CHAPTER – VII
EFFECT OF METHANOLIC LEAF EXTRACT OF
Pimenta dioica ON THE HISTOPATHOLOGICAL
ALTERATIONS OF STREPTOZOTOCIN INDUCED
DIABETIC RATS

7.1 INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from faults in insulin secretion, insulin action, or both (Amos, 2010). Diabetes is becoming the third killer disease of mankind, after cancer and cardiovascular disease, because of its high prevalence, morbidity and mortality (Li, 2004). Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting the eyes, kidneys, nerves and arteries (Sharma, 1993). Recently, the search for appropriate hypoglycemic agents has been focused on plants used in traditional medicine because of many advantageous effects provided by traditional medicine that may act as better treatments than currently used synthetic drugs (Rates, 2001). Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases. There is an increasing interest in evaluation of pharmacological active compounds from numerous plants utilized in Indian traditional medicine (Lima, 2010).
Dietary control and traditional therapeutic plant as mentioned in Ayurvedic system and other known indigenous systems of medicinal practice are being used generally in India. Many indigenous found Indian medicinal plants have been found to be useful in successful managing of diabetes and some of these have been screened for their identification active compounds.

The liver has an important role in metabolism (Lavoie, 2005) and also maintains the glucose level in the body by storing the glucose as glycogen. More oxidative stress affects the liver cells. Hence the activity of liver is impaired and therefore it is very important that the drug should have the protective effect on liver to maintain the normal metabolic activity. Treatment of the diabetic rats with Ipomea pes-caprae reduced the activity of these enzymes in plasma when compared to the diabetic untreated group and consequently alleviated liver damage caused by streptozotocin-induced diabetes (Suhasini, 2014). The histology of the pancreas in TC treated diabetic rats showed a similar architecture to that of diabetic rats. There was no considerable change in the architecture of the islets of Langerhans after the TC treatment. It appears that there was no regeneration of β-cells after the TC treatment and therefore, it appears that the anti-hyperglycemic
activity of TC is not through the insulin secretion and is independent of insulin secretion by pancreatic β- cells (Puranik, 2010).

The blood glucose lowering effect of 95% ethanolic extract of *Cinnamomum tamala* (Hamm.) leaves were studied at a single oral dose of 250mg/kg body weight in normal, fasted, fed, glucose loaded and streptozotocin-induced diabetic induced albino rats. Further the effect of crude extract was studied on the levels of muscle glycogen content and histology of pancreatic β-cells granularity of normal rats to investigate its mechanism (Rahul Gupta, 2009). This animal model has hyperglycemia and hyperlipidemia, with symptoms of diabetic nephropathy, such as increased urine protein and BUN, decreased Ccr, glomerular hypertrophy, increased mesangial cells and renal tissue fibrosis. Reduction in the mass of pancreatic β-cells is the main reason of type1 diabetic pathophysiology (Eizirik and Darville, 2001). ROS mediated apoptotic death is not only seen in pancreatic β-cells, but also includes hepatocytes (Jaeschke, 2000) and splenic cells (Rashid et al., 2012) of the diabetic animals. Electron microscopic studies also showed that the hyperglycemia in diabetes in the advent of high oxygen environments lead to hypertrophy of endoplasmic reticulum and golgi complex, increased free ribosomes, degranulation and the
margination of secretory granules in the β-cells (Hamaguchi et al., 1986).

Streptozotocin (STZ) was originally developed as an antibiotic and antitumour agent and is now widely used to increase insulin–dependent diabetes mellitus in rats because of its toxic action on islet β-cells. However, it is also noted that STZ has acute toxic effects on many organs in some animal species (Levin et al., 1980) and leads to delayed type toxicity in rats (Okawa and Doi, 1983). This suggests that full attention should be paid to histological changes in other organs as well as the pancreas when STZ–induced animals are used for studies on diabetes. There are only a few reports, however, on the systemic histopathology of streptozotocin-induced diabetic animals (Arison et al., 1967).

Pancreatic lesions induced by STZ leads to full blown diabetes which were reversed upon the treatment with CiREE which possibly stimulate the regeneration of pancreatic islet cells. STZ is known to induce chemical diabetes by selective destruction of pancreatic cells through three processes viz: DNA nitric oxide production, alkylation and free radical generation (Szkudelsk, 2001). It was observed in the above study that diabetic control rat pancreas showed markedly reduced and shrunken islet mass, infiltrated by
lymphocytes with general fibrosis. This was observed from various reports in literature due to STZ damage to the pancreas (Bolkent, 2000; Noor, 2008) and the diabetogenic agent alloxan. Liver disease is one of the leading causes of death in persons with type 2 diabetes. The standardized mortality rate for death from liver disease is greater than that of cardiovascular disease (Keith et al., 2004). Meager work has been done in *Pimenta dioica* on diabetes mellitus with respect to histopathological studies.

Hence the present study was aimed to investigate the methanolic leaf extracts of *Pimenta dioica* in streptozotocin induced diabetic rats by conducting histopathological studies in the liver and pancreas.

### 7.2 MATERIALS AND METHODS

Histopathological studies were carried out by the method of Gurr (1958). At the end of 45 days of the experiment the diabetic and treated animals were sacrificed by cervical decapitation. Pancreas and liver were dissected out, washed in ice-cold saline to remove the blood and were immediately transferred to the fixative of 10% formalin. After the completion of the fixation period, tissues were dehydrated to remove the water. The tissues were dehydrated by passing through different percentages of alcohol-water mixture. For stepwise dehydration, the tissues were kept in the progressive alcohol-grades for the following
duration: in 30% and 50% grades for 5 to 10 hours: in 70% grade for 10 to 12 hours: in 90% grade for about 30 minutes and in absolute alcohol (100%) for 20 minutes. After dehydration, the tissues were embedded in paraffin wax to make it firm for the purpose of section cutting. The thickness of the section was adjusted at 6 micron (μ) and then stained with hematoxylin and eosin dye which is mounted in neutral deparaffinized xylene (DPX) medium for microscopic observations. Scanning electron micrographs were taken and discussed.

7.3 RESULTS

The pancreatic cells of the normal control rats were all present in their normal proportions. The acinar cells that seems to be stained strongly were arranged in lobules with prominent nuclei. The islet cells were seen embedded within the acinar cells, which were surrounded by a fine capsule. It showed lobules of exocrine acini, interlobular ducts and occasional islets of Langerhans. The β - cell mass was normal with original diameter in the islet cells possessing actual average number of β - cells (Fig.27 - A )

The histopathological observations in the pancreas of the STZ-induced diabetic rats revealed a decrease in the size of the islets and degranulation and consequently a decrease in the β - cells. Well granulated β-cells disappeared almost completely in all the islets. The
size of the islets had also decreased more obviously at the diabetic group. It was observed that the islet cells were shrunken having inflammatory cellular infiltration with fibrosis. The size of the islet cells and the number of β-cells in the islet cells diminished dramatically due to diabetic induction. The islet cells were also depleted (Fig. 27 - B)

In the diabetic rats administered with 75 mg/kg b.w. of the methanolic extract of *P. dioica* leaves, the pancreatic cells showed moderate recovery when compared with that of the diabetic induced animals. The acinar cells were seen to be normal. The islets were present with heavy lymphocytic infiltration in and around the islet cells which is normally termed as insulinitis. Normal islet cells were also present. Inhibition of shrinking of islets of Langerhans, inhibition of inflammatory cellular infiltration and reversion to normal pancreatic cells occurred after the plant extract treatment. The necrosis of β - cells also started recovering. The diminished islet size and β-cell number also recovered after the treatment (Fig. 27 - C)

In the pancreas of the diabetic rats after treatment with 150 mg/kg b.w. of the methanolic leaf extract of *P. dioica*, the acinar cells were seen to be absolutely normal. The islets of Langerhans were located with a large proportion of islet cells in their original form
though with a smaller volume when compared with that of the normal control group. There were very scanty inflammatory cell infiltration and eosinophilic deposits were seen (Fig. 27 - D). It seems that the plant extract led to the regeneration of the islets which is documented by the presence of stable cells in the islets with the ability of regeneration. The quiescent cells had been proliferated to replace the lost cells due to STZ- induced diabetic injury. The shrinkage of islet cells, inflammatory cellular infiltration and enlarged pancreatic cells were all reverted to normal condition after the plant extract treatment. The recovery of necrotic β-cells was especially more pronounced after treatment with 150 mg/kg b.w. of P. dioica leaf extract. Considerable restoration of β-cell mass was also observed. The diameter of the islets and the number of β-cells were also restored significantly. The standard drug glibenclamide also revealed the reversion to normal cellular architecture (Fig. 27- E).

The liver cells of the normal control rats revealed sinusoidal cords of hepatocytes with central vein and portal tracts. The portal tracts exhibited portal triad with portal vein, hepatic artery and bile duct which were very compact in the histoarchitecture (Fig. 28 - A). The hepatocytes were normal with non-dense cytoplasm located with normal quantum of eosinophilic granules. There was no cytoplasmic
invagination and nucleus was evidenced to be absolutely normal. These were no documentation of any phagocytic process occurred in the livers of normal animals.

The characteristic histological alteration in the liver of STZ-induced diabetic rats was hypertrophy of hepatocytes (Fig 28 -B). It was clearly seen in almost all rats of the group. Many hypertrophic hepatocytes showed dense cytoplasm with an increased amount of eosinophilic granules. In certain cases, hepatocytes were swollen with lucent cytoplasm. Moreover, cytoplasmic invagination into the swollen nucleus was found sporadically among hypertrophied hepatocytes in some markedly hyperglycemic rats. In addition, phagocytosis of yellowish brown pigments by Kupffer’s cells was generally seen in the liver of STZ-induced diabetic rats. The arrangement of the cells around the central vein in the hepatocytes were distorted and had periportal fatty infiltration with focal necrosis of hepatocytes (Fig.28 - B)

The administration of the methanolic extract of *Pimenta dioica* leaves at the dose of 75 mg/kg b.w. of the STZ-induced diabetic rats, brought back the cellular arrangement towards normalcy. The histoarchitecture of the cells around the central vein in the hepatocytes reverted back from their distorted nature and the focal necrosis of the
hepatocytes also tended to change back. It also helped to bring the blood vessels to normal condition (Fig 28- C). The hypertrophic condition of the hepatocytes changed with loose normal cytoplasm and original number of eosinophilic granules. The swollen hepatocytes reverted, the invaginated cytoplasm changed and the swollen nucleus became normal. Whereas, the administration of the methanolic leaf extract of *P. dioica* at the dose 150 mg/kg b.w. in the STZ - induced diabetic group completely changed the hypatrophied hepatocytes to near normal condition. The hepatocytes showed normal cytoplasm with the original eosinophilic granulas. The lucent cytoplasm with swelling of hepatocytes reverted back. In such treated normoglycemic rats, the invagination of the cytoplasm came back and the swollen nature of the nucleus changed. Moreover, the phagocytic stature of the yellowish brown pigments by Kupffer’s cells was brought back to normal condition. The arrangement of cells around the central vein in the hepatocytes was normal and the periportal fatty infiltration with necrotic hepatocytes were reverted back to normalcy (Fig. 28- D). The standard drug glibenclamide also reverted back the distorted nature of the hepatocytes, necrotic condition and the hypertrophic stature also changed to normal (Fig. 28- E).
A-Control pancreas (ISL-islets of langerhans)
B-Diabetic pancreas (DISL-Damaged islets of langerhans)
C-Diabetic + Pimenta dioica@75 mg/kg b.w. (RISL-Recovered islets of Langerhans, A-Acini cells)
D- Diabetic + Pimenta dioica@150 mg/kg b.w. (BV- blood vessel)
E- Glibenclamide treated pancreatic tissue (RISL-Recovered islets of Langerhans, ILD-intra lobular duct)

Fig. 27. Histopathological changes in the pancreatic tissues of control, diabetic induced and treated rats.
A - Control liver tissue (NhP: Normal hepatocytes)
B - Diabetic liver tissue (Dhp: Damaged hepatocytes)
C - Diabetic + *Pimenta dioica* @ 75 mg/kg b.w. (Rhp: Recovered hepatocytes)
D - Diabetic + *Pimenta dioica* @ 150 mg/kg b.w. (Rhp: Recovered hepatocytes)
E - Gilbenamide treated liver tissue (Rhp: Recovered hepatocytes)

**Fig. 28.** Histopathological changes in the liver tissues of control, diabetic induced and treated rats.
7.4 DISCUSSION

Cumulative evidence has shown that inadequate and erratically controlled hyperglycemia can lead to the production of high reactive oxygen species (ROS) levels, which can react with essential molecules like lipids, proteins and DNA, thus causing histological changes and functional alterations (Wang et al., 2010). STZ is a toxin frequently used to induce diabetes in experimental animals through its ability to induce selective destruction of pancreatic β-cells, resulting in insuling deficiency and hyperglycemia (Alice et al., 2010).

Most of the islet cells are composed of β-cells which producing insulin. Depletion of damage β-cells will, thus cause in deficiency of insulin, which will result in abnormalities in carbohydrate, protein and fat metabolisms causing the resultant hyperglycemia. In this study, streptozotocin which selectively destroy β-cells of the islets was used to induce diabetes mellitus. Insulinitis and loss of β-cells were observed which may be seen in type-I diabetes. Insulinitis is evidenced by heavy lymphocytic infiltration in and around the islets. Similar observations were obtained by Eliakin Ike.chukwer and Obri (2009) in alloxan induced diabetic animals.

This is commonly observed in islets containing residual β-cells and it may also support the possibility of a specific, immunologically
mediated destruction of \( \beta - \) cells and cause of Type I diabetes mellitus (Anderon, 1985). There is no evidence of immune involvement in the pathogenesis of Type 2 diabetes (Kumar and Clark, 2005). Insulinitis was observed in the group treated with \( \textit{P. dioica} \) leaf extract showing that some of the scanty cells seen in the islets are \( \beta - \) cells.

Pancreas is the primary organ involved in sensing the dietary and energetic states. In histological study, STZ-induced rats revealed necrosis and reduction in the number of islet cells due to concomitant decrease in the antioxidant defense. The \( \textit{P. dioica} \) leaf extract treated animals showed decreased cellular necrosis and regeneration. Diabetic rats exhibited degeneration cells in the islets of Langerhans of pancreas. It was also identified the existence of shrunken islets and inflammatory cellular infiltration with fibrosis. Treatment of these diabetic rats with the drug, glibenclamide inhibited STZ-induced shrinking of islets of Langerhans of the pancreas, inflammatory cellular infiltration and enlarged pancreatic cells (Sandhya Rani et al., 2013). Treatment with \( \textit{P. dioica} \) leaf extract reversed all the effects of streptozotocin dose dependently.

The present histopathological study clearly indicated that in STZ-induced diabetic rats, the endocrine region of pancreas revealed the presence of shrinkage, necrosis and damaged \( \beta - \) cell population. The
diabetic animals which were treated with the methanolic leaf extract of *P. dioica* showed an increase in the number of islets, lesser degree of shrinkage and necrosis of β- cells of pancreas. Similar results have been reported by Irudayaraj et al., (2012) and Gohil et al., (2010). This implies that the decreased blood glucose and increased insulin secretion are due to the insulin released from the regenerated β-cells of pancreas.

From the results of the present investigation, it was observed that treatment with *P. dioica* methanolic leaf extract decreased the serum glucose and increased serum insulin in STZ induced diabetic rats. It is perhaps due to the stimulation of insulin secretion from remnant pancreatic β-cells which in turn enhances glucose utilization by the peripheral tissues of diabetic animals, either by promoting glucose uptake and metabolism or by inhibiting hepatic gluconeogenesis. This is further confirmed by the histopathological observations which show that the structural integrity of islets of langerhans was restored towards normalization (Hassan et al., 2015).

A decrease in the size of the islets and degranulation and a decrease in the number of β-cells were observed in STZ- induced diabetic rats. Well granulated β-cells disappeared almost completely in all islets at the diabetic control animals. The size of the islets had
also decreased more obviously. Similar observations were also reported in two strains of STZ-treated mice (Honja et al., 1986).

The pathology and pathogenesis of pancreatic islet lesions induced in male mice by multiple injections of a subdiabetogenic dose of STZ had been described in detail by Like et al., (1978). Similar histopathological findings of the pancreas in the chronic stage were also observed in the female mice in the above study. In addition, the liver was also involved in the lesions. The hepatic changes were similar in the two strains of mice and were characterized by hypertrophy of hepatocytes due to an increase in intracytoplasmic eosinophilic granules. In some cases, cytoplasmic invagination into hepatocyte nuclei was observed simultaneously. These hepatic changes were not found in spontaneous diabetes in mice (Makino et al., 1980; Unchida et al., 1985), and they differed from the direct hepatotoxic changes such as necrosis and vacuolation caused in mice by a single injection of a large dose of STZ (Levine et al., 1980). Jones and Hunt (1983) reported that the cytoplasmic invagination into hepatocyte nuclei was frequently found in aged rodents. The mice examined in this study were under 20 weeks of age and such changes were never detected in any control mice. As their severity corresponded well with the duration and degree of hyperglycemia, the present hepatocytic alterations seem to be secondary to persistent metabolic disorders.
Large deposits of a homogenous eosinophilic material largely occupying the islet and around blood vessels are visualized in diabetic control group. This may be the formation of localized amyloidosis, which has been previously documented in the pancreas in most diabetics (Eliakim-Ikechukwu and Obri, 2009). This is deposited extracellularly and foremost appears in the walls of small vessels. It is also deposited in reticulin fibres and basement lamina. When present in more amount, it causes pressure atrophy of the surrounding cells (Anderson, 1985). Thus, the scanty atrophic cells are found in this group can worsen the situation. This is thus associated with chronic inflammatory disorder (Kumar and Clarke, 2005). Islet cells of P. dioica leaf extract treated group has regenerated considerably suggesting the presence of stable cells in the islets with the regenerating ability. (De fronzo et al., 1997). This also suggests that the plant leaf extract at this dose has the ability of inducing the quiescent cells to proliferate to replace the lost cells. Though the exact mechanism is yet to be known, it has been documented that the flavonoid fraction of the plant leaf extract decreases blood glucose and increases the number of β-cells (Chakravathy et al., 1980).

The present findings revealed the significant decrease in the serum glucose level in the P. dioica leaf extract treated diabetic rats. Streptozotocin not only destroys the pancreatic β-cells but also causes
liver damage, which is however reversible while streptozotocin also selectively destroys pancreatic insulin secreting β-cells (Gillman, 1990). The degenerative changes in the histology of liver and pancreas brought about by streptozotocin and alloxan administration are similar to the earlier observations made (Ghosh and Surawanshi, 2001; Thakran et al., 2004). Histologically, the liver section of streptozotocin induced diabetic rats showed marked structural alterations in the liver as a result of absence of insulin. The major alterations were periportal fatty infiltration and necrosis of hepatocytes. This damage was partially reversed by the P. dioica leaf extract treatment and is similar to that observed by Gymnema sylvestre therapy in alloxan- induced diabetic rabbits (Shanmugasundram et al., 1983), Vinca rosea extract in alloxan- induced diabetic rats (Ghosh and Surawanshi, 2001) and Terminalia arjuna stem bark extract in alloxan induced diabetic rats (Ragavan and Krishnakumari, 2006).

The present study revealed the immediate action of streptozotocin induced diabetes by destroying the β-cells even at minimum exposure. The ultrastructure of streptozotocin induced diabetic pancreas showed considerable reduction in the islets of Langerhans and depleted islets. The above observations are in agreement with earlier reports made by Ghosh and Suravanshi, (2001), Gholamali et al., (2005) and Ragavan and Krishnakumari (2006).
The diabetic rats exhibited pancreatic islet regeneration after the administration of *P. dioica* leaf extract. The regenerative effect of the pancreatic cells by *P. dioica*, via the exocrine cells of pancreas may enlighten the positive effects of these agent on the production of insulin.

It had been reported that there are several models of STZ-induced diabetes. Multiple subdiabetic doses of STZ cause a subtle β-cell damage, activating apoptosis, and thus triggering an autoimmune reaction in the islets that is followed by insulin deficiency (Inaba et al., 1992). Whereas a single high dose of STZ induces type I diabetes and cause a rapid and complete destruction by the necrosis of β-cells, but a single moderate dose of STZ is an intermediate situation of persistent diabetes by both toxic and inflammatory mechanisms (Rodriques et al., 1997). Probably the pancreatic damage caused by STZ was halted, insulin synthesis and secretion were both enhanced and the beta cell damage was repaired with plant extract treatment. Such a stimulation of STZ-induced damage might have occurred in the present study as well, which could have been reverted upon the treatment with the two doses of the methanolic extract of *P. dioica* leaves.

Liver is a vital organ that play an important role in the defense mechanism of the postprandial hyperglycemia and involved in the glucose metabolism. In liver, glucose is converted into glucose-6-
phosphatase by the help of hexokinase (Baquer et al., 1998; Latha and Pari, 2003). STZ-induced diabetic rats decrease glycolysis, disrupt the capacity of the liver to synthesis glycogen and decrease the level of hexokinase. Decreased level of hexokinase show an effect on glycolysis and inhibits the utilization of glucose for energy production (Raju et al., 2001). Such an disturbance to the liver and the accumulation of glucose lead to alterations in the tissue structure and hence such histopathological changes in the liver could be taken as a marker for any tissue damage due to hyperglycemic condition.

Histopathological evaluation of the liver cells showed necrosis and reduction in the cells showed necrosis and reduction in the number of cells due to decrease in the antioxidant defense after inducing diabetes with streptozotocin. Treatment with 75 and 150 mg/kg b.w. of P. dioica leaf extracts reversed all the effects of streptozotocin dose dependently. The literature reports reveal that certain phytoconstituents present in the plant extract might have been known to possess antioxidant activity (Rani et al., 2013). In the present investigation also the observed antidiabetic potential of test plant extracts may be due to the presense of similar phyto constituents harboured in P. dioica.

The findings of Yadav et al. (2013) states that the liver and pancreas histology showed endogenous chronic oxidative stress in the
STZ-induced diabetic group due to the resulting hyperglycemia. STZ was found enough for the complete demolition of the liver and pancreatic cells within 24 h injuring the hepatic cells and causing injury to the islets (Ito et al., 2001). The results also disclosed that the pathological effects of rat liver and pancreatic tissues by diabetes due to oxidative stress were reversible and improved by plant extract treatment. The treated diabetic group showed reverted hepatocytes and pancreatic islet regeneration. This could also be evident from the histological examination of the present study.

It has also been observed that the phenolic constituent of therapeutic plants contributes primarily to the antioxidant activity of the plants. The phenolic component of *P. dioica* might have blocked the further damage to the remaining β-cells by scavenging the circulating reactive oxygen species, which are generated by the streptozotocin to destroy the β-cells followed by other phytochemicals present in the plant to trigger regenerative activities in the pancreas.

 Though the exact mechanism is unknown, it is obvious that *P. dioica* leaf extract is able to cause regeneration of pancreatic β-cells at a dose of 150 mg/kg b.w. either by its active constituents acting single or together.