Bulletin of Pure and Applied Sciences
Vol.40A (Zoology), No.1,
January-June 2021: P.57-62

Print version ISSN 0970 0765
Online version ISSN 2320 3188
DOI 10.5958/2320-3188.2021.00008.5

Content Available online at: https://www.bpasjournals.com/zoology

Original Research Article

Histo-Pathological Alterations Associated Metabolic Alterations in STZ Induced Diabetic Rats with Leaves Extract of *Sapindus saponaria*

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Article Info:
Received on 17.11.2020
Accepted on 16.03.2021
Published on 15.06.2021

ABSTRACT:
In the present study oral administration of aqueous extract of *Sapindus saponaria* (SS) to streptozotocin (STZ) induced diabetic rats secluded the rats from the changes in Histopathological tissues of rats. STZ induces diabetes that resembles human hyperglycemic non-ketotic diabetes mellitus. Further rats treated with STZ display many of the features in human subjects with uncontrollable DM and are invaluable when studying the mechanisms by which hyperglycemia may contribute to microvascular complications such as neuropathy, nephropathy and retinopathy. The functioning of pancreas, liver and kidney may be affected due to decreased levels of insulin, hyperglycemia and its consequences. In the present investigation the histological changes in these tissues of diabetic rats and the effect of *Sapindus saponaria* SS aqueous extract on these was studied.

Keywords: *Sapindus saponaria*, Pancreas, Liver, Kidney and STZ.

INTRODUCTION

Diabetes mellitus (DM) which is an inter-metabolic disorder which is found globally and fast increasing worldwide. This disorder is quite alarming in most of the developing countries like India. India has more than 40 million diabetic individuals which represent nearly 20 % of total diabetes population worldwide. Liver is considered to be consisting of large number of hexagonal lobules. Each lobule consists of a central vein, from which cords or rows of liver cells radiate like spokes of a wheel. Each lobule is delineated by a connective tissue (Chaudhari. 1998). Microscopic examination of normal liver shows the glycogen granules as reddish purple material in hepatocytes with Periodic acid- Schiff (PAS) staining (Mitra *et al*., 1996). But diabetic liver shows decreased deposits of glycogen granules. Histology of liver during diabetes shows structural alterations in the liver as a result of absence of insulin. In liver cells the sinusoidal spaces and the vein lumen are enlarged. The major alterations are thickening of the wall of the blood vessels and capillaries in diabetic state. The distortion in the usual arrangement of hepatic cells may be brought about by the

How to cite this article: Naik, M.J., Naik, R.N.R. (2021). Histo-Pathological Alterations Associated Metabolic Alterations in STZ Induced Diabetic Rats with Leaves Extract of *Sapindus saponaria. Bulletin of Pure and Applied Sciences-Zoology,* 40A(1), 57-62.

increase in the lumen of the veins which might have pushed the surrounding cells. (Anil & Paulose, 1995; Anil *et al*., 1996) The fibrosis observed in diabetic liver shows the extensive damage of liver cells which is replaced by a fibrous tissue (Balazs & Halmos, 1985).

Pancreas is a compound tubular alveolar, partly exocrine and partly endocrine gland. The exocrine part of the pancreas is in the form of serous acini, secreting the secretions into intralobular duct. The endocrine part of the pancreas is in the form of numerous rounded collections of cells known as Islets of Langerhans, embedded within the exocrine part. Each Islet is separated by the surrounding alveoli by a thin layer of reticular tissue. The average islet in rats is 150μm in diameter and contains about 45ng of insulin. There are four major endocrine cell types in mammalian islets, the insulin-producing β-cells, the glucagon-producing alpha cells, the somatostatin producing gamma cells and pancreatic polypeptide-producing cells. The β-cells are polyhedral, being truncated pyramids and are usually well granulated with secretary granules 250-300 nm in diameter. It has been estimated that each rat β-cell contains about 10,000 granules. There are two forms of insulin granules electron dense mature granules and moderately dense immature granules (Bonner-Weir & Smith, 1994).

Microscopic examination shows abundant patches of β-cells in the pancreas of normal rats, which are absent in diabetic pancreas. (Anil *et al*., 1996) Selective destruction of β-cells is observed in alloxan or streptozotocin induced diabetic rats. Lytic and vascular changes of cellular components are also observed in diabetes. Small and shrunken islets and destruction of β-cells are observed in the diabetic condition. (Mitra *et al*., 1996) Insulitis, with heavy lymphocytic infiltration in and around the islets may be present and is more commonly seen in islets containing residual β-cells in type I diabetes.

Streptozotocin is well known for its selective pancreatic islet β cell cytotoxicity and in many animal species; STZ induces diabetes that resembles human hyperglycemic non-ketotic diabetes mellitus (Weir *et al*., 1981). Further rats treated with STZ display many of the features in human subjects with uncontrollable DM and are invaluable when studying the mechanisms by which hyperglycemia may contribute to microvascular complications such as neuropathy, nephropathy and retinopathy (Obrosova *et al*., 2005). The functioning of pancreas, liver and kidney may be affected due to decreased levels of insulin, hyperglycemia and its consequences. In the present investigation the histological changes in these tissues of diabetic rats and the effect of SS aqueous extract on these was studied.

MATERIALS AND METHODS

Animals

Male albino rats (Wistar strain, weighing 180-200g) were purchased and housed under standard husbandry conditions (30ºC ± 2ºC, 60-70 % relative humidity and 12hr day night cycle) and allowed standard pelleted feed and water ad libitum.

Plant material and extraction preparation

The *Sapindus saponaria* leaves were harvested and shade dried for 20 days. Then grinded mechanically and 100g of Coarse powder was extracted by using water in soxhlet apparatus. Extract was concentrated to semi-solid water free Material and final extract yield was 9.5%.

Induction of diabetes mellitus in rats

Diabetes was induced in male Wistar albino rats aged 2–3 months (180–200 g body weight) by intraperitoneal administration of STZ (single dose of 50 mg/kg b.w.) dissolved in freshly prepared 0.01M citrate buffer, pH 4.5 [Gupta et al., 2004]. After 72 h rats with marked hyperglycemia (FBG ≥250 mg/dl) were selected and used for the study.

Experimental design

Animals were divided in to six groups of six animals each. Group I served as a control: group II had normal + SS (60mg/ kg bw) rats; group III had normal + SS (100 mg/kg bw) and Group IV acts as diabetic control, group V as diabetic + SS (60 mg/ kg bw) and group VI comprised the diabetic + SS (100 mg/kg bw) rats treated with *Sapindus saponaria* aqueous

roots extract 60 and 100 mg/Kg bw/day respectively for 6 weeks, by oral incubation method. Rats were sacrificed at the end of 6 weeks and the blood samples were collected to analyze the effect of SS leaves extract on biochemical parameters. Collection and processing of blood for estimation of glucose and other biochemical parameters. Total hemoglobin was estimated by the cynomethaemoglobin method by Drabkin (1932) and glycosylated hemoglobin (HbA1C) was estimated by the method Nayak and Pattabiraman (1981); Bannon 1982). Serum total cholesterol, triglycerides and serum HDL cholesterol were using commercial kits (Dialab, Austria).

Tissue homogenate preparation

Tissue sampling for histopathological study: For histopathological study, three rats from each group were perfused with cold physiological saline, followed by formalin (10% formaldehyde). The liver, kidney and pancreas were excited immediately and fixed in 10% formalin.

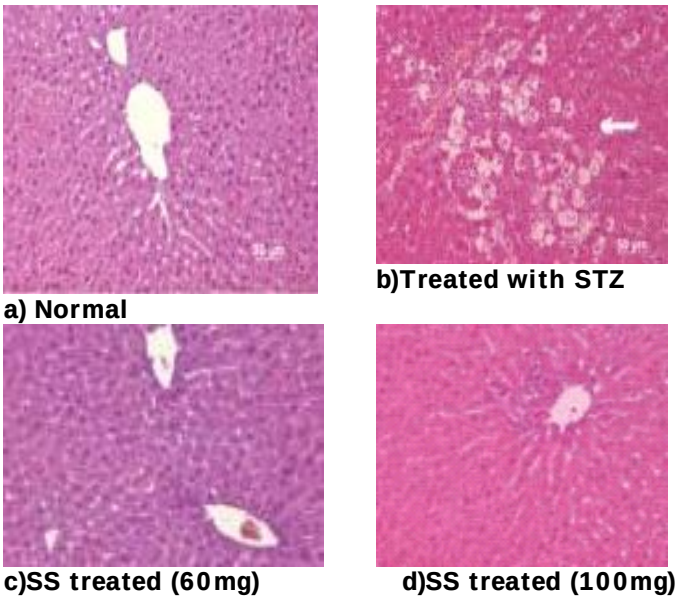
Light microscopic studies-Paraffin method: The light microscopic study was done by the method of Humason, (1979).

Reagents: 1. Physiological saline (0.9%) 2. Bouin-Hollande fixative 3. Ehrlich's hematoxylin 4. Eosin

RESULTS

Histological changes in liver and pancreas in normal rats, diabetic rats and diabetic rats treated with SS leaves extract are given below.

Liver: (Figure 1a) is the photomicrographs of the liver of a normal rat showing the normal hepatic architecture with normal central vein, prominent nucleus and normal hepatocytes. Figure 1b is the photomicrographs of the liver of diabetic untreated rats, which show degenerative liver with severe congestion of central vein, hemorrhages in the sinusoidal spaces and granular appearance of the hepatocytes (degenerative change) with cloudy swelling (hazzy nucleus). Figure 1c and 1d are the photomicrographs of the liver of diabetic rats treated with SS showing normal liver architecture with slight congestions in central vein, normal sinusoidal spaces and normal hepatocytes.

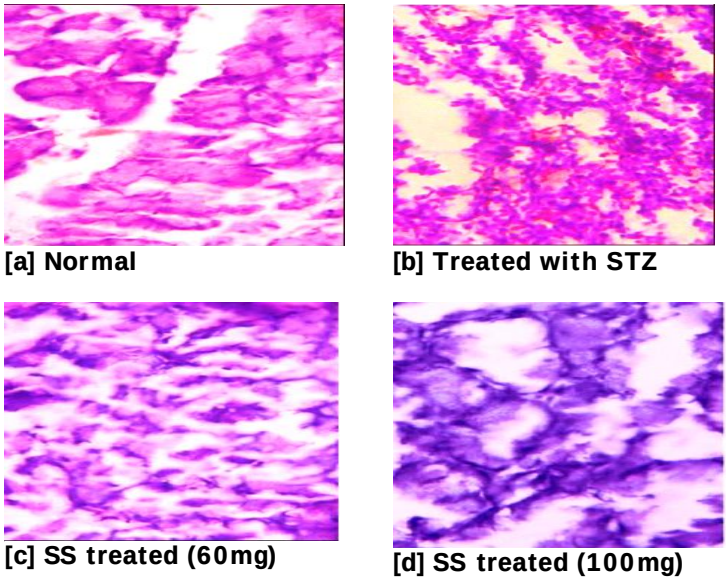


a) Normal **b)Treated with STZ**

c)SS treated (60mg) **d)SS treated (100mg)**

Figure 1(a, b, c, d): Histopathological panels of rat Liver prepared from 4 different groups of rats a. Control; b. Diabetic; c. Diabetic - SS (60 mg/kg bw); d. Diabetic - (100 mg/kg bw). Liver tissue showing with (100 X & 400).

Pancreas: The histology and ultrastructure demonstrated that most of the islets were affected and showed observed changes in structures. The β-cells showed degranulation and swelling of the intracellular organelles. All these vital intracellular structures were affected thus inhibiting the synthesis and release of insulin. Microscopic examination shows abundant patches of β cells in the pancreas of normal rats which are absent in diabetic pancreas. Oral administration of SS leaf extract for 30 days effectively restored the pathological changes in STZ induced diabetic rat pancreatic tissues (Figure 2a, b, and c, d). (Shanmugasundaram *et al.,* 1990) have reported that sections of pancreatic islets of GS leaf extract-treated diabetic rats showed its ability to regenerate the damaged endocrine tissue and increase β-cell numbers partially.



[a] Normal **[b] Treated with STZ**

[c] SS treated (60mg) **[d] SS treated (100mg)**

Figure 14 (a,b,c,d): Histopathological panels of rat pancreas prepared from 4 different groups of rats a. Control; b. Diabetic; c. Diabetic - SS (60 mg/kg bw); d. Diabetic - (100 mg/kg bw). It is showing with (100 X & 400 X)

DISCUSSION

The histological sections of the liver and pancreas tissues were observed to know the effect of SS fed in non-diabetic and diabetic rats. This was done to observe any protective or harmful effect of SS on non-diabetic and diabetic rats. The changes in the liver in diabetic rabbits induced by streptozotocin have been reported earlier (Mitra *et al.,* 1996). The diabetic liver showed degeneration and congestion. In diabetes, degradation of liver glycogen and gluconeogenesis are increased while glucose utilization is inhibited. Glucose 6-phosphatase increases in the liver, facilitating glucose release into the blood. The opposing enzymes which phosphorylate glucose is hexokinase, which is unaffected by insulin and glucokinase, which decrease in diabetes. As a result, the liver continues to produce glucose even with severe hyperglycemia. Under these circumstances the normal liver would shut off and deposit glycogen (Sherlock & Dooley, 1993). In the present study Sinusoidal haemorrhages, Vasculations in the hepatocytes (fatty changes), Granular appearance of the hepatocytes (degenerative change) and cloudy swelling (hazy nucleus) and inflammation were noticed in the liver of diabetic rats. These changes were reduced in SS fed rats. This may be due to beneficial and protective effect of SS aqueous extract on liver tissues of diabetic rats. Our histological findings are in agreement with the

degenerative structural changes reported in liver tissues as result of insulin depletion (Can *et al.*, 2004) in neonatal STZ (100 mg/kg) - induced type-II diabetic rat models. Can *et al*., (2004) observed an increase in degeneration in central veins to portal veins, excess vacuolization, granular appearance in the cytoplasm, dilations in the sinusoids and moderate hyperemia. In the present study, the histology and ultra structure demonstrated that most of the islets were affected and showed observed changes in structures. The β-cells showed degranulation and swelling of the intracellular organelles. All these vital intracellular structures were affected thus inhibiting the synthesis and release of insulin. Microscopic examination shows abundant patches of β cells in the pancreas of normal rats which are absent in diabetic pancreas (Anil *et al.,* 1996). Selective destruction of β cells is observed in STZ induced diabetic rats (Susan Bonner and Smith 1994). Small and shrunken islets and destruction of β cells are observed in the diabetic condition (Mitra *et al.,* 1996).

Oral administration of SS leaf extract for 30 days effectively restored the pathological changes in STZ induced diabetic rat pancreatic tissues. In this context, treatment of STZ induced diabetic animals with. (-)-epicatechin and N-acetyl-l-cysteine (NAC) well-known saponin, oligoglysodes and terpenoids and bio active compounds prevented hyperglycemia through reduced oxidative stress and restored β-cell function (Sheehan and Zemaitis, 1983). Shanmugasundaram *et al.,* (1990) have reported that sections of pancreatic islets of *Gymnema sylvestre* leaf extract-treated diabetic rats showed its ability to regenerate the damaged endocrine tissue and increase β-cell numbers partially. Our results are in agreement with the above observations of Nagappa *et al.,* (2003) showed that the regeneration of beta cells in the pancreas of *Terminalia catappa* fruit extract-treated diabetic rats, due to β-carotene, which is a constituent of *T. catappa* fruit. Sharma *et al.,* (2003) have reported that oral administration of *Eugenia jambolana* seed extract reversed the abnormalities in the islet of Langerhans of STZ-induced diabetic rabbits. The histopathological study reveals that decreased blood glucose concentration of diabetic rats by SS leaf extract treatment is due to the regeneration/proliferation in the pancreatic β-cells.

CONCLUSION

From this study it can be concluded that the administration of aqueous extract of *Sapindous saponaria* (SS) leaves is beneficial in normalizing the alterations in different tissues. The histopathological study reveals that decreased blood glucose concentration of diabetic rats by SS leaf extract treatment is due to the regeneration/proliferation in the pancreatic β-cells.

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