CHAPTER – V

ANTIHYPERLIPIDEMIC ACTIVITY OF

*Pimenta dioica* METHANOLIC LEAF EXTRACT IN

STZ – INDUCED DIABETIC RATS

5.1 INTRODUCTION

Diabetes mellitus is described as a glucose imbalance and impaired insulin secretion or action (WHO, 1985) and the diabetic condition damages vital organs like the eyes, kidneys, nerves, heart and blood vessels. Diabetes mellitus is the main disorder which causes most of the disabilities and death in the world (Ramesh, 2007). Sedentary life style, changing food habits, stress, obesity (Nagappa, 2003), genetical alterations and environmental factors are the main causes for diabetes mellitus. Diabetes is recognized as one of the leading causes of morbidity and mortality in the world.

Currently available antidiabetic drugs are associated with numerous side effects. Although a wide range of synthetic drugs is available for diabetes, most of them do not execute all requirement and their numerous side effects and potential interferences with drug metabolism are common. Thus, a screening of medicinal herbs is very important and may provide an useful source for new therapeutic and/or alternative simple dietary adjuncts to currently available
therapy. It has been reported that traditional systems have immune potential against various diseases (Sindhu, 2011).

Diabetes affects about 5% of the global population (Chakraborty and Rajagopalan, 2002) and management of diabetes without any side effects is still a challenge to the medical system (Kameswara Rao et al., 2003). Type II diabetes accounts for 90–95% of all diabetic cases (Harris, 1987). Long-term complications such as cardiomyopathy, angiopathy, nephropathy, etc. are the major causes of morbidity in patients with diabetes mellitus. Hyperlipidemia and oxidative stress frequently co-exist with diabetes mellitus (Dennery, 2006). For many decades, plants are being used worldwide as sources of treatment for different health problems. The indigenous knowledge on medicinal plants and their uses are currently gaining recognition worldwide.

Medicinal plants possess various physiologically active phytochemicals and have been exploited for years in traditional medical practice for the treatment of various ailments (Adebanjo, 1983). Four thousand years ago, the medical intelligence of the Indian subcontinent was termed as Ayurveda. The literal meaning of Ayurveda is “science of life,” which remains an imperative system of medicine and drug therapy in India. The diseases evolved in the body due to external factors were reported in Ayurveda medicine. It also
covered all aspects of diseases, pharmacy and therapeutics in Sanskrit literature (Ramar, 2008). More than thirteen thousand plants have been studied for various pharmacological properties. The major merits of herbal medicines seem to be their efficacy, low incidence of side effects and low cost (Haldar, 2010).

*Pimenta dioica* (L.) Merril (Fam: Myrtaceae) is commonly known as Allspice in culinary. It takes its name from the aroma of dried berries, which smells like the combination of spices, especially cinnamon, cloves, ginger and nutmeg. Allspice owes its characteristic odour due to the presence of essential oil in the pericarp of the seeds. The plant allspice is mentioned in the Wealth of India (Wealth of India 1969). The essential oil obtained from the berries of *Pimenta dioica* has been reported to contain the following components such as, limonene, 1,8 cineole, terpinolene, β-caryophyllene, β-selinene and methyl eugenol. The Jamaican Pimento leaf oil contains eugenol, methyl eugenol, myrcene and β-caryophyllene (Tucker et al., 1991). The therapeutic properties of the allspice berry oils are anesthetic, analgesic, antimicrobial, antioxidant, antiseptic, acaricidal and also as carminative, muscle relaxant, rubefacient, stimulant and tonic. Pimento oil can be helpful for the digestive system, forcramp, flatulence, indigestion and nausea. Further, the essential oil can help in cases of
depression, nervous exhaustion, tension, neuralgia and stress and is also used as a natural repellent. The essential *P.dioica* leaf and fruit oil is also used in perfumes, aftershaves and commercial food flavoring (Seidemann, 2005; Sharma, 2003). Although there are known synthetic antidiabetic medicines available in the pharmaceutical market, diabetes and its related complications are continued to be a major medical problem. Various medicinal plants have been reported to be very useful in treating diabetes worldwide and have been used as antidiabetic as well as antihyperlipidemic remedies (Mitra et al, 1996).

Diabetes mellitus is commonly linked with abnormalities such as lipid metabolism, dyslipidemia, hyperlipidemia and alterations in lipoprotein composition (Umesh et al., 2004; Sophia and Manoharan, 2007). Hyperglycemia is a symptom of diabetes mellitus which causes glycation of proteins that leads to functional and morphological changes in eyes, kidneys, nerves and arteries (Kameswara Rao, 1999). The chronic hyperglycemic condition of diabetes is linked to long term damages and complications such as dysfunction and failure of various organs (Babu, 2003). In diabetic rats, the impaired carbohydrate metabolism leads to accelerated lipolysis and resulting in hyperlipidemia (Bajpay, 1993). Diabetes mellitus is well known to cause hyperlipidemia, via. various metabolic dysregulations. Amongst
several metabolic dysregulations, insulin deficiency is primarily known to stimulate lipolysis in the adipose tissue and lead to hyperlipidemia as well as fatty liver. Thus, in diabetes hypercholesterolemia and hypertriglyceridemia are very common occurring abnormalities (Hou, 2005). Dyslipidemia [elevated plasma levels of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and triglycerides (TG) and a low concentration of high-density lipoprotein-cholesterol (HDL-C)] is one of the most common complications of DM. It plays a major role in the development of atherosclerosis, coronary insufficiency as well as myocardial infarction (Tang, 2004). Lipid profile abnormalities in diabetes are mediated through derangements in a variety of regulatory processes, especially insulin deficiency, thereby rendering diabetic patients more prone to hypercholesterolemia and hypertriglyceridemia. (Nesto, 2005)

Impairment in insulin sensitization because of high concentration of lipids in the cells may be regarded as the prime risk leading to elevated cardiovascular risk in diabetes mellitus patients (Krishna Kumar et al., 2000; Mironava et al., 2000). The liver is responsible for the oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids as well as in the secretion of specific classes of plasma lipoproteins. Erythrocyte membranes as
well as liver cells showed alterations in the level of lipids in diabetes (Bhandaru et al., 1982; Pari and Latha, 2002). Membrane fluidity is known to be dependent on the molar ratio of cholesterol to phospholipids (Kolanjiappan et al., 2002).

Cholesterol enrichment in red cells resulting in the loss of membrane fluidity has been reported (Cooper, 1977). Erythrocyte membrane composition (total cholesterol, phospholipids) is altered both in hyperglycemic and hyperlipidemic conditions and may provide an useful model for evaluating lipid carbohydrate abnormalities of membrane structures in diabetes mellitus (Bhandaru et al., 1982).

Liver has an important role in glucose metabolism and as a consequence of increased glucose and insulin deficiency in plasma, hepatic regulation of lipid metabolism is greatly altered. The liver is the major organ that can catabolize and excrete quantitatively significant amounts of cholesterol. Insulin administration to diabetic rats normalized the lipid and lipoprotein patterns (Pathak et al., 1981). High concentration of cholesterol in plasma and liver in diabetes are mainly due to the defect in insulin secretion and function. Accumulation of triglycerides in diabetic liver is due to increased synthesis or decreased output from liver as VLDL or combination of both (Mutlu et al., 2003). Hyperlipidemia is one of the major
cardiovascular risk factors. It has been demonstrated that insulin
deficiency in diabetes mellitus leads to a variety of derangements in
metabolic and regulatory processes, which in turn leads to the
accumulation of lipids such as TG and TC in diabetic patients
(Goldberg, 1981).

Apart from the regulation of carbohydrate metabolism, insulin
also plays an important role in the metabolism of lipids. Insulin is a
potent inhibitor of lipolysis since it inhibits the activity of the hormone
sensitive lipases in adipose tissue and suppresses the release of free
fatty acids (Gilman, 1990). *Enicostemma litorale, Azadirachta indica, Tinospora cordifolia* and *Curcuma longa* are also reported to produce
antihyperlipidemic activity in various animal experiments. The
possible mechanism for the decreased lipid levels could be either
insulin releasing effect or insulin sensitizing activity, because insulin
has been proved to inhibit the activity of the hormone sensitive lipases
in adipose tissue and suppress the release of lipids. The HDL-
cholesterol is involved in the transport of cholesterol from peripheral
tissues to liver and thereby it acts as a protective factor.

Hyperlipidemia is the metabolic complication of both clinical
and experimental diabetes (Solano and Goldberg, 2006). Impairment of
the biological action of insulin at the cellular level is believed to be a
cardinal and possibly primary underlying metabolic defect in the development of the characteristic dyslipidemia observed in type 2 diabetes. The key components of diabetic dyslipidemia are elevated plasma low density lipoproteins (LDL), very low-density lipoproteins (VLDL), triglycerides (TGs), circulating free fatty acids (FFAs) and lowered high density lipoprotein – cholesterol (HDL-C) (Ansari et al., 2011).

Lipoproteins are an independent risk factor for the development of atherosclerotic disease. In the earlier studies an increase in the plasma LDL and VLDL fractions along with a decrease in HDL were observed in STZ–induced diabetic rats. This increased LDL concentration in the plasma of diabetic rats might be due to the defect in insulin secretion. In particular, many studies have found LDL to be the most dangerous among the plasma lipids and the oxidation of LDL leads to its increased penetration of arterial walls (Arcari et al., 2011). When there is excess LDL in the blood it may accumulate in the extracellular sub endothelial space of arteries and are highly atherogenic and toxic to vascular cells thereby leading to atherosclerosis, hypertension, obesity, functional depression in some organs, etc. (Schneider et al., 2011). HDL carries cholesterol and CE from the peripheral tissues and cells to the liver, where cholesterol is
metabolized into bile acids (Singh et al., 2007). This pathway plays a very important role in reducing the cholesterol levels in the blood and peripheral tissues and in inhibiting atherosclerotic plaque formation in the aorta. Low level of plasma HDL has been observed in adults with type 2 diabetes mellitus (Garvey et al., 2003).

Cellular cholesterol homeostasis is very important for the prevention of cardiovascular disease. The degree of hypercholesterolemia is directly proportional to the severity of diabetes. The increase of blood glucose is accompanied by a rise in TC, TGs and a fall in HDL-C (Chen et al., 2011). In diabetes, since blood glucose is not utilized by the tissues, the fatty acid from adipose tissue are mobilized for energy purpose and excess fatty acids are accumulated in the liver and converted to TGs. Cholesterol levels were increased due to decreased cholesterol absorption and increased cholesterol biosynthesis (Kim et al., 2008). Hyperlipidemia contributes significantly in the manifestation and development of atherosclerosis and coronary heart diseases (CHD).

Atherosclerosis is the most common cause of mortality and morbidity worldwide. Although several factors, such as diets high in saturated fats and cholesterol, age, family history, hypertension and life
style play a significant role in causing heart failure, the high levels of cholesterol particularly TC, TG and LDL cholesterol is mainly responsible for the onset of CHDs. 20% reduction of blood cholesterol level can decrease about 31% of CHD incidence and 33% of its mortality rate (Zamani Marzyieh et al., 2007). The underlying process leading to these disorders is the development of atherosclerosis which is characterized by endothelial dysfunction, vascular inflammation and the progressive accumulation of lipids, cholesterol, calcium and cellular debris in the intima of the vessel wall. The elevation of lipids in plasma leads to the deposition of lipids especially cholesterol on the arterial walls, subcutaneous tissues, tendons and cornea. The important manifestation of hyperlipidemia is due to the accumulation of lipids on arterial walls and resultant pathological changes leading to atherosclerosis (Vasudevan and Sreekumari, 1995).

Obesity contributes to the development of insulin resistance which may underlie a number of manifestations and cardiovascular complications of diabetes and metabolic syndromes (Reaven, 1988). Abdominal obesity involves in insulin resistance, a metabolic abnormality linked to the development of type 2 diabetes mellitus and cardiovascular disease (CVD). Insulin resistance is associated with increased cardiovascular risk and early intervention to treat insulin
resistance, an important preventive health strategy (Daniel, 2007). Most of the herbal medicines are having potential glucose lowering activity and lipid lowering effect. In this context, the present study was aimed to investigate the efficacy of the methanolic leaf extract of *Pimenta dioica* on the serum lipid profile in streptozotocin induced diabetic rats.

### 5.2 MATERIALS AND METHODS

#### 5.2.1 Extraction of lipids

The method of Folch *et al.*, (1957) was adopted for lipid extraction. A known volume of tissue homogenate was homogenized with three to four times of cold methanol extracts and were filtered in whatman No. 1 filter paper and the residue on the filter paper was scrapped off and then it was homogenized in chloroform with twice the volume of methanol. The residue was again homogenized in chloroform methanol mixture (2:1 v/v) and the extract was filtered. The lower chloroform layer containing lipids was evaporated to dryness and finally suspended in a known volume of chloroform-methanol mixture (2:1 v/v). To the lipid extract, 1 ml of 0.1 M potassium chloride was added, shaken well and centrifuged. The chloroform layer obtained was mixed with chloroform: methanol:
potassium mixture (10:10:1 v/v). The washed layer was made upto known volume and aliquots were used for the analysis of lipids.

5.2.2 Total cholesterol

Total cholesterol was estimated by the method of Parekh and Jung (1970).

Reagents

1. Ferric chloride-uranyl acetate:

10 ml of water and 3 ml of concentrated ammonia was added to 500 mg of ferric chloride. The precipitate obtained was washed several times with distilled water, dissolved in glacial acetic acid and made upto 1 litre with acetic acid. 100 mg of uranyl acetate was then added and the contents were shaken well and left over night in a brown bottle.

2. Ferrous sulphate and sulphuric acid reagent:

100 mg of anhydrous ferrous sulphate in 100 ml of glacial acetic acid was made upto 1 litre with concentrated sulphuric acid.

3. Cholesterol standard: 2mg/ml chloroform.

Procedure

A known volume of the serum/lipid extract was added to 10 ml of ferric acetate-uranyl acetate reagent. The mixture was kept for 5 minutes and centrifuged at 3000 rpm for 5 minutes and 2 ml of ferrous
sulphate-sulphuric acid reagent was added to 3.0 ml aliquot of the supernatant. After 20 minutes, the color was read at 540 nm along with a series of standard cholesterol solutions (925-100 µg) and a blank containing the reagent processed in a similar manner. Values were expressed as mg/dl for serum.

5.2.3 Triglycerides

Triglycerides were estimated by the method of Foster and Dunn (1973). The triglycerides were extracted by isopropanol which upon saponification with potassium hydroxide, yields glycerol and soap. The glycerol liberated is treated with metaperiodate which release formaldehyde, formic acid and iodide. The formaldehyde released reacts with acetyl acetone and ammonia forming yellow colored compound which was read in a spectronic 20 at 45 nm.

Reagents

1. Isopropanol

2. Activated alumina (neutral)

3. Saponification agent: 5.0 g of potassium hydroxide was dissolved in 60 ml distilled water and 40 ml isopropanol was added to it.

4. Acetyl acetone reagent: 0.75 ml of acetyl was dissolved in 60 ml distilled water and 40 ml isopropanol was added to it.
5. Sodium metaperiodate reagent: 77 g of anhydrous ammonium acetate was dissolved in about 700 ml of distilled water and 60 ml of glacial acetic acid was added to it followed by 650 mg sodium metaperiodate. The mixture was dissolved and diluted to one litre with distilled water.

6. Standard solution of triolein: 1.0 g of triolein was dissolved in 100 ml of isopropanol. 1.0 ml of the stock standard was diluted to 100 ml to prepare working standard containing 100 µg of triolein/ml.

**Procedure**

To an aliquot of serum/lipid extract evaporated to dryness, 0.1 ml methonal was added followed by 4.0 ml isopropanol, 4.0 g of alumina to all the tubes and shaken well for 15 minutes, centrifuged and then 2 ml of the supernatant fluid was transferred to appropriately labeled tubes. The tubes were placed in a water bath at 65 °C for about 15 minutes for saponification after adding 0.6 ml of the saponification reagent followed by 0.5 ml of acetyl acetone reagent. After mixing, the tubes were kept in a water bath at 65 °C for an hour. The contents were cooled and the absorbance was read in a spectronic 20 at 420 nm. A series of standard concentrations from 8 to 40 µg triolein were treated similarly along with a blank containing only the reagent. All the tubes
were cooled and read in a spectronic 20 at 405 nm. The triglyceride content was expressed as mg/dl for serum.

5.2.4 HDL

The HDL cholesterol was estimated by the heparin-manganese chloride precipitation method of Gidez and Webb (1950).

Reagents

1. Heparin
2. Manganese chloride
3. Heparin-manganese chloride: 3.1167 g of manganese chloride and 1.0 ml of heparin were made upto 8.0 ml with distilled water.

Procedure

To 1.0 ml of serum, 0.18 ml of heparin manganese chloride reagent was added and mixed. After standing in an ice bath for 30 minutes, the contents were centrifuged at 2500 rpm for 30 minutes to get the HDL fraction. An aliquot of the HDL supernatant was used for cholesterol estimation by the method of Parekh and Jung (1970). HDL cholesterol was expressed as mg/dl serum.
5.2.5 VLDL AND LDL

VLDL and LDL was calculated by using the formula of Friedewald et al., (1972).

VLDL=TG/5 and

LDL was calculated by using the formula,

LDL= Total Cholesterol- (HDL + VLDL).

Statistical Analysis

All data are expressed as mean ± S.E. Statistical analysis was performed using one-way ANOVA followed by Tukey’s multiple tests using SPSS (version 18) computer software. In all cases, P-value of less than 0.05 was considered to be significant.

5.3 RESULTS

To evaluate the effects of the methanolic extracts of *Pimenta dioica* leaves on the serum lipid profile in the STZ-induced diabetic rats, the leaf extracts were administered to the diabetic rats apart from the standard antidiabetic drug, glibenclamide. The serum lipid profile was analysed in the control and the diabetic rats and are exhibited in Table- 3. The total cholesterol levels of the diabetic control rats showed an abnormally high concentration (140.33±1.20 mg/dl) due to diabetic induction. However, the administration of the two doses of the leaf extracts of *P.dioica* revealed drastic reduction in the level of total
cholesterol. A prominent reduction (104.18±2.31 mg/dl) was observed in the higher dose of treatment ie.150 mg/kg bw. The standard drug glibenclamide also showed a decline in the total cholesterol level.

Table. 3. Effect of methanolic leaf extract of *P. dioica* on the serum lipid profile (mg/dl) in the normal, STZ induced diabetic and treated rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Total Cholesterol</th>
<th>Triglycerides</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
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<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>86.34±2.10&lt;sup&gt;e&lt;/sup&gt;</td>
<td>69.30±0.41&lt;sup&gt;e&lt;/sup&gt;</td>
<td>36.90±1.10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>39.53±2.10&lt;sup&gt;e&lt;/sup&gt;</td>
<td>17.20±1.45&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>140.33±1.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>132.20±1.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.96±0.85&lt;sup&gt;e&lt;/sup&gt;</td>
<td>94.50±3.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.90±2.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>Diabetic + <em>P. dioica</em>&lt;br&gt;(75mg/kg b.w)</td>
<td>112.14±2.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.32±3.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.18±1.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>46.69±3.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.50±2.27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic + <em>P. dioica</em>&lt;br&gt;(150mg/kg b.w)</td>
<td>104.18±2.31&lt;sup&gt;d&lt;/sup&gt;</td>
<td>90.68±2.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>49.26±1.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>49.43±1.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.90±2.30&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td>Diabetic + glibenclamide&lt;br&gt;(0.6mg/kg b.w)</td>
<td>110.10±3.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>89.58±1.31&lt;sup&gt;d&lt;/sup&gt;</td>
<td>39.25±1.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>42.50±2.90&lt;sup&gt;d&lt;/sup&gt;</td>
<td>21.67±1.89&lt;sup&gt;b&lt;/sup&gt;</td>
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Values are expressed as mean ± S.E (n=6), significantly different at P<0.05 when compared with control group.
Fig. 12. Effect of *P. dioica* leaf extract on the total cholesterol level in the normal, diabetic induced and treated rats

A two-fold elevation in the level of triglyceride (132.20±1.20 mg/dl) was found in the diabetic control rats when compared with that of the normal control rats (69.30±0.41 mg/dl). Supplementation of *P. dioica* at the doses of 75 and 150 mg/kg b.w to the STZ-induced diabetic rats resulted in significant diminution of the triglycerides level, i.e. 92.32±3.20 mg/dl and 90.68±2.20 mg/dl, respectively and the triglyceride level was restored to near normal level. The drug glibenlamide also markedly reduced the level of triglycerides in the diabetic animals (89.58±1.31 mg/dl). A prominent decline in triglycerides was observed during the 4th week of treatment.
**Fig.13. Effect of P. dioica leaf extract on the triglycerides level in the normal, diabetic induced and treated rats**

The low density lipoprotein cholesterol (LDL) also revealed a similar trend like that of triglycerides in which the diabetic control rats showed a multifold increase in the level of LDL cholesterol (94.50 ±3.20mg/dl) after diabetic induction by STZ when compared with that of normal control rats (39.53± 2.10mg/dl). Administration of *P.dioica* at 75 and 150 mg/kg b.w led to a drastic decline in the level of LDL as 46.59±3.21 and 49.43 ± 1.90mg/dl respectively. The drug glibenclamide reduced the LDL cholesterol level to near normal level.
Fig.14. Effect of *P. dioica* leaf extract on the low density lipoprotein level in the normal, diabetic induced and treated rats

The level of very low density lipoprotein cholesterol (VLDL) was at its minimum (17.20±1.45 mg/dl) in the normal control rats whereas it shoot up remarkably (37.90±2.32 mg/dl) after the induction of diabetes by STZ. However, the above parameter decreased significantly in the *P. dioica* leaf extract supplemented groups of about 20.50±2.27 mg/dl at 75 mg/kg b.w dosage and 18.90±2.30 mg/dl at 150 mg/kg b.w dosage respectively when compared with that of the diabetic rats and were almost near control level. The standard antidiabetic drug, glibenclamide also showed a marked decline in the VLDL level in the diabetic induced animals, ie. (21.67±1.89 mg/dl).
Fig. 15. Effect of *P. dioica* leaf extract on the very low density lipoprotein level in the normal, diabetic induced and treated rats

Unlike all the above studied parameters, the high density lipoprotein cholesterol (HDL), the beneficial lipoprotein, was decreased in the diabetic induced group (30.96±0.85mg/dl) with respect to the normal control (36.90± 1.10 mg/dl) group. However the methanolic leaf extract of *P. dioica* significantly elevated the HDL level in both the treatments (43.18±1.32 and 49.26 ±1.38mg/dl) respectively which were markedly higher than that of the normal control animals. A similar elevation in the level of HDL of about 39.25 ±1.28mg/dl was attained after treating the STZ- induced diabetic rats with the drug glibenclamide.
Fig.16. Effect of *P. dioica* leaf extract on the high density lipoprotein level in the normal, diabetic induced and treated rats

The overall results of the present study revealed a significant increase in serum total cholesterol, triglycerides, low density lipoproteins and very low density lipoproteins while a decrease in the high density lipoproteins in the diabetic control animals.

The methanolic extract of *P. dioica* treatment to the diabetic induced rats showed a significant decrease in total cholesterol, triglycerides, low density lipoprotein and very low density lipoprotein whereas an increase in the high density lipoprotein. Standard drug glibenclamide also exhibited a similar trend as that of the extract dosages. However the antihyperlipidemic effect of *P. dioica* was found to be more potent than the standard drug.
5.4 DISCUSSION

In diabetes mellitus, a primary derangement in carbohydrate metabolism along with metabolic alterations in lipid contribute to a great extent to the development of secondary complications (Truel et al., 1980), affecting the various organs. Thus the dietary management should not only aim at normalizing blood sugar level, but also normalizing serum lipid levels. Besides the food rich in dietary fiber, other plant foods such as spices and condiments (Sharma, 1996) as well as other medicinal plants exhibit beneficial effects in reducing hyperlipidemia.

Cardiovascular diseases are listed as the cause of death in 65% of people suffering from diabetes (Geiss et al., 1995). Since lipid abnormalities accompanied with premature atherosclerosis are the major causes of cardiovascular disease in diabetic patients, an ideal treatment for diabetic, in addition to glycemic control, should seek to achieve a favorable lipid profile. There was a significant decrease in TC, TG, LDL and VLDL levels as well as an enhancement in the level of HDL in diabetic rats following extract treatment. Increase in HDL-cholesterol is associated with a decrease in coronary risk and most of the drugs that decrease total cholesterol also decrease HDL-cholesterol (Wilson, 1990). In the present study, the methanolic leaf extract of
*P. dioica* not only decreased total cholesterol but also enhanced HDL-cholesterol significantly.

The abnormally increased level of serum lipids are mainly due to the abnormal actions of lipolytic hormones on the fat depots due to the lack of insulin. In normal conditions, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. Thus, in diabetic state lipoprotein lipase is not activated due to deficiency in insulin, which result in the condition of hypertriglyceridemia (Pushparaj, 2007) and insulin deficiency is also know to be associated with hypercholesterolemia attributable to the metabolic abnormalities. Dyslipidemia is characterized by an increase in TC, TG, LDL, VLDL and fall in HDL. This altered serum lipid profile was restored to near normal after treatment with an aqueous root extract of *I. senegalensis*.

The most characteristic features of diabetic dyslipidemia are a high plasma triglyceride concentration, low HDL cholesterol concentration and increased concentration of small dense LDL-cholesterol particles (Mooradian, 2009). Abnormal glucose utilization leads to hyperglycemia accompanied with mobilization of fatty acids from adipose tissue for energy generation (Shih, 1997). The lipid level changes associated with diabetes mellitus are attributed to increased flux of free fatty acids into the liver, which is secondary to insulin
deficiency/ resistance found in diabetes (Solano, 2005; Chahil, 2006). Reduced secretion of insulin as well as defect in the functioning of insulin contributes to enhanced metabolism of lipids from adipose tissue to the plasma (EL-Hazmi and Warsy, 1999; Frayn, 2002).

The prevalence of all forms of cardiovascular disease is multifold higher in diabetics when compared to non-diabetics. The vascular disease occurred in diabetic patients probably due to the disturbance in lipoprotein metabolism which causes acceleration of atherosclerosis. In diabetic condition, increased level of total cholesterol, triglycerides and reduced level of HDL along with altered composition of LDL cholesterol were commonly reported (Howard et al., 2000). In the present study, the altered lipid profile due to STZ induction were reversed to near normal after the treatment with P.dioica leaf extract and also glibenclamide in STZ-induced diabetic rats. This lipid lowering action may be due to proper stabilization of glucose level and increase in insulin level after the administration of the plant leaf extract which in turn might have normalized the disturbed lipid metabolism in diabetic rats. Therefore the hypolipidemic effect of P.dioica in diabetic rats substantiates its potential to inhibit the cardiovascular disease associated with diabetes (Ramachandran et al., 2012).
The lipoprotein levels in the STZ induced diabetic rats in the present study revealed a significant alteration in the lipoprotein metabolism. The serum total cholesterol content increased significantly in diabetic animals. The elevated hypertriglyceridemia resulted in the synthesis of triglyceride rich lipoprotein particles in the liver due to diminished catabolism in diabetic rats (Ginsberg, 1991). Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver (Ohno, 1970). The increased levels of low-density lipoprotein (LDL) in the diabetic animals might be due to the over production of LDL by the liver due to the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx (Coppack, 1994). The high density lipoprotein (HDL) was significantly reduced in the diabetic rats which indicate a positive risk factor for atherosclerosis (Bopanna, 1997).

In recent years, substantial attention has been directed towards the accumulation of plasma lipids and lipoproteins in diabetic subjects due to the fact that abnormal lipid level leads to the formation of coronary artery disorder in diabetics (Scheen, 1997). Decreased secretion of insulin and defects associated with insulin function results in enhanced lipid metabolism from the adipose reserves to the plasma.
Impairment in insulin sensitivity due to the high concentration of lipids is responsible for the elevated cardiovascular risk in diabetes mellitus (Shukla et al., 2006). Thus the altered lipid and lipoprotein pattern noticed in diabetic animals could be due to any defect in insulin secretion and/or action. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats. Accumulation of cholesterol and phospholipids in the liver due to elevated plasma free fatty acids have been observed in diabetic rats. In the present study, the methanolic leaf extract of *Pimenta dioica* had significantly reduced the total cholesterol, triglycerides, LDL and VLDL and markedly elevated the HDL which poses protective function over the heart when compared with that of the diabetic control group (Maurya et al., 2012).

The levels of serum lipids are usually raised in diabetes mellitus and such elevation represents a risk factor for coronary heart disease (Kannel and Mcgee, 1979). The similar results were observed in the present study, where serum total cholesterol and total triglyceride levels were significantly elevated in comparison to the normal control groups. It has been observed that the abnormally high concentration of serum lipids is mainly due to the increase in the mobilization of the fatty acids from the peripheral depots (Ahmed et al., 2001).
Supplementation of *P. dioica* leaf extract to the STZ-induced diabetic rats resulted in significant diminution of the lipid parameters and the levels of these parameters were restored to the normal level.

Nearly 40% of the diabetic cases are found to have abnormal lipid profile as one of their major complications (Ravi et al., 2005). Increased mobilization of free fatty acids from the peripheral fat depots could be observed in diabetic condition, which leads to an abnormal serum lipid concentration. Hence, diabetes causes an increase in total cholesterol, triglycerides and LDL and VLDL cholesterols (Soltani et al., 2007). The subsequent administration of *Pimenta dioica* leaf extract in the present study increased the level of serum HDL cholesterol and decreased the levels of total cholesterol, triglycerides, LDL and VLDL cholesterol respectively. These changes might be due to the inhibitory action of insulin on a key rate-limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles (Pitchai and Manikkam, 2012).

High levels of total cholesterol and more importantly LDL-cholesterol are major coronary risk factors (NCEPEP, 1994). Administration of *P. dioica* leaf extract to diabetic rats lowered the total cholesterol and LDL cholesterol by 30% and 50% respectively. This is very remarkable as diabetes is often associated with coronary
complications which constitute the major cause of morbidity and death in diabetic subjects. Biochemical studies suggest that TG itself is independently related to coronary heart disease (Bainton et al., 1992) and most of the anti hypercholesterolemic drugs do not decrease the TG levels but the plant leaf extracts lowered it by 40% after treatment. The level of glycemic control is the major determinant of very low density lipoprotein and triglyceride concentration (Taskinen, 1986). Improved glycemic control following extract treatment also resulted in the decreased levels of serum VLDL and total triglyceride. This progressive change in the lipid profile of the treated diabetic rat sheds some light on the mode of action of the extract as a positive stimulator of lipid homeostasis in streptozotocin induced diabetic rats.

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Shamony et al., 1994). The abnormally higher concentration of serum lipids in diabetic animals are mainly because of the enhanced movement of free fatty acids from the peripheral fat reserves of the tissues (Logmani and Zari, 2009). The serum cholesterol, triglycerides, LDL and VLDL cholesterol levels increased substantially and the serum HDL level declined drastically in the diabetic rats. Administration of *P. dioica* leaf extract reversed all the above parameters which ultimately could be
beneficial in preventing diabetic complications such as coronary heart disease and atherosclerosis existing during diabetic state.

In diabetes, hyperglycemic condition is normally accompanied with dyslipidemia representing major risk factor for coronary heart disease. The abnormally high level of serum lipids is mostly attributed to the uninhibited actions of lipolytic hormones on the fat depots, mainly due to the lack of insulin. Under normal circumstances, insulin influence the activation the enzymes lipoprotein lipase, which hydrolyzes triglycerides. However, during diabetic state, lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities (Girija et al., 2011). The characteristic features of diabetic dyslipidemia are increase in serum triglyceride, total cholesterol, LDL – C and decline in HDL – C levels (Karim et al., 2013). The results of the present study is in correlation with the findings of Sharma et al., (2008). The altered lipid profile was reversed towards normal after the administration of the leaf extract of *P.dioica*. The extract helps in improving the altered lipid metabolism and this consequently could prevent the diabetic complications (Hassan et al., 2015).
Insulin levels higher than those of the control group may result in the inhibition of lipolysis and decreased plasma triglyceride and cholesterol levels (Kumar, 2012). Some studies suggest that the antihyperglycemic actions of traditional antidiabetic plant extracts may be due to decreased glucose absorption in vivo. This mechanistic explanation may also apply to the actions of *P. dioica* leaf extracts in lowering the triglyceride and cholesterol levels.

In the present study, the feeding of *P. dioica* leaf extract resulted in significantly decreased total cholesterol and serum triglycerides and significantly increased HDL-cholesterol level. These findings correlate well with the results of many previous studies. Investigation on *G. sylvestre* produced a substantial lowering of cholesterol in a hypertension model. Insulin is a potent inhibitor of lipolysis, since it inhibits the activity of the hormones sensitive to lipases in adipose tissue and suppresses the release of triglycerides. The increase in HDL-cholesterol levels may be beneficial owing to the negative correlation between HDL cholesterol levels and cardiovascular diseases. This could be due to the presence of other hypolipidemic agents in the extracts of the respective plant leaves (Mall et al., 2009).

Hypercholesterolemia, hypertriglyceridemia and hyperuricemia have been reported to occur in STZ induced diabetic rats. In the present
study as well, a significant increase was obtained that was in accordance with the earlier findings (Joy and Kuttan, 1999; Resmi et al., 2001). Repeated administration of the methanolic leaf extracts of *P. dioica* had decreased the total cholesterol and triglycerides significantly.

Many previous findings brought out the beneficial effects of plants and their parts on treating diabetic subjects and the allied disorders of diabetes, such as *Abroma augusta*, *Coccinia indica*, *Curcuma longa*, *Azadirachta indica* and so on (Shukla et al., 2000; Hasim Eshrat and Ali Hussain 2002; Halim Eshrat, 2003). The above plant extracts possesses not only antihyperglycemic effect but also hypolipidemic effect. The improvement in the lipid profile is suggestive of the action of plants on the enzymes and concurrently the lipid metabolism which is responsible for the strong anti-hyperlipidemic effect of the plant extracts in streptozotocin induced diabetic animals.

The altered lipid profile in the serum of diabetic animals, happened to act as a major factor in the development of premature atherosclerosis and lead to an increase in the total cholesterol and triglycerides levels. In the present investigation, the methanolic leaf extract of *P. dioica* significantly reduced the triglyceride levels in the treated diabetic rats when compared with that of the untreated diabetic
rats. The methanolic leaf extract also markedly reduced the total cholesterol concentration in the treated diabetic animals when compared to that of the untreated diabetic animals. These reductions could be beneficial in preventing diabetic complications and also improving lipid metabolism in diabetic subjects (Cho et al., 2002).

Diabetes is also associated with profound alterations in the plasma lipid and lipoprotein profile and consequently an increased risk of coronary heart disease. Based on the literature, it was known that the STZ-induced diabetes forms hyperglycemia resulting from insulin deficiency that in turn leads to a variety of disorders in metabolic and regulatory processes (Choi et al., 1991; Ceriello, 2003). These alterations ultimately lead to increased levels of serum cholesterol and triglycerides (Bagri et al., 2009). This significant elevation of serum cholesterol, triglycerides and LDL-C and the concomitant reduction of HDL-C in the present study, seemingly were in accordance to the earlier findings as the STZ-induced diabetic rats exhibited definite defects in lipid metabolism (Muruganandan et al., 2005). However, treatment with the methanolic leaf extracts of *P. dioica* induced significant reduction in TC, TG and LDL-C level and significant increase in HDL-C level among the drug treated diabetic rats. These results indicate that the leaf extracts of *P. dioica* have a remarkable lipid lowering effect on the diabetic animals.
Based on the above results, *P. dioica* seem to exert its cholesterol-lowering effect by binding with bile acids within the intestine and increasing bile-acid secretion, thus decreasing cholesterol absorption from the intestine (Kritchevsky, 1978; Kerly and Tsai, 1978). In addition, the observed hypotriglyceridemic effect in the present study, may be due to a decrease in fatty acid synthesis (Bopanna et al., 1997) and enhanced catabolism of LDL (Sancheti et al., 2010).

The present results obtained with the methanolic leaf extracts of *P. dioica* on diabetic rats have clearly indicated the potential beneficial effects of *P. dioica* leaves in improving the lipid profile in the serum and reducing the lipids in the tissues respectively. The major constituents in the *P. dioica* leaves might have assisted in exerting the hypolipidemic effect. The present observations will provide the platform for further studies with clinical trials and also in the characterization of the active principles associated with the hypolipidemic action.