

Original Research Article

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## Pathomorphological Changes in Type-2 Diabetes in Rat Model

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### ABSTRACT

#### Keywords

Type-II diabetes, Rats, Gross and histopathological changes, Kidney, Pancreas

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The present study was aimed for studying gross and histopathological changes in various organs of STZ-NAD induced Type –II diabetic rat model. In diabetic group rats, the gross changes included congestion in liver, kidneys, heart and brain, atrophic changes in liver, heart, pancreas, spleen, thymus, testes and epididymes, edematous mesenteric lymph nodes and ulcerations in gastrointestinal tract in comparison to control group. The histopathological changes consisted of reduction in number /size / cellular components of islets of Langerhans and necrotic /degenerative changes in pancreas while coagulative necrosis, tubular mineralization and vacuolar degeneration in kidneys.

### Introduction

Type 2 diabetes mellitus is a non-autoimmune and polygenic metabolic disease in which the body fails to produce enough insulin resulting in abnormal glucose homeostasis.

The pathogenesis appears to involve complex interactions between genetic and various environmental factors (Gupta *et al.*, 2008).

It occurs due to impaired insulin effectiveness at cellular level (Permutt *et al.*, 2005) and is accompanied by a number of side effects involving almost all the organs resulting in diabetic retinopathy, neuropathy, nephropathy and macrovascular/ microvascular

complications (*viz.*, coronary artery disease, cerebrovascular disease and peripheral vascular disease), dyslipidemia, oxidative stress, cardiomyopathy (Anonymous, 1991; Aaron and Vinik, 2001) and poor wound healing (Lin and Sun, 2010).

### Materials and Methods

#### Chemicals and instruments

Diabetes inducing agents i.e. Streptozotocin and Nicotinamide, Hemotoxylin and eosin stains and micro slides were purchased from Himedia Pvt. Ltd. Mumbai. The tissues were processed on Automated tissue processor (Yorko Ltd.) and stained manually.

## **Experimental animals**

The rats were procured from Zydus Research Centre, Ahmedabad, Gujarat and Laboratory Animal Resources (LAR), Sun Pharma Advanced Research Company Limited, Vadodara, Gujarat, India. Ethical clearances for performing the experiments on the rats were obtained from Institutional Animal Ethical Committee (IAEC) (No. 030-VCN-VPP-2015.)

## **Induction of Type-II diabetes in rats**

Type-II diabetes mellitus was induced in rats by administering Nicotinamide (NAD) and Streptozotocin (STZ). The animals were first subjected to intraperitoneal injection of NAD @ 230 mg/kg (dissolved in normal saline) followed by the administration of STZ @ 65 mg/kg (dissolved in 0.1 Molar citrated buffer) intraperitoneally after 15 minutes (Masiello *et al.*, 1998; Bisht, 2013).

Two days post STZ-NAD administration blood glucose level was estimated by glucometer to confirm diabetes.

Ten male and ten female rats with glucose level  $\geq 180 \pm 8$  mg/dl were considered as diabetic and were selected for the study. Control group rats were injected normal saline followed by 0.1Molar citrated buffer.

## **Grouping and treatment provided**

Gr.1 consisted of 10 male and 10 female healthy rats. Gr.2 consisted of 10 male and 10 female diabetic rats. The rats were numbered, colour marked on tail and given cage card number for easy identification.

## **Necropsy and organ collection**

At the end of the study all the rats covering different groups were subjected to necropsy.

Prior to sacrifice the animals were fasted overnight, weighed and examined externally. The rats were sacrificed by over dose of Ether inhalation followed by cervical dislocation.

## **Results and Discussion**

Various gross and histopathological changes in different organs were observed in the rats of different groups (Gr.1 to 5) and are described as below.

### **Group 1 (Vehicle /Non Diabetic Control)**

#### **Gross Findings**

Gross lesion of pathological significance could not be observed in any rat belonging to this group except atrophy of thymus (2 males and 2 females) which might be attributed to increasing ageing process.

It has been well established that as the animal ages atrophy of thymus occurs (Boorman *et al.*, 1990).

#### **Histopathological Findings**

Histopathological examination of liver (2 males) and kidneys (1 male) and thymus (2 males and 2 females) revealed changes. Foci of mononuclear cells infiltration were seen in liver and kidneys whereas lymphoid depletion characterised by 'starry sky appearance' was discernible in thymus.

Liver and kidneys are considered to be important detoxifying organs and during detoxifying process micronecrotic foci would have been developed. 'Starry sky appearance' in thymus could have developed due to lymphophagocytic process as a result of individual cell necrosis (Stephan *et al.*, 1990). Further, these findings have been considered as incidental findings without any etiological agent.

## Effect of diabetes

### Group 2 (Streptozotocin (STZ) - Nicotinamide (NAD) Induced Diabetic Rats)

#### Gross findings

Liver (6 males and 4 females), kidneys (5 males and 5 females), heart (4 males and 3 females) and brain (2 males and 2 females) revealed varying degree of congestion while liver (5 males and 3 females), heart (2 males and 2 females), pancreas (6 males and 4 females), spleen (6 males and 5 females), thymus (6 males and 5 females), testes (5 males), epididymides (4 males) and seminal vesicle (5 males) were found to be atrophied as compared to Gr.1. Mesenteric lymph nodes were edematous (6 males and 6 females) and gastrointestinal tract showed ulcerations (5 males and 5 females) in the present study.

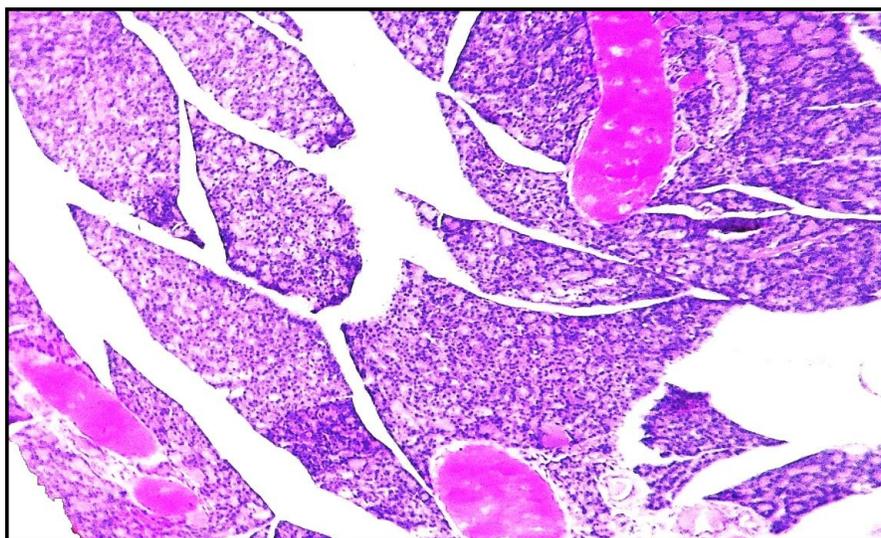
Our findings were in support of observations made by Barr *et al.*, (2007) who mentioned that the diabetes is associated with long-term complications affecting pancreas, heart, eyes, kidneys and nerves. Reduction in the cardiac size in present study might be due to reduction in diabetic cardiac mass over the time as

opined by Renuga *et al.*, (2013) who further assigned atherosclerosis and myocardial congestion as the hallmark of diabetic macrovascular disease. Further, changes in liver and kidneys of diabetic rats supported the observations of Sachi *et al.*, (2002), Enas *et al.*, (2004) and Olurishe *et al.*, (2013).

#### Histopathological findings

##### Pancreas

Varying degree of congestion (6 males and 5 females), mononuclear cells infiltrations, degenerative /necrotic changes in exocrine pancreas, reduction in the number, size and cellular components of the islets, loss of architectural details in exocrine pancreas (6 males and 4 females) were observed on histopathological examination. A well circumscribed uniform/homogeneous eosinophilic material with complete loss of cellular details at some places were noted in pancreatic sections (6 males and 6 female). Comparable findings in terms degeneration in pancreatic acini, reduction in pancreatic islets diameter, islets count and islet cells were reported in the STZ-induced diabetic rats in comparison to control animals in earlier study done by Jana *et al.*, (2002).



**Fig.1** Histopathological findings

Atrophy of pancreatic beta cells in alloxan induced (150 mg/kg ip) diabetic rats was also reported by *Khaled et al.*, (2009). Our findings strengthened the report of *Fazil et al.*, (2010) who recorded extensive damage in the islets of Langerhans and reduced dimensions of islets in alloxan induced diabetic rats. Our findings were also in agreement with the report of *Singh and Gupta* (2007) who described disorganization and degranulation of islet cells, acinar hyperplasia with ballooning of cells, hydropic degeneration and fibroblastic replacement in alloxan induced diabetic rats.

The pathomorphological features seen in cattle (*Taniyama*, 1993), dogs and cats (*Hess et al.*, 2000; *Davison et al.*, 2003) were reported to be comparable to those seen in insulin dependent diabetes mellitus in human beings. Our findings were also in linearity with the report made by *Barr et al.*, (2007) who observed amyloid deposition within islets, around capillaries and between pancreatic cells in type-2 diabetic human patients. Comparable findings like reduction in the number and size of pancreatic islets (*Kaneko and Rhode*, 1964; *Von and Wittwer*, 1986) along with vacuolar degeneration of beta cells (*Baker et al.*, 1983) were also reported in diabetes mellitus. The histopathological changes observed in pancreas in the present study might be due to direct cytotoxic action of streptozotocin (nitrosourea moiety) used to induce diabetes as opined by *Junod et al.*, (1967) and *Ozturk et al.*, (1996) or due to oxidative stress associated apoptosis of pancreatic beta cells (*Jana et al.*, 2010). We felt two possibilities about genesis of the eosinophilic structure in pancreatic sections; one might be localized amyloidosis and other the hyalinization followed by necrotic lesion. As such we could not ascertain the exact identity of the said structure without special staining. The

uniform eosinophilic material occupying the islets of Langerhans in alloxan induced diabetic Wistar rats was also observed by *Eliakim-Ikechukwu and Obri* (2009). Nicotinamide (NAD) used STZ-NAD in diabetes induction in present study could have acted as an antioxidant thus partially protected pancreatic beta cells from cytotoxic action of streptozotocin but needs further research.

### **Kidneys**

Kidneys showed varying degree of patchy area of congestion and mononuclear cells infiltration (5 males and 5 females), coagulative necrosis (5 males and 4 females), tubular mineralizations (5 males and 5 females), cloudy swellings (6 males and 5 females), vacuolar degenerations (6 males and 5 females), desquamation and denucleation of tubular epithelium (5 males and 5 females) and total absence of tuft of capillaries in some of the glomeruli also known as 'ghost glomeruli' in 5 males and 5 females rats belonging to this group.

Our findings supported the histopathological observations made by *Enas* (2004) who described dilatation and severe engorgement of blood vessels, atrophy and swelling of glomerular tuft and cloudy swelling accompanied by hyaline cast in convoluted tubules, vacuolar degenerative changes in renal tubules, diffuse extravagations of red blood cells between degenerated renal tubules in alloxan induced diabetic rats. *Sachi et al.*, (2002) also described nephropathy and the changes including diffuse glomerulosclerosis, thickening of basement membrane, mesangial proliferation and fibrin cap in diabetic fatty rats. In diabetic patients urinary tract infections are usually more severe associated with increased risk of complications. Patients both with Type 1 and Type 2 diabetes

mellitus are at increased risk of urinary tract infections. Kakadiya *et al.*, (2010) attributed hyperglycaemia as most probable contributing factor in the development of ischaemic renal failure in diabetes.

In diabetic group rats the gross changes included congestion in liver, kidneys, heart and brain, atrophic changes in liver, heart, pancreas, spleen, thymus, testes and epididymes, edematous mesenteric lymph nodes and ulcerations in gastrointestinal tract in comparison to Gr.1/control group. The histopathological changes in diabetic control group consisted of reduction in number /size / cellular components of islets of Langerhans, necrotic /degenerative changes in pancreas (uniform/homogeneous eosinophilic material with complete loss of cellular details) while coagulative necrosis, tubular mineralization and vacuolar degeneration in kidneys.

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