Discussion
5. DISCUSSION

Within the context of present study, antidiabetic, antihyperlipidemic and antioxidant activities of the 70% alcoholic extract and its fractions of seeds of *Bryonia laciniosa* were investigated by using *in vivo* assay techniques in order to assess the validity of the use of this plant.

Results of our investigation have established that seeds of *Bryonia laciniosa* possess antidiabetic, and antihyperlipidemic activities mediated through antioxidant activity. The activity could be attributed to the presence of bitter principles and saponins present in the seeds of *Bryonia laciniosa*. As traditional to phytopharmacological work, we first authenticated the plant. The plant was authenticated by comparison with voucher specimen no. VSM502 and ARM 2174 at the Prof. G.L.Shah Herbarium of S.P.University, Vallabh Vidyanagar, Anand, Gujarat, India. Quantitative limit tests like ash and extractive values are other parameters to standardize the herbal drugs (Bhutani, 2000). *Bryonia laciniosa* used in the study showed that the seeds contained 15.25 % of total ash, 1.95% of acid insoluble ash, 0.67% of water soluble ash, 17.2 % of ethanol soluble extractives, 11.2 % of water soluble extractives, 4.7% of moisture content in the sample.

After having authenticated the samples of *Bryonia laciniosa* used in the present study, 70% alcoholic extract was prepared for carrying out the pharmacological activities. The yield of 70% alcoholic extract was found to be 11.7%. The 70 % alcoholic extract was further fractionated to ethyl acetate soluble fraction and ethyl acetate insoluble fraction. Ethyl acetate insoluble fraction was fractionated to n-butanol soluble and n-butanol insoluble fractions. The yield of ethyl acetate soluble fraction and n-butanol soluble fraction were 4.5% and 2.5% respectively. The 70% alcoholic extract of *Bryonia laciniosa* and its fractions were checked for their antidiabetic and antihyperlipidemic potential.

Phytochemical evaluation was done for presence of various phytoconstituents. Preliminary chemical tests indicated presence of saponins, carbohydrates, steroids, and triterpenoids.

Although herbal drugs are generally considered to be safe, there are reports of toxicity including hepatic toxicity and renal toxicity by several of these preparations (Langmead and Rampton, 2001; Corns, 2003; Colson and De Broe, 2005). In view of such reports and to ascertain the safe use of extract of *Bryonia laciniosa*, present
study was carried out to evaluate its effect on the different biochemical parameters. Administration of 70% alcoholic extract of *Bryonia laciniosa* (125, 250 and 500 mg/kg, p.o.) for a period of 21 days did not affect body weight, food intake and water intake. Further, treatment of normal rats with 70% alcoholic extract of *Bryonia laciniosa* (125, 250 and 500 mg/kg, p.o.) for a period of 21 days did not produce a significant change in the serum levels of glucose, triglycerides, HDL-C, VLDL-C and LDL-C. Our study did not show any change in the levels of serum urea, creatinine, SGOT and SGPT in normal rats suggesting thereby that the kidney and liver functions were not affected by crude extract of seeds of *Bryonia laciniosa*. Consequently, the extract showed significant antihyperglycemic activity in glucose-hyperglycaemic, while no significant effect was observed on normoglycaemic rats.

Intraperitoneal injection of STZ produced various cardinal symptoms of diabetes such as hyperglycemia, loss of body weight, polyphagia and polydipsia. These findings are consistent with earlier studies (Prince et al., 1998; Yadav et al., 2002). The loss of body weight could be due to dehydration and catabolism of fats and proteins (Hofteizer and Carpenter, 1973). Treatment with 70% alcoholic extract prevented the loss in body weight, polyphagia and polydipsia. Diabetic control rats showed significant rise in glucose levels associated with decrease in insulin levels. Treatment with 70% alcoholic extract produced significant fall in blood glucose levels which was not associated with rise in serum insulin levels. This effect was comparable to the effect of insulin (5 I.U. /kg, i.p.) on STZ-induced diabetic group. The possible underlying mechanism could be either increased peripheral glucose utilization or stimulation of secretion of insulin at pancreatic level in response to increased glucose that is already reported by Kar et al (1999).

OGTT is a closed loop method that is simple and directly measures the action of endogenous insulin in response to a glucose stimulus (Alford et al., 1971). In this method, \( \text{AUC}_{\text{glucose}} \) (mg/dl.min) indicates insulin stimulated glucose disposal. During OGTT, when further glucose load was given STZ-induced type 1 diabetic rat, there was further rise in glucose levels observed at 0, 30, 60 and 120 min when compared with non diabetic control rats. Treatment with 70% alcoholic extract (250, 500mg/kg, p.o.) and its n-butanol fraction significantly lowered serum glucose levels at 30 min as compared to diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group. There was a significant increase in
Discussion

AUC\textsubscript{glucose} value in diabetic rats as compared to non-diabetic control rats. Treatment with 70% alcoholic extract (250, 500 mg/kg, p.o.) and its n-butanol fraction significantly reduced AUC\textsubscript{glucose} in STZ induced type 1 diabetic rats when compared with diabetic control rats. Delaying or inhibiting glucose absorption at intestinal level or decrease in the endogenous glucose production in the liver could be the underlying mechanism for observed decrease in fed serum glucose levels.

Abnormalities in lipid profile are the most common complications in diabetes mellitus found in 40% of diabetic cases (Ravi et al., 2005). Diabetes causes an increase in the cholesterol, triglycerides, LDL and VLDL (Soltani et al., 2007). High levels of total cholesterol and more importantly LDL-cholesterol in blood are major coronary risk factors. The abnormal high concentration of serum lipids in the diabetic subject is due mainly to the increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Insulin deficiency or insulin resistance may be responsible for dyslipidemia, because insulin has an inhibitory action on HMG-coA reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. This results in increased production of cholesterol rich LDL particle (Ravi et al., 2005; Soltani et al., 2007). The administration of 70% alcoholic extract of \textit{Bryonia laciniosa} elevated the level of serum HDL-cholesterol and lowered the levels of total cholesterol, triglycerides, VLDL and LDL-cholesterol. The glucose lowering action of \textit{Bryonia laciniosa} can be due to the consequence of an improved lipid metabolism apart from the direct interaction with glucose homeostasis. The triglycerides lowering property of extract could indirectly contribute to the over all antihyperglycemic activity through a mechanism of so called glucose--fatty acid cycle (Randle et al., 1963). According to the Randle’s glucose--fatty acid cycle, increased supply of plasma triglycerides per se could constitute a source of increased free fatty acid (FFA) availability and oxidation that can impair insulin action, glucose metabolism and utilization leading to development of hyperglycemia. Therefore, the reduction of triglycerides following treatment with extract would also facilitate the glucose oxidation and utilization and subsequently the reduction of hyperglycemia.

The atherogenic index of STZ diabetic rats was found to be higher than normal rats. The atherogenic index represents the ratio of LDL and VLDL to HDL.
Higher the value, higher will be chances of atherogenesis, which lead to cardiovascular complications (Grover et al., 1999). In the present study, treatment with insulin brought down the levels of increased lipoproteins and total cholesterol. Administrations of 70 % alcoholic extract of *Bryonia laciniosa* (250, 500 mg/kg, p.o.) and its n-butanol fraction also lowered the levels of circulating lipids and also produced significant reduction in atherogenic index in STZ-induced diabetic rats when compared with diabetic control. Treatment with 70 % alcoholic extract of *Bryonia laciniosa* and its n-butanol fraction has also lowered the atherogenic index which indicates the balance of lipoprotein fraction in favor of HDL that may reduce the cardiovascular complications.

The liver, a major site of insulin clearance and production of inflammatory cytokines, plays an important role in maintaining normal glucose concentrations during fasting and postprandially (Leclercq et al., 2007). Loss of insulin effect on the liver leads to glycogenolysis and an increase in hepatic glucose and free fatty acid production. The excess free fatty acids found in the insulin- deficient state are known to be directly toxic to hepatocytes. Putative mechanisms include cell membrane disruption at high concentration, mitochondrial dysfunction, toxin formation, and activation and inhibition of key steps in the regulation of metabolism (Neuschwander-Tetri, 2003). SGOT and SGPT measure the concentration of intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury. It is hypothesized that elevation in SGOT and SGPT are considered as predictors of diabetes (Harris, 2005). Further, the elevation in the levels of these gluconeogenic enzymes whose gene transcription is suppressed by insulin could indicate impairment in insulin signaling rather than purely hepatocyte injury (O'Brien, 1991). Other potential explanations for elevated SGOT and SGPT in insulin- deficient states include oxidative stress from reactive lipid peroxidation, peroxisomal β-oxidation, and recruited inflammatory cells. The insulin-deficient state is also characterized by an increase in proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), which may also contribute to hepatocellular injury (Grove, 1997). On the other hand, in the present study, treatment of the diabetic rats with 70% alcoholic extract (250, 500 mg/kg, p.o.) caused reduction in the activity of these enzymes in serum when compared to the diabetic group and consequently alleviated liver damage caused by STZ-induced diabetes.
Urea is the main end product of protein catabolism in the body. Accumulation of urea nitrogen in experimental diabetes may be due to the enhanced breakdown of both liver and plasma proteins (Green and Miller, 1960). Alterations in nitrogen homeostasis may lead to increased hepatic elimination of urea nitrogen and increased peripheral release of nitrogenous substances. Thus, the observed negative nitrogen balance may partly because of changes occurring within the hepatocytes (Almdal et al., 1986). The oral administration of 70% alcoholic extract (250 and 500 mg/kg) to diabetic rats significantly decreased the elevated levels of blood urea suggesting the prophylactic role of *Bryonia laciniosa* in protein metabolism.

Creatinine is a byproduct of the breakdown of creatine and phosphocreatine, which are considered as energy storage compounds in muscle. The serum creatinine concentration may vary based on a number of factors including diet composition, muscle mass and gender. Serum creatinine values also depend on the ability of the kidney to excrete creatinine. An elevation in creatinine levels in blood usually occurs simultaneously with an increase in blood urea nitrogen. Creatinine concentration is often used as a variable not only to assess impairment of kidney function but also as clinical end point to detect treatment related toxic effects of compounds on the kidney in experimental animals (Travlos et al., 1996). In the present study, oral treatment with *Bryonia laciniosa* for 21 days significantly reduced the serum creatinine levels. Therefore, it may be concluded that the early renal changes which occurred in the diabetic rats were significantly reversed by the oral administration of 70% alcoholic extract of *Bryonia laciniosa* and its n-butanol fraction. Hence, it appears to be beneficial since it provides some protection against diabetic nephropathy.

Since the 70% alcoholic extract and n-butanol fraction of *Bryonia laciniosa* showed beneficial effects in hyperglycemia and hyperlipidemia in STZ induced type 1 diabetic rats, we carried studies with extract of *Bryonia laciniosa* in STZ induced type 2 diabetic rats.

Studies in type 2 diabetes showed a significant increase in fasting glucose as well as insulin levels in diabetic control rats. This is consistent with earlier reports (Shinde et al., 2003; Vishwakarma et al., 2003). Hyperinsulinemia with low hepatic excretion and hypersecretion of beta cells are also reported in mild glucose intolerant obese subjects (Bonora et al., 1983). The high insulin concentration found in neonatal STZ-diabetic rats need not be of pancreatic origin. It could be due to metabolic
alterations at extra pancreatic levels. In these rats, the metabolic clearance rate of insulin might have been altered. Insulin degradation following hormone receptor binding (Gliemann and Sonne, 1978) and reduced binding of insulin to its receptor have been reported in mild glucose intolerance (Olefsky, 1981). Therefore, the hyperinsulinemia in neonatal STZ-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. Various studies with insulin sensitizers have shown improvement in glycemic control in conditions of diabetes associated with hyperinsulinemia. The antihyperglycemic effect by insulin sensitizers has been shown to be associated with improvement in insulin sensitivity and thereby glucose disposal by peripheral tissues (Murami et al., 1998; Shinkai, 2001; Chakrabarti et al., 2003). The agents which decrease fatty acid synthesis and reduce synthesis and secretion of triglycerides are shown to ameliorate insulin resistance by improving insulin sensitivity (Boden, 1994). In the present study, when animals were subjected to OGTT, the AUC_{glucose} of NIDDM control rats was significantly greater when compared with the non-diabetic rats. Despite high insulin levels, the AUC_{glucose} of the diabetic animals was greater than non-diabetic rats. However, AUC_{glucose} of NIDDM rats treated with *Bryonia laciniosa* was found to be significantly lower as compared to NIDDM control rats.

Our results of the OGTT study demonstrated that 70% alcoholic extract of *Bryonia laciniosa* and its n-butanol fraction have hypoglycemic activity. The significantly elevated blood glucose concentrations at 30, 60 and 120 minutes of the OGTT in diabetic rats were lowered by the administration of the 70% alcoholic extract and its n-butanol fraction. However, the concentrations of serum insulin were significantly elevated in diabetic rats, with or without extract. Although we did not determine the insulin sensitivity of the cells, the unchanged insulin profile and improved glucose response during OGTT suggest that the extract may have improved glucose tolerance via increased insulin sensitivity (Carnevale et al., 2003). In considering this, the antihyperglycemic activity of the *Bryonia laciniosa* may be due to an improved glucose uptake, insulin resistance in cells or inhibiting the glucose absorption through gastrointestinal tract. Our study suggests that the hypoglycaemic activity of *Bryonia laciniosa* is not related to the insulin secretion and that post prandial glucose reduction is probably effected without any extra load on pancreatic
Discussion

β-cells and/or stimulation of glucose uptake by peripheral tissues. Hence, the hypoglycaemic effect may be probably brought about by an extra pancreatic mechanism. There are medicinal plants, which are known to exert antidiabetic activity without the stimulation of insulin secretion (Hannan et al., 2003; Maghrani et al., 2003; Sachdewa and Khemani, 2003). In vitro studies on Hep G2 cell lines have shown that berberine is able to exert an insulin independent glucose lowering effect in hepatocytes similar to that of metformin (Yin et al., 2002).

Levels of plasma triglycerides and cholesterol in individuals with various types of diabetes are higher than those of normal subjects (Chase and Glasgow, 1976). However, the levels of lipids depend on the severity of diabetes, its therapeutic control and the composition of diet. It is known that dietary ingredients affecting glucose metabolism may also influence the lipid metabolism (Jenkins and Joose, 1995; Jayasooriya et al., 2000). Lipid profile, which is altered in the serum of diabetic patients (Betteridge, 1994) appears to be significant factor in the development of premature atherosclerosis and includes an increase in triglyceride and total cholesterol levels. In the present study, insulin-resistant STZ animals also showed abnormalities in lipid metabolism as evident from increased plasma triglycerides and plasma total cholesterol levels, as in case of human type 2 diabetic patients. This might contribute to various cardiovascular complications. In the present study, there was significant increase in serum cholesterol, triglyceride, VLDL, LDL levels while decrease in HDL levels in STZ-induced diabetic control rats. Treatment with 70 % alcoholic extract of Bryonia laciniosa significantly lowered cholesterol, triglyceride, VLDL and LDL levels but failed to improve the HDL cholesterol levels in diabetic rats. The observed hypolipidaemic effect may be because of decreased cholesterogenesis and fatty acid synthesis. Significant lowering of total cholesterol and rise in HDL cholesterol level is a desirable biochemical state for prevention of atherosclerosis and ischaemic conditions.

The prevalence of insulin resistance and associated diseases has sharply risen around the world (Yach et al., 2006). The general view of insulin action places this hormone at the point of multiple organ adaptations to the ingested nutrients, in particular, dietary carbohydrates (Bessesen, 2001). It has been established that insulin resistance, impaired glucose tolerance, hyperinsulinemia, hypertension and hyperlipidemia are associated with fructose intake in animal models (Basciano et al.,
2005; Le and Tappy, 2006). Consumption of high dietary fructose might be one of the factors responsible for the development of obesity and the accompanying insulin resistance syndrome. Thus, rats receiving fructose-rich diet for three weeks could be serve as a reliable model for the investigation of insulin resistance (Liu et al., 2005). The results of our study are consistent with those of others indicating that fructose feeding produces hyperlipidaemia, insulin resistance, and hyperinsulinemia (Reaven et al., 1990; Brands and Hall, 1992; Martinez et al., 1994; Patrick et al., 1998), and the increase in circulating triglycerides (Zavaroni et al., 1986). Hyperlipidemic rats showed significant elevated levels of cholesterol, triglyceride, LDL-cholesterol, VLDL cholesterol and increased body weight as compared to normal control rats. Treatment with 70% alcoholic extract and its n-butanol fraction of *Bryonia laciniosa* produced a significant reduction in cholesterol, triglyceride, VLDL-cholesterol, body weight and increased HDL-cholesterol as compared to hyperlipidemic control rats. The 70% alcoholic extract and n-butanol fraction also produced a significant reduction in glucose and LDL-cholesterol but failed to alter serum insulin levels to significant extent.

Insulin is the leading hormone that regulates blood glucose and fat metabolism. Insulin resistance is a condition in which the responsiveness to insulin of target tissues, namely skeletal muscle, adipose tissue, and the liver decreases despite higher insulin levels. In type 2 diabetes, the sensitivity of insulin decreases due to β-cell dysfunction, insulin receptor mutation, and obesity factors. Insulin sensitivity may also be affected by many circulating lipids, which include triglycerides and free fatty acids (Boden and Shulman, 2002). Raised plasma free fatty acids level is an important inductor of both peripheral and hepatic insulin resistance because it inhibits insulin signaling. In addition, hypertriglyceridemia is also an important marker of insulin resistance (Steiner, 1994). In our study, insulin resistance developed by providing fructose as dietary supplement for three weeks. However, treatment with 70% alcoholic extract and its n-butanol fraction of *Bryonia laciniosa* failed to ameliorate insulin resistance to a significant extent.

In the present study, oral administration of the plant extract and its n-butanol fraction was found to reduce the serum glucose concentration of fructose-rich chow-fed rats in a dose-dependent manner, showing the beneficial action of *Bryonia laciniosa* in rats with insulin resistance. However, our study has shown that, the
treatment with the 70% alcoholic extract and its n-butanol fraction does not alter serum insulin levels in fructose-induced insulin resistant rats.

The main causative factor for atherothrombotic diseases is the disturbances occurring in lipid metabolism. Despite the presence of different hypolipidaemic drugs in the market, their therapeutic application is usually associated with severe side effects (Ghatak and Asthana, 1995). Cholesterol is an essential structural element of the biological membranes. In addition, it is the precursor of many compounds such as the starting material for the synthesis of bile acids, steroid hormones, and vitamin D among others. However, high concentration of serum cholesterol increases the risk of developing coronary heart disease (CHD) (Libby et al., 2000). The results of present study have demonstrated that rats fed a high-fat diet shows higher concentration of serum cholesterol as compared to rats fed a standard laboratory diet. Oral administration of 70% alcoholic extract of *Bryonia laciniosa* reduced the high level of serum cholesterol. It has already been reported that low plasma triglyceride levels are associated with lower risks of CHD (Davignon and Cohn, 1996). In our study, the administration of the extract to hyperlipidaemic rats significantly lowered their serum triglyceride levels. This reduction might be related to decreased triglyceride absorption from the gastrointestinal tract (Shukla et al., 2004). It is widely accepted that elevation of serum LDL levels is major risk factor for CHD. Direct correlation between LDL and atherosclerosis and also the reversibility of the related pathological events by lowering the serum level of LDL have already been reported by many research groups (Bbrliner and Hetnecke, 1996; Ross, 1999). Our data suggest that, high concentrations of LDL-C in hypercholesterolaemic rats are significantly reduced by oral administration of the extract and its n-butanol fraction. Therefore, seeds of *Bryonia laciniosa* might prove to be useful for the treatment of atherosclerosis by lowering serum LDL-C level. Another risk factor for developing atherosclerosis is the reduced serum levels of HDL-C. The effect of HDL is largely attributed to its central function in the reverse cholesterol transport, a process whereby excess cell cholesterol is taken up and processed by HDL particles for further delivery to the liver for metabolism (Martinez et al., 2004). Therefore, it is logical that an increase in HDL level can contribute to lower risk of atherosclerosis (Nofer et al., 2002). Our results show that the 70% alcoholic extract and its n-butanol fraction are capable of increasing the serum level of good cholesterol (HDL-C) in the rats. Atherosclerotic
index (AI) is believed to be one of the risk factors for development of atherosclerosis. Our data suggest that the 70% alcoholic extract and its n-butanol fraction are capable of decreasing this risk factor after 10 days of drug treatment. The decrease in cholesterol balance, which indicates the total change in body pools of cholesterol, may be due to the compensatory mechanisms, such as a decrease in resorption of endogenous cholesterol or an increase in the rate of secretion into intestinal tract or both.

Free radical-induced oxidative stress has been implicated in the pathogenesis of a variety of clinical disorders resulting usually from deficient natural antioxidant defenses. Potential antioxidant therapy therefore, should include either natural free radical scavenging antioxidant enzymes or agents which are capable of augmenting the activity of these enzymes. Reactive oxygen species (ROS) have received considerable attention in the recent past because of their role in several pathological conditions including cancer, aging, neurodegenerative disorders and atherosclerosis. Since, human disease is believed to be due to the imbalance between oxidative stress and antioxidative defense, it is possible to limit oxidative tissue damage and hence prevent the progression of disease by antioxidant defense supplement.

Oxidative stress has been shown to play a role in the causation of diabetes 1 and 2 and as such, antioxidants may have a role in the alleviation of diabetes (Baynes, 1991). Oxidative stress is produced under diabetic condition and it is likely involved in progression of pancreatic β-cell dysfunction (Kajimoto and Kaneto, 2004). Also, because of the relatively low expression of antioxidant enzymes such as catalase and superoxide dismutase, pancreatic β-cells may be vulnerable to ROS attack when the system is under oxidative stress (Lenzen et al., 1996; Tiedge et al., 1997). Similarly, elevated levels of free radicals, due to insufficiency of the antioxidant defense system by glycosylation, may lead to disruption of cellular function and oxidative damages to membranes, and also enhance their susceptibility to lipid peroxidation (Baynes, 1991). Streptozotocin impairs the oxidation of glucose and thus insulin synthesis and its secretion by causing damage to β-cells of pancreas (Szkudelski, 2001). Streptozotocin-induced hyperglycemia induces free radical generation which thereby leads to DNA damage, protein degradation, lipid peroxidation and finally damage to various organs of the body like liver, kidney, brain and eyes. These elevated free radicals and depressed antioxidant defense may lead to cell disruption and oxidative
damage to the cell membranes and may hence increase the susceptibility to lipid peroxidation. Therefore, if excessive generation of free radicals like \( \text{O}_2^- \), \( \text{OH}^+ \) can be checked, protection from free radical-induced damage can probably be accorded. It is believed that plants with antioxidant property can prevent or protect tissues against damaging effect of free radicals (Yazdanparast et al., 2007). In the present study, alterations in the antioxidant levels in liver tissue due to diabetes were restored to a significant extent on treatment with the extract of *Bryonia laciniosa*. This effect may be due to strong antioxidant and stimulatory action on \( \beta \)-cells of Langerhans in pancreas that could contribute to the antihyperglycemic action of the extract (Yazdanparast et al., 2007).

In the present study, reduction in the activity of antioxidant enzymes (SOD, CAT) and reduced glutathione (GSH) in the liver of diabetic rats supports the view expressed earlier by various authors (Anuradha and Selvam, 1993; Stanely et al., 2001; Rajasekaran S et al., 2005). The decrease in antioxidant enzyme activity in liver may be due to the activation or inhibition of the enzymes by increased production of free radicals during diabetes. Liver is considered to be more susceptible to oxidative stress as it consumes high amount of oxygen, has high level of polyunsaturated fatty acids and low level of antioxidant enzymes (Ugochukwu and Babady, 2002). Thus, increase in the antioxidant enzyme level could be a part of compensatory mechanism against oxidative stress and/or it may be a result of over expression of antioxidant genes (Solomon et al., 2002). GSH and catalase activity in tissues of diabetic rats was found to be reduced which could be due to the increased endogenous production of superoxide anions. \( \text{H}_2\text{O}_2 \) is toxic and can react with \( \text{O}_2^- \) in the presence of metal ions to form \( \text{OH}^+ \) that will result in higher levels of malondialdehyde (MDA) (Prakasam et al., 2005). High levels of unscavenged free radicals result in oxidative deterioration of polyunsaturated lipids leading to MDA formation. These products may be important in pathogenesis of vascular complications of diabetes (Haliwell, 2000). Increased peroxidative damage reported in diabetic rats disturbs the membrane function by affecting the membrane fluidity and changing the activity of membrane bound enzymes and receptors (Xiang and Benny, 2000). Liver contains low levels of vitamin E and selenium dependent glutathione peroxidase activity and hence the liver is more prone to lipid peroxidation (Rajasekaran et al., 2005). Difference in lipid peroxidation in liver due to oxidative stress is region specific. In the present study, increase in the
level of MDA in diabetic rats and restoration on treatment with the extract in liver was in accordance with some recent reports (Anuradha and Selvam, 1993; Stanely et al., 2001; Rajasekaran et al., 2005).

Reduced glutathione (GSH), a non-enzymatic antioxidant is known to accord protection against reactive O$_2$ species by effectively scavenging free radicals and other ROS directly and indirectly through enzymatic reactions. Deficiency of GSH may lead to various complications like neuropathy, myopathy and cataract in diabetic rats (Meister, 1991; Amr et al., 2006). Decrease in GSH content the liver tissue of diabetic rats can also be due to NADPH depletion or its increased utilization in removal of peroxides produced due to oxidative stress (Xiang and Benny, 2000; Kamalakkannan and Stanely, 2006). The decrease in GSH may be due to low GPx activity in diabetic tissues, as GSH is a cofactor and also a substrate of this enzyme. The elevated levels of GSH by the extract may protect many cellular proteins against oxidation through glutathione redox cycle and may detoxify the generated free radicals (Latha and Pari, 2003b).

In the present study, the reversal of reduction in the antioxidant enzyme and peroxidative damage in tissues by 70% alcoholic extract and its n-butanol fraction suggests antioxidant and anti-peroxidative property of the plant and hence reveals the potential of the plant to play some role in defense against free radicals. These activities on treatment with the extract were comparable to the standard drugs, glipizide and insulin. Our results confirm that *Bryonia laciniosa* could be responsible for the restoration of metabolic activities and according protection against streptozotocin in diabetic rats. The mechanism could be related to scavenging activity. However, possible involvement of other mechanisms cannot be ruled out at this stage. Thus, *Bryonia laciniosa* appears to have potential at least as adjunct therapy to possibly inhibit the complications due to diabetes.

For the measurement of the reductive ability, we investigated the Fe$^{3+}$ - Fe$^{2+}$ transformation in the presence of the 70% alcoholic extract of *Bryonia laciniosa* using the method of Athukorala (2006). The reducing capacity of the 70% alcoholic extract of *Bryonia laciniosa* compound may serve as a significant indicator of its potential antioxidant activity. Like the antioxidant activity, the reducing activity of the extracts increased with increasing concentrations. The antioxidant activity has been attributed to various mechanisms, among which are the prevention of chain initiation, the
binding of transition metal ion catalysts, decomposition of peroxides and the prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Jianxiong, 2008).

The effect of antioxidant on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts electron of hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals is determined by the decrease in its absorbance at 517 nm induced by the antioxidant. It is visually noticeable as a discolouration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. In our study, a significant decrease in the concentration of DPPH radical was observed due to the scavenging ability of Bryonia laciniosa and standard (ascorbic acid). Free radical scavenging activity was also increased by increasing the concentration of the extract.

Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but is an intracellular precursor of hydroxyl radical which is toxic to the cell (Jianxiong, 2008). Thus, scavenging of H₂O₂ is taken as a measure of the antioxidant activity. The 70% alcoholic extract of Bryonia laciniosa scavenged hydrogen peroxide. This may be attributed to the presence of saponins that could donate electrons to hydrogen peroxide, thereby converting it into water.

In vitro inhibition of nitric oxide radical is a measure of antioxidant activity of plant drugs. Nitric oxide is a free radical which plays an important role in the pathogenesis of pain, inflammation, etc. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent (Marcocci et al., 1994). The absorbance of the chromophore is measured at 546 nm. The 70% alcoholic extract of Bryonia laciniosa decreased the amount of nitrite generated from the decomposition of sodium nitroprusside in vitro. This may be due to the antioxidant principles in the extract which compete with oxygen to react with NO thereby inhibiting the generation of nitrite.

In the β-carotene bleaching assay, linoleic acid produces hydroperoxides as free radicals during incubation at 50 °C. The presence of antioxidants in the extract may possess ability to minimize the oxidation of β-carotene by hydroperoxides. In the present study, we evaluated the antioxidant activity of the 70% alcoholic extract of
Discussion

*Bryonia laciniosa* by the β-carotene–linoleate bleaching method as β-carotene shows strong biological activity and is a physiologically important compound (Sarkar et al., 1995; Kumazawa et al., 2002; Sakanaka et al., 2005). Thus, the degradation rate of β-carotene–linoleate depends on the antioxidant activity. There is a correlation between degradation rate and the bleaching of β-carotene. The substance with the lowest β-carotene degradation rate exhibits the highest antioxidant activity. In the present study, 70% alcoholic extract of *Bryonia laciniosa* showed lower antioxidant activity than ascorbic acid. Linoleic acid hydroperoxides attack the β-carotene molecule and, as a result they undergo rapid decolourisation. The corresponding decrease in absorbance was monitored spectrophotometrically. The presence of antioxidant principles in the extracts may hinder the extent of β-carotene bleaching by acting on the free radicals formed in the system (Jayaprakasha et al., 2001; Suja et al., 2005).

Phytochemicals, especially the saponins and bitter principles found in medicinal plants have been proposed as the major bioactive compounds providing the health benefits associated with diets rich in plant-foods. Saponins are class of secondary plant metabolites found ubiquitously which acts as pharmacologically active compounds in many medicinal plants (Waller and Yamasaki, 1996). Many biological actions of saponins and bitter principles have been attributed to their powerful antidiabetic and antihyperlipidemic properties (Ajabnoor, 1990; Oishi, 2007; Firdous et al., 2009; Koneri and Balraman, 2008).

In our study, alleviation of diabetes and hyperlipidemia was observed in the rats treated with *Bryonia laciniosa* extract. We have confirmed the presence of saponins and bitter principles in 70% alcoholic extract and n-butanol fraction by performing HPLTC fingerprinting analysis. Because of the presence of saponins and bitter principles in the extract, our results suggest that the antidiabetic and antihyperlipidemic activities of this plant could be attributed to the presence of the saponins and bitter principles. It might be possible that these active constituents reverse the complications associated with hyperglycemia and hyperlipidaemia.

In the light of above discussion, it is suggested that prolonged exposure to the 70% alcoholic extract is well tolerated, as shown by the normal control group of rats treated for 3 weeks, with no change in any of the markers assayed, compared with normal control. Taken together, the results show that 70% alcoholic extract and its n-butanol fraction is a safe treatment for diabetes mellitus and hyperlipidemia that can
prevent marked changes in metabolic parameters implicated in the etiology metabolic syndrome. All these results indicate that 70% alcoholic extract and its n-butanol fraction do not induce any regeneration of pancreatic β cells. The extract and its fraction might be acting through increasing insulin sensitivity. Although no report is available, other possible mechanism for the antihyperglycemic activity of the plant might be through delaying or inhibiting glucose absorption at intestinal level. The antidiabetic and antihyperlipidemic actions of *Bryonia laciniosa* may be related to its antioxidant effect and future studies are needed to determine the mechanism of these effects.