested that *E.ribes* extract treatment enhance the antioxidant defense mechanism in diabetes and in this way may have improved blood glucose, oral glucose tolerance and lipid profile.

Between the two doses *i.e.* 100- and 200 mg/kg bw of ethanolic extract of *E.ribes*, the dose 200 mg/kg dose was found to be more effective in reducing fasting blood glucose levels, hepatic glucose-6-phosphatase activity, hepatic fatty degeneration, oxidative changes; restoring the elevated blood pressure and liver glycogen; and reversal of circulating levels of serum lipids, lipase, insulin, leptin and adiponectin towards normal in diabetic rats. The results were comparable to the standard drug metformin (180 mg/kg bw, p.o.).

In the present study, well documented and established animal models of diet-induced obesity and type 2 diabetes. In HFD-induced obese model, rats become obese, hyperleptinemic, hyperinsulinemic, hyperglycemic, hypertriglyceridemic and insulin resistant following oral HFD-feeding. Treatment with ethanolic extract of *E.ribes* (100 mg/kg bw) showed a preventive effect on body weight gain, visceral fat accumulation and elevated blood pressure. The extract treatment elicited a significant reduction in serum levels of leptin, insulin, glucose, total cholesterol, and triglycerides. Further, extract treatment decreased the myocardial lipid peroxidation and increased antioxidant levels in obese rats.

In HFD-fed and low dose STZ-treated rat model exhibits stable, long lasting hyperglycemia, insulin resistance and the symptoms of type 2 diabetes like polyuria, polydipsia, and polyphagia and diabetic complications such as hypertension, hyperlipidemia and nephropathy. Treatment with ethanolic extract of *E.ribes* effectively reduced the body weight, hyperglycemia, insulin resistance, hyperlipidemia, elevated blood pressure and oxidative stress in adult diabetic Wistar rats. Further, ethanolic extract of *E.ribes* prevent progression of diabetic nephrotoxicity by improving renal function and amelioration of renal
histopathological changes in diabetic rat model. In future, if these data will be validated in clinical trials, *E.ribes*-being plant origin medicine, without any apparent toxicity, seems to offer potential for alleviating ‘metabolic syndrome’.