

# Nephroprotective effects of ethanolic extract of *Sesamum indicum* seeds (Linn.) in streptozotocin induced diabetic male albino rats

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**Background:** Hyperglycaemia is the important hallmark of Diabetes mellitus and it is the world's most common endocrine disorder. The aim of the present study was to evaluate the effect of ethanolic extract of *Sesamum indicum* (Linn.) on kidney function in Streptozotocin Nicotinamide induced diabetic rats. **Materials and Methods:** Twenty four Wistar albino rats of 200-250 g each were grouped into 4 groups with six rats in each group (control, diabetic, diabetic treated and standard drug -glibenclamide treated). After treatment for 8 weeks, the animals were sacrificed and the biochemical parameters like serum total protein, albumin and globulin, urea, uric acid and creatinine were measured for the evaluation of kidney damage. **Results:** STZ-induced diabetic rats showed a significant decrease in the levels of serum total protein, albumin and globulin and significant increase in the levels of blood urea, serum creatinine and uric acid when compared to normal rats. These levels were reverted after the treatment regimen. **Conclusion:** From the study, the *Sesamum indicum* extract administration to the diabetic rats resulted in normalizing the marker enzymes in serum and the histopathological results also reveal the protective effect of the plant extract. The above results shows that the ethanolic extract of *Sesamum indicum* seeds has a potential effect to control hyperglycemia in streptozotocin induced diabetic rats.

**Key words:** Creatinine, diabetes, histopathology, *sesamum indicum*, urea, uric acid

## INTRODUCTION

Diabetic nephropathy (DN), one of the most serious micro vascular complications of diabetes, is a major cause of end stage renal disease.<sup>[1]</sup> It occurs approximately in one third type 2 diabetic patients<sup>[2]</sup> and is on rise. Kidney excretes the metabolic wastes, which includes urea, uric acid and creatinine and other ions. By the removal of these metabolic wastes it maintains the optimum balance in the body fluids. In the renal damage associated with diabetes, the increased levels of these metabolites were observed.<sup>[3]</sup> Due to the uncontrolled blood glucose levels, these metabolites may deposit in the vital organs such as kidneys, the toxic concentration of blood sugar damages the kidney tissue. This leads to altered kidney function in the patients, causing Diabetic nephropathy.

Diabetes causes renal damage due to abnormal glucose regulation, including elevated glucose and glycosylated

protein tissue levels, haemodynamic changes within the kidney tissue and increased oxidative stress.<sup>[4]</sup> DM is also grossly reflected by profound changes in protein metabolism and by a negative nitrogen (N) balance and loss of nitrogen from most organs.<sup>[5]</sup> Increased urea nitrogen production in diabetes may be accounted for by enhanced catabolism of both liver and plasma proteins.<sup>[6]</sup>

Ayurveda, the ancient system of Indian medicine, has identified hepatic and renal diseases quite early and recommended a number of herbal drugs, which are a good source of natural antioxidants believed to exert their effects by reducing the formation of the final active metabolite of the drug induced systems or by scavenging the reactive molecular species to prevent their reaching a target site.<sup>[7]</sup> The majority of the plants that are used in popular medicine for treatment of diabetes have been shown to possess biologically active chemical constituents (alkaloids, carbohydrates, coumarins, flavonoids, terpenoids, phenolic substances, and other constituents) that can be used as new hypoglycemic agents.<sup>[8,9]</sup>

Sesame (*Sesamum indicum* L.) is one of the world's most important oilseed crops. Sesame is mainly cultivated in the developing countries of Asia and Africa for its high content of good quality oil (42–54%) and protein

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(22–25%).<sup>[10]</sup> Its meal protein is composed of globulins (67.3%), albumins (8.6%), prolamine (1.4%), and lutelin (7%).<sup>[11]</sup> Sesame protein is high in methionine (3.2%), which is unusual for most plant proteins, and these unique properties render sesame seed an excellent protein source for supplementing soybean, peanut, and other vegetable proteins (which lack sufficient methionine) to increase their nutritive qualities.<sup>[12]</sup> The seed contains appreciable amounts of various bioactive components including tocopherols, phytosterols, resveratrol and flavonoids, and the lignans sesamin and sesamol.<sup>[13]</sup> The identification and management of early stage diabetic kidney disease is important, but the majority of people exhibit no symptoms until the disease is more advanced. Therefore, we have investigated therapeutic agents for the prevention of early-stage diabetic renal damage using short-term streptozotocin induced diabetic rats.

## MATERIALS AND METHODS

### Collection of Plant Material and Preparation of Plant Extract

*Sesamum indicum* (Linn.) (Family- Pedaliaceae) seeds were collected from Tirupur district, Tamilnadu, India. Taxonomic authentication was done by Taxonomist Dr.V.S.Ramachandran, Associate Professor, Department of Botany, Bharathiar University, Coimbatore, Tamilnadu, India. The seeds were shade dried and powdered using mixer grinder. The powdered material (50 g) was extracted with 250 ml of ethanol using Soxhlet apparatus and filtered. The filtrate was concentrated and dried under reduced pressure and controlled temperature.

### Chemicals and Reagents

The chemicals and solvents used in the study were of highest purity and analytical reagents grade. They were purchased from SD Fine Chem., Himedia and Qualigens, India.

### Experimental Animals

Male Wistar strain Albino rats (200-250 g) of about two months were obtained from the National Institute for Mental Health and Neuro Sciences, Bangalore, India. They were housed in polypropylene cages under the standard laboratory condition (25 ± 2° C, humidity 60-70%, 12 hours light/dark cycles). The animals were fed with commercial rat pellet diet (AVM feeds, Coimbatore) and water was provided *ad libitum*. The animal care and handling were done according to the regulations of Council Directive CPCSEA no: 659/02/a about Good Laboratory Practice (GLP) on animal experimentation. All animal experiments were performed in the laboratory according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC).

### Grouping of Animals

After one week of acclimatization period, the animals were divided into four groups with six animals in each.

Group I: Control rats fed with standard pellet diet and water.

Group II: Rats induced with single dose of 65 mg/kg body weight streptozotocin (i.p.) 15 minutes after administration of 110 mg/kg body weight nicotinamide (i.p.). Animals whose blood glucose level exceeded 200 mg/dl at 24 hours after treatment were considered diabetic. These animals served as untreated diabetic control.

Group III: Diabetic rats treated with ethanolic extract of *Sesamum indicum* (500 mg/kg body weight for 8 weeks)

Group IV: Rats treated with standard drug glibenclamide (600 µg/kg body weight for 8 weeks)

### Sample Collection

After the experimental regimen, the animals were sacrificed by cervical dislocation under mild chloroform anaesthesia. Blood was collected by an incision made in the jugular veins and the serum was separated by centrifugation at 2000 rpm for 20 minutes.

### Analytical Procedures

Serum total protein, albumin and globulin were measured by the method of Wolfson (1948),<sup>[14]</sup> urea was measured by the method of Natelson *et al.* (1951),<sup>[15]</sup> uric acid was measured by the method of Caraway, 1963<sup>[16]</sup> and creatinine was measured by the method of Owen *et al.*, (1954)<sup>[17]</sup> for the evaluation of kidney damage.

### Histopathological Examination of Kidney

The kidney tissues of the experimental animals were isolated immediately after sacrificing the animal and washed with ice-cold saline. They were then fixed in 10% formalin solution. Sections of 3 µm thickness were stained with haematoxylin and eosin (H and E) for histopathological analysis.

### Statistical Analysis

The data was expressed as mean ± SD for six rats in each group. Statistical analysis was performed using SPSS software, version 16.0. The values were analyzed by one - way analysis of variance (ANOVA) followed by Least Significant Difference (LSD). *P*<0.05 were considered as statistically significant.

## RESULTS

Table 1 shows the effect of oral administration of *Sesamum indicum* extract on body weight in normal and streptozotocin induced diabetic rats. In the present study diabetic control

group rats showed significant loss of body weight when compared with the normal group. All animals treated with *Sesamum indicum* and the standard drug glibenclamide showed significant prevention of the loss in body weight throughout the study.

Table 2 depicts the effect of *Sesamum indicum* on serum total protein, albumin and globulin in normal and diabetic rats. STZ-induced diabetic rats showed a significant decrease in the levels of serum total protein, albumin and globulin when compared to normal control rats. The lowered levels of total protein, albumin and globulin in serum of STZ-induced diabetic rats were reverted to near normal levels due to plant extract treatment. Glibenclamide treated rats significantly increased the levels of total protein, albumin and globulin in serum after treatment regimen.

The levels of blood urea, serum creatinine and uric acid in normal and STZ induced diabetic rats are shown in Table 3. Rats induced with STZ, showed a significant increase in the levels of blood urea, serum creatinine and uric acid when compared to normal rats. Diabetic rats treated with *Sesamum indicum* and the standard drug glibenclamide showed significant reduction in the levels of blood urea, serum creatinine and uric acid when compared with diabetic rats.

Figure 1a-d shows the histopathological architecture of kidney in normal and experimental rats.

From the figure, it is evident that in Group I normal rats, the kidney section showed normal architecture. Group II diabetes induced rats the kidney tissue under microscope showed minimal diffuse mesangial thickening, very minimal lymphocytic infiltrate in the interstitium and only very few glomeruli appear normal. In Group III and IV Diabetic rats treated with the ethanolic extract of *Sesamum indicum* and glibenclamide respectively, the kidney tissue showed similar features of group I (normal rats) with slightly more number of normally appearing glomeruli when compared to diabetic kidney.

## DISCUSSION

Diabetes mellitus is a chronic metabolic disorder of multiple aetiologies, characterized by a state of insulin deficiency that leads to a rise in glycemia,<sup>[8]</sup> initially involving changes in carbohydrate metabolism and secondarily of lipids and proteins.<sup>[9]</sup> Despite progress in the management of diabetes mellitus by synthetic drugs most of these drugs have side effects in the long run. So, the search for improved and safe natural anti diabetic agents is on-going and World Health Organization has also recommended the development of herbal medicine in this concern.<sup>[18]</sup> Distinct metabolic renal

**Table 1: Effect of oral administration of *Sesamum indicum* extract on body weight in normal and streptozotocin induced diabetic rats**

Groups	Body weight (gms)	
	Initial	Final
I	251.67±1.50	271.50±3.08
II	253.50±2.25	208.67±2.87a*
III	252.33±1.96	233.16±5.34b*
IV	252.00±2.00	236.50±3.01c* d <sup>ns</sup>

\*Indicates P < 0.05; ns – Non-significant; Values are expressed as mean±SD of six animals in each group statistical comparison: a – Group I and II; b – Group II and III; c – Group II and IV; d – Group III and IV

**Table 2: Effect of oral administration of *Sesamum indicum* extract on serum total protein, albumin and globulin in normal and streptozotocin induced diabetic rats**

Groups	Protein	Albumin	Globulin
I	7.36±0.27	4.90±0.05	2.63±0.20
II	4.94±0.33 a*	2.79±0.19 a*	2.23±0.18 a*
III	6.89±0.05 b*	3.80±0.13 b*	3.09±0.11 b*
IV	6.84±0.22 c*d <sup>ns</sup>	3.82±0.15 c*d <sup>ns</sup>	3.01±0.23 c*d <sup>ns</sup>

\*Indicates P < 0.05; ns – Non-significant; Values are expressed as mean±SD of six animals in each group statistical comparison: a – Group I and II; b – Group II and III; c – Group II and IV; d – Group III and IV

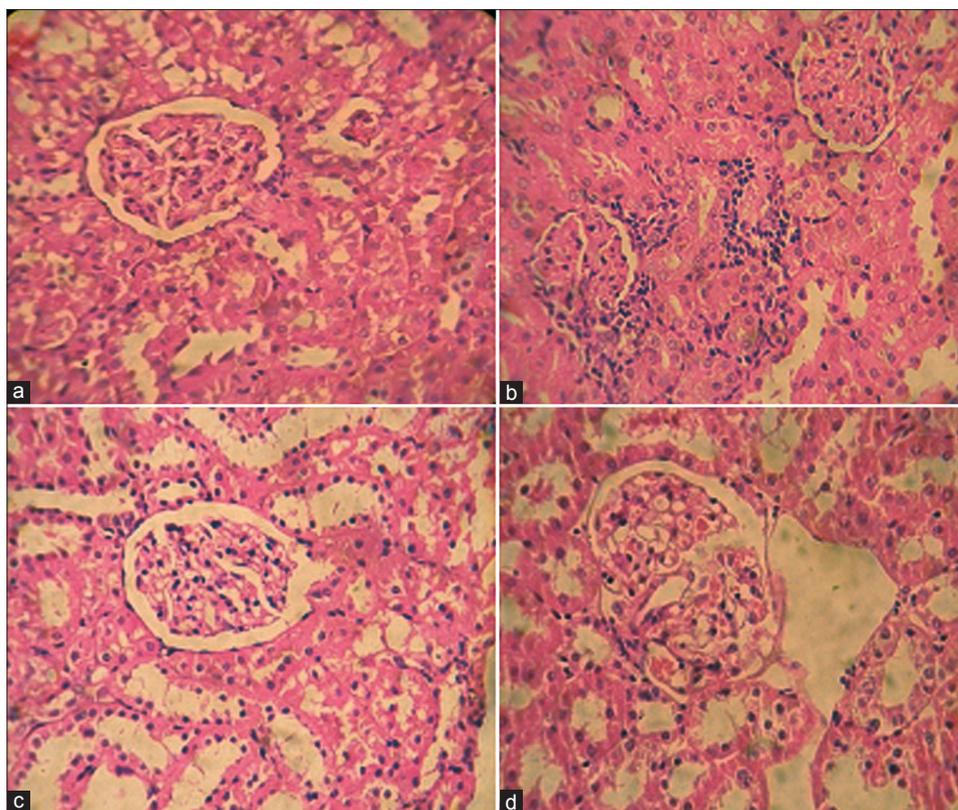
**Table 3: Effect of oral administration of *Sesamum indicum* extract on serum urea, uric acid and creatinine in normal and streptozotocin induced diabetic rats**

Groups	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
I	30.83±1.92	1.30±0.11	1.23±0.15
II	44.47±1.55 a*	2.65±0.27 a*	2.16±0.12 a*
III	35.10±1.00 b*	1.85±0.13 b*	1.57±0.13 b*
IV	35.72±1.21 c*d <sup>ns</sup>	1.87±0.21 c*d <sup>ns</sup>	1.56±0.05 c*d <sup>ns</sup>

\*Indicates P < 0.05; ns: non-significant; Values are expressed as mean±SD of six animals in each group statistical comparison: a – Group I and II; b – Group II and III; c – Group II and IV; d – Group III and IV

alterations are demonstrable in experimental diabetes, leading to a negative nitrogen balance, enhanced proteolysis and lowered protein synthesis.<sup>[19]</sup>

The decrease in body weight in diabetic rats clearly shows a loss or degradation of structural proteins due to diabetes as the structural proteins are known to contribute to body weight. The structural proteins are known to contribute for the body weight.<sup>[20]</sup> Ravi *et al.*<sup>[21]</sup> reported that the characteristic loss of body weight associated with STZ-induced diabetes is due to excessive breakdown of tissue proteins and an increased muscle wasting in diabetes. Dhanraj *et al.*<sup>[22]</sup> reported that in the absence of insulin, protein production is not favored. Additionally, several studies showed that the levels of serum total protein were declined in diabetic animals.<sup>[23-25]</sup> Plant extract administration improved the body weight in diabetic rats. This prevention of loss in body weight by *Sesamum indicum* extract may be due to increasing glucose uptake in peripheral tissues or inhibiting catabolism of fat and protein or by glycemic control. Thus the plant potentials in



**Figure 1:** (a) Group I – Normal rats; (b) Group II – Diabetes induced rats; (c) Group III – Diabetic rats treated with; (d) Group IV – Diabetic rats treated with *Sesamum indicum* treated with glibenclamide

the *Sesamum indicum* seeds improve the protein production by stimulating the insulin secretion.

Distinct metabolic renal alterations are demonstrable in experimental diabetes, leading to a negative nitrogen balance, enhanced proteolysis and lowered protein synthesis (Bhavapriya *et al.*).<sup>[19]</sup> Changes in protein metabolism include a reduced uptake of amino acids by tissues, a higher rate of proteolysis and a fall in protein synthesis, leading to an increase in the production of urea by the liver.<sup>[26]</sup> The overload of urea, glucose and other compounds in the kidney, together with renal vascular changes arising from the increased glycosylation of blood proteins, can damage the kidney and thus promote a loss of protein in the urine (Viberti *et al.*).<sup>[27]</sup>

An overall reduction in serum total protein in diabetic animals and subsequent reduction in the albumin was observed in the present study. This corroborates earlier reports.<sup>[28]</sup> The levels of serum total proteins were found to be decreased in diabetic rats may be ascribed to (i) decreased amino acid uptake; (ii) greatly decreased concentration of variety of essential amino acids; (iii) increased conversion rate of glycogenic amino acids to carbon dioxide and water; and (iv) reduction in protein synthesis secondary to a decreased amount and availability of mRNA.<sup>[29]</sup> Also, this decline may be due to the inhibited oxidative

phosphorylation processes which lead to decrease in protein synthesis, increase in the catabolic process and reduction of protein absorption.<sup>[30,31]</sup> Increased protein catabolism in diabetes might have induced a direct adverse effect on the synthesis and secretion of albumin. Hypoalbuminemia is a common problem in diabetic animals and is generally attributed in the presence of nephropathy. On the other hand, in the *Sesamum indicum* extract treated and standard drug glibenclamide treated diabetic rats the protein levels were reverted to the normal range which might be due to the stimulation of insulin which results in decreasing protein catabolism activation of protein synthesis via oxidative phosphorylation. The reversal of these changes by ethanolic *Sesamum indicum* extract therapy proved that insulin deficiency had been grossly corrected.

The main function of the kidneys is to excrete the waste products of metabolism and to regulate the body concentration of water and salt. Insulin dependent diabetes is usually accompanied by high urinary glucose concentration, which produces an osmotic diuresis and therefore polyuria. One of the most sensitive and dramatic indicators of kidney injury is to increase the creatinine and urea level in serum. Plasma uric acid and creatinine can be used as a rough index of the glomerular filtration rate.<sup>[32]</sup> High levels of uric acid and creatinine indicates several disturbances in kidney.<sup>[33]</sup>

In the present investigation the changes in STZ diabetic rats is associated with significant increase in the levels of urea, uric acid and creatinine, indicating impaired renal function of diabetic rats. The diabetic hyperglycemia induces elevation of the serum levels of urea, creatinine and uric acid which are considered as significant markers of renal dysfunction.<sup>[5]</sup> Lehninger (1998)<sup>[34]</sup> reported that in severe diabetic condition there is an elevated excretion of urea whose concentration may be five times higher than the normal value. *Sesamum indicum* extract treatment significantly decreased the levels of blood urea and serum uric acid and creatinine in diabetic rats, which could be due to the prevention of protein and nucleic acid degradation. Similar reduction in the levels of blood urea and serum uric acid and creatinine were observed in the glibenclamide treated rats.

Creatinine is the major waste product of creatine metabolism. In the kidney, it is filtered by the glomerulus and actively excreted by the tubules. Moreover, free creatinine appears in the blood serum (Stevenes *et al.*)<sup>[35]</sup> urea is the principal waste products of protein catabolism. They are synthesized in the liver from ammonia, produced as a result of the deamination of amino acids. High-serum creatinine level is also the marker of muscle wastage.<sup>[36]</sup> Kidney function tests help to determine if the kidney is performing their task adequately. The diabetic rats had increased levels of creatinine and urea which are considered as significant markers of renal function and this is in agreement with the present result.<sup>[37]</sup>

Renno *et al.*<sup>[38]</sup> demonstrated that green tea extract provides a beneficial effect on long-term diabetic nephropathy via suppressing hyperglycemia and preventing glycogen accumulation in the proximal tubules. The diabetic hyperglycemia induces elevation of the plasma levels of urea, uric acid and creatinine which are significant markers of renal dysfunction and reflecting a decline in the glomerular filtration rate.

STZ-induced diabetes in rats had been shown to be associated with functional and/or morphological changes in the kidney.<sup>[39]</sup> All structural changes in kidneys resulting from STZ administration in rats can thus be attributed to altered metabolism in diabetes.<sup>[40]</sup> Present observations on the kidney sections showed minimal diffuse mesangial thickening and feature of mild chronic and non-specific interstitial nephritis and only very few glomeruli appear normal, indicating a progressive damage in streptozotocin induced diabetic rats, which increased with the duration of time and the severity of hyperglycaemia. Progressive glomerulosclerosis associated with decreased kidney function, resulting in end stage renal failure is the major finding in diabetic nephropathy. After the administration of *Sesamum indicum* extract the kidney showed many similar

features of normal kidney with many normally appearing glomeruli when compared with diabetic kidney. Standard drug treated rats also show similar morphology as that of normal animal with minimal diffuse mesangial thickening. Thus, the treatment with the plant extract causes the recovery of body weight and certain altered biochemical parameters and restored the histology of the diabetic rat kidney.

## CONCLUSION

Thus, the therapeutic property of *Sesamum indicum* seems propitious in improving nephropathy by significantly improving serum parameters and histopathological evidence also suggests the same. In conclusion, the present study demonstrated that *Sesamum indicum* ameliorates the renal damage and increases the protein levels in the diabetic rats after the treatment regimen.

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