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Protective Effect of Chitosan Extracted from Freshwater Crab *Sartoriana spinigera* (Wood-Mason, 1871) on the Histological Changes in the Kidneys of Streptozotocin-Induced Diabetic Rats

Tigga Khushboo

Department of Zoology, S.P. Mahila College, Dumka, S.K.M. University, Dumka, Jharkhand, India

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Abstract: The aim of this study was to investigate the protective effect of chitosan extracted from exoskeleton of freshwater crab *Sartoriana spinigera* on streptozotocin induced diabetic rat kidney. *S. spinigera* is a freshwater edible crab consumed as a delicacy as well as used in folk medicine by the tribal people of Jharkhand. Chitosan was extracted from chelate legs, characterized by FTIR and average DD% was found as 80.156%. Male albino rats were injected streptozotocin (i.p.) 40 mg/kg b wt. to induce diabetes. The rats were divided into three groups (n=10): I (control), II (diabetic untreated), and III (diabetic rats treated with chitosan 100 mg/kg b wt.). Rats with blood glucose levels of ≥ 220 mg/dl were considered diabetic. In GR-III, daily administration of *Sartoriana spinigera* chitosan for 30 days improved histo-architecture of the kidney and almost restored its functionality in comparison to GR-II diabetic group. 90.28% glomeruli were normal and only 9.52% were in degenerated (shrunken glomeruli) condition. Reappearance of Bowman's space with distinct outer parietal layer and inner visceral layer was observed. Proximal collecting tubule (PCT) as well as distal collecting tubule (DCT) were surrounded by a single layer of cuboidal epithelial cells with a lumen. The epithelial cell height of PCT and DCT was significantly decreased to 14.84 ± 1.08 μ m in PCT ($p < 0.001$) and 14.99 ± 2.07 μ m in DCT ($p < 0.01$), respectively in comparison to GR-II. There was no sign of pyknosis and necrosis in PCT and in DCT. There was no infiltration of leucocytes in renal convoluted tubules as well as in tubulo-interstitial space. No tubulo-interstitial space was observed in chitosan treated diabetic rats' kidney. The relative weight of kidney in GR-II increased significantly than GR-I ($p < 0.05$). In case of GR-III relative weight of kidney was increased significantly to only $0.76 \pm 0.04\%$ ($p < 0.05$) in comparison to GR-I but there was decrease in relative weight of kidney in comparison to GR-II.

Keywords: Chitosan, *Sartoriana spinigera*, Streptozotocin, Cuboidal cell height, Diabetes mellitus, Kidney

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Introduction

The term Diabetes mellitus describes a metabolic disorder of multiple etiology characterized by

chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism

resulting from defect in insulin secretion, insulin action or both (World Health Organization, 1999). Diabetes mellitus (DM) is associated with a number of common symptoms, such as polyurea and polydipsia and long-term complications, including retinopathy, neuropathy, nephropathy and atherosclerosis (Abolfati *et al.*, 20120).

Sartoriana spinigera freshwater edible crab is listed as least concern (Wood-Mason, 1871) in view of its wide distribution, presumed large population and because it is unlikely to be declining fast enough to qualify for listing in a more threatened category. It is widely distributed in Bangladesh, Pakistan and India (Wood-Mason, 1871). In India, it is commonly found in Uttar Pradesh, West Bengal, Uttarakhand, Bihar, Rajasthan, Orissa and Assam. *Sartoriana spinigera* is not only a local delicacy but is also a great ethnobiological significance as it is used by tribal peoples of Jharkhand for treatment of various diseases.

Chitosan is useful in a wide application in various industries such as pharmaceuticals, biochemistry, biotechnology, cosmetics, biomedical, paper industry, food and textile industries and others (Muzzarelli *et al.*, 1985). Chitin the precursor to chitosan was first discovered in mushroom by Henri Braconnot (1811). In the 1820's chitin was isolated from insects (Odier, 1823). Chitin is a polysaccharide made up of β -(1 $\rightarrow$ 4) linked N-acetyl-D-glucosamine that is widely found in nature forming exoskeletons of crustaceans such as crabs, lobsters, krill and shrimps and insects as well as components of bacterial cell wall (Rinaudo, 2006). Different preparation and animal species source results in different physiochemical properties specially the degree, solubility, pH and even yield % of chitosan (Cho *et al.*, 1998). Chitosan can be extracted from various marine crustaceans' species among these freshwater crab shells is also a source of chitosan. Although freshwater crab meat is of huge importance in food industry but crab shell is not considered of any use.

The present study was undertaken to observe the histological changes in kidney of streptozotocin-induced diabetic male rats fed with chitosan as compared to those which were either normal or untreated. This study on the histology of the kidney highlights the protective action of the chitosan extracted from exoskeleton of *S. spinigera*.

Materials and Methods

Extraction and characterization of chitosan:

Methods of Takiguchi (1991 a, b) (Demineralization, deproteinization and deacetylation) was followed to extract chitosan from exoskeleton (chela legs) of *S. spinigera*. Extracted chitosan was sent to CIF, BIT Mesra, Ranchi for FTIR analysis and DD (%) was calculated using the formula of Brugnerotto *et al.* (2001).

Experimental animals:

Male albino rats (Wistar rats) weighing 140-230 g were used in this study. The rats were purchased from Jaz Scientific Store, Ranchi, Jharkhand. The animals were housed in polypropylene cages under hygienic conditions inside a well-ventilated room with dry rice husk as bedding material. Each cage consisted of not more than 3 rats. Rats were allowed to acclimatize to the laboratory condition of the University Department of Zoology, Ranchi University, Ranchi, animal experiment room at 24-28°C, and 12 h photoperiod and relative humidity of 50 $\pm$ 20% for two weeks before being used. They were fed standard diet and water *ad libitum*. The standard diet consisted of carbohydrate, protein, fat, salt mixture and adequate minerals and vitamins (Kumar *et al.*, 2009). General health condition and body weight of animals were investigated regularly throughout the entire treatment schedule. Clinical and behavioral observations were also monitored during the experimental period. Animals were maintained according to guidelines of Institutional Ethical Committee, Ranchi University, India.

Induction of diabetes to experimental animals:

Male albino rats were rendered diabetic (Prabhu and Natarajan, 2013) by a single intraperitoneal injection of Streptozotocin (SRL company-14653) (40 mg/kg b. wt.) in freshly prepared citrate buffer (0.1 M, pH 4.5) after an overnight fast. Streptozotocin (STZ) injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality. STZ injected animals exhibited massive hyperglycemia and diabetes in STZ rats was confirmed by measuring the fasting blood glucose concentration, 72 h after injection with STZ. The rats with blood glucose more than 220 mg/dl were considered diabetic and used for the experiment. The rats were randomly allotted into three groups (n=10): I (normal control rats), II (STZ-induced diabetic rats), III (Chitosan-100 mg/kg b wt. treated STZ-induced diabetic rats). All STZ-induced diabetic animals were treated with chitosan for 30 days.

Effect of chitosan on histology of rat kidney:

Tissue samples were taken from the kidney tissues of the animal groups (GR-I, GR-II and GR-III), fixed in freshly prepared Bouin's fluid, for 24 h, dehydrated in graded series of ethanol, cleared in xylene, and embedded in paraffin. Tissues were sectioned at 3-5 μ m, and the sections were stained with Hematoxylin and Eosin (HE) for histopathological examinations.

Statistical analysis:

Results are expressed as Mean $\pm$ SD and the difference between the groups were tested by student's t-test. $p < 0.05$ was considered as statistically significant.

Results

Chitosan was successfully extracted from chelate legs, FTIR analysis confirmed the extracted chitosan and average DD% was found 80.156%.

Induction of diabetes to experimental animals:

In the present study, average fasting blood glucose in normal male albino rats (n=10) was found as

69.10 $\pm$ 6.20 mg/dl, it ranged between 58-79 mg/dl. Average non-fasting/random blood glucose level was found as 112.90 $\pm$ 7.79 mg/dl, it ranged between 96-120 mg/dl. Rats exhibited hyperglycemic condition after 9th day of STZ (40 mg/kg b wt.) intraperitoneal injection. The animals with blood glucose more than 220 mg/dl were considered diabetic and used for the experiment. In the present study, hyperglycemic rats exhibited blood glucose level between 273-500 mg/dl.

Histopathological changes in kidney of chitosan treated diabetic rats:

Relative kidney weight:

Table 1 shows that the average body weight of rats in GR-I (normal control rats) was 215.00 $\pm$ 15.00 g, in GR-II (STZ-induced diabetic rats) average body weight of rats decreased to 176.66 $\pm$ 15.27g and in case of GR-III (chitosan treated STZ-induced diabetic rats) body weight of rats increased to 183.33 $\pm$ 11.54 g as compared to group II). The entire dissected kidney weight in GR-I (normal control rats) was 1.35 $\pm$ 0.05 g, in GR-II (STZ-induced diabetic rats) it increased to 1.49 $\pm$ 0.04 g, in GR-III (chitosan treated STZ-induced diabetic rats) kidney weight increased to 1.39 $\pm$ 0.10 g (in comparison to GR-I). When relative weight of kidney and % change was calculated, it was observed that in GR-II (STZ-induced diabetic rats) % increase was 33.33%, whereas in GR-III (chitosan treated STZ-induced diabetic rats) % increase was only 17.10%. Statistical analysis showed that the relative weight of kidney in GR-II (STZ-induced diabetic rats) increased significantly than GR-I ($p < 0.05$). But in case of GR-III (chitosan treated STZ-Induced diabetic rats) relative weight of kidney was increased significantly to only 0.76 $\pm$ 0.04% ($p < 0.05$) in comparison to GR-I but there was decrease in relative weight in comparison to GR-II (Table 1).

Histopathological observations:

Histopathological observations of GR-I (normal control rats) kidney: Figures 1 and 2 show kidney

Table 1: Effect of chitosan on relative weight of kidney in control rats, STZ-induced diabetic rats and chitosan treated STZ-induced diabetic rats

Groups	Average body wt. (g)	Absolute wt. of kidney (g)	Relative wt. of kidney (%)	% change in Relative wt. of kidney (%)
GR-I (control rats)	215.00±15.00	1.35±0.05	0.63±0.01 ^a	-
GR-II (STZ-induced diabetic rats)	176.66±15.27	1.49±0.04	0.84±0.09 ^{b*}	33.33 ↑
GR-III (chitosan treated STZ-induced diabetic rats)	183.33±11.54	1.39±0.10	0.76±0.04 ^{b*}	17.10 ↑

Values are given as Mean ±SD in each group. The same letters in the same column means that there is no significant difference between groups. The different letter in the same column means that there is a significant difference between groups at p<0.05. * = significantly different than GR-I at 5%.

Table 2: Damage in kidney in normal control rats, STZ-induced diabetic rats and chitosan treated STZ-induced diabetic rats

Groups	Normal Glomeruli (%)	Damaged glomerulus		
		Atrophied Glomeruli (%)	Shrunkened Glomeruli (%)	Diffused Glomerulosclerosis glomeruli (%)
GR-I (control rat)	97.78	-	2.22	-
GR-II (STZ-induced diabetic rat)	20.50	15.50	36.00	28.00
GR-III (chitosan treated STZ-induced diabetic rat)	90.28	-	7.22	2.50

of control rat. It showed normal architecture of cellular components. Many intact and round glomeruli with Bowman's capsule were seen. Renal corpuscle was a round or irregular structure which forms the glomerulus that was enveloped by Bowman's capsule (Fig. 2). The intact glomerulus was observed with well-defined cellularity. The Bowman's capsule was formed of two thin layered, outer parietal layer and inner visceral layer. The parietal layer consisted of flat single layer of squamous epithelium enclosing a narrow space i.e. Bowman's space. The visceral layer surrounds the glomerulus (Fig 2). No infiltrations of leucocytes were observed around the Bowman's capsule. 97.78 % of glomerulus

were in normal condition whereas only 2.22% of glomerulus were in shrinkage condition (Tables 2, 3). Proximal convoluted tubule (PCT) was surrounded by a single layer of cuboidal epithelial cells. The average cell height of cuboidal cells was 11.40±2.07 µm in PCT (Table 3). Cuboidal epithelial cells contained eosinophilic granular cytoplasm. PCT has centrally placed lumen. Distal convoluted tubule (DCT) was also surrounded by a single layer of cuboidal epithelial cells with a lumen (Fig. 2). The average cell height of cuboidal cells was 12.02±1.67 µm in DCT (Table 3). PCT were narrower than DCT (Fig. 2). Renal corpuscles and renal convoluted tubules were closely packed without any space in the interstitium. No

Table 3: Histomorphometric observations of kidney in control rats, STZ-induced diabetic rats and chitosan treated STZ-induced diabetic rats

Parameters Groups	Normal glomeruli (%)	Damaged glomeruli# (%)	Diameter of glomerulus (μm)	Epithelial Cell height of PCT (μm)	Epithelial Cell height of DCT (μm)
GR-I (control rat)	97.78	2.22	144.63 $\pm$ 16.50 b***	11.40 $\pm$ 2.07 b***	12.02 $\pm$ 1.67 b***
GR-II (STZ-Induced diabetic rat)	20.50	79.50	62.84 $\pm$ 14.89 a	19.96 $\pm$ 2.11 a	19.90 $\pm$ 0.71 a
GR-III (chitosan treated STZ-Induced diabetic rat)	90.28	9.52	177.40 $\pm$ 10.75 c***	14.84 $\pm$ 1.08c***	14.99 $\pm$ 2.07 c**

Damaged glomeruli (atrophied, shrunken and diffused glomerulosclerosis). Values are given as Mean $\pm$ SD in each group. The same letters in the same column means that there is no significant difference between groups. The different letter in the same column means that there is a significant difference between groups. ** = significantly different than GR-II at 1%, *** = significantly different than GR-II at 0.1%.

edematous changes and no infiltration of leucocytes was observed in the interstitium, in PCT and in DCT (Figs. 1, 2).

Histopathological observations of GR-II (STZ-induced diabetic rats) kidney: A lot of abnormalities were seen in the kidney histology of STZ-induced diabetic rat (Figs. 3, 4, 5, 6). In STZ-induced diabetic rat only 20.50% of glomeruli were normal whereas 79.50% glomeruli were damaged (Table 3). Some of the glomeruli were atrophied (Fig. 3) and atrophied glomeruli was 15.50 % (Table 2). Some of the glomeruli were in shrunken condition (Fig. 6). Shrunken glomeruli were total 36% (Table 2). Some glomeruli showed diffused glomerulosclerosis condition (Fig. 5). Diffused glomerulosclerosis condition of glomeruli was 28% (Table 2). Cellularity of glomerulus was less as compared to normal glomerulus (Fig. 5). The diabetic glomerular tuft showed diffused glomerulosclerosis. Squamous epithelial lining of parietal and visceral layer was degenerated (Figs. 4,5,6). Cuboidal epithelial cells of PCT and DCT became columnar due to thickening of epithelial cells (edematous changes)(Fig. 4). In diabetic condition cell height increased significantly from cuboidal to columnar in PCT and in DCT as 19.96 $\pm$ 0.27 μm and 19.90 $\pm$ 0.71 μm , respectively (Table 3). Some renal tubules showed pyknotic nuclei (Fig. 4). Widening of tubulo-interstitial

space was observed (Fig. 6). Infiltration of leucocytes in the tubulo-interstitial space was observed (Fig. 5).

Histopathological observations of GR-III (chitosan treated STZ-induced diabetic rats) kidney: Fig 7 shows kidney of STZ-Induced diabetic rat treated with chitosan. Daily administration of *Sartoriana spinigera* chitosan (100 mg/kg b wt.) for 30 days to STZ-induced diabetic rats improved histo-architecture of the kidney and almost restored its functionality. Chitosan treatment caused features of healing. 90.28% glomeruli were in normal condition and only 9.52% were in degenerated condition. Degenerated glomeruli were in shrunken condition. Normal glomerulus was round shaped. Bowman's capsule was clearly visible. Outer parietal layer and inner visceral layer were made up of squamous epithelial cells. These layers were intact and no damage was observed (Fig. 8). Glomerulus was made up of glomerular tuft which did not showed any fibrosis. No tubulo-interstitial space was seen (Figs. 7, 8). PCT was surrounded by a single layer of cuboidal epithelial cells with a lumen. DCT was also surrounded by a single layer of cuboidal epithelial cells with a lumen (Fig. 8). The epithelial cell height of PCT and DCT was significantly decreased to 14.84 $\pm$ 1.08 μm in PCT and 14.99 $\pm$ 2.07 μm in DCT at 0.1% and 1%, respectively in comparison

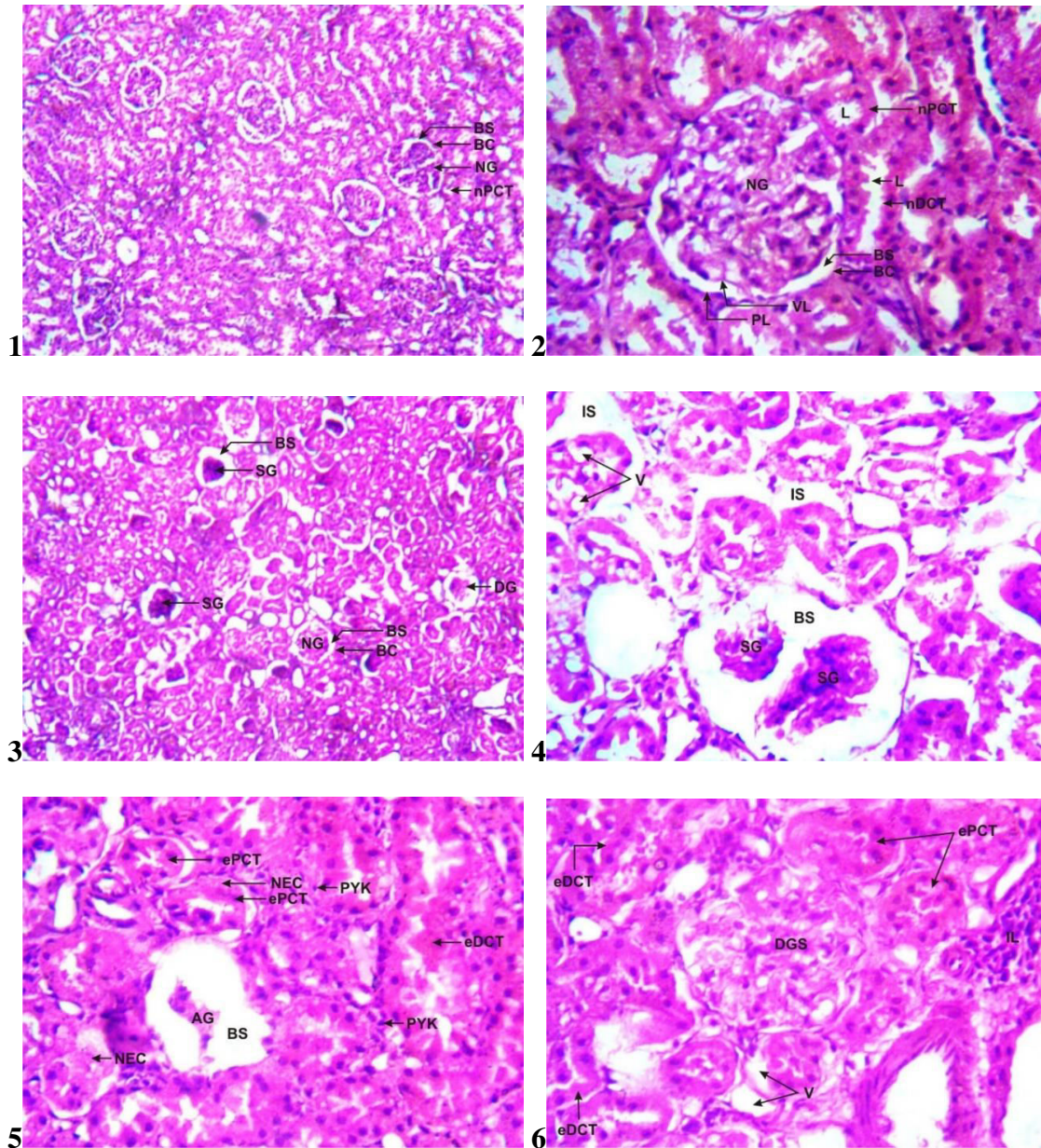


Fig. 1: Photomicrograph of control rat kidney showing normal architecture with intact round glomeruli, Bowman's capsule, Bowman's space, normal proximal convoluted tubule and distal convoluted tubule. HE X100.
 Fig. 2: Photomicrograph of control rat kidney showing normal architecture with intact round glomeruli, Bowman's capsule, Bowman's space, normal proximal convoluted tubule and distal convoluted tubule. HE X400.
 Fig. 3: Photomicrograph. of STZ-Induced diabetic rat kidney showing shrunken glomerulus and damaged glomerulus with increased Bowman's space. HE X100.
 Fig. 4: Photomicrograph. of STZ-Induced diabetic rat kidney showing shrunken glomerulus with wide Bowman's space, wide tubulo-interstitial space. HE X400.
 Fig. 5: Photomicrograph of STZ-Induced diabetic rat kidney showing atrophied glomerulus, edematous PCT and DCT, necrosis in PCT cells and DCT cells. HE X400.
 Fig. 6: Photomicrograph of STZ-Induced diabetic rat kidney showing diffused glomerulosclerosis, vacuolization in the cytoplasm of epithelial cells of proximal convoluted tubules, edematous proximal convoluted tubule and distal convoluted tubules, infiltration of leucocytes in interstitial space. HE X400.

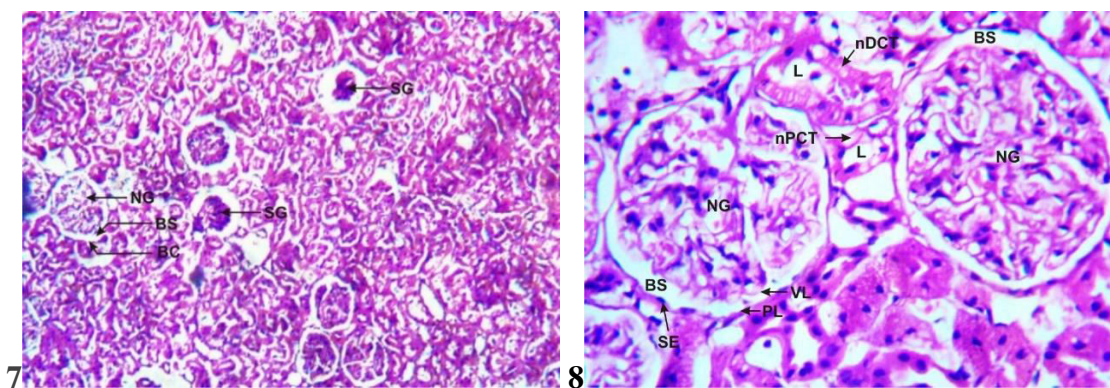


Fig. 7: Photomicrograph of chitosan treated STZ-Induced diabetic rat kidney showing normal round intact glomeruli and shrunken glomerulus. HE X100.

Fig. 8: Photomicrograph of chitosan treated STZ-Induced diabetic rat kidney showing round shaped intact glomerulus surrounded by Bowman's capsule, presence of Bowman's space with well-defined parietal layer and visceral layer, normal cuboidal epithelial cells of proximal convoluted tubule and distal convoluted tubule with lumen. HE X400.

Abbreviations for Figs. 1-8: NG- normal glomerulus, SG- shrunken glomerulus, DGS-Diffused glomerulosclerosis, BC-Bowman's capsule, BS-Bowman's space, VL- Visceral layer, PL-Parietal layer, nPCT- Normal PCT, nDCT- Normal DCT, L-Lumen, IS-interstitial space, V-vacuolation, ePCT- edematous PCT, eDCT-edematous DCT, IL-infiltration of leucocytes, NEC-necrosis, PYK-pyknotic nucleus.

to STZ-induced diabetic rats (Table 3). There was no sign of pyknosis and necrosis in PCT and in DCT. In Figures 7 and 8 there was no inflammatory signs i.e. absence of infiltration of leucocytes in PCT and in DCT and in tubulo- interstitial space. No tubulo- interstitial space was observed in chitosan treated diabetic rat kidney. The above observations seen were due to curative and nephroprotective effect of chitosan of *Sartoriana spinigera*.

Discussion

Hyperglycemia is the principal factor responsible for structural alterations at renal level. STZ-induced diabetic rodents are seen to develop kidney disorders similar to the early stage of human diabetic-associated disorders of the kidney (Teoh *et al.*, 2010). This study was undertaken to assess the nephroprotective and possible reversible effect of chitosan of *Sartoriana spinigera* on cyto-architectural alterations induced by administration of STZ (40 mg/kg b wt.). 30 days of daily administration of *Sartoriana spinigera* chitosan (100 mg/kg b wt.) caused a significant histopathological effect on kidney tissue.

In this study there was a significant increase in relative kidney weight of the diabetic rats in comparison to the kidneys of normal control rats. The renal enlargement is a characteristic feature of diabetic kidney. It might have occurred due to glomerular hypertrophy and nephromegaly. Kiran *et al.* (2012) reported that the increase of kidney weight initiated from the first month of diabetes mellitus and was exaggerated at the end of the fourth month. Teoh *et al.* (2010). stated that kidney enlargement in diabetes mellitus is attributed to certain factors like glucose over administration, glycogen accumulation, lipogenesis and protein synthesis in the diabetic kidney.

In the present investigation, histological study of normal control rat kidney showed normal cellular architecture. The intact round glomeruli were with well-defined cellularity surrounded by Bowman's capsule. This observation is similar to the results of Aboonabi *et al.* (2014). They also reported normal structure of glomerulus surrounded by the Bowman's capsule in normal rat kidney. Outer parietal layer of Bowman's capsule was lined by single layer of squamous epithelial cell and inner visceral layer surrounding

glomerular tuft. In present study, narrow Bowman's space was clearly visible which is in agreement with the result of Komolafe *et al.* (2013). They also reported normal Bowman's space surrounding the glomeruli in control rat. In present study, PCT was surrounded by a single layer of cuboidal epithelial cells. The average cell height of cuboidal cells was $11.40 \pm 2.07 \mu\text{m}$ in PCT. Cuboidal epithelial cells contained eosinophilic granular cytoplasm. PCT has centrally placed lumen. DCT was also surrounded by a single layer of cuboidal epithelial cells with a lumen. The average cell height of cuboidal cells was $12.02 \pm 1.67 \mu\text{m}$ in DCT. Inflammatory cells were absent in renal tubules. These findings are in agreement with AL-Samawy (2012). He also reported that PCT exhibited a small, uneven lumen and a single layer of cuboidal cells with eosinophilic granular cytoplasm in normal albino rats.

In the present study diabetic rat kidney showed degenerated and shrunken glomeruli with widening of Bowman's space. The above findings are in agreement with Teoh *et al.* (2010). They reported degenerated glomeruli in diabetic kidney. According to Ayelagbe and Adele (2015) some glomeruli were damaged and atrophied in STZ-induced diabetic rat. AL-Quraishy *et al.* (2015) also reported shrunken or completely lost glomeruli in STZ-induced diabetic rat kidney. The present study is in agreement with the observations of Yassin *et al.* (2004) who noticed that in diabetic rats the glomerular tufts were obviously contracted, lobulated and degenerated. Some glomeruli showed diffused glomerulosclerosis condition. Diffused glomerulosclerosis condition of glomerulus was 28%. Progressive glomerulosclerosis associated with decreased kidney function, resulting in end stage renal failure is the major finding in diabetic nephropathy (Zafar *et al.*, 2009). In this study in the kidney of STZ-induced diabetic rat, squamous epithelial lining degeneration of parietal and visceral layer of Bowman's capsule was observed. The above findings are in agreement with findings of Komolafe *et al.* (2013). They also reported

increased Bowman's space due to degeneration of parietal and visceral layer of Bowman's capsule in STZ-induced diabetic rat kidney.

In this study cuboidal epithelial cells of PCT and DCT became columnar due to thickening of epithelial cells (edematous changes) which is in conformity with the result of Bassey *et al.* (2014) and Teoh *et al.* (2010). They also reported edematous changes in PCT. The observed thickening of convoluted tubules specially in PCT was also observed by Casalena *et al.* (2012). Histological study of kidney in STZ-induced diabetic rat showed tubular necrosis. Signs of degeneration in the cells of renal tubules was observed in the form of karyolysis (dissolved chromatin in the cell nucleus) and pyknosis. This might be due to condensation and fragmentation of nucleus due to chromosomal dissolution and condensation. This is in conformity with Zafar *et al.* (2009) as they have also noticed necrosis with loss of brush border in proximal tubular area in diabetic rats. The present investigation noticed vacuolar degeneration in the proximal convoluted tubular cells. These vacuolation may be due to altered permeability of the cell membrane that would allow increasing fluid uptake (Johnson, 1982). In the present study, STZ-induced diabetic rat kidney showed pyknotic nuclei in some renal tubules. Similar reports were given by Bassey *et al.* (2014). They also reported tubular necrosis. In this study widening of tubulo-interstitial space was observed in diabetic kidney which derives support from observations of Ibrahim *et al.* (2016) as they have also noticed tubulo-interstitial changes in diabetic rat kidney. In present study infiltration of leucocytes was observed in tubulo-interstitial space. Najafian *et al.* (2011) and An *et al.* (2015) also noticed interstitial space with mononuclear cell infiltration in diabetic rat kidney.

Protective effect of chitosan (100 mg/kg b wt.) of *Sartoriana spinigera* treatment was observed in the histology of STZ-induced diabetic rat kidney. 9.52% glomeruli were in degenerated condition. Degenerated glomeruli were in shrunken

condition. Reappearance of normal cellularity of glomerulus and reappearance of Bowman's space with inner visceral layer and outer parietal layer with single layer of squamous epithelium was seen. Bassey *et al.* (2014), also reported appearance of intact glomerular capillaries and squamous lining of Bowman's capsule in alloxan induced diabetic rats when treated with *Allium sativum* extract. According to Komolafe *et al.* (2013), administration of *Psidium guajava* extract to STZ-induced diabetic Wistar rats improved histo-architecture of the kidney and restores its functionality due to regenerative capacity induced by *Psidium guajava* extract. In the present study, the tissue necrosis in kidney was also observed to decrease in group treated with chitosan. Edematous condition was almost reversed to normal in PCT and in DCT in chitosan treated group. Similar findings were also noticed by Mestry *et al.* (2016). They also reported that when STZ-induced diabetic rats were given methanolic extract of *Punica granatum*, it significantly reduced degeneration of tubules. In the present study there was no inflammatory signs i.e. absence of infiltration of leucocytes in PCT, in DCT and in tubulo-interstitial space in the kidney of rats treated with chitosan (100 mg/kg b wt.). Aboonabi *et al.* (2014) also reported that the STZ-induced diabetic rat treated with pomegranate demonstrated normal architecture without any inflammatory cells.

Conclusion

From the present study it is concluded that freshwater edible crab *Sartoriana spinigera* is an excellent source of chitosan. Chitosan from this local crab of Jharkhand has not only shown a high degree of deacetylation but this research also gave significant evidence to prove that chitosan extracted from exoskeleton of *Sartoriana spinigera* has curative effect on histology of streptozotocin-induced diabetic rat kidney.

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