Antidiabetic effects *of Setaria italica* seeds aqueous extract in STZ induced diabetic rats. A Histopathological study

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Abstract-
Background: *Setaria italica* is commonly known as Foxtail millet. In India it is chiefly cultivated in Andhra Pradesh, Telangana and Tamil Nadu. It can be taken as sweet or savoury food in all ways that rice is used. Due to the presence of high fibre content, it is suggested as a food for diabetic patients.
Objectives: To evaluate the protective effects of SISAE on STZ induced diabetic rats.
Materials and Methods: The rats were divided into five groups (six rats in each group).
The animals in group 2 and 4 were given daily oral dose of 300mg of SISAE /kg b.w/day while groups 1 and 3 rats were given water alone and group 5 rats were treated with Glibenclamide at a dose of 20mg/kg b.wfor a period of 30 days. All the five groups were sacrificed on the last day of treatment by cervical dislocation and then blood, pancreas, liver and kidney were collected and stored in 10% formalin after washing 3 times with normal saline. Serum was separated and stored for further biochemical investigations. The tissues were washed, dehydrated with alcohol, cleared with xylene and paraffin blocks were made. Serial sections of 5 micrometres thickness were cut using a rotary microtone. The sections were then deparaffinised with xylene and hydrated in descending grades of alcohol. The slides were then transferred to haematoxylin for 10 minutes, followed by rinsing with water, differentiated in 1% acid alcohol, rinsed in water, blueing in running tap water or 1% lithium carbonate differentiated in 1% alcohol. Later they were counterstained with eosin, rinsed with water, dehydrated with ascending grades of alcohol, cleared with xylene and mounted on glass slides. These slides were examined under 10X and 40X using microscopes.
Results: In histopathological examination, Stained sections of pancreas of diabetic untreated rats showed destruction of beta cells with lymphocytic infiltrations and atrophy. Diabetic treated rats with SISAE showed regenerative changes with tissue architecture. Kidneys sections of diabetic untreated rats showed tubular damage and haemorrhage. But after treatment with SISAE damage of the kidney tissue was controlled. Stained sections of the diabetic untreated rats showed degenerative liver with severe congestion of central vein, haemorrhage in the sinusoidal spaces with hazy nucleus. Whereas, treatment with SISAE in diabetic rats showed improvement in histological structure of liver sections of diabetic rats with normal appearance of the liver lobules.

***Key words*: *Setaria italica*, seeds aqueous extract, (SISAE), Diabetes mellitus (DM), Streptozotocin (STZ), hyperglycemia, histopathology.**

INTRODUCTION

Diabetes mellitus is a metabolic disorder affecting a huge number of populations in the world. It is characterized by chronic hyperglycemia, resulting from defects in insulin secretion or insulin action. It is predicted that the number of diabetic persons in the world could reach 366 million by the year 2030. From the data available through literature, it was found that plant having antidiabetic activity is mainly due to the presence of secondary metabolite. Oxidative stress is involved in development of diabetes and a lot of other diseases. Diabetes mellitus is one of the oxidative stress conditions in which free radicals are increased and antioxidant mechanisms are inhibited. Plants impose their activity through this mechanism and can reduce the toxic effects of drugs. Ayurveda is an ancient Indian form of medicine which deals with plants and plant extracts. Plant drugs are frequently considered to be less toxic and freer from side effects than synthetic ones. Many herbs have been shown to have hypoglycaemic action in animals and humans**.** *Setaria italica* belongs to family Poaceae commonly known as Foxtail millet. The edible part of the plant is seed. The millet has protein and fiber content compared to other millets. In previous studies anti hyperglycemic and hypolipidemic activity of aqueous seed extract of *Setaria italica* in streptozotocin induced diabetic rats (Sireesha et al. 2011) was reported with improved activities of carbohydrate metabolic and antioxidant enzymes. (Sireesha, et al. 2023). In this paper, the protective effects of SISAE against diabetes induced damage are presented.

HISTOLOGY OF PANCREAS

Pancreas is a pistol shaped gland located inferior and deep to the stomach. It's about 6 inches or 15 cms long. It has both endocrine and exocrine functions. Its exocrine functions are to produce digestive enzymes. Its edoncrine functions pertain to regulating blood glucose. Pancreas produce insulin and glucagons, two hormones that regulate sugar levels in blood. Insulin and glucagons are secreted from pancreas directly into blood.

The part of the pancreas with endocrine function is made up of approximately a million cell clusters called islets of Langerhans. Four main cell types exist in the islets. They are alpha-cells which secrete glucagon (increase glucose in blood), beta-cells secrete insulin (decrease in blood), delta-cells secrete somatostatin (regulate /stop alpha and beta cells) and pp-cells or gamma-cells, secrete pancreatic polypeptide. The islets are a compact collection of endocrine cells arranged in clusters and cords and are cris-crossed by a dense net work of capillaries. The capillaries of the islets are lined by layers of endocrine cells in direct contact with blood vessels and either by cytoplasmic process or by direct apposition.
The islets are destroyed in type 1 diabetes mellitus. Heavy lymphocytic infilterates appear in and around islets. The number and size of islets are eventually reduced, leading to decreased insulin production and glucose tolerance. The islets are normal in number or somewhat reduced with type 2 DM. Fibrosis and deposition of amylin polypeptide within islets are most characteristic of the chronic states of type 2 DM (Tomita, 2012).

HISTOLOGY OF LIVER

Liver is the largest gland which receives both venous blood, through the portal vein and arterial blood, through the hepatic artery. It is surrounded by a well-defined but thin capsule of connective tissue. The connective tissue extends into the liver parenchyma and divides it into the basic structural units of the liver, the classical liver lobules. It functions as an exocrine gland, because it secretes bile. The portal vein, hepatic artery and bile duct enter the liver through the portal. These groups of three tubes are called portal triads. Portal triads are the key features of the organization of the liver. Portal triads are embedded in interlobular connective tissue.
Liver lobule is a six-sided prism about 2mm long and 1mm diameter. It is delimited by inter lobular connective tissue. In its corners we find the portal triads. In cross sections, the lobule is filled by cords of hepatic parenchyma cells, hepatocytes, which radiate from the central vein and are separated by vascular sinusoids.
Hepatocytes are large polyhedral epithelial cells, with large round centrally located nuclei (two or more). Hepatocytes are grouped in interconnected plates that are arranged into thousands of small polyhedral lobules. Hepatocytes are separated from the blood stream by thin discontinuous simple squamous epithelium, which lines the sinusoids. Sinusoidal endothelial cells are from the wall of the blood vessels (sinusoids) that carry blood throughout the liver from a single layer with spaces between each cell known as fenestra, that allow an efficient flow of essential materials to pass from blood to hepatocytes and vice versa. They are rich in lysozomal enzymes needed for degrading endocytosed material. Between the hepatocytes and epithelial cells is a narrow peri sinusoidal space. Contents of the blood plasma can freely enter the peri sinusoidal space, through the openings in the epithelium lining the sinusoids. Fixed macrophages, Kupffer cells, are attached to the epithelium. They clear the blood of ingested bacterial pathogens that may enter portal blood from the gut and also, they remove aged erythrocytes and free heme for reuse.
The liver lobule is drained by the central vein, which opens into the intercalated or sub-lobular veins of the liver. These in turn coalesce to form the hepatic veins. They run alone through the tissue, are usually covered by connective tissue, and eventually empty into the inferior vena cava.
The best indication of a liver lobule are the large central veins and the strands of hepatocytes, which seem to radiate out from the central veins. The slides show the macrophages adhere to the wall of the liver sinusoids. They are represented by the accumulations of small black dots.
Hepatocytes make up about 80% of the cells in the liver. Hepatocytes function in the storage of glucose in the form of glycogen. They participate in the turnover and transport of lipids. They synthesize some of the plasma proteins.
The liver of diabetic rat shows perivenular inflammatory infiltration filling over the sinusoidal vacuolation of the hepatocyte nuclei.
Tissue section shows distortion in the arrangement of cells around the central vein, peri portal fatty infiltration with focal necrosis of hepatocytes. Degeneration with diffuse vacuolations of the hepatocytes was observed (Elamin et al., 2018).

HISTOLOGY OF KIDNEY

The essential tissue composition of kidney is that of a gland with highly modified secretory units and highly specialized ducts. The secretory units of kidney are called renal corpuscles, comprise a relatively small portion of the kidney. The bulk of the kidney consists of highly specialized tubules, which correspond to the duct tree. Together the renal corpuscles and its associated tubule is called a nephron.
In kidney each renal corpuscle is a highly modified secretory acinus. Each corpuscle secretes a filtrate of blood plasma which drains into its associated renal tubule. Renal tubules in turn function like exaggerated striated ducts, modifying the filtrate by reabsorbing everything that is not waste. Renal tubules have convoluted tubules, loops of Henle, Collecting ducts.
The cortex consists of convoluted tubules together with renal corpuscles. The medulla consists of loops of Henle and collecting ducts. The cortex and medulla together comprise millions of individual nephrons, all packed together. Renal corpuscles produce a filtrate of blood plasma. Renal corpuscles are also called as Malpighian capsules.
STZ is one of the most used substances to induce diabetes in rat. This compound was found to be selectively toxic to the beta cells of the pancreatic islets, which regulate blood glucose level by producing the hormone insulin. This molecule causes the death of beta-cells by alkylation of DNA. Furthermore, this toxin has been shown to be involved in the fragmentation of DNA as well as other deleterious effects by means of the production of reactive oxygen species (Szkudelski, 2001). The functioning of pancreas, liver and kidney may be affected due to decreased levels of insulin, hyperglycemia and its consequences. Rats administered with STZ provide a useful model for investigating the effects of type 2 diabetes. In the present study, using STZ induced diabetic rats the histological changes in these tissues of diabetic rats & the effect of SISAE on these was studied.

MATERIALS AND METHODS

Collection of Plant material:

Dry seeds of *Setaria italica* were purchased and identified by the Botanist, Department of Botany, S.V. University, Tirupati. A voucher specimen (Herbarium Accession No.1842) was deposited in the herbarium, Department of Botany, S.V. University, Tirupati. These seeds were powdered and the powder was used for the extraction of antidiabetic active principle(s).

Preparation of aqueous extract

The seed powder was soaked in glass jar for 48h at room temperature and solvent was filtered This was repeated 3-4 times until filtrate gave no coloration. The filtrate was concentrated to dryness under reduced pressure in Buchi Rotavapor R-200 and finally freeze dried. The yield of the extract was 21% (w/w).

Induction of diabetes

Diabetes was induced in male wistar albino rats aged 2-3 months (180-200g body weight) by intraperitoneal administration of STZ (single dose of 55mg/kg b.w) dissolved in freshly prepared 0.01M citrate buffer, pH4.5 (S. Gupta et al.,2004). After 72 hours rats with marked hyperglycemia (fasting blood glucose ≥ 250 mg/l) were selected and used for the study. All the animals were allowed free access to tap water and pellet diet duct and maintained at room temperature in plastic cages, as per the guidelines of Institute Animal Ethics committee (Regd. no. 470/01/a/CPCSEA, dt. 24th August 2001).

Experimental design

Rats with the fasting blood glucose levels of ≥ 250mg/dl were taken for the experiment. The rats were divided into 5 groups, 6 rats in each group.

Group 1:Normal rats
Group 2:Normal rats treated with 300 mg/kg bw of SISAE
Group 3: Diabetic control rats
Group 4: Diabetic rats treated with 300 mg/kg b w of SISAE
Group 5:Diabetic rats treated with 20 mg glibenclamide /kg. b.w

The animals in group 2 and 4 were given daily oral dose of 300 mg/kb b w of SISAE, while groups 1 and 3 rats were given water alone and group 5 rats were treated with glibenclamide at a dose of 20 mg/kg.b.w at morning time for a period of 30 days.. All the 5 groups were sacrificed on the last day of treatment by cervical dislocation and then blood, pancreas, liver, and kidney were collected and stored in 10% formalin after washing 3 times with normal saline. Serum was separated immediately and then stored for further biochemical investigations.

Histological studies

Histopathological parameters were studied at the Department of Pathology, S.V. Veterinary University, Tirupati, A.P, India. The tissues were washed, dehydrated with alcohol, cleared with xylene and paraffin blocks were made. Serial sections of 5 um thickness were cut using a rotary microtone. The sections were then deparaffinised with xylene and hydrated in descending grades of alcohol. The slides were then transferred to haematoxylin for 10 min, followed by rinsing with water, differentiated in 1% acid alcohol, rinsed in water, bluing in running tap water or 1% lithium carbonate. Later counter stained with eosin, rinsed with water, dehydrated with ascending grades of alcohol, cleared with xylene and mounted on glass slides. These slides were examined under 10X and 40X using microscope

RESULTS

Histopathology

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

Pancreas

Photographs (P1 & P2) of normal rats showing exocrine acini and endocrine islets with scattered beta cells and RBC are visible in the vicinity. The cellular integrity and architecture were intact in the normal. Normal arrangement of islets of Langerhans of various sizes scattered throughout the exocrine tissue within the visible lesion (Ebong, et al. 2009).

Photographs (P3 & P4) of diabetic rats showed damaged beta -cells, depleted islets due to necrosis and fewer beta-cells. Severe necrotic changes of pancreatic islets especially in the centre of islets nuclear changes, Karyolysis, disappearance of nucleus and residue of destroyed cells were visible in some places. Relative reduction of size and number of islets especially around the central vessel and severe reduction of beta- cells were seen. Marked degeneration of the islets of the Langerhans with severe vacuolation of the exocrine tissue restored the diameter of the islet cells.

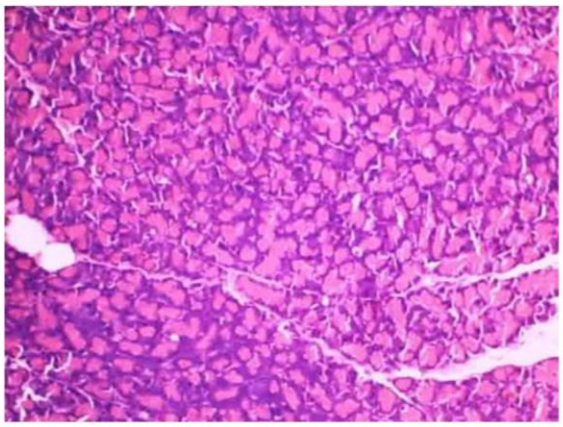

Fig P1. Normal rat pancreas 10X

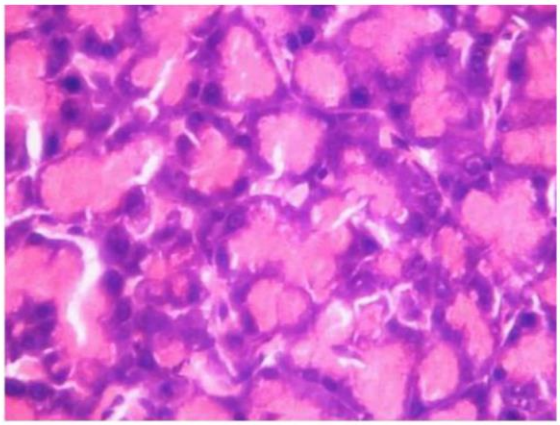

Fig P2. Normal rat pancreas 40X

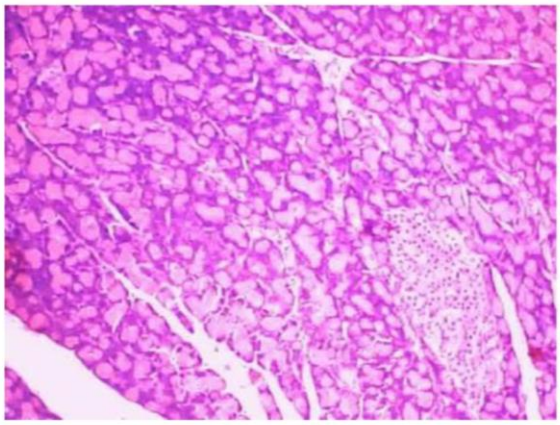

Fig P3. Normal rat treated pancreas 10X

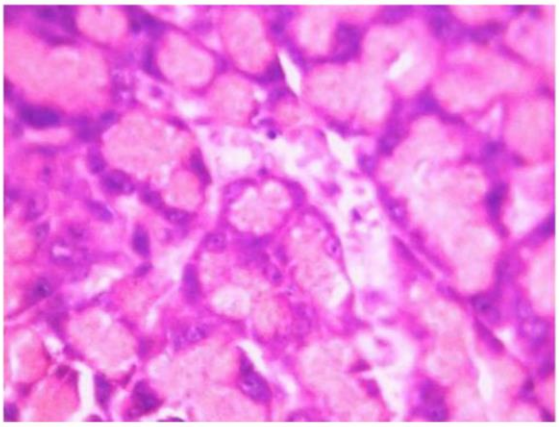

Fig P4. Normal rat treated pancreas 40X

Photographs (P5 & P6) of the diabetic treated rats shows evenly distributed beta -cells and an increased number of beta -cells. Even in severely diabetic animals with reduced beta- cell mass, recovery of the damaged islets and an improvement in number of beta -cells was observed after treatment

Photographs (P7 & P8) of normal treated pancreas showing exocrine acini and small preserved islets, no significant change was observed when compared to normal pancreas.

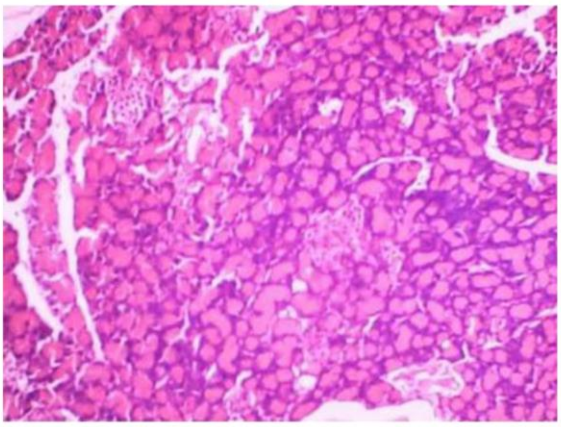

Fig P5. Diabetic rat pancreas 10X

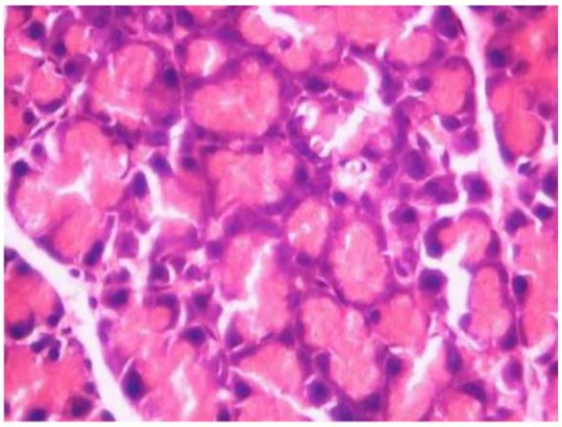

Fig P6. Diabetic rat pancreas 40X

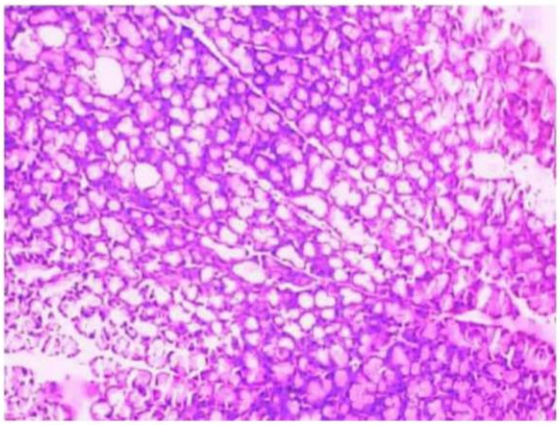

Fig P7. Diabetic rat treated pancreas 10X

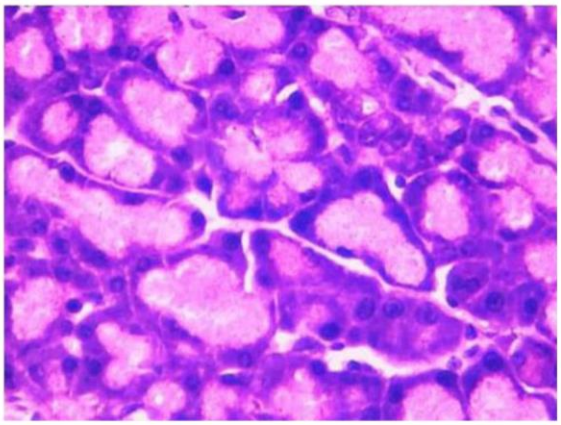

Fig P8. Diabetic rat treated pancreas 40X

Liver

Photographs (L1 & L2) of normal liver section shows normal architecture that is essentially formed of hepatic lobules. Each lobule is made up of radiating plates, strands of cells forming a net work around a central vein. The liver strands are altering with sinusoids. These sinusoids have irregular boundaries composed of only a single layer of fenestrated endothelial cells and large irregularly phagocytic cells, which are known as Kupffer cells.

Outside the hepatic lobule at certain angles, lie the portal areas of connective tissue each including a hepatic portal vein, a branch of hepatic artery and a bile ductile.

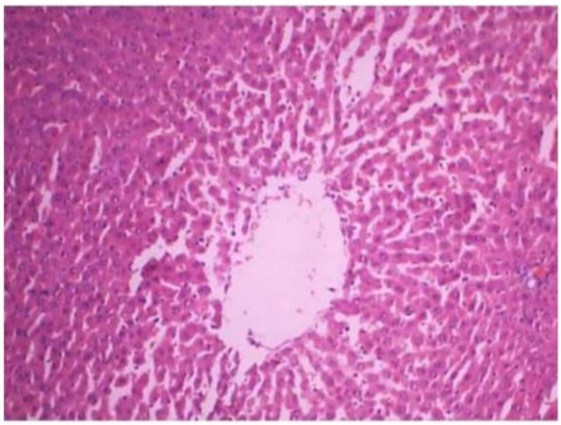

Fig L1. Normal rat liver 10X

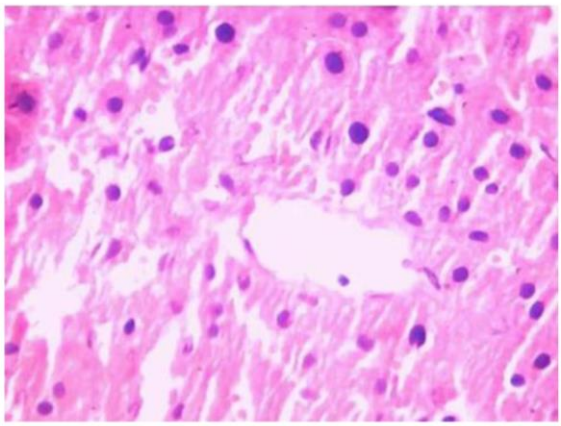

Fig L2. Normal rat liver 40X

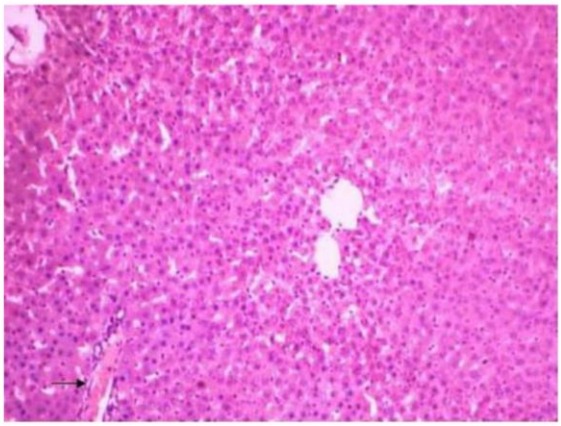

Fig L3. Normal rat treated liver 10X

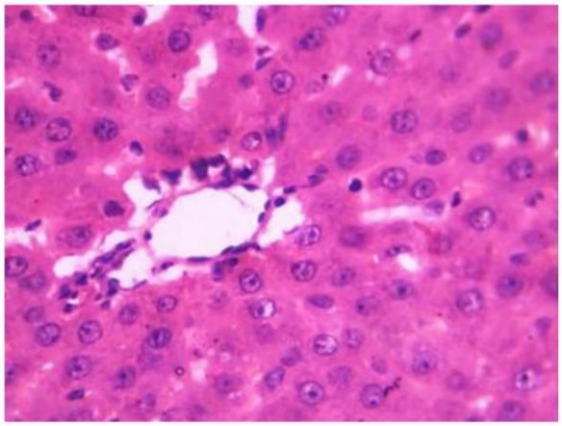

Fig L4. Normal rat treated liver 40X

Photographs (L3 & L4) of liver of STZ induced diabetic rats showed hepato cellular injury pronounced in loss of the normal architecture of the liver, cord like arrangement of the normal hepatocytes were not well distinct. Dilatation and inflammation in central and portal vein. There was severe fibrosis and leucocytic infiltration around the portal veins which appeared congested with blood with dilatation in blood vessels. The sinusoids between the hepatic cells were markedly dilated with increase in Kupffer cells. The hepatocytes appeared to be suffering from certain degree of cloudily swelling with marked cytoplasmic vacuolations, fatty infiltration, nuclei of most cells revealed clear signs of pyknosis and karyolysis

In the photographs (L5 & L6) of the diabetic treated rats the portal areas showed portal veins still distended and engorged with blood, but the reversed towards control sections were considerably higher in SISAE treated rats. The hepatocytes observed some degree of histological regeneration, reduction in fat accumulation, less sinusoids dilatation with decrease number of Kupffer cells, less necrotic cells were observed.

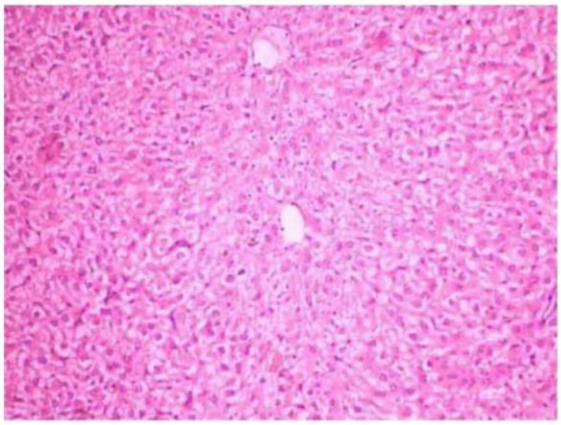

Fig L5. Diabetic rat liver 10X

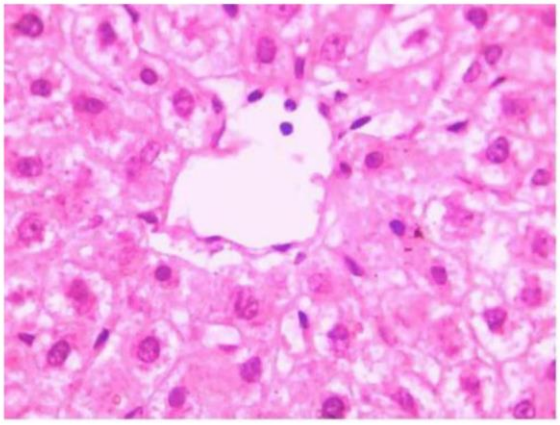

Fig L6. Diabetic rat liver 40X

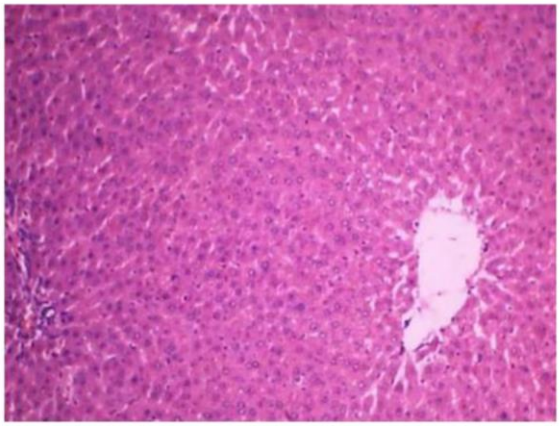

Fig L7. Diabetic rat treated liver 10X

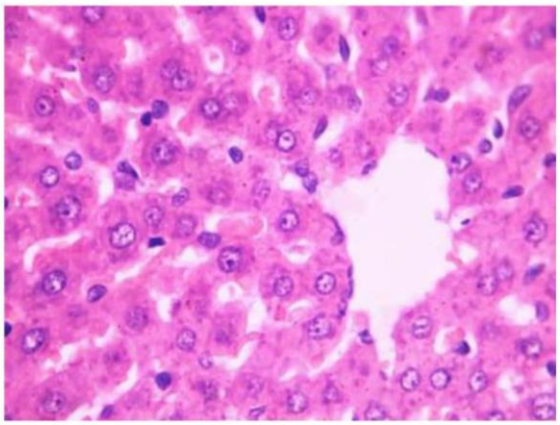

Fig L8. Diabetic rat treated liver 40X

The photographs (L7 & L8) of liver section of normal treated rats have no significant change when compared to liver sections of normal rats

Kidney

Photographs (K1 & K2) of the kidney sections of normal rats, collecting tubules are lined with relatively low simple cubic epithelium. The thick well demarcated cortex & medulla, and intact capsule with well formed glomerular tuft. The glomeruli were surrounded by narrow spaces of Bowman's capsules.

Kidney sections (K3 & K4) of diabetic animals showed thickening on the walls of the nephron filling their lumen along with glomerulopathy. Kidney sections of diabetic rats showed tubular damage, proteinuria, and hemorrhages. Hemorrhages was seen with in the Bowman's space due to glomerular damage. Degeneration of glomeruli with wider Bowman's spaces and diffuse vacuolation of the tissues.

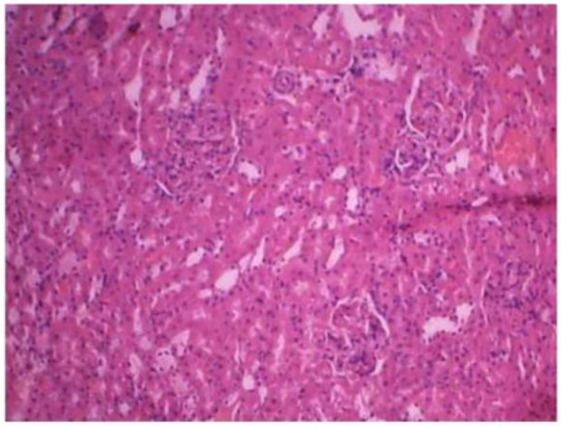

Fig K1. Normal rat kidney 10X

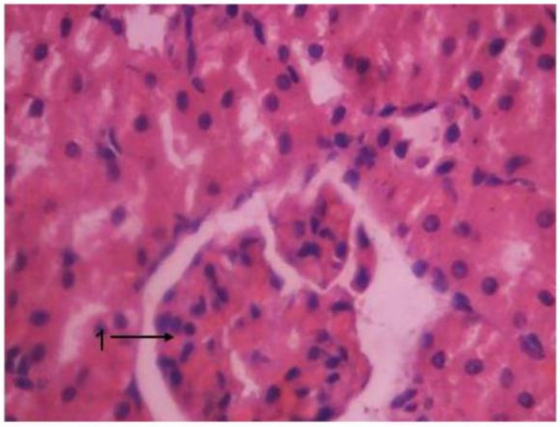

Fig K2. Normal rat kidney 40X

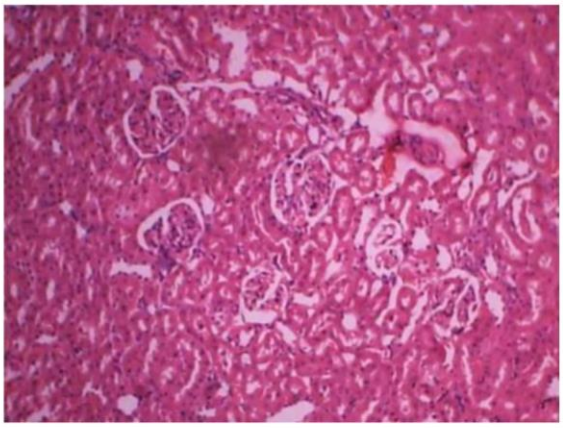

Fig K3. Normal rat treated kidney 10X

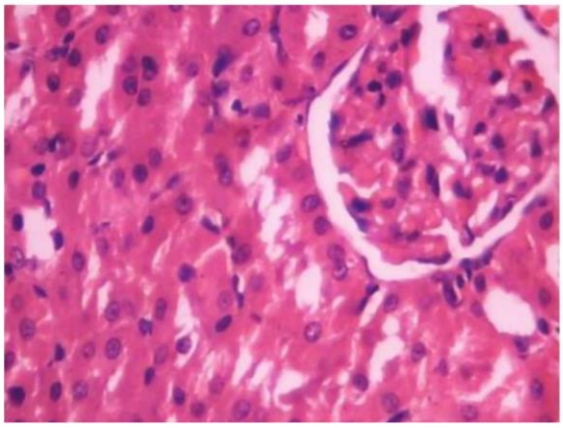

Fig K4. Normal rat treated kidney 40X

Kidney sections (K5 & K6) of diabetic treated rats showed the damaged capillary loops with increase in the thickness of the wall; glomeruli and tubules without proteinuria and haemorrhage.

Kidney sections (K7 & K8) of the normal treated rats shown no changes in the histology of kidney when compared to normal rats.

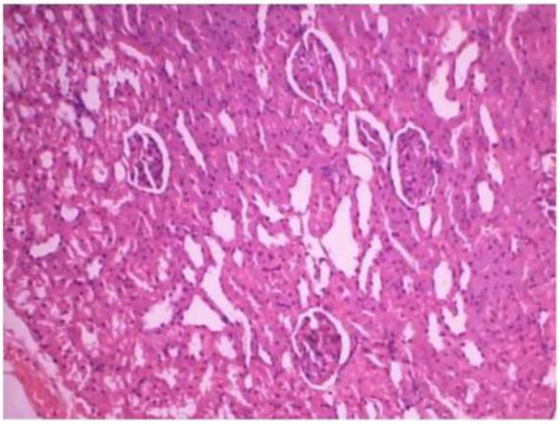

Fig K5. Diabetic rat kidney 10X

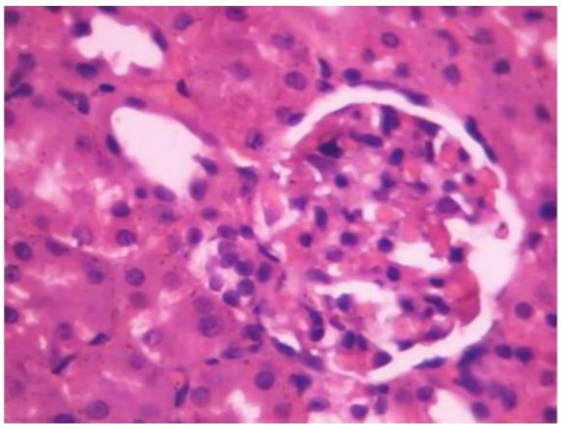

Fig K6. Diabetic rat kidney 40X

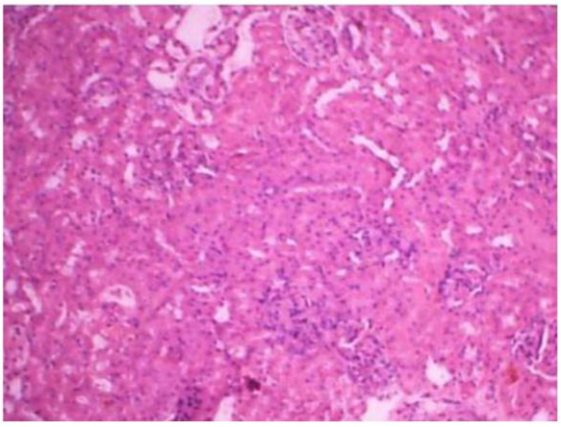

Fig K7. Diabetic rat treated kidney 10X

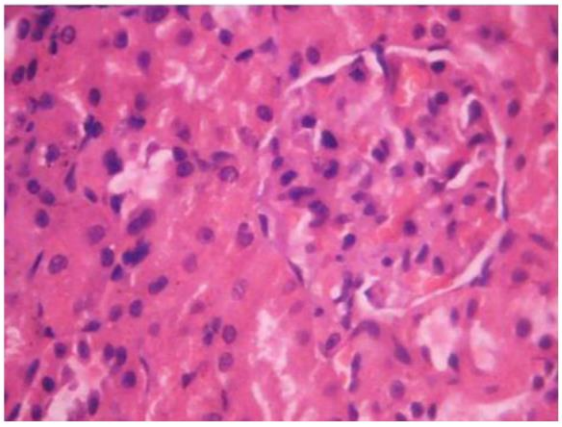

Fig K8. Diabetic rat treated kidney 40X

DISCUSSION

Diabetes mellitus is one of the oxidative stress conditions in which free radicals are increased and antioxidant mechanisms are inhibited. Free radicals induce oxidative stress and can lead to injury of cellular membrane (Tiedz, et al. 1998). Free radical formation has been reported to be a direct consequence of hyperglycemia (Giugliano, et al. 1996). Antioxidant enzyme expression was lowest in islets of Pancreas than liver and kidney. Increased free radicals may lead to degradation of cellular functions and oxidative damage to membranes by consuming antioxidant defense components (Baynes, et al. 1991; Van Dam, et al. 1995). Plants with antioxidant activity and some of the active ingredients obtained from these are thought to be useful for the treatment of Diabetes mellitus. The active metabolites of plants have positive effect on glucose metabolism and provide protective role against oxidative stress caused by hyperglycemia (Nicolle, et al. 2011). Research studies revealed that these compounds have a sweeping effect on free radicals and have a strong antioxidant role (Veeraraghavan, et al. 2015; Kasi Ravi, et al. 2004).

STZ is a glucosamine-nitrosourea compound. Streptozotocin was found to be selectively toxic to the beta-cells of the pancreatic islets, which regulate blood glucose level by producing the hormone insulin. STZ is toxic to cells by causing damage to the DNA, though other mechanisms also contribute. DNA damage induces activation of poly ADP-ribosylation, which is likely more important for diabetes induction than DNA damage itself. Streptozotocin is similar enough to glucose to be transported into the cell by glucose transport protein GLUT2, but is not recognized by other glucose transporters. This explains its relative toxicity to beta cells, since these cells have relatively high levels of GLUT2. STZ induces diabetes that resembles human hyperglycemic non-ketotic DM. The functioning of pancreas, liver and kidney may be affected due to decreased levels of insulin, hyperglycemia, and its consequences. In the present investigations the histological changes in these tissues of diabetic rats and the effect of SISAE on these was studied.

In diabetic rats the reduction in number of beta-cells within islets of Langerhans reflects the cytotoxicity of STZ. This agrees with the reports submitted by (Szaleczky, et al. 1999). STZ liberates toxic amounts of Nitric oxide that inhibits aconitase activity and participates in DNA damage. The regeneration of beta-cells of STZ-destructed islets is because pancreas contain stable cells for regeneration (Kumar, et al. 1992).

Pancreas is the primary organ involved in sensing the organisms dietary and energetic states via glucose concentration in blood. In response to elevated blood glucose, insulin is secreted (Uyar, et al. 2016; Yaman, 2016). Diabetic rats showed degeneration of

pancreatic islet cells, which propably gave rise to insulin deficiency. Insulin deficiency causes excessive elevation of blood glucose and underutilization leading to hyperglycemia. (Subha, et al. 2004: Yadav, et al. 2008)
The diabetic treated group indicated increased volume density of islet cells & increased percentage of beta-cells, which may be a sign of regeneration. Signs of regeneration of beta cells, potentiation of insulin secretion from surviving beta–cells of islets of Langerhans & decrease of blood glucose have been reported following the consumption of SISAE (Prakash, et al. 2012; Raghavan, et al. 2006).

The changes in the liver in diabetic rats induced by STZ have been reported earlier (Khan et al., 1990; Kholoud, et al., 2013; Mohammed Zafar et al., 2009). Hepatocytes were markedly swollen and suggested that these abnormalities may represent an intermediate leision between fatty steatosis (excessive build up of fat inside liver cell) and cirrhosis. These changes were reduced in SISAE fed rats. This is due to the antihyperlipidemic effect of SISAE on liver tissues of diabetic rats. Our histopathological changes agree with those of (Can et al., 2004).
The effect of SISAE showed improvement in histological structure of liver sections of diabetic rats, pronounced in normalized appearance of liver lobules with strains of hepatocytes comparing with section of diabetic rat liver (Maisaa and Rawi, et al. 2007).
The main function of the kidney is to excrete the waste products of metabolism and to regulate the body concentration of water and salt. In STZ induced Type2 DM, alterations in the structural integrity of the apical membrane of proximal tubules of the kidney tissue in diabetic rats was observed (Abdollah, et al. 2010). The improvement in renal morphology and function in STZ diabetic rats after treatment with SISAE could be due to its antidiabetic action.
The effect of SISAE showed improvement in histological structure of kidney of diabetic rats, the damaged capillary loops with increase in the thickness of the wall; glomeruli and tubules without proteinuria and haemorrhage.
The findings indicate the presence of some antihyperglycemic agents in the seeds of *Setaria italica*, which have been concentrated in extracts. The hypoglycemic effects of plants may be due to insulin like substance in plants (Krishna Kumari, et al.1998), stimulation of beta-cells to produce more insulin (Chang & Johnson, 1980) increasing glucose metabolism (Broadhust, 1997) or regenerative effect of plants on pancreatic tissue (Sundaram, et al. 1990).

CONCLUSION

SISAE has been shown to protect the Pancreas, Liver and Kidney from the diabetes induced damage due to its antidiabetic and antioxidant activity. Therefore, SISAE seems to be useful in the treatment of diabetic related complications.

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