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# Biochemical and Histomorphological Study of Streptozotocin-Induced Diabetes Mellitus in Rabbits

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**Abstract:** This study was designed to investigate the biochemical and histomorphological changes occurring due to streptozotocin-induced diabetes mellitus in rabbits. Twelve New Zealand male rabbits of uniform age were divided into two Groups. Group I was made diabetic by single intravenous injection of streptozotocin (@ 65mg/kg.b.w.) whereas Group II served as control. Significant increase in blood sugar ($P<0.01$), blood urea ($P<0.10$) and serum creatinine ($P<0.10$) was recorded in Group I rabbits compared to Group II rabbits. Further, histomorphological alterations of pancreas, kidneys, liver, heart, lungs and brain were observed. However, no pathological features were observed in alimentary canal. From the study, it is concluded that streptozotocin-induced diabetes causes disturbances in biochemical and histological features in rabbits and serves as a model in studying the various complications arising due to this illness.

**Key words:** Streptozotocin, diabetes mellitus, biochemistry and histopathology

## Introduction

Streptozotocin induced diabetes mellitus in many animal species has been reported to resemble human hyperglycemic nonketotic diabetes mellitus (Weir *et al.*, 1981). This effect has been extensively studied and appears to be mediated through a lowering of beta cell nicotinamide adenine dinucleotide (NAD+) and results in histopathological alteration of pancreatic islet beta cells (Karunanayake *et al.*, 1974). West *et al.* (1996) reported that streptozotocin at first abolishes the beta cell response to glucose. Temporary return of responsiveness then appears which is followed by its permanent loss and cells are damaged. Kidney hypertrophy is observed in both the glomerular basement membrane and capillaries of diabetics (Heidland *et al.*, 1996; Rabkin *et al.*, 1996) and may contribute to end-stage renal damage. Vascular hypertrophy may be one of the causes of diabetic hypertension (Hulthen *et al.*, 1996). Hepatomegaly is also associated with streptozotocin-induced diabetes (Kume *et al.*, 1994).

The present experiment was designed to investigate the biochemical, behavioural and histopathological disturbances that occur in rabbits after the evolution of streptozotocin-induced diabetes.

## Materials and Methods

Twelve New Zealand white rabbits of almost uniform age were selected for the study. Prior to the start of experiment rabbits were acclimatized to standard laboratory conditions for fifteen days and received human care according to the guidelines outlined in the "Guidelines for the Care and Use of Animals in Scientific Research" prepared by the Indian Science Academy, New Delhi (Anonymous, 2000). Blood sugar, blood urea, serum creatinine, body weight and physical activities of all the rabbits were recorded before start of the experiment.

The rabbits were randomly divided into two groups of six each. Group I received intravenous injection of streptozotocin @ 65mg/kg.b.w. following twelve hours fasting whereas Group II received normal saline and served as control.

Development of induced diabetes mellitus was confirmed by examining the fasting glucose level along with blood urea and serum creatinine in the blood taken from marginal ear vein. This was verified by postmortem histological examination of pancreas, kidneys, liver, lungs, heart, brain and alimentary canal of diabetic rabbits.

**Analytical Procedure:** The blood sugar of rabbits was estimated by Glucometer Gx (Bayer Diagnostic India, Ltd.), blood urea by "DAM Method" and serum creatinine by 'Alkaline Picrate Method" using commercially available kits. Histological examination was done by fixing the organs of the rabbits in 10% formalin, processed and embedded in paraffin wax. Tissue blocks were sectioned 5 micron thick and stained with Harris Haematoxylin and Eosin (Luna, 1968). However, to demonstrate pancreatic islet cells, Gomoris modified stain (Halami, 1952) was used with a modification of substituting the Lugol's iodine by equal parts of 0.5% $KMNO_4$ and 0.5% sulphuric acid, and sodium thiosulphate by 2% sodium bisulphite respectively.

For quantitative assessment of beta cells in the islets of Langerhan's, cells of approximately 4 islets on each tissue and 40 islets of each group were counted.

**Statistical Analysis:** All values were expressed as mean and analyzed using students 't' test (Prasad, 2000). 'P' value was obtained from the distribution of 't' probability chart.

## Results

The induction of diabetes mellitus in rabbits by intravenous administration of streptozotocin @ 80 mg/kg.b.w. was confirmed on day 2nd of the experiment. Table 1 shows the changes in biochemistry and body weight of rabbits used in the experiment. Further, Group I rabbits exhibited frequent urination, decreased physical activities and sluggishness in comparison to Group II rabbits.

The blood sugar (F), blood urea and serum creatinine levels of group I rabbits were highest on day 2nd with mean values of 233 ± 9.17 mg/dl, 39.75 ± 0.80 mg/dl and 2.25 ± 0.32 mg/dl respectively compared to the values of Group II rabbits which remained almost constant for these parameters till the end of the experiment. In consonance with biochemical parameters body weight of Group I rabbits started to fall on day 2nd which was recorded to be 1.227 ± 0.10 kg from an initial value of 1.275 ± 0.14 kg in comparison to Group II rabbits which showed an increased trend in body weight throughout the experiment.

Pancreatic sections stained with Haematoxylin and Eosin showed slight congestion and mild degenerative changes in the acini. The acinar epithelium was swollen. The islets of Langerhan's revealed decreased cellularity and in some islets the cells appeared to be fusiform (Fig. 1). However, using special stain (Halami, 1952) for pancreatic sections reduction in the number of beta cells were observed in Group I rabbits (Fig. 2). The lung sections of Group I rabbits showed congestion and haemorrhage in alveoli and branchioles (Fig. 3), congestion in kidneys (Fig. 4), degeneration and congestion in liver (Fig. 5), haemorrhage and myopathy in heart (Fig. 6) and mild neuronal damage in brain (Fig. 7). However, H&E stained sections of alimentary canal did not reveal any histopathological finding.

## Discussion

Streptozotocin is well known for its selective pancreatic islet beta-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms (Papaccio *et al.*, 2000). Intravenous administration of streptozotocin (65 mg/kg b.w.) in the present study effectively induced diabetes mellitus in rabbits and is in consonance with earlier methods of induction (Kedar and Chakrabarti, 1983; Tawfeeg and Sherif, 2001). The elevation of blood sugar level on day 2nd confirmed the establishment of diabetes mellitus in rabbits which is attributed to its selective cytotoxicity on beta cells and subsequently impairs glucose oxidation (Bedoya *et al.*, 1996). Two hours after injection of streptozotocin, the hyperglycemia is observed with a concomitant drop in blood insulin followed by hypoglycemia about six hours due to decrease in blood insulin levels (West *et al.*, 1996). The blood sugar level of the rabbits was on peak on day 2nd after streptozotocin administration followed by changes with a decreasing tendency. The changes in blood glucose and insulin concentrations reflect abnormalities in beta cell function (Bedoya *et al.*, 1996). The fluctuations in the blood sugar might also be attributed to the sensitivity to streptozotocin that varies with species, strain, sex and nutritional state and there are batch differences in activity (Okamato, 1981). When administered intravenously, plasma levels of streptozotocin rapidly decrease within 15 minutes and concentrate in the liver and kidneys (Sicor Pharmaceticals, 2003). Twenty percent of the drug is metabolized and/or excreted by the kidneys (Sicor Pharmaceuticals, 2003). The changes in blood urea and serum creatinine observed in the present study could be attributed to the functional and/or morphological changes in the kidneys (Alderson *et al.*, 2004). Kedar and Chakrabarti (1983) had reported elevated levels of blood sugar to 340 mg percent associated with glycolysis, ureamia, hypercholesterolemia, hypertriglyceridemia and loss of body weight in rabbits by a single intravenous injection of streptozotocin (65 mg/kg). Further, a significant increase of total protein excreted, albuminuria, glycosuria and urinary urea levels indicating impaired renal function have been reported (Alderson *et al.*, 2004).

Streptozotocin effectively induced diabetes in rabbits characterized by polydipsia, polyuria, weight loss, decreased physical activities and hyperglycemia, which is in agreement with earlier findings (Calabresi and Chabner, 1985; Shenoy and Ramesh, 2002). In streptozotocin induced diabetes there is excess of fatty acids in the serum, which promotes conversion of excess fatty acids into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins. The abnormal high concentration of serum lipids in the diabetic subject is due, mainly to increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase (Bopanna *et al.*, 1997).

The decrease in cellularity within islets of Langerhan's observed in the present study reflects the cytotoxity of streptozotocin (Papaccio *et al.*, 2000; Szkudelski, 2001). The reduction in the number of beta cells was also confirmed in rabbits using special stains. Streptozotocin destroys beta cells selectively and a single adequate dose produces lasting hyperglycemia and insulin deficiency (Szaleczky *et al.*, 1999). Previous studies have reported that streptozotocin enters the beta cells via a

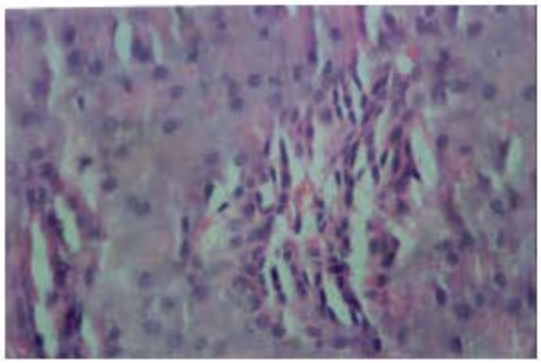

Fig. 1: Pancreatic section showing fusiform shape of cells within the islets of Langerhan's (H&E x 400)

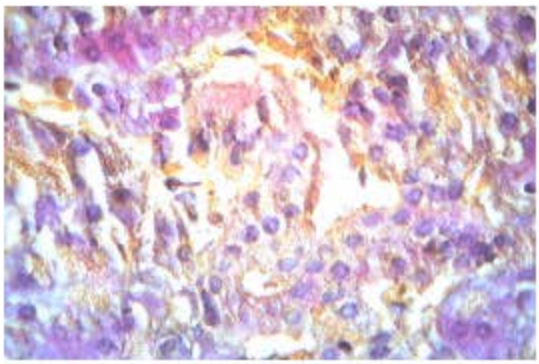

Fig. 2: Pancreatic section showing alpha (yellow) and beta Purple) cells (within the islets of Langerhan's (Halami, 1952 x 1000)

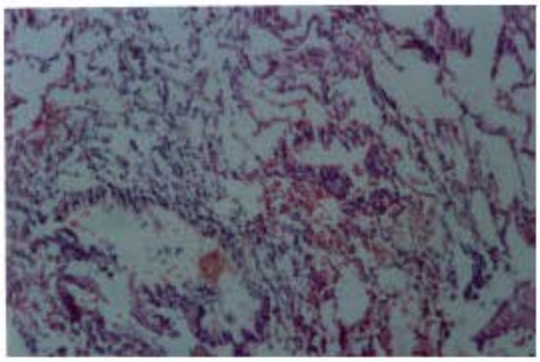

Fig. 3: Lung section showing haemorrhage in alveoli and bronchioles (H&E x 400)

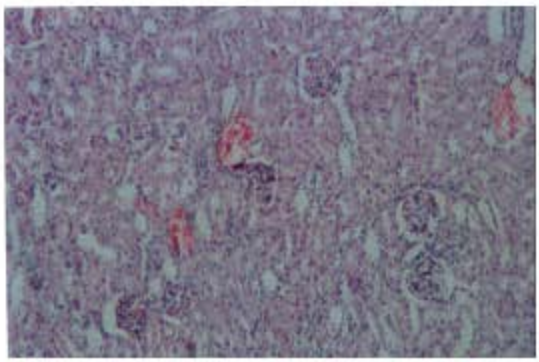

Fig. 4: Kidney section showing congestion (H&E x 400)

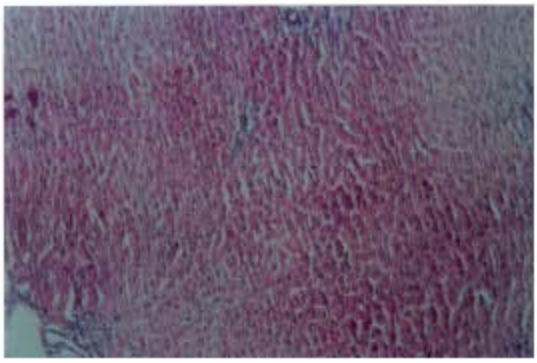

Fig. 5: Liver section showing congestion and degeneration (H&E x 400)

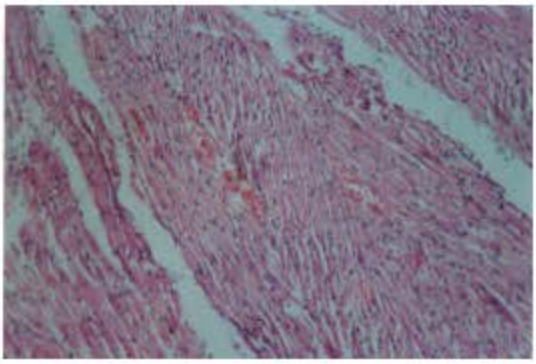

Fig. 6: Heart section showing haemorrhage and myopathy (H&E x 400)

glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenecity of steptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular $NAD^+$ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of super oxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the streptozotocin action, beta cells undergo destruction by necrosis (Szkudelski,

Table 1: Changes in Blood Sugar (F), Blood Urea, Serum Creatinine and Body Weight of Streptozotocin-Induced Diabetic Rabbits

| | | | Parameters | | | |
|---|---|---|---|---|---|---|
| | | | Blood Glucose (mg/dl) | Blood Urea(mg/dl) | Serum Creatinine (mg/dl) | Body Weight(Kg) |
| Initial Value | | C | 100±5.08 | 18.75±0.62 | 0.93±0.07 | 1.177±0.06 |
| | | T | 96.25±8.16 | 20.5±1.32 | 1.02±0.12 | 1.275±0.14 |
| DAYS | 2nd | C | 102±3.37 | 17.5±0.86 | 0.94±0.04 | 1.242±0.07 |
| | | T | 239±9.17 | 39.75±0.80 | 2.25±0.32 | 1.227±0.10 |
| | 5th | C | 99±2.68 | 18.5±0.64 | 0.95±0.09 | 1.302±0.05 |
| | | T | 198±6.97 | 36.75±1.10 | 1.98±0.21 | 1.251±0.07 |
| | 10th | C | 100±3.32 | 17.5±1.01 | 0.92±0.08 | 1.320±0.05 |
| | | T | 182±8.21 | 28.25±1.51 | 1.87±0.18 | 1.270±0.08 |
| | 15th | C | 101±2.73 | 17.7±1.46 | 0.95±0.087 | 1.377±0.06 |
| | | T | 159±6.51* | 25±2.07** | 1.54±0.19** | 1.380±0.06 |

C = Control (Saline-treated normal rabbits); T= Treated (Streptozotocin-induced diabetic rabbits). Values are mean ± SEM; *p < 0.01, **p < 0.10 compared to control.

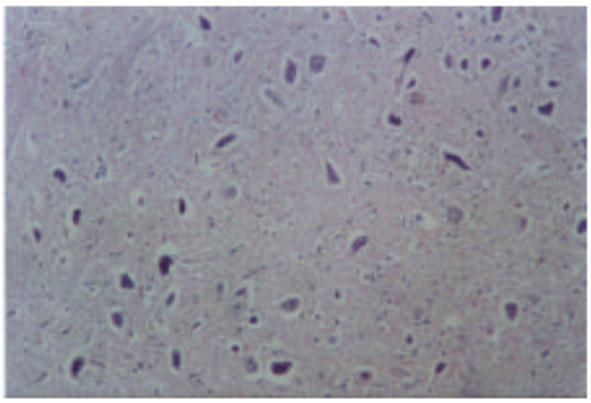

Fig. 7: Brain section showing mild neuronal damage (H&E x 00).

2001). Other studies indicated that cytotoxic effects of streptozotocin are dependent upon DNA alkylation by site-specific action with DNA bases (Benneth and Pegg, 1981) and by free-radical generation during streptozotocin metabolism (Bolzan and Bianchi, 2002). In the present study beta cells in some islets were found to be fusiform. The change in the shape of cells can be attributed to the partial damage of streptozotocin to some beta cells. Aybar *et al*. (2001) have reported that use of lower dose of streptozotocin produced an incomplete destruction of pancreatic beta cells even though rats became permanently diabetic.

The histomorphological study of the lungs observed in the present study indicated alterations such as congestion and haemorrhage in alveoli and bronchioles. Lung damage in streptozotocin induced diabetic hamsters has been reported (Popov and Simionescu, 1997). It is postulated that hyperglycemia affects the lungs by damaging capillaries and by the non-enzymatic glycosylation of collagen (Bell *et al*., 1988). Hyperglycemia appears to cause cellular stress by a number of mechanisms, which could be detrimental to the lung (Brownlee, 2001). Firstly, hyperglycemia increase movement of glucose through polyol pathway and sorbitols are produced which in turn causes osmotic stress to cells and dihydronicotine amide adenine dinuleotide phosphate (NADPH) is consumed, depleting intracellular glutathione. Secondly, hyperglycemia increases concentrations of advanced glycation end products. These golycosylated proteins are formed by non-enzymatic reactions and changes in protein structure may alter their cellular functions. Thirdly, glucose activates various isomers of protein kinase C which in turn affects the expression of nitric oxide, endothelin, nuclear factor kappa B and plasminogen activator inhibitor. Finally, hyperglycemia increases the flux of glucose through the hexasomine pathway effecting inflammatory mediators and insulin resistance. The combined effect of these mechanisms results in over-production of mitochondrial superoxides, causing cellular stress and damage (Brownlee, 2001).

The morphological study in kidneys of streptozotocin induced diabetic rabbits did not show any significant alteration. It has been reported that streptozotocin does not possess any significant nephrotoxic potential (Floretto *et al*., 1998). However, the kidney sections showed congestion in the present study, which can be attributed to altered metabolism in diabetes (Rasch, 1980). The changes in the liver in diabetic rabbits induced by streptozotocin have been reported earlier (Mitra *et al*., 1996). The diabetic liver showed degeneration and congestion. In diabetes, degradation of liver glycogen and gluconeogenesis are increased while glucose utilization is inhibited. Glucose 6-phosphatare increases in the liver, facilitating glucose release into the blood. The opposing enzymes which phosphorylate glucose is hexokinase, which is unaffected by insulin and glucokinase, which decrease in diabetes. As a result, the liver continues to produce glucose even with severe hyperglycemia. Under these circumstances the normal liver would shut off and deposit glycogen (Sheila and James, 1993).

The histopathological changes in the heart of streptozotocin-induced diabetic rabbits showed haemorrhage and cardiomyopathy which could be attributed to the hyperglycemia, which by the formation of oxygen free radicals induces degenerative changes in

the tissues along with cardiomyopthy and nephropathy (Oberley, 1988).

In the present study the nervous system of streptozotocin-induced diabetic rabbits showed mild neuronal damage. Diabetes accelerates maturation of neuronal damage, increases infarct volume and induces postischemic seizures (Muranyi *et al.*, 2003).

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