Validation of Histopathological changes in control and streptozotocin induced diabetic rats treated with *N. crenulata* plant extracts

**INTRODUCTION**

Histopathological studies on animal models are useful techniques to evaluate the potential curative effect of the herbal drugs especially for diabetes mellitus. The liver is a vital organ performing specific functions like detoxification, synthesis of several components of blood plasma, glycogen storage and release of glucose to the blood. Liver involves in lipid metabolism and the lipoproteins are produced in liver. 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. The spectrum of liver disease in type 2 diabetes ranges from nonalcoholic fatty liver disease to cirrhosis and hepatocellular carcinoma (Keith *et al.*, 2004). Diabetes mellitus and advanced liver disease are associated with each other more frequently than expected by chance and such an association carries a significant risk of morbidity and mortality. A metabolic pathway leading to advanced liver disease via fatty liver and steatohepatitis has been demonstrated, further supporting the possibility that cirrhosis may be a late complication of diabetes (Simona *et al.*, 2007). Liver and kidney are important for glucose and lipid homeostasis, they participate in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. Thus, it is expected to have changes in liver and kidney during diabetes (Seifter and England, 1982).

Diabetes mellitus is associated with generation of ROS leading to oxidative damage particularly in liver and kidney (Mohamed *et al.*, 1999). The elevated blood glucose levels in
diabetes mellitus are thought to induce cell death through free radical formation that occurs as common sequel of diabetes induced non-enzymatic modification of sugar moieties on proteins and lipids (Donnini et al., 1996). The kidney is mainly concerned with the removal of waste materials from the body. In severe diabetic condition, excess glucose is excreted through the kidney. Diabetic nephropathy is the most important cause of death in type 1 diabetic patients, of whom, 0-40% eventually develop end stage renal failure (Giorgino et al., 2004). The number of functionally intact β-cells in the islet region is of decisive importance for the development course and outcome of diabetes mellitus. The renewal of β-cells in diabetes has been studied in several animal models (Nagappa et al., 2003). STZ act as a diabetogenic agent owing to its ability to destroy pancreatic β-cells, possibly by a free radical mechanism (Halliwell and Gutteridge, 1994; Simmons, 1984). It is selectively uptake by β-cells by structural analogue uniformity to that of glucose. Antidiabetic agents have generally been shown to decrease the levels of serum biomarker of hepatic injury (Harris, 2005) but these agents can produce serious side effects (Akhtar and Iqbal, 1991).

Plants play a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances including anti-oxidation, anti-hyperglycemia and anti-hyperlipidemia (Li-Qin, 2006). The treatment with herbal drugs has an effect on protecting β cells and smoothing out fluctuation in glucose levels (Jia et al., 2003 and Elder, 2004). Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for hyperglycemia and liver toxicity. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown. Beneficial effect of freshly prepared aqueous extracts of Psidium guajava, Momordica charantia, and Coccinia indica leaves and their combination in STZ-induced diabetes rats has been proved by Rafiq et al. (2009). The pathological effects of diabetes mellitus on liver, kidney and
pancreas have been studied by several workers (Murugan and Pari, 2006, Neveen et al., 2007, and Anusuya et al., 2003). In the present study, STZ induced diabetic animals and consecutive _N.crenulata_ treated diabetic animals were subjected for histopathological studies which were carried out in liver, kidney, and pancreas.

**MATERIALS AND METHODS**

Histopathological studies are carried out by the method of Gurr, (1958). At the end of 45 days of experimental diabetic and treated animals were sacrificed by cervical decapitation. Pancreas, kidney 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 through passing different percentage of alcohol-water mixture. For stepwise dehydration, the tissues were kept in the progressive alcohol-grades for the following duration; 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 section was adjusted at 6 micron (μ) and then stained with hematoxylin and eosin dye which is mounted in neutral deparaffinated xylene (DPX) medium for microscopic observations.

**Scanning Electron Microscopic studies (SEM)**

For Scanning Electron Microscopy (SEM), tissues were and fixed in Karnovsky solution (2% paraformaldehyde and 2.5 glutaraldehyde in 0.2M sodium (cacodylate buffer). The tissues were then dehydrated in a graded ethanol series (70%-100%) and in 100% ethanol acetone (1:1) solution, followed by four washes in 100% acetone. After drying, the samples were assembled on aluminum stubs, coated with gold were examined and photographed with JOEL JSM-P15 Scanning Electron Microscope.
RESULTS

Histopathology of Liver

The liver of treated diabetic animals exerted highly significant results, tissue samples of these experimental groups were undertaken for histopathological studies. Results of histopathology revealed that control animals showed ideal hepatocytes in liver tissue (Plate-I: A) whereas, streptozotocin treated animals caused a drastic tissue damage in liver hepatocytes and major blood sinusoids were underwent degeneration and internal blood cysts were characterized in streptozotocin induced diabetic animals (Plate-I: B). In the mean time, plant extract and glibenclamide drug treated group showed a reversible tissue regeneration with prominent hepatocytes (Plate-I: A-D). Scanning Electron Micrography of Liver histology revealed the same of course (Plate IV- A-D)

Histopathology of Pancreas

Pancreas, the target tissue of diabetic etiology has severe damage in the case of streptozotocin treated animals. (Plate: II-A). The sinusoids and islet cells in the control animals were intact whereas, in the STZ induced animals showed necrotic stages in the tissues (Plate: II-B-D). Plant administered groups effected with regeneration of islet cells with blood vessels and the same effect was encountered in standard glibenclamide treated group (Plate: V-A-D).

Histopathology of Kidney

STZ induces severe kidney necrosis with damaged glomeruli (Plate:III-B). Plant samples of *N.crenulata* treated group reflected the tissue recovery to considerable extent and the significant results are comparable to that of standard drug glibenclamide treated group (Plate: VI-B-D).
Plate-I:
A- Control liver (Nhp: Normal hepatocytes)
B- Diabetic liver (STZ treated) (Dhp: Damaged hepatocytes)
C- N.crenulata treated liver (Rhp: Recovered hepatocytes)
D- Glibenclamide treated liver (Standard Drug)
Plate-II:
A- Control Pancreas (NI: Normal Islets)
B- Diabetic Pancreas (DI: Damaged Islets)
C- *N.crenulata* treated Pancreas (Rbv: Recovered blood vessels)
D- Glibenclamide treated Pancreas (PI: Prominent Islets)
Plate-III:

A- Control Kidney (Normal saline treated)
B- Diabetic Kidney (Nbs: Necrosized sinusoids; Dg: Damaged glomeruli)
C- *N.crenulata* treated Kidney (Rg: Regenerative glomeruli)
D- Glibenclamide treated Kidney (Mrg: Moderate regenerative glomeruli)
A- Control Liver
B- Diabetic Liver
C- *N.crenulata* treated Liver
D- Glibenclamide treated Liver
PLATE: V

A- Control Pancreas
B- Diabetic Pancreas
C- *N.crenulata* treated Pancreas
D- Glibenclamide treated Pancreas
A- Control Kidney
B- Diabetic Kidney
C- *N.crenulata* treated Kidney
D- Glibenclamide treated Kidney
DISCUSSION

Medicinal plants play an important role in the treatment of diabetes mellitus, especially in the developing countries due to their cost effectiveness. Diabetes mellitus, a metabolic disorder, is becoming a serious threat to mankind health. The prevalence of diabetes mellitus is expected to reach up to 4.4% in the world by 2030. Among all type of diabetes, type 2 diabetes is main complication. Currently available treatment options in modern medicine have several adverse effects. Therefore, there is a need to develop safe and effective treatment modalities for diabetes. In this regard, plants provide the best option for search of desired safe and effective medications. Since ancient times, plants have been an exemplary source of medicine. Various plants have been an exemplary source of medicine. Various plants have been found to possess significant anti-diabetic property after their preclinical and clinical evaluation. The present study evaluates the effect of *N.crenulata* extract on the histology of STZ diabetic rats.

Liver is the vital involving in maintaining the optimum blood glucose levels within narrow limits. The liver and kidney exhibit numerous morphological and functional alterations during diabetes (Sochhar *et al.*, 1985). The liver of diabetic showed periportal necrosis of hepatocytes near the portal areas with dilated and congested portal vessels as well as areas of inflammatory cell infiltration and pericentral glycogen depletion (Neveen *et al.*, 2007). Free radical toxicity is very high in diabetic condition both in human and animal models (Nourooz – Zedeh *et al.*, 1997; Wohaieb and Godin, 1987). Hyperglycemia induced free radical toxicity causes severe damage to vital organs especially liver. Previous studies support that the liver damage in diabetic rats may be due to fatty changes in the portal triad by free radical toxicity (Balakrishnan *et al.*, 2007). Liver histology in non alcoholic fatty
liver disease showed (a) Steatosis (fatty infiltration >5% hepatocytes) (B) Necroinflammation (perisinusoidal, perivenular, bridging, cirrhosis (Simona et al., 2007). Streptozotocin injection in mice caused hepatotoxicity such as necrosis and vacuolization to some extent (Levine et al., 1980).

In the present study, it has been observed that diabetic rat liver showed much necrosis in liver cells and was previously reported by Mansour et al. (2002). Diabetic liver showed dilations in hepatic sinusoids and kupffer cell hyperplasia is coincided with the findings of Evelson et al. (2005). The changes in the serum enzyme activities are normal in uncomplicated diabetes. However, when tissue damage caused by metabolic and circulatory alterations occur, their activities are increased. Hence, the liver and kidney damage are primarily caused by congestion and metabolic disorders might be the cause of these enzymatic changes (Geidam et al., 2004). The restoration of damages in liver of diabetic rats treated with N.crenulata extracts and glibenclamide may improved glycemic control and thereby control over free radical production and glycation of protein.

Histopathological studies of kidney showed damaged tubules, lessions and fatty infiltration in the diabetic rats. The kidney of diabetic rats showed an increase in mesangial cell and matrix of glomeruli with increase in glycogen deposition and hyalinization of arterioles with thickened basement membranes of proximal and distal convoluted tubules (Neveen et al., 2007). Hyperglycemia and glycation of proteins are the main causes for the damage occurred in diabetic rats. In addition, fatty acid composition also altered in diabetic condition leading to increase severity of damages in kidney structure. These results were well supported by the previous investigators (Balakrishnan et al., 2007). Most of the tissue damage is caused by free radicals by attacking membranes through peroxidation of
unsaturated fatty acids (Kasi et al., 2004). Hyperglycemia was responsible for dilation of proximal and distal tubules in the cortex (Leegwates et al., 1984). Diuresis is a common feature associated with diabetes which may be the reason for structural changes observed with glomerulus (Das et al., 1996). The developed albuminaria in diabetic animals may be due to impaired tubular reabsorption or leakage of albumin due to damaged glomerular membrane which lead to alteration in size or changes in selective barriers of the glomeruli (Gomes et al., 1997). Untreated diabetes mellitus will develop irreversible hepatic and renal deterioration (Shalini et al., 2004). The recovery of damages in kidney of diabetic rats treated with N.crenulata and glibenclamide may be due to controlled free radical toxicity and prevention of disintegration of tissue membrane.

β- cell is the most abundant cell type in the endocrine pancreas; β- cell number is the most important factor that determines islet area. Pancreas of diabetic rats showed ruptured islet and decreased β- cell (Jiyin et al., 2009). The area of islet was reduced markedly in diabetic rats which were restored in N.crenulata extract and glibenclamide treated diabetic rats. The restoration of damages in pancrease may be improved control over the toxic activity of free radical by N.crenulata extracts. Diabetic rats showed shrunken islets and fatty infiltration may be due to STZ induced highly reactive free radicals can deplete GSH (Reduced Glutathione) which also destroy hepatic and renal cell (Blum and Fridovich, 1985). Glycation reaction in diabetes occurs in various tissues including β- cell (Myint et al., 1995 and Tajiri et al., 1997). The activity of antioxidant enzymes such as superoxides dismutase (SOD), catalase and glutathione peroxidase which is low in islet cells when compared to other tissues under diabetic conditions (Tiedge et al., 1997). Glycation mediated reactive oxygen species leads to apoptosis of β- cell (Kaneto et al., 1996) and reduced insulin gene
transcription (Matsuoka et al., 1997). The treatment of diabetic rats with *N. crenulata* extract prevents the glycation process by its antihyperglycemia activity and thus it protects the β-cell of pancreas from hyperglycemia induced injuries.

*N. crenulata* extract have the tissue repairing potential from diabetic related complications and its action is much comparable with the standard drug, glibenclamide. The improved glycemic control, lipid lowering effect, hepatoprotective effect and antioxidative potential of *N. crenulata* extract showed its tissue repair capability from diabetes mellitus. Thus, the present findings are also in agreement with those of earlier workers for their protective effect on the pancreas by *Momordica charantia* (Ahmed et al., 1998) *Aegle marmelos* (Kamalakkannan and Prince, 2005) and *Eugenia jambolana* (Grover et al., 2001) respectively.

**SUMMARY AND CONCLUSION**

Diabetes mellitus is a disorder of carbohydrate, fat and protein metabolism mainly caused due to attenuate production of insulin or its inhibitory action. Before there was no synthetic drug, natural cure was used and they can still be used today. Diabetes is a metabolic disorder which can be considered as a major cause of high economic loss. Further, uncontrolled diabetes leads to many chronic complications such as blindness, heart failure, and renal failure. In order to prevent this alarming health problem, the research and development of new hypoglycaemic plants are undergoing. More than 400 medicinal plants are available globally for the medication of the diabetes. Their therapeutic effectiveness varied from one study to other depending upon the methodologies and the experimental models used.
The pathogenesis of type 2 Diabetes Mellitus involves insulin resistance, as well as insulin secretion from the pancreatic β-cell. Diabetic nephropathy is the most frequent cause of terminal kidney failure in developed countries. The manifestation of Diabetic Nephropathy is associated with a poor prognosis of affected patients. It is a major health concern in both rural and urban populations of the Indian subcontinent. Development of novel therapeutic agents inhibiting mentioned factors is of particular interest as they represent potential treatments for the prevention of diabetic complications.

Present results showed that oral administration of *N.crenulata* extracts for 45 days had protective effect against the damage caused by STZ as evidenced by the presence of more number of viable β-cells of islets of Langerhans in pancreas and practically normal architecture of liver and kidney tissues on histological examination. The appearance of more number of darkly stained granules may be due to activation of survival/ dormant β-cells which were not functional in normal pancreas. Some workers, however, also pointed out the regeneration of the β-cells of islets of Langerhans with medicinal plants. Hence, the *N.crenulata* extract might be effective therapeutic agent against diabetes. However, this study provides the basis for further study on the detailed pharmacological effects of the extracts of *N.crenulata* and their active component(s).