Discussion
6. DISCUSSION

Diabetes mellitus has been identified as the refractory disease for which an alternate system of medicine has to be considered for the treatment. There is worldwide epidemic of diabetes. Besides this, the emergence of the concept of Insulin Resistance and the description of diabetes as the ‘Metabolic Dyslipidaemic Cardiovascular Syndrome’ has warranted a need for the development of different strategies in the investigation of newer anti-diabetic drugs, although a large number of plant preparations have been reported to possess anti-diabetic activity over last several decades. In the present investigation we have studied the antidiabetic effect of *E. officinalis* in animal models of type1 and 2 diabetes mellitus.

The authentication of the fresh fruits of *E. officinalis* is usually carried out by comparing them morphologically and chemically as mentioned in different standard texts and floras. The sample used in our study was found to be authentic. The fruits of *E. officinalis* were spherical, smooth and have six ridges extending from the base to the apex that divide it into six segments. The average diameter of the present sample was 4 cm, although much larger fruits were grown in other parts of India (Ammal and Raghavan, 1958). In Florida, the diameter ranged from 2.5 to 3.2 cm (Morton, 1960). The skin which encloses the crisped juicy pulp is thin and translucent, and the core of the fruit is hexagonal in shape, contains six small seeds.

The chemical analysis of the fresh fruit pulp was carried out for pH, moisture content, titrable acidity, ash and total water-insoluble solids, soluble carbohydrate, total ash, reducing substances, total flavanoids and phenol content. All these values were found to comply with previously reported data (Barthakur and Arnold, 1991). The total polyphenol content of *E. officinalis* fruit shows slight differences from that of reported values. The total polyphenol content of the *E. officinalis* fruit was very high, such that the only 1 ml of fruit juice contains approximately 29.04 mg/ml gallic acid equivalent total polyphenol. The difference between the total polyphenol content reported in this study and reported value may be attributed to the diversity in environmental and cultural conditions in which the fruits were grown and fruit size. Some fruits came from nurseries and botanical gardens, whereas other fruits were collected from untended trees from different parts of India. The difference in fruit size
also seems to be important. All these results of the present investigation have authenticated the quality of fruits of *E. officinalis* (amla) used in the study.

After morphological and chemical authentication the extraction of the fresh juice of *E. officinalis* was carried out and the yield of fresh juice was found to be 750ml/kg of fresh fruit. Successive fractionation of 1500ml of fresh fruit juice with solvents like petroleum ether, toluene, chloroform, ethyl acetate and n-butanol obtained a yield of 0.1% with petroleum ether, 0.01% with toluene, 0.03% with chloroform, 0.05% with ethyl acetate, 65.28 % with n-butanol and 34.64 % with residual fraction (Table 5.1).

Standardization of the fractions of the herbal drug can be performed by estimation of marker compounds that may be chemical markers or biomarkers using modern analytical techniques like HPTLC. HPTLC analysis has emerged as one of the tools for the quantitative and qualitative assessment of the herbal drugs (Bhutani, 2000).

Phytochemical investigations of fruits of *E. officinalis* show that it is having high amount of polyphenol content like low and high molecular weight gallotannins such as gallic acid, ethyl gallate, digallic acid, ellagic acid, phyllembic acid, chebulinic acid, L-malic acid 2-O-gallate, mucic acid 2-O-gallate, chebulagic acid, putrajivain A, elacocarpusin, mucic acid, 1-O-galloyl-β-D-glucose, mucic acid 6-methyl ester 2-O-gallate, mucic acid 1,4- lactone 2-O-gallate, mucic acid 1-methyl ester 2-O-gallate, mucic acid 2-O-gallate, mucic acid 1,4-lactone 6-methyl ester 2-O-gallate, mucic acid 1,4-lactone 3-O-gallate, mucic acid 1,4-lactone 3,5-di-O-gallate, emblicanin A and B, punigluconin, pedunculagin, methyl gallate, corilagin, furosin and geraniin. (Zhang *et al.*, 2001; Kumaran and Karunakaran, 2006; Anila and Vijayalakshmi, 2003; Ghosal *et al.*, 1996; Sukh, 2006).

In the present study fruit juice and its fractions were subjected to HPTLC analysis for the estimation of gallic acid (free form of gallotannin), which we used as the marker compound present in *E. officinalis*. On performing HPTLC analysis of the fractions of *E. officinalis* only ethyl acetate fraction found to contain gallic acid in free form. But after performing hydrolysis study of other fractions N-butanol fraction showed maximum amount of gallic acid as compared to residual fraction. Other fractions did not showed presents of gallic acid in free or bound form. *Thus, our data show that the n-butanol fraction contains higher concentration of gallotannins (esters of gallic acid)*.
acid) as compared to residual fraction. Among various other fractions of fruit juice did not contain gallotannins.

*In vitro* hydrolysis studies concerning the availability of gallic acid after hydrolysis from gallotannins present in fruit juice of *E. officinalis* and its fractions are in agreement with their potential therapeutic effects. The pharmacological effects have been mainly attributed to the contents of gallotannins present in fruit juice of *E. officinalis* and its fractions.

Many polyherbal formulations like Trifala, Cogent db and Diasulin contain *E. officinalis* as one of the ingredient useful for its antidiabetic, antioxidant and antihyperlipidemic activity commercially (Naik *et al.*, 2006; Saravanan and Pari, 2005; Pari and Saravanan, 2002). It has been reported that extracts of *E. officinalis* reduce the blood sugar levels in alloxan induced diabetic rats (Sabu and Kuttan, 2002; Tripathi *et al.*, 1979). The fruit juice of *E. officinalis* is reported to produce hypolipidemic activity in cholesterol-fed rabbits (Ritu *et al.*, 1996). Our preliminary clinical study on diabetic patients also showed that fresh juice *E. officinalis* produces significant antidiabetic, antihyperlipidemic and antioxidant activities (unpublished data).

Medicinal plants like *Terminalia catappa* fruit, *Helichrysum plicatum* (leaves), *Lagerstroemia speciosa* (leaves), *Punica granatum* (flower), *Pterocarpus marsupium* (bark), green tea, *Prunus amygdalus* (seeds), red grapes are rich in polyphenolic compounds like gallotannins that are reported to be antidiabetic in various experimental models (Nagappa *et al.*, 2003; Mustafa *et al.*, 2007; Liu *et al.*, 2001; Huanga *et al.*, 2005; Ahmad *et al.*, 1989; Kao *et al.*, 2002; Teotia and Singh, 1997; Al-Awwadi *et al.*, 2004). Gallotannins are reported to produce antidiabetic activity not only in diabetic animals but also in diabetic patients (Najim *et al* 2004; Gin *et al.*, 1999). Results obtained from phytochemical investigations revealed that the fruit juice possessed higher concentration of gallotannins. A good correlation of antidiabetic activity and total gallic acid concentration in fruit juice of *E. officinalis* was found in our study.

Gallotannin is a mixture of esters of gallic acid depending on the plant source. The relationship between the diet and prevention of diseases in man has been the topic of
numerous investigations during recent years. Gallotannins are polyphenolic compounds found in legumes, vegetables, beverages and fruits are the most abundant antioxidants in our diets of plant origin (Scalbert and Williamson, 2000; Okuda et al., 1995). Gallotannins were reported to possess multiple biological activities including anticancer (Okuda et al., 1995); antioxidant (Hagerman et al., 1998) and antimicrobial activities (Cowan, 1999) and their consumption may contribute to prevent stroke, cardiovascular disease, neurodegenerative diseases or diabetes (Scalbert et al. 2002; Williamson and Manach, 2005). In recent years gallotannins have been also studied for their antihyperglycemic, lipid lowering (Yunsheng et al., 2005; Suzuki et al., 1999) and antioxidant properties (Croft, 1998). Gallotannins from Lagerstroemia speciosa (banaba) are reported to produce antidiabetic activity in in vivo model of diabetic db/db mice and obese ob/ob mice and in in vitro studies they also produce glucose transport stimulatory activity in 3T3-L1 adipocytes and adipocyte differentiation inhibitory activity in preadipocytes (Liu et al., 2001). Several anti-diabetic small molecules with direct or indirect insulin releasing effect on islet cell, direct insulin-like and insulin mimetic effect have been reported (Zhang et al., 2000; Manchem, et al., 2001; Barbera et al., 1997; Nadareddy et al., 2005; Rieusset, et al., 2002). Most of these compounds bind or activate the insulin receptors and have hypoglycemic and hypolipidemic effects in animals (Zhang et al., 1999; Manchem, et al., 2001; Zhang et al., 2000; Rocchi, et al., 2001). The antidiabetic activity of gallotannins of E. officinalis appears to be mediated through peripheral utilization of glucose in diabetic conditions. We believe that gallotannins may provide the molecular basis for a new generation of orally deliverable, anti-diabetic small molecule with antihyperlipidemic activity. They distinguish themselves from other drugs by the fact that they not only stimulate glucose transport, but it also decreases lipid level in diabetes. It is possible that, in hyperinsulinemic type 2 diabetic rats, gallotannins may produce antidiabetic activity due to increased insulin sensitivity which correlates well with previous report which indicates that gallotannins increase insulin sensitivity in adipocytes (Liu et al., 2001). Gallic acid present in Punica granatum are reported to enhance cardiac PPAR-γ mRNA expression and restore the down-regulated cardiac glucose transporter (GLUT)-4 in Zucker diabetic fatty rats (Huanga et al., 2005). Further, gallotannin produce lipid lowering activity along with glucose lowering activity. In previous reports it has been proved that gallotannins increase peripheral insulin sensitivity in rat adipose tissue by inhibiting lipogenesis (Ong et al., 1995) and produced increased
PPAR-\(\gamma\) dependent mRNA expression and increased activity of lipoprotein lipase (LPL) in human THP-1-differentiated macrophage cells in \textit{in vitro} studies in \textit{Punica granatum} flower extract (Huanga \textit{et al.}, 2005). In various \textit{in vivo} and \textit{in vitro} animal models oxygen free radical scavenging activity of different form of gallates, such as gallyl esters, methyl gallate, propyl gallate, gallocatechin, gallotannins, has been documented (Buttemeyer, 2003; Ajay 2003; Calvin and Mattill \textit{et al.}, 1942). Thus, from all these previous reports we can speculate that gallotannins (esters of gallic acid) may be responsible for antidiabetic, antihyperlipidemic and antioxidant activities in various medicinal plants and that by virtue of these properties gallotannins may be beneficial in diabetes mellitus. It is possible that gallotannins present in \textit{E. officinalis} might be having antidiabetic, antihyperlipidemic and antioxidant activities because gallotannins have been reported to produce significant antidiabetic activity; we carried out similar studies for fruit juice of \textit{E. officinalis} in STZ-induced type 1 diabetic rats.

STZ, a \(\beta\)-cytotoxin, induces ‘chemical diabetes’ in a wide variety of animal species including rat, by selectively damaging the insulin-secreting \(\beta\)-cells of the pancreas. Intravenous injection of STZ produces fragmentation of DNA of \(\beta\)-cells of pancreas leading to destruction of \(\beta\)-cells and it is evidenced by clinical symptoms of hyperglycemia, hypoinsulinaemia, loss of body weight and other cardinal signs of STZ diabetes viz., polyphagia, polyuria and polydipsia (Kawashima \textit{et al.}, 1978; Rodrigues \textit{et al.}, 1986; Goyal \textit{et al.}, 1985; Sevak and Goyal 1996; Umrani and Goyal 2002; Hofteizer and Carpenter 1973; Oakley, 1968; Vadlamudi \textit{et al.}, 1982; Tahiliani \textit{et al.}, 1983). We first investigated in the present study diabetic rats were found to have loss of body weight, increased food and water intake which could be due to excessive break-down of tissue proteins and dehydration and catabolism of fats and proteins (Chatterjea and Shinde, 2002; Hakim \textit{et al.}, 1997; Rajkumar \textit{et al.}, 1991). Chronic treatment with fruit juice of \textit{E. officinalis} to diabetic rat decreased food and water consumption and prevented loss of body weight and this could be due to decrease in catabolic reaction and a better control of the hyperglycemic state in the diabetic rats. The fruit juice of \textit{E. officinalis} produced decrease in blood glucose levels in STZ induced type 1 diabetic rats. The fruit juice of \textit{E. officinalis} produced decrease in glucose levels but failed to prevent STZ induced decrease in serum insulin levels. This suggests that the fruit juice possesses antidiabetic activity which may be due to
increased sensitivity of peripheral tissue to insulin or to a direct insulin-like effect. The result of OGTT also substantiates the findings that fruit juice produced improvement of glucose metabolism in diabetic rat.

Earlier studies have shown that STZ-induced diabetic rats are associated with hypercholesterolemia and hypertriglyceridemia (Rodrigues et al., 1986). In the present study concentrations of lipids, such as cholesterol, triglyceride, LDL cholesterol and VLDL cholesterol were significantly increased in diabetic rats as compared to normal rats which correlates with previous studies in diabetic patients and diabetic rats which showed high levels of triglycerides, VLDL, LDL, and total cholesterol (Guerci et al 1999; Andersen et al., 1983; Kaleem et al., 2006). These could be due to variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency or resistance which may be responsible for dyslipidemia, because insulin has an inhibitory action on HMG-CoA reductase, a key enzyme that is rate limiting in the metabolism of cholesterol rich LDL particles. In diabetic rats there were also decrease in LPL activity (Nikkila et al., 1977) resulting in impaired clearance of VLDL and chylomicrons from plasma (Bagdade et al., 1968) which is responsible for the observed accumulation of lipids (Rajalingam et al., 1993). In the present study chronic treatment with fruit juice of *E. officinalis* exhibited more potent lowering effect on triglyceride, total cholesterol, LDL, VLDL. These may be due to inhibition of lipolysis in adipose tissue by insulin sensitizing or insulinmimetic effect of fruit juice of *E. officinalis* because insulin sensitizers and insulinmimetics reported to inhibit lipolysis by inhibiting the activity of the hormone sensitive lipases in adipose tissue (Loci et al., 1994; Pathak et al., 1981) indicates that fruit juice of *E. officinalis* may possess lipid lowering activity by acting on insulin receptors.

STZ-diabetic rats have been shown to exhibit elevated SGOT and SGPT levels in liver (Domingo et al., 1991; Cam et al., 1993; Dai et al., 1993). Earlier reports from our laboratory have showed a significant elevation in SGOT and SGPT levels in STZ-induced type 1 diabetic rats (Umrani et al., 2003; Vishwakarma et al., 2003; Shinde et al., 2004). In the present study a significant elevation in SGOT and SGPT levels were observed with type 1 diabetic rats. Treatment with fruit juice of *E. officinalis* significantly reduced elevated SGOT and SGPT levels and suggests their beneficial effects on altered liver functions in conditions of diabetes.
STZ diabetes is also associated with a significant elevation in serum creatinine and urea levels indicating impaired renal function of diabetic animals. Increase in serum creatinine and urea has been observed in diabetic patients (Mulec et al., 1990). Earlier reports from our laboratory have shown a significant elevation in serum urea and creatinine levels in STZ-induced type 1 diabetic rats (Umrani et al., 2003; Vishwakarma et al., 2003, Shinde et al., 2004). The increase in serum creatinine and urea levels may be due to hyperglycemia that causes osmotic diuresis and depletion of extracellular fluid volume (Ritz et al., 1989) and several studies also have shown an increased correlation between serum creatinine and urea in diabetic patients (Ritz et al., 1989; Mogenson and Christensen, 1985). Treatment with fresh juice found to decrease serum creatinine and urea levels. This may be correlated with decrease in glucose levels by fresh juice and thereby decrease in osmotic diuresis and depletion of extracellular fluid volume which indicated its beneficial effect on altered kidney functions.

Since fruit juice of *E. officinalis* showed similar actions like gallotannin in STZ-induced type 1 diabetic rats, we carried studies with fruit juice of *E. officinalis* in STZ-induced type 2 diabetic obese rats.

Diabetes, particularly obesity and metabolic syndrome related type 2 diabetes (T2D) which is characterized by a reduced sensitivity to insulin signaling and a reduced efficiency of glucose transport, primarily in adipocytes and muscle cells, leading to hyperglycemia and hyperinsulinemia (Kahn and Flier, 2000). The hyperinsulinemia in type 2 diabetic rats could be due to either decreased hepatic clearance of insulin or decreased number of insulin receptors, resulting in decreased insulin binding and lowered insulin degradation (Olefsky, 1981; Bonora et al., 1983; Gliemann and Sonne, 1978). Insulin resistance and hyperinsulinemia develop in Sprague-Dawley rats fed a high-fat diet (Kraegen et al., 1986; Storlien et al., 1986; Kraegen et al., 1991). Rats fed such fat-enriched diets do not become hyperglycemic, presumably because of the compensatory hyperinsulinemia but STZ injection produce slight decrease in serum insulin concentration would no longer enable insulin resistant, fat-fed rats to maintain euglycemia, and that hyperglycemia would develop in these rats at higher insulin levels or comparable to those seen in chow-fed, normoglycemic rats. The transition from insulin resistance to hyperglycemia in this situation would be
analogous to the decline in compensatory hyperinsulinemia and development of hyperglycemia that occurs in human type 2 diabetes (Lillioja et al., 1993; Soeldner et al., 1990). Insulin resistance and compensatory hyperinsulinemia have been shown to predict the development of type 2 diabetes (Lillioja et al., 1993; Soeldner et al., 1990). Serum glucose concentrations in STZ-diabetic obese rats are higher than values in chow-fed rats either fasting or post glucose challenged condition. However, because fasting and post glucose challenge insulin concentrations were significantly higher in fat-fed rats, it appears likely that insulin-mediated glucose disposal was decreased in these animals. This conclusion is consistent with previous reports that high-fat diets (HFD) induce insulin resistance in rodents (Kraegen et al., 1986; Storlien et al., 1986; Kraegen et al., 1991) and is further supported by the K_ITT in HFD/STZ rats which exhibited significant reduction in the glucose disappearance rate (K-value) as compared to control rats. The frank hyperglycemia in the presence of hyperinsulinemia together with reduced K-value indicated the persistence of insulin resistance even after STZ injection in HFD rats (Saltie and Olefsky, 1996; Portha et al., 1995).

In present study HFD/STZ diabetic rats showed a significant increase in fasting glucose as well as insulin levels. This is consistent with earlier reports (Viswanad et al., 2006; Reed et al., 2000). Treatment with fruit juice of E. officinalis reduced glucose levels, however unlike in type 1 diabetes there was decrease in insulin levels. This could be because in type 2 diabetes there is high insulin levels associated with insulin resistance. In oral glucose tolerance test HFD/STZ diabetic control rats showed a significant increase in AUC_glucose and AUC_insulin values and in insulin sensitivity test diabetic rats showed decrease in K-value indicates decreased insulin sensitivity in HFD/STZ diabetic control rats. Treatment with fruit juice of E. Officinalis produced decrease in AUC_glucose and AUC_insulin values and increased reduced K-value in diabetic rats during K_ITT. The results of OGTT and K_ITT further substantiates the contention that the reduction in elevated glucose levels on treatment with extracts of E. officinalis may be due to improvement in insulin sensitivity.

Various studies with insulin sensitizers have shown improvement in glycemic control in conditions of diabetes associated with hyperinsulinemia. The antihyperglycemic effect by insulin sensitizers was shown to be associated with improvement in insulin
sensitivity and thereby effective glucose disposal by peripheral tissues (Murami et al., 1998; Shinaki et al., 2001; Chakrabarti et al., 2003). Insulin receptors are potential targets that may be helpful to restore/increase insulin signaling and enhance glucose transport (Moller, 2001). Small molecules that have insulin sensitizing activity (insulin mimetics) have been proposed as potential therapeutic agents in the promotion of glucose transport and glucose metabolism, and the prevention and treatment of diabetes (Moller, 2001; Zhang and Moller, 2000). While studying medicinal plants, we previously found that fruit juice of *E. officinalis* exhibited significant antidiabetic, antihyperlipidemic and antioxidant activities in preliminary clinical study on type 2 diabetic patients. Since fruit juice of *E. officinalis* showed glucose and lipid lowering activities in STZ-induced type 1 diabetic rat. More recently, we found reports on the presence of gallotannin, comprises naturally occurring esters of gallic acid that belong to the larger group of plant polyphenols known as gallotannins (Okuda et al., 1995) is a major component of the fruit juice of *E. officinalis* (Ying-Jun et al., 2001; Kumaran and Karunakaran, 2006; Anila and Vijayalakshmi, 2003; Ghosal et al., 1996). Studies with gallotannins from *Lagerstroemia speciosa* and *Punica granatum* are reported to produce antidiabetic activity in *in vivo* diabetic animal models (Liu et al., 2001; Huanga et al., 2005). Since gallotannin was found to exhibit promotion of glucose transport and glucose metabolism and the prevention and treatment of hyperlipidemia in type 2 diabetes in various medicinal plants, we hypothesized that active compounds in fruit juice of *E. officinalis* utilize the insulin-mediated signaling pathway for the induction of glucose transport in HFD/STZ diabetic rats which were reported to improve insulin sensitivity and hence produce antidiabetic activity (Liu et al., 2001; Huanga et al., 2005). In HFD/STZ diabetic rat, the hepatic glycogen content was found to be decreased as compared to control animal. These could be due to decreased conversion of glucose in glycogen in liver and decreased glycogen level have been reported in mild glucose intolerance (Olefsky, 1981). Hyperinsulinemia with low hepatic glycogen content are also reported in mild glucose intolerant obese subjects (Bonora et al., 1983). The antihyperglycemic effect by fruit juice of *E. officinalis* in both type 1 and 2 diabetes may be associated with improvement in insulin sensitivity and thereby effective glucose disposal by peripheral tissues and increased conversion of glucose to glycogen in liver.
Apart from glucose, these fat-fed, insulin-resistant STZ animals also showed abnormalities in lipid metabolism as evidenced from increased serum triglyceride and total cholesterol levels, as in case of human type 2 diabetic patients. The hypertriglyceridemia observed in these HFD/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 triglyceride-enriched hepatic VLDL and decreased triglyceride 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 (Shafrir, 2003; Colca et al., 1991). Diabetic obese rat has been shown to induce insulin resistance by different mechanisms one of them is through glucose-fatty acid cycle (Randle et al., 1963). 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 (Belfiore and Iannello, 1998; Iwanishi and Kobayashi, 1993; Rosholt et al., 1994). The agents which decrease both fatty acid synthesis and reduce the synthesis and secretion of triglycerides are shown to ameliorate insulin resistance by improving insulin sensitivity (Boden, 1994).

Treatment with fruit juice of *E. officinalis* significantly decreased both cholesterol and triglyceride levels in HFD/STZ rats. The reduction in elevated cholesterol and triglyceride levels in diabetic rats by treatment with fruit juice of *E. officinalis* can be supported by earlier reports which show that gallotannin produce lipid lowering activity along with glucose lowering activity (Liu et al., 2001). In previous reports it has been proved that gallotannins increase peripheral insulin sensitivity in rat adipose tissue by inhibiting lipogenesis (Ong et al., 1995) and produced increased PPAR-γ dependent mRNA expression and increased activity of LPL in human THP-1-differentiated macrophage cells in *in vitro* studies (Huanga et al., 2005) which suggests that PPAR-γ receptors may be involved in lipid lowering activity of gallotannins in fruit juice of *E. officinalis*. 
Adipogenesis is a process within which clonal expansion of preadipocytes precedes adipocyte differentiation (Kopelman, 2000). It leads to an increase in both the number and size (volume) of adipocytes (Hotamisligil, 2001; Camp et al., 2002). Overweight and obesity are the result of excessive adipogenesis which is one of the most important contributing risk factors to T2D (Bays et al., 2004; Palou et al., 2000; Camp et al., 2002). Adiposity is frequently associated with insulin resistance (Hansen et al., 1997). In present study we have found that the increased body weight in HFD/STZ 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). Fruit juice administration reduces weight gain in HFD/STZ diabetic rats. This could be due to either inhibition of fat accumulation or decreases in body fat or increase in energy expenditure.

In the present investigation we have also found that after the high-fat diet weight of total fat pads and adiposity index were significantly increased in HFD/STZ diabetic rats which may results in adiposity and insulin resistance. The results of present study correlates well with previous reports which have demonstrated that rats fed a high-fat diet develops adiposity and insulin resistance (Cohen and Teitelbaum, 1968; Hallfrisch et al., 1979; Storlien et al., 1986). Oral administration of fruit juice protects against the development of adiposity and decreases adiposity index associated with high-fat feeding. This finding is consistent with previous studies of E. officinalis in several rodent models of obesity (Mathur et al., 1996; Anila and Vijayalakshmi, 2002; Anila and Vijayalakshmi, 2003). In addition, it has been shown that dietary tea rich in gallotannin reduce the deposition of visceral fat in mice fed a high-fat diet (Muramatsu et al., 1986, Murase et al., 2002) and it was also reported the anti-obesity effect of gallic acid in an animal model of diet-induced obesity. Thus, the present results suggests that gallotannin the main components of fruit juice of E. officinalis would be related to the currently observed anti obesity effect of E. officinalis and thereby decreases insulin resistance.

Obesity associated type 2 diabetes mellitus, is characterized at the cell biological level by an increase in the number and size of adipocytes differentiated from fibroblastic preadipocytes (Furuyashiki et al., 2004). Elevations in the plasma free fatty acid
Discussion

(FFA) concentration which stored as triglycerides in adipocytes have been documented in individuals with obese type 2 diabetics (Groop et al., 1989; Reaven et al., 1988). Furthermore, Increase in intracellular triglyceride synthesis is considered as an index of adipocyte differentiation (Kraegen et al., 1986; Storlien et al., 1986; Kraegen et al., 1991). Increased intracellular triglyceride content, is a better predictor of adiposity and impaired insulin action (Goodpaster et al., 1997). Conversely, a decrease in intracellular lipid content brought about by decrease in adipocyte differentiation is strongly correlate with improved insulin sensitivity (Goodpaster et al., 1999; Kelley et al., 1999; Greco et al., 2002). Chronic treatment with fruit juice of E. officinalis to HFD/STZ diabetic animals decreased triglyceride content in adipocytes isolated from epidydimal fat pads of diabetic rats. Our results correlate well with previous studies which show that gallotannins from an extract from Lagerstroemia speciosa L. (banaba) possessed adipocyte differentiation inhibitory activity (Liu et al., 2005). Gallic acid, a free form of gallotannin produces proliferative inhibition of 3T3-L1 preadipocytes to adipocytes (Hsu et al., 2006). The results of present studies clearly demonstrate that gallotannins present in fruit juice of E. officinalis may be responsible for adipocyte differentiation inhibitory activity by decreasing intracellular triglyceride synthesis and there by inhibiting adipogenesis process.

Increase in serum creatinine and urea levels has been observed not only in patients with diabetes but also in animal model of STZ-induced type 2 diabetic rats (Mulec et al., 1990; Umranı et al., 2003; Vishwakarma et al., 2003, Shinde et al., 2004). In present investigation a significant elevation in serum urea and creatinine levels has been observed in HFD/STZ diabetic rats. Treatment with fruit juice of E. officinalis produce significant decrease in these levels suggesting treatment produce beneficial effects on altered kidney functions.

Numerous experimental and clinical observations have indicated that hyperglycemia may directly or indirectly contribute to an increased formation of free radicals. Oxidative stress in diabetes coexists with a reduction in antioxidative enzymes like SOD, GSH, catalase (Sukalski et al., 1993). There is a significant decrease in GSH, SOD and catalase level was observed in the liver of STZ-induced type 1 diabetic rats indicates hyperglycemia decreases antioxidant capacity due to the accumulation of
superoxide anion radicals and hydrogen peroxide. Treatment with fruit juice produced increase in levels of antioxidant enzymes like SOD, catalase, and GSH may results in reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals. Study also showed that the increased level of LPO, a marker of fatty chain peroxydation, was significantly increased in diabetes. The treatment with fruit juice of *E. officinalis* decreased LPO level significantly in diabetic rats. Many plant extracts and plant products have been shown to possess antidiabetic activity having significant antioxidant activity (Anjali and Manoj, 1995). It is possible that since *E. officinalis* contains a large amount of gallotannins and found to possess significant antioxidant activity may be due to the presence of gallotannins which are responsible for the antidiabetic activity of *E. officinalis*. These findings further suggest a correlation among the gallotannins and antidiabetic, antihyperlipidemic and antioxidant activities.

The decreased antioxidant enzymes activities and increased LPO observed in the present study also substantiates the existence of oxidative stress in the HFD-fed insulin resistant rat model. Treatment with fruit juice of *E. officinalis* increased the levels of endogenous antioxidant enzymes and decreased LPO in diabetic rats. Phenolic principles of fruit juice of *E. officinalis* are reported to have strong antioxidant property (Bhattacharya *et al.*, 1999; Ghosal *et al.*, 1996; Kumar *et al.*, 2006; Jose and Kuttan, 2001; Naik *et al.*, 2005). Moreover, research has evidence supporting the role of gallic acid for radical scavenging, chelating and antioxidant activity (Heim *et al.*, 2002). The relationship analysis of the present study indicated that gallotannin present in fruit juice of *E. officinalis* tested for anti obesity activity were well correlated to their antioxidant activity.

The effect of fruit juice of *E. officinalis* on biochemical parameters was observed with significant decrease in the serum insulin levels, and thus suggesting its mechanism of action might be through improving whole body insulin sensitivity rather than stimulating the beta cell insulin secretion. Further, fruit juice of *E. officinalis* might have reduced the serum glucose level by sensitizing the insulin action in the target tissues mainly through diminishing lipolysis in adipose tissue or subsequent reduction of glucose production in liver or enhancement of insulin-mediated glucose disposal in skeletal muscle (Srinivasan *et al.*, 2004). Further more, gallic acid present in *Punica*
granatum flower extract produced increased PPAR-γ dependent mRNA expression which indicates that the actions of gallotannins a component of fruit juice of *E. officinalis* might to be mediated by interaction with nuclear peroxisome proliferator-activated receptor (PPAR)-γ receptors in target tissues (Huang *et al.*, 2005). Further, the attenuating effect of fruit juice of *E. officinalis* on hyperlipidemia might result either from the inhibition of triglyceride synthesis in liver or increased triglyceride clearance in the periphery by stimulating the enzyme LPL and/or inhibition of dietary cholesterol absorption from the intestine. Thus, there are several mechanisms are proposed for antidiabetic and anti obesity activity of gallotannins from fruit juice of *E. officinalis* including decreased energy/food intake and increased energy expenditure, decreased preadipocyte differentiation and proliferation, decreased lipogenesis, oxidative stress and increased lipolysis and fat oxidation.

In vitro hydrolysis studies concerning the availability of gallic acid from gallotannins present in fruit juice of *E. officinalis* are in agreement with their potential therapeutic effects. These pharmacological effects have been mainly attributed to the content of esters of gallic acid (gallotannins) present in fruit juice of *E. officinalis*. In the present study, the concentration of total gallic acid was 2.34 %w/v in fruit juice of *E. officinalis*. However, data presented here indicated that fruit juice produced better pharmacological activities at these gallic acid concentrations.

*In nutshell study with fruit juice of E. officinalis in type 1 and type 2 diabetic rats revealed significant antidiabetic activity, associated with significant reduction in elevated lipid levels. A good correlation of antidiabetic activity and gallotannin concentrations in fruit juice of E. officinalis was found in the above study.*

Since, fruit juice of *E. officinalis* showed greater antidiabetic effect in both the models of diabetic rats, we decided to go for its fractionation to have a more active fraction for the antidiabetic activity of fruit juice of *E. officinalis*. The fruit juice of *E. officinalis* was fractionated using the solvents of varying polarity like petroleum ether, toluene, chloroform, ethyl acetate, n-butanol and residual fraction. The activity of n-butanol and its residual fractions were studied for their activity in STZ-induced type 1 and type 2 diabetic rats.
Chronic treatment with n-butanol and residual fraction of *E. officinalis* could prevent the loss of body weight in STZ-diabetic rats, but treatment did not alter the normal gain in body weight of control rats. Treatments with n-butanol and residual fractions were able to produce significant reduction in the elevated water intake and food intake of type 1 diabetic rats.

Treatment with n-butanol fraction of *E. officinalis* showed significant decrease in fasting serum glucose level and slight increase in insulin levels as compared to diabetic rats but it was not statistically significant. Residual fraction produced decrease in glucose level but it was not significant. The antidiabetic activity of n-butanol fraction of *E. officinalis* further supported by decrease in GHb. STZ-diabetic rats were found to have significantly elevated GHb. Treatment with n-butanol produced a significant reduction in elevated GHb, which are a measure of long-term mean glycemia that predicts risks for the development and/or progression of diabetic complications in patients with type 1 and type 2 diabetes.

Similar results were obtained in OGTT which measures the action of endogenous insulin in response to a glucose stimulus (Alford et al., 1971). Treatment with n-butanol fraction showed significant decrease in $\text{AUC}_{\text{glucose}}$ and slight increase in $\text{AUC}_{\text{insulin}}$ which was not statistically significant. Residual fraction did not show any significant effect on these parameters. All these results indicate that n-butanol fraction possesses antidiabetic activity and that of residual fraction may have either weak antidiabetic potential. Further, our studies also indicate that the mechanism involved in glucose lowering effect may not be the release of insulin by *E officinalis* but may be due to increase in the sensitivity of insulin. That is further supported by the observation that the decrease in hepatic glycogen content in liver of diabetic rats significantly increased by treatment with n-butanol fraction of *E. officinalis*. Residue did not produce any significant effect on liver glycogen levels.

Treatment with n-butanol produced decrease in serum cholesterol and triglyceride levels in diabetic rats. There were also significant decreased in serum LDL and VLDL levels by treatment with n-butanol fraction of *E. officinalis* in STZ-diabetic rats. No significant changes were observed in serum HDL level in STZ-diabetic rats or treatment with n-butanol fraction of *E. officinalis* to STZ-diabetic rats. These results
indicate that *E. officinalis* prevent STZ induced hyperlipidemia and might play an important role in a complication associated with the diabetes.

n-Butanol fraction decreased urea, creatinine levels significantly in diabetic rats as compared to those of control indicating n-butanol fraction is effective in improving kidney function in type 1 diabetes. Significant decrease in serum SGOT and SGPT levels was observed in type 1 diabetic rats. No significant changes were observed with n-butanol or residual fraction as compared to control rats. Thus, n-butanol fraction does not seem to have a direct effect on STZ-induced damage to liver.

Treatments with n-butanol fraction of *E. officinalis* significantly decreased LPO and increase in SOD, catalase, and GSH levels. These, results of our study indicate a potent antioxidant and LPO inhibiting activity of the n-butanol fraction of *E. officinalis* and the antioxidant activity of n-butanol fraction of *E. officinalis* may be due to antioxidant properties of the gallotannins present in n-butanol and residual fraction.

The alterations in glucose and insulin levels were found to be produced with both the fractions of fresh juice of *E. officinalis*. N-butanol fraction produced a greater reduction in elevated glucose levels and other biochemical parameters as compared to residual fraction in type 1 diabetes. The differences in the effects among fruit juice and the fractions of *E. officinalis* can be explained on the basis of corresponding differences in gallotannin concentration in both the fractions. As mentioned before the concentration of gallotannin was in the order of residual fraction (12.9%), n-butanol fraction (23.4%) which correlate well with pharmacological activities.

Studies in type 2 diabetes treatments with n-butanol fraction produced greater decrease in glucose and insulin levels as compared to residual fraction on altered glucose and insulin levels in diabetic rats. In oral glucose tolerance test treatment with n-butanol fraction produced greater effect (decrease in AUC$_{\text{glucose}}$ and decrease in AUC$_{\text{insulin}}$ values) as compared to residual fraction on altered AUC$_{\text{glucose}}$ and AUC$_{\text{insulin}}$ values. The results of OGTT and K$_{\text{ITT}}$ further substantiate the contention that the reduction in elevated insulin levels on treatment with n-butanol fraction of *E. officinalis* may be due to improvement in insulin sensitivity.
Discussion

Treatment with n-butanol fraction reduced glucose and insulin levels. This may be due to decrease in insulin resistance. Treatment with n-butanol fraction of fruit juice of *E. officinalis* significantly decreased lipid levels in diabetic rats. Decrease in lipid levels was found to be greater with n-butanol fraction as compared to residual fraction.

In the present study the administration of n-butanol fraction of fruit juice of *E. officinalis* was able to reduce significantly body weight gain, weight of body fat pads, adipocyte triglyceride content and adiposity index indicating its potent anti obesity and hypolipidemic activity. Residual fraction able to produce slight decrease in all these parameters but it was not statistically significant.

Treatment with n-butanol fractions of fruit juice of *E. officinalis* significantly reduced elevated serum urea and creatinine levels which suggest their beneficial effects on altered kidney functions in conditions of type 2 diabetes. However, residual fraction failed to produce any significant change in these levels suggesting treatment did not produce beneficial effects on altered kidney functions. Treatment with n-butanol and residual fractions significantly increased antioxidant enzymes and reduced elevated LPO suggest their beneficial effects in oxidative stress in conditions of type 2 diabetes.

**In nutshell study with n-butanol and residual fractions of fruit juice of *E. officinalis* in type 1 and type 2 diabetic rats revealed significant antidiabetic activity, associated with significant antihyperlipidemic and anti obesity activity. An n-butanol fraction of fruit juice of *E. officinalis* was found to produce greater antidiabetic activity as compared to residual fractions of fruit juice of *E. officinalis*. A good correlation of antidiabetic activity and gallotannin concentrations in n-butanol and residual fractions were found in the above study.**

The improvement in glucose and lipid profile was significantly greater in case of n-butanol fraction as compared to residual fractions. If gallic acid is considered to be the active constituent responsible for such an activity, higher concentration of total gallic acid in fruit juice of *E. officinalis* and its n-butanol fraction fraction may be responsible for greater antidiabetic activity in both type 1 and type 2 diabetic rats. Our data suggest a good correlation of gallic acid concentration and antidiabetic activity in
fruit juice of *E. officinalis* and its fractions. It was found from the pharmacological work using normoglycemic, STZ-induced type 1 and type 2 diabetic rats, that the concentration of total gallic acid in fruit juice of *E. officinalis* and fractions of fruit juice of *E. officinalis* correlate well with pharmacological activity. Thus, one of the objectives of the present research work was to find out concentration of gallic acid (gallotannin) the active compound from fruit juice of *E. officinalis* and its fractions for diabetes and to carry out *invitro* hydrolysis of gallotannin from fruit juice of *E. officinalis* in simulated gastric and intestinal juice so as to get a direct evidence for the hypothesis that gallic acid is responsible for pharmacological activity in all above models. The estimation of free form of gallic acid from fruit juice of *E. officinalis* and fractions was performed by rhodanine assay. The bound form of gallic acid (gallotannin) was estimated after acid hydrolysis with 2N HCl at 100°C for 10 hours. The yield of the total gallic acid estimated from fruit juice of *E. officinalis*, n-butanol and residual fractions was 2.43mg/ml, 24.3mg/100mg,12.9mg/100mg respectively.

The biological properties and bioavailability of gallotannins depends on their chemical structure and it is important to study the effect of gastrointestinal fluids (Manach *et al.*, 2004; Manach *et al.*, 2005). Gallotannins present in fruit juice of *E. officinalis* consists of several galloyl esters, and a small amount of free gallic acid. It has been demonstrated by several workers that pH is one of the most important factors affecting the hydrolysis of gallotannins. These workers indicated that there is an optimum pH for this property (Van Buren and Robinson, 1969; Brenbaum, 1980; Martin *et al.*, 1985). In the present study, acidic and alkaline pH of GI tract caused dissociation of the bond between gallic acid and carbohydrate, however hydrolysis with acidic pH of gastric juice was much faster than alkaline pH of intestinal fluid.

The results of our study were consistent with previous findings (Osawa and Walas, 1993). Thus, it can be postulated that, if fruit juice was consumed, free form of gallic acid would be readily liberated from the ester complexes in the acidic stomach or the alkaline small intestine and these could be available for pharmacological effect. However, our results suggest that the situation may be more complex if gallic acid released from the complexes could rebind with dietary and/or endogenous proteins (i.e., mucus, digestive enzymes, etc.). In this study, we obtained some detailed information about the bioavailability of gallotannins of fruit juice in *in vitro* system. Further *invivo* studies are required to find out how much free form of gallic acid bioavailable in plasma for pharmacological activity.
It was found from the pharmacological effect of type 1 and type 2 diabetic rats, that the concentration of gallic acid in fruit juice of *E. officinalis* and fractions of fruit juice of *E. officinalis* correlate well with pharmacological activity thus, the gallic acid was tested in both type 1 and type 2 diabetic rats. In type 1 diabetic study treatment with gallic acid (25mg/kg, 50mg/kg, 100mg/kg p.o.) for 8 weeks was carried out.

In the present study, we monitored animals individually for cardinal signs of diabetes like loss of body weight, increase in food consumption, and water intake on a daily basis, and accordingly the dose was titrated to observe effect of different doses of gallic acid. Chronic treatment with gallic acid to diabetic rats decreased food and water consumption and improved loss of body weight in diabetic rats in dose dependent manner and this could be due to decrease in catabolic reaction in hyperglycemic state in the diabetic rats because it has been reported that all cardinal signs of diabetes are due to excessive break-down of tissue proteins and dehydration and catabolism of fats and proteins which leads to reduced weight gain and increase in food and water intake by diabetic rats (Chatterjea and Shinde, 2002; Hakim et al., 1997; Rajkumar et al., 1991).

Treatment with gallic acid significantly reduced the serum glucose levels and produced slight increase in serum insulin levels of STZ-diabetic rats. However, the insulin levels were still statistically non significant as compared to control groups. This indicates gallic acid may produce glucose lowering effect either due to increased sensitivity of peripheral tissue to insulin or direct insulin like effect. The results of the OGTT also clearly indicate improved glucose tolerance with treatment of gallic acid to diabetic rats. The results of the present study indicates that polyphenolic compounds like gallic acid may able to reduce blood glucose level and produced antidiabetic activity in diabetic animals. Chronic treatment with gallic acid in diabetic rats decreased elevated lipid profiles. These results of present study correlates with the previous reports that gallic acid increase peripheral insulin sensitivity in rat adipose tissue by inhibiting lipogenesis and produced increased expression and activity of LPL in *in vitro* studies (Huanga et al., 2005; Ong et al., 1995). In previous studies it has been proved that treatment of Zucker diabetic rats with PPAR-γ agonists reversed lipotoxicity and increased glucose metabolism (Golfman et al., 2005; Zhou et al., 2000). This therapeutic approach had two major metabolic consequences. First,
increase in glucose transport and second, decrease in lipid level. Thus it is possible that gallic acid decreased glucose levels and a reduction in lipid level may be due to acting on PPAR-γ receptor. Thus antidiabetic and antihyperlipidemic activities may be one of the important reasons of the effectiveness of gallic acid because carbohydrate and lipid metabolic abnormalities have been attributed to development of complications in diabetes mellitus (Dhalla et al., 1985; Tomlison et al., 1992).

In the present study treatment with gallic acid did not produce any significant effect on elevated SGOT and SGPT levels in type 1 diabetic rats. Treatment with gallic acid found to decrease serum creatinine and urea levels thereby decrease in osmotic diuresis and depletion of extracellular fluid volume because the increase in serum creatinine and urea levels may be due to hyperglycemia that causes osmotic diuresis and depletion of extracellular fluid volume (McCance and Widdowson, 1939).

In present investigation, chronic administration of gallic acid produced antioxidant effect by increase in SOD, GSH and catalase levels these could be due to decreased accumulation of superoxide anion by SOD and reduction of hydrogen peroxides by GSH and catalase because SOD is proved to be an important defense enzyme that catalyses the dismutation of superoxide radicals (McCord et al., 1976) and GSH and catalase are also important biomolecule which can participate in the elimination of reactive intermediates by reducing hydroperoxides (Meister, 1984; Nicotera and Orrenius, 1986; Vucic et al., 1997). STZ diabetic rat also showed increased level of MDA, a marker of fatty chain peroxydation because high concentration of lipid was found to be present in liver of diabetic rat which results in the activation of NADPH dependent microsomal LPO in liver (Cohn and Roth, 1996). The treatment with gallic acid decreased MDA level significantly in diabetic rats indicates protection against LPO. The results of the present study indicate that the antioxidant effects of gallic acid may be due to inhibition of LPO and increase in antioxidant enzymes by gallic acid.

In HFD/STZ diabetic rats treatment with gallic acid produced a significant reduction in elevated glucose and insulin levels in HFD/STZ rats. It has been demonstrated that gallic acid is capable of inducing insulin sensitivity by activation or induction of PPAR-γ receptors (Huanga et al., 2005). The discovery of glucose and insulin lowering activity of gallic acid may also helps to explain antidiabetic activity as a
result of improvement in insulin sensitivity by activation of PPAR-γ receptors. In oral glucose tolerance test and insulin sensitivity test also treatment with gallic acid produced a significant reduction in $AUC_{\text{glucose}}$ values as well as $AUC_{\text{insulin}}$ and increase reduced $K$-value in diabetic rats compared to diabetic control rats. Treatment with gallic acid significantly reduced the plasma glucose levels in HFD/STZ rats after glucose challenge, demonstrating an improvement by gallic acid of glucose uptake by the tissues, this is consistent with effect of gallic acid on insulin sensitivity test in HFD/STZ rats, further strengthen the conclusion that the gallic acid may activates PPAR-γ, which is involved in improvement of impaired glucose tolerance in HFD/STZ rats.

Insulin resistance in type 2 diabetic rats could be mainly due to resistance to insulin action at the peripheral tissue which is the main site of glucose disposal (Pedersen and Beck-Neilsen, 1987; DeFronzo, 1997). In present investigation elevated body weight and total body fat pads, adipocyte triglyceride content and adiposity index were decreased in HFD/STZ rats by the treatment with gallic acid at all three doses in dose dependent manner. The results of present investigation clearly demonstrate that gallic acid decreases adiposity and there by may improves insulin sensitivity in fat fed diabetic rats. Elevations in concentration of stored triglycerides in adipocytes have been documented in individuals with obese type 2 diabetics (Groop et al., 1989; Reaven et al., 1988). Chronic treatment with gallic acid to HFD/STZ diabetic animals decreased triglyceride content in adipocytes isolated from epidydimal fat pads of diabetic rats. Thus, gallic acid appears to improve glucose homeostasis in diabetic obese animals by enhancing the action of insulin by decreasing intracellular triglyceride synthesis and by increasing the reduced peripheral glucose uptake.

Treatment with gallic acid found to decrease elevated serum creatinine and urea levels indicates gallic acid produces improvement in kidney function in HFD/STZ diabetic rats. Treatment with gallic acid at all doses produced significant improvement in antioxidant parameters.

The results of our study suggest that gallic acid possesses beneficial effects on elevated glucose, insulin, lipids, creatinine, urea, body weight, weight of body fat pads, adipocyte triglyceride content and adiposity index in HFD/STZ rats and produces improvement in antioxidant enzymes in liver of diabetic obese rat. The results of our
study not only suggest that gallic acid possesses beneficial effects on hyperglycemia, hyperinsulinemia, hyperlipidamia, but also provides strong support to the hypothesis that the antidiabetic activity of E. officinalis is due to polyphenolic compound like gallic acid.

As mentioned above gallotannins have been reported to possess antidiabetic, antihyperlipidemic and antioxidant activities. Thus, it is possible that gallotannins present in E. officinalis might be having antidiabetic, antihyperlipidemic and antioxidant activities and these activities of E. officinalis may be effective in prevention or delaying the development of cardiomyopathy because hyperlipidemia associated with hyperglycemia is considered to be major cause for cardiovascular risk factors (Garg et al., 1992; Howard et al., 1998). Diabetes mellitus increases the risk of heart failure independently of underlying coronary artery disease and leads to cardiomyopathy (Boudina and Abel, 2007). The pathogenesis of diabetic cardiomyopathy is multifactorial. Several hypotheses have been proposed, including metabolic derangements like hyperglycemia and hyperlipidemia (Garvey et al., 1993; Nakayama, et al., 2001). Sustained hyperglycemia in turn leads to glycation of interstitial proteins such as collagen, result in myocardial stiffness and impaired contractility (Capasso et al., 1989; Berg et al., 1999). There may be decrease in insulin sensitivity linked to autonomic dysfunction, hypertension and LV hypertrophy (Eiro et al., 2003; Nakano et al., 2003; Hirayama et al., 2001) and increase oxidative stress contributes to the characteristic morphological and functional abnormalities that are associated with diabetic cardiomyopathy (Cai et al., 2006). Among various therapeutic strategies, treatments effective for preventing or delaying the development of above derangement in diabetic cardiomyopathy includes improvement of diabetic control, prevention of hyperlipidemia, oxidative stress and the use of insulin-sensitizing drugs. Taking this into consideration we conducted a study to determine whether chronic treatment of fruit juice of E. officinalis has beneficial effects on glycemia, lipidemia and cardiac dysfunction in the STZ induced type 1 diabetic rat.

Many plant derived drugs used in modern medicine are developed by ethnomedical leads and subsequent ethnopharmacological studies. Plants containing polyphenols have been reported to possess strong glucose lowering, lipid lowering and antioxidant activities. Phytochemical investigations of fruits of E. officinalis show that it is having
high amount of polyphenols like gallotannins. Gallotannins present in various medicinal plants have been reported to have cardioprotective effect (Michael et al., 2005; Karthikeyan et al., 2007). Several studies have suggested that carbohydrate and lipid metabolic abnormalities such as hyperglycemia and hyperlipidemia may contribute to the development of cardiac dysfunction in diabetes mellitus (Dhalla et al., 1985). Diabetic hearts have a primary defect in the stimulation of glycolysis and glucose oxidation (Mokuda et al., 1990). Increasing evidence suggests that altered glucose supply and utilization by cardiac myocytes could be the primary injury in the pathogenesis of this specific heart muscle disease (Rodrigues et al., 1998). A significant reduction in myocardial glucose supply and utilization has been observed in isolated diabetic cardiomyocytes (Chen et al., 1984) and diabetic patients (Ohtake et al., 1995). Therefore it is necessary to increase glucose utilization or increase the rate of glucose transport in the diabetic heart. STZ diabetic rats were found to exhibit significant hyperglycemia and hypoinsulinaemia. The response to hypoglycemic therapy further confirms the correlation of myocardial functional and structural changes with glycemic control (Pogatsa et al., 1979). Earlier studies have shown that in STZ-diabetic rats, hyperglycemia is associated with hypercholesterolemia and hypertriglyceridemia (Rodrigues et al., 1986). Abnormalities in lipid metabolism have been demonstrated in cardiomyopathy in which the rate of free fatty acid (FFA) uptake by myocardium is inversely proportional to the severity of the myocardial dysfunction (Yazaki et al., 1999). Elevated lipid levels are believed to be one of the major contributing factors in the pathogenesis of diabetic cardiomyopathy. These changes are characterized by elevation of circulating FFA caused by enhanced adipose tissue lipolysis, as well as hydrolysis of augmented myocardial triglyceride stores which results in decrease in peripheral insulin sensitivity. Moreover, in addition to the FFA-induced inhibition of glucose oxidation, high circulating and cellular FFA levels may result in abnormally high oxygen requirements during FFA metabolism and the intracellular accumulation of potentially toxic intermediates of FFA, all of which lead to impaired myocardial performance and severe morphological changes (Rodrigues et al., 1998; Nakayama et al., 2001). Thus strategy to be employed to produce improvement in cardiac function is to improve upon these metabolic disarrangements. Thus both hyperglycemia and hyperlipidemia leads to impaired myocardial performance and severe morphological changes (Rodrigues et al., 1998; Nakayama et al., 2001) and previous studies have demonstrated that normalization of
glycemia and correction of lipid abnormalities can improve heart function in STZ-diabetic rats (Yuen et al. 1993). Thus strategy to be employed to produce improvement in cardiac function is to improve upon these metabolic disarrangements. In the present study it was found that the oral administration of fruit juice of *E. officinalis* decreases glucose levels and elevated lipid profiles. Thus, it is possible that gallotannins relatively abundant in fruit juice of *E. officinalis* might be responsible for glucose lowering and lipid lowering effects thereby produces cardioprotective effect.

Hypertension is secondary to diabetes and is associated with LV dysfunction in patients with diabetes, because hyperglycemia has been shown to increase blood pressure in humans and in animal models of type 1 diabetes (Fitzgerald, 2002). Diabetes induced myocardial dysfunction associated with increased force of contraction and development of bradycardia (Rodrigues et al. 1986; Zarich and Nesto, 1989; Hayashi et al., 2001). Medicinal plants containing gallotannins has been shown hypotensive effect by decreasing blood pressure and increasing heart rate (Mohammad, 2005; Hantamalala, 2004). In our study treatment of fruit juice of *E. officinalis* for 8 weeks significantly reduced blood pressure, force of contraction and increased heart rate as compared to diabetic control group indicate cardioprotective effect of fruit juice of *E. officinalis*.

Various studies have shown that experimentally induced diabetes causes increase in collagen formation and accumulation of protein within the interstitium, which in turn results in anatomic and physiological changes in the myocardium (Regan et al., 1981; Indu et al., 2006). Further, various studies have demonstrated that increase in left ventricular collagen and protein content may produce cardiac stiffness and fibrosis resulting in cardiac dysfunction (Weber and Brilla 1991; Weber et al. 1994; Nagai et al. 1988). In the present study, increases in left ventricular collagen and protein content in diabetic rats was found to be decreased by treatment of fruit juice of *E. officinalis*. Thus, fruit juice of *E. officinalis* may produce protection against cardiac stiffness and fibrosis in cardiac dysfunction.

In the present study, diabetic rats were found to exhibit increased left ventricle weight and heart weight. Both the ratio of LV/BW and HW/BW serve as an index of cardiac hypertrophy. In our study LV/BW and HW/BW found to be increased in diabetic hearts. These are in agreement with previous reports in which it has be shown that
there is increase in fibrous tissue formation and accumulation of collagen in diabetic rats causing increase in the left ventricular mass (Joffe et al., 1999). A correlation with biopsy study in diabetic patients has also supported these findings in animals (Shimizu et al., 1993). The left ventricular dysfunction has been also associated with increased LV/BW and HW/BW (Balakumar, 2006). Treatment of fruit juice of E. officinalis produced improvement in LV/BW and HW/BW may be due to increased breakdown of collagen suggesting that cardioprotective effect is associated with the improvement in LV hypertrophy.

Serum LDH and CKMB activities were found to be increased in STZ diabetic rats, this may be due to myocardial dysfunction because previously it has been reported that serum LDH and CKMB activities were found to be increased in cardiomyopathy (Hall, 1991). Serum CKMB and LDH levels are also reported to increase in diabetic patients and may serve as a cardiovascular risk-related marker and cardiac muscular damage (Huang et al., 2006; Hagar, 2002). In this study there was a significant decrease in serum LDH and CKMB levels were observed with treatment of fruit juice of E. officinalis indicates good cardioprotection.

Increased ROS production in the diabetic heart is a contributing factor in the development and progression of diabetic cardiomyopathy (Cai, 2006). Increased ROS amplifies hyperglycemia-induced activation of protein kinase C isoforms, increased formation of glucose-derived advanced glycation end products, and glucose flux through the aldose reductase pathways (Brownlee, 1995; Koya and King, 1998). All these may contribute to the development of cardiac complications in diabetes mellitus. Increased ROS generation may activate maladaptive signaling pathways, which may lead to cell death, which could promote abnormal cardiac remodeling, which ultimately may contribute to the characteristic morphological and functional abnormalities that are associated with diabetic cardiomyopathy (Kwon et al., 2003). Thus, the levels of antioxidant enzymes like GSH, SOD, or catalase are decreased in diabetic heart (Matkovics et al., 1997; Aliciuzel et al., 2003). The strategies either to reduce ROS or augment myocardial antioxidant defense mechanisms might have therapeutic efficacy in improving myocardial function in diabetes mellitus (Oberley, 1988). Treatment with fruit juice of E. officinalis increased the levels of endogenous antioxidants and decreased LPO in diabetic rats. Gallotannin active principal of fruit
juice of *E. officinalis* are reported to have strong antioxidant property and this can be beneficial in prevention of diabetes induced cardiomyopathy (Bhattacharya *et al.*, 1999; Ghosal *et al.*, 1996; Kumar *et al.*, 2006; Jose and Kuttan, 2001; Naik *et al.*, 2005). In our study increase in levels of antioxidant enzymes in the heart were observed after treatment with fruit juice of *E. officinalis* in diabetic animal which can reverse diabetic cardiomyopathy. Thus diabetics, who have an increased risk of cardiac dysfunction, may benefit both from an improvement in glucose and lipid homeostasis as well as from the antioxidant cardioprotective effect of fruit juice of *E. officinalis*. In conclusion, treatment of fruit juice of *E. officinalis* ameliorates hyperglycemia, hyperlipidemia, oxidative stress and development of cardiac dysfunction associated with STZ-diabetes. Thus result of our study demonstrates the potential use of fruit juice of *E. officinalis* for treatment of diabetic cardiomyopathy.

Since fruit juice of *E. officinalis* showed greater cardioprotective effect in diabetic cardiomyopathy, we also studied the effect of n-butanol and residual fractions of fruit juice of *E. officinalis* on diabetic cardiomyopathy. Treatment with n-butanol fraction of fruit juice of *E. officinalis* produced significant reduction in serum glucose, cholesterol, triglyceride, VLDL-cholesterol, LDL-cholesterol as compared to diabetic control rats and improved tolerance to glucose in OGTT, which leads to enhancement of glucose disposal without increasing insulin levels. Residual fraction also produced improvement in all these parameters. Elevated glucose and lipid levels are believed to be one of the major contributing factors in the pathogenesis of diabetic cardiomyopathy (Rodrigues *et al.*, 1998; Nakayama *et al.*, 2001). Thus improvement in both hyperglycemia and hyperlipidemia by n-butanol fraction can improve heart function in STZ-diabetic rats.

Treatment with n-butanol reverted back the elevated blood pressure, force of contraction and heart rate near to normal level indicating that this fraction produces a cardioprotective effect. Treatment with n-butanol fraction also decreased the LW/BW and HW/BW which was increased in diabetic animals which indicates that there may be decrease in fibrous tissue formation and accumulation of collagen in diabetic heart because it is reported that left ventricular hypertrophy index was greater in patients with hypertension and diabetes mellitus due to fibrous tissue formation and accumulation of collagen in diabetic heart than in those without diabetes mellitus.
(Grossman et al., 1992; Joffe et al., 1999) suggesting that cardioprotective effect is associated with the improvement in left ventricular hypertrophy. Residual fraction altered these parameters but it was not significant.

LDH and CKMB levels begin to rise in cardiomyopathy and extremely sensitive index of myocardial necrosis, cardiac injury or ischemia (Roberts et al., 1978, Lott et al., 1980, Wagner et al., 1973). Diabetic animals showed significantly increased LDH and CKMB levels and treatment with n-butanol and residual fraction decreased both the levels but decrease was not statistically significant with residual fraction as compared to diabetic control. This indicates that n-butanol fractions has cardioprotective action. It may be further proved by the significantly decreased collagen content and protein content in the left ventricle which was increased in the diabetic rat's heart.

Treatments with n-butanol fraction of *E. officinalis* significantly decreased LPO and increase in SOD, catalase, and GSH levels in diabetic hearts. These, results of our study indicate a potent antioxidant and LPO inhibiting activity of the n-butanol fraction of *E. officinalis*. The cardioprotective activity may be due to antioxidant properties of the gallotannins present in n-butanol and residual fraction. In conclusion, our data suggest that n-butanol and residual both fractions of *E. officinalis* have a cardioprotective effect and the cardioprotective effect appears to be present more in n-butanol fraction as compared to residual fraction. It is possible that cardioprotective effect of n-butanol fraction may be due to higher amount of gallotannin present in this fraction. The results of our study not only suggest that fruit juice and fractions of *E. officinalis* possesses beneficial cardioprotective effect but also provides strong support to the hypothesis that the cardioprotective effect of *E. officinalis* is due to gallic acid present in bound form in fruit juice and fractions of *E. officinalis*. The concentration of total gallic acid in fruit juice of *E. officinalis* and fractions of fruit juice of *E. officinalis* correlate well with cardioprotective effect thus, the gallic acid was tested in type 1 diabetic cardiomyopathy and treatment with gallic acid (25mg/kg, 50mg/kg, and 100mg/kg p.o.) was carried out for 8 weeks.

It has been reported that cardiac dysfunction may be corrected by insulinmimetics or insulin sensitizer which produce increase in glucose utilization in the diabetic heart and increase in rate of glucose transport across the sarcolemmal membrane into the
myocardium, probably due to the cellular depletion of glucose transporters (GLUTs) 1 and 4 (Eckel and Reinauer, 1999; Garvey et al., 1993; Russell et al., 1998). To meet the high energy demands of the contracting heart muscle, the heart needs to produce a constant and plentiful supply of energy which is primarily produced by the metabolism of carbohydrates and fatty acids. Glucose is the principle carbohydrate metabolized by the heart. Glucose is taken up by the cardiomyocytes by GLUT-4 and GLUT-1 isoforms, in which GLUT-4 is insulin-dependent (Abel, 2004; Young et al., 1997). Myocardial glucose transport has been shown to be defective in both diabetic humans and GSH-induced diabetic animals (Stanley et al., 1999). In the GSH-induced diabetic animal model, the hearts were found to have a decreased level of GLUT-4 content (Hall et al., 1995). Desrois et al. have reported that GLUT-4 protein level was 28% lower in the heart of Goto- Kakizaki rat, another model of diabetes, compared with its age-matched control. In isolated perfused hearts, insulin-stimulated glucose uptake rate was decreased by 23% in male Goto-Kakizaki rat heart (Desrois et al., 2004). Insulin-stimulated glucose uptake was also reduced in cardiomyocytes from insulin-resistant type 2 diabetic db/db mice (Carley et al., 2004). PPAR-γ plays an important role in regulating GLUT-4 expression in the heart (Abel, 2004). A stimulatory effect of PPAR-γ activation on GLUT-4 gene expression has been described in adipocytes (Wu et al., 1998) and chronic oral administration of PPAR-γ agonist like rosiglitazone improved the reduction in insulin-stimulated glucose uptake in db/db mice (Carley et al., 2004; Sidell et al., 2002). In the present study, our result demonstrated that gallic acid produced antidiabetic activity may be due to increase in GLUT-4 protein expression by activation of PPAR-γ because it has been reported that gallic acid produced glucose lowering activity by activation of PPAR-γ receptors and restores the down-regulated cardiac glucose transporter (GLUT)-4 in Zucker diabetic fatty rats (Huanga et al., 2005; Liu et al., 2001). Thus, results of present investigation suggests that diabetes results in progressive, marked changes in the myocardium due to decrease of glucose transporters into the myocardium that can be prevented by gallic acid treatment may be by increasing glucose utilization or glucose transport by activation of PPAR-γ receptors in diabetic heart. Thus response to hypoglycemic therapy further confirms the correlation of myocardial functional and structural changes with glycemic control.
Chronic treatment with gallic acid in diabetic rats decreased elevated lipid profiles. These results of present study correlates with the previous reports that esters of gallic acid increase peripheral insulin sensitivity in rat adipose tissue by inhibiting lipogenesis and increased activation of PPAR-γ activity of LPL in human THP-1-differentiated macrophage cells in invitro studies (Huanga et al., 2005; Ong et al., 1995). In previous studies it has been proved that treatment of Zucker diabetic rats with PPAR-γ agonists restored cardiac function and reversed lipotoxicity in addition to an increase in glucose metabolism (Golffman et al., 2005; Zhou et al., 2000). Thus it is possible that gallic acid may produce increased glucose use and a reduction in lipid level by activation of PPAR-γ receptor may have major contribution in improvement of cardiac function. Thus antidiabetic and antihyperlipidemic activities may be one of the important reasons of the effectiveness of gallic acid in preventing cardiac dysfunctions because carbohydrate and lipid metabolic abnormalities have been attributed to development of cardiac dysfunction in diabetes mellitus (Dhalla et al., 1985; Tomlison et al., 1992).

Increase in blood pressure after treatment with STZ has been reported by several workers and prevalence of hypertension is approximately doubled in diabetic patients compared with nondiabetic controls (Bunag et al., 1982; Cavaliere et al., 1980; Funakawa, 1980; Sowers and Epstein, 1995). Treatment with gallic acid significantly reduced blood pressure and force of contraction and increased heart rate as compared to diabetic control group and this indicates gallic acid has beneficial effect in diabetic cardiomyopathy associated with increased blood pressure and force of contraction and bradycardia.

Collagen accumulation in the diabetic myocardium may be due to impaired collagen degradation resulting from glycosylation of the lysine residues on collagen (Fiordaliso et al., 2001). Treatment with gallic acid decrease left ventricular collagen and protein content. Thus, gallic acid may produces protection against cardiac stiffness and fibrosis in cardiac dysfunction because glycosylation of the lysine residues on collagen results in myocardial fibrosis and myocyte hypertrophy are the most frequently proposed mechanisms to explain cardiac changes in diabetic cardiomyopathy.
After treatment with gallic acid produced improvement in the wet LV/BW and HW/BW may be due to increased breakdown of collagen due to decrease in glycation of lysine residue on collagen in left ventricle suggesting that good diabetic control is associated with the improvement in left ventricular hypertrophy.

In this study there was a significant decrease in serum LDH and CKMB levels were observed by treatment of gallic acid indicates beneficial effect in reducing the cardiovascular risk in diabetes mellitus.

Hyperglycemia also results in the production of reactive oxygen species (ROS), which coexists with a reduction in antioxidant enzymes like SOD, catalase, GSH and increase in LPO which ultimately may contribute to the characteristic morphological and functional abnormalities that are associated with diabetic cardiomyopathy (Kwon et al., 2003; Sukalski et al., 1993; Tanaka et al., 1999). Treatment with gallic acid increased the reduced levels of endogenous antioxidants and decreased LPO in diabetic rats. Numerous studies which have demonstrated that polyphenolic compounds of plant origin are good antioxidants inhibit the LPO processes in vitro and in vivo (Bhattacharya et al., 1999; Scartezzini et al., 2006; Anilakumar et al., 2004) and therapy may be beneficial in preventing the development of diabetic cardiomyopathy (Oberley, 1988; Cuvrelier et al., 1992). Antioxidant effect of polyphenols may also reduce β-cell toxicity of STZ in diabetic rats (Ihara et al., 1999). Thus, this study shows that gallic acid exhibits protection against myocardial dysfunction associated with type 1 diabetes which may be due to antioxidant activity of gallic acid. Thus diabetics, who have an increased risk of cardiac dysfunction, may benefit both from an improvement in glucose and lipid homeostasis as well as from the antioxidant effect of gallic acid. In conclusion, gallic acid treatment ameliorates hyperglycemia, hyperlipidemia and development of cardiac dysfunction associated with STZ-diabetes.

Results of various in vivo models mentioned above clearly suggest that E. officinalis produces significant antidiabetic, antihyperlipidemic, antioxidant and cardioprotective activity. The antidiabetic activity by E. officinalis appears to be mediated through increase in peripheral utilization of glucose in hypoinsulinemic diabetic conditions, however the antihyperglycemic effect produced by E. officinalis in conditions of insulin resistance is possibly mediated through enhanced insulin
sensitivity. The results of our study not only suggest that gallic acid possesses beneficial effects on hyperglycemia, hyperlipidamia, but also provides strong support to the hypothesis that the antidiabetic activity of *E. officinalis* is due to gallotannins present in its fruit juice.