**ORIGINAL ARTICLE**

# Effect of Streptozotocin on the Morphology of Proximal Convoluted Tubules in Albino Rats

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## ABSTRACT

**Aim:** To observe the nephrotoxic effect of STZ on the morphology of proximal convoluted tubules (P.C.T) of albino rats for three weeks & eight weeks duration.
**Study design:** Prospective experimental study.
**Place and duration of study:** This study was conducted in Department of Anatomy, Post Graduate Medical Institute, Lahore from February to April, 2012.
**Material and methods:** 45 male albino rats were obtained from the animal house of BMSI and divided into three groups (gps) - control gp A and STZ treated gp B and C, made diabetic by single dose STZ(37mg/Kg b/w).Serum Glucose level of all the rats were measured before the experiment and twice weekly.Gp B animals were sacrificed at the end of 3rd week and gp C animals at the end of 8th week for a detailed morphological examination of the P.C.T in the Periodic Acid Schiff—Haematoxylin(PAS-H) stained sections under the light microscope. Serum creatinine levels were also determined in all the gps for the assessment of renal function.
**Results:** Microscopically, gp B slides showed dilated proximal tubules and some of them appeared to be distorted. Kidney sections of gp C showed signs of tubular necrosis with disrupted brush borders and basement membranes. Serum glucose levels were significantly elevated in both gp B and C. Serum creatinine was not significantly elevated in gp B and increased significantly in gp C.
**Conclusion:** STZ at a dose of (37mg/Kg b/w) is severely nephrotoxic. It produces significant hyperglycaemia leading to oxidative damage.
**Key words:** Streptozotocin, proximal convoluted tubules, oxidative stress, serum creatinine.

## INTRODUCTION

STZ is a naturally occurring nitrosurea used in cancer chemotherapy[1]. It is used in metastatic carcinoid tumors, Hodgkin's disease, and treatment of `advanced Islet cell carcinoma[2]. The effect of STZ on different organs has been extensively studied. It is diabetogenic, hepatotoxic, nephrotoxic and also causes gastric ulceration[3]. STZ is a pancreatic beta cell toxin which induces rapid and irreversible necrosis of these cells[4]. The mechanism of STZ induced beta cells injury involves excessive Reactive Oxygen Species (ROS) production, lipid peroxidation, Protein oxidation and DNA damage leading to beta cells death[5]. Formation of ROS is thought to be a primary mediator of the cytotoxic actions of STZ, leading to oxidative stress[6]. STZ has broad spectrum antibiotic activity[7] and is often used to induce Diabetes Mellitus in experimental animals through its toxic effects on pancreatic beta cells[8]

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Drug overdoses, whether accidental or from chemical overloads, such as antibiotics and chemotherapeutics may cause the onset of acute renal injury as one of the major functions of the kidney is concentration and excretion of toxic metabolites. Renal toxicity is the major dose limiting side effect of STZ[9]. The site of STZ injury involves both the glomerulus and the tubules, based on histological changes[10]. STZ alters various metabolic and enzymatic functions of kidney resulting in various pathologic lesions[1]. The proximal renal tubular cells vulnerability to the toxic actions of chemicals is largely due to the role played by this portion of the nephron in absorption and secretion.

The purpose of this study was to observe the nephrotoxic effect of STZ on the morphology of P.C.T given at a dosage of 37mg/Kg b/w for duration of 3 weeks and 8 weeks. The effect of this drug on serum glucose and serum creatinine was evaluated in all the three groups.

## MATERIAL AND METHODS

This study was conducted in the Department of Anatomy, Post Graduate Medical Institute, Lahore for a period of 8 weeks .In this study, 45 healthy male

albino rats 90—120 days old, weighing around 250-290gms were obtained from the animal house of BMSI and divided into three gps, each gp containing 15 animals. All the animals were kept under observation for 1 week prior to the commencement of study for the assessment of their health status. All the animals were marked by ear punching and weighed. They were kept in propylene cages equipped with drinking water bottles and wood chip floor bedding under natural environment. Food and water was supplied ad libitum. Serum glucose and creatinine of all the animals was determined from tail vein at the start of the experiment and once weekly for the determination of blood glucose and renal functions.

Gp A was taken as control. The animals of gps B and C were fasted over night and administered STZ intraperitoneally in a dose of 37mg/Kg dissolved in one ml of citrate buffer at 4pH, only on the first day of experiment. Serum glucose of both gp B and C were closely monitored throughout the experimental period. Serum creatinine was determined by colorimetric method using ELISA kit and was measured as a parameter of renal functions once weekly throughout the experiment.

Gp B animals were sacrificed at the end of 3rd week and gp C animals were sacrificed at the end of 8th week. Both the kidneys were exposed and dissected. After washing with normal saline they were fixed in buffered neutral formalin for 24 hours and then kept in 70% alcohol overnight. Dehydration of the tissues were done with ascending strength of alcohol, cleared in xylene and infiltrated with paraffin at 59 degrees. Paraffin blocks of tissue were made and 5 micron thick longitudinal sections were cut by a rotatory microtome, mounted on labelled glass slides and stained with PAS-H[11] for a detailed examination of the P.C.T of the cortex under the light microscope.

## RESULTS

Group A rats showed normal architecture of the P.C.T with intact brush border and basement membranes (Fig 1). The serum glucose and serum creatinine levels were within normal range (table 1). Group B rats showed dilated proximal tubules with epithelial casts in the lumina and some of the tubules appeared to be distorted (Fig 2). The mean glucose levels reached 412±17mg/dL which were highly significant as compared to control, whereas the serum creatnine level (1.2±0.05mg/dL) did not increase significantly 3 weeks after STZ administration (table 1). Group C: Kidney sections of this group showed distorted architecture with dilated tubules and flattened epithelia. Variable number of proximal tubules showed signs of tubular necrosis. Their epithelial linings were disrupted with vacuolated cytoplasm and loss of brush borders and basement membranes (Fig 3). Both the serum glucose (528±43mg/dL) and serum creatinine levels (2.16± 0.24mg/dL) were significantly elevated as compared to control.

Table 1: Mean serum glucose and serum creatinine level in different groups of Albino rat.

| Groups | Treatment Received | Serum Glucose (mg/dL) | Serum creatinine (mg/dL) |
|---|---|---|---|
| A | Control | 125±15 | 0.95 ±0.07 |
| B | STZ(3 weeks) | 412 ±17** | 1.20±0.05* |
| C | STZ (8 weeks) | 528±43** | 2.16±0.24** |

Values are mean ± SEM of 15 animals in each group.
* P<0.01 as compared to control **P<0.05

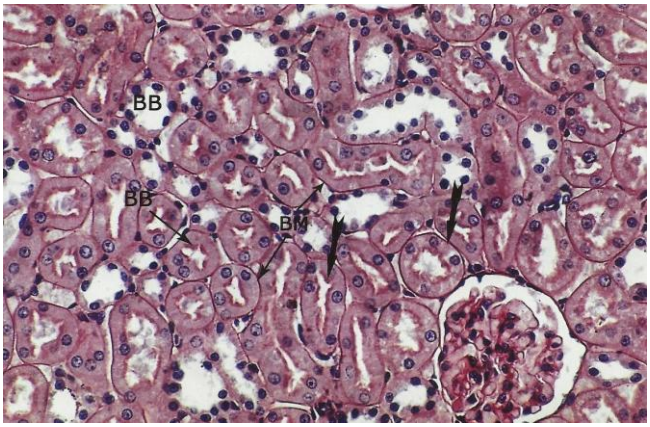

Fig 1: Photomicrograph of 5 microns thick PAS-H stained section of Group A (control) rat showing normal architecture of proximal convoluted tubules with intact brush borders and basement membranesx400

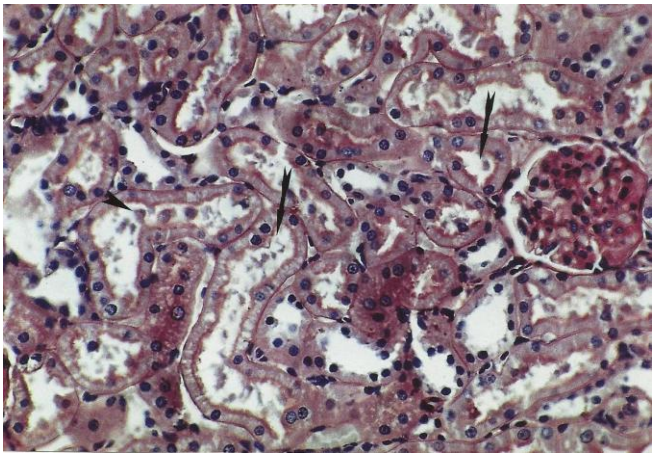
Figure 2: Photomicrograph of 5 micron thick PAS-H stained section of group B (3 weeks STZ treated) rats showing dilated proximal tubules with epithelial casts in the luminax400.

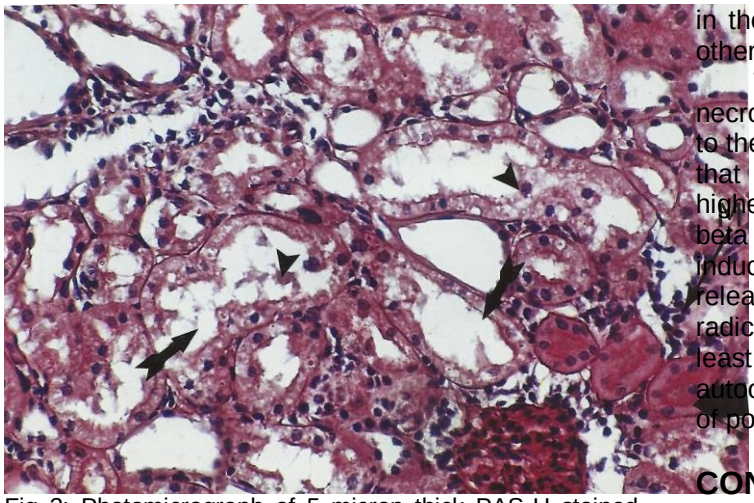

Fig 3: Photomicrograph of 5 micron thick PAS-H stained sections of group C (8 weeks STZ treated) rats showing distorted architecture, renal tubules with flattened epithelia, nuclear and epithelial debris in the luminax400

## DISCUSSION

Present observations on the kidney sections showed progressive damage which increased with duration of time and severity of hyperglycaemia. There was a significant rise in serum glucose levels in group B and group C animals. STZ induced hyperglycaemia could be a causative factor for the damage of renal tubules and for a significant rise in serum creatinine in gp C animals exposed to a prolonged hyperglycemic state.In a four weeks study conducted by N.V Thakker in 2010[12]a single dose of STZ (70mg/Kg b/w) caused marked hyperglycaemia and a significant rise in serum creatinine in albino rats.

In the present study STZ was administered in a dose of 37mg/kg b/w, while Zafar et al (2010)[1] used 45mg/kg and Oscika et al(2000)[13]used STZ in dose of 50mg/kg b/w in rats to produce hyperglycaemia. The selection of lower dose was adopted to avoid mortality of rats. We observed progressive wide spread tubular necrosis with loss of brush border membranes in most of kidney sections in gp C animals. This is in agreement with the findings of Ramesh et et al(2008)[14] and Reno et al(2008)[15] who showed renal tubular epithelial changes, enlargement of lining cells of tubules and accumulation of glycogen in kidney tubules with STZ administration. Similarly Zafar et al in 2009[1] demonstrated the effect of STZ (45mg/kg b/w) on the morphology of renal cortex of albino rats and the severity of renal damage with increasing time duration.

Oxidative stress plays a major role in the pathogenesis of STZ induced diabetes[16]. Although it is generally accepted that cytotoxicity produced by STZ depends on DNA alkylation[17].several lines of evidence indicate that free radicals play essential role in the mechanism of DNA fragmentation and evoke other deleterious changes in the cells[18].

The results of our study indicate the presence of necrosis of the cells of PCT in gp C animals exposed to the prolonged effects of STZ. Saimi et al [19] showed that STZ at lower doses induces apoptosis and at higher doses causes necrosis in murine pancreatic beta cells. One of the primary pathways of drug induced apoptosis is the pathway that involves the release of Cytochrome C from mitochondria [20].Free radicals caused by hyperglycemia may occur via at least 3 different routes-Nonenzymaticglycation[21], autooxidation of glucose[22] and intracellular activation of polyol pathway.

## CONCLUSION

It may be concluded that STZ at a dose of 37mg/kg b/w causes significant hyperglycemia resulting in renal damage and impairment of renal functions in albino rats who are under the effects of this drug over a prolonged period of time.

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