Research article

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Alteration of testicular cytomorphology in albino rats in alloxan induced diabetes: A histological study

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ABSTRACT:

Diabetes mellitus (DM) is a constellation of biochemical abnormalities resulting in disorder of carbohydrate, lipid and protein metabolism. Altered testicular structure and function have been observed in diabetic men and animal models of diabetes with impaired reproductive function. Male infertility is a common threat now a days and it has increased rapidly because of hyperglycaemia which results in the generation of reactive oxygen species (ROS) leading to oxidative stress in testicular tissues. The objective of the present study was to examine the histopathology of rat testis after treatment of alloxan. Examination of sections of testis of treated rat showed that testis had lost its characteristic architecture compared with the control group. There is marked distortion of seminiferous tubules with almost complete disintegration of connective tissue between them. Cells in seminiferous tubules showed nuclear pyknosis. Sertoli cells lost their integrity and hence can be identified with difficulty. The present findings may provide a novel approach to elucidate the mechanisms of action of alloxan on testis in diabetes. But further experiments are in progress to solve this paradox.

KEY WORDS: Diabetes Mellitus, Testis, Alloxan

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INTRODUCTION:

Diabetes mellitus (DM) affects a significant number of humans and is a serious disease that has metabolic complications that leads to early morbidity and mortality (Hassen NS *et al*., 2007; Feldman, Stevens and Greene, 1997). Colling and Dicarlo (1995) and Sousa Lion *et al* (2004) stated that DM is a constellation of biochemical abnormalities resulting in disorder of carbohydrate, lipid and protein metabolism. Furthermore, Salahdeen and Alada (2007) added that DM is a genetically determined chronic disorder of

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carbohydrate metabolism that results in clinical syndrome combining several pathological events into a common clinical image. Altered testicular structure and function have been observed in diabetic men and animal models of diabetes with impaired reproductive function (Hassen NS *et al*., 2007). Previous researches revealed testicular abnormalities included suppression of mating behaviour, oligospermia and involution of secondary sex organs which in part reflected depressed levels of serum testosterone (Hassen NS *et al*., 2007). Thus, the testicular atrophy and infertility are common in untreated or poorly-controlled diabetics (Sainio *et al*., 1997).

Diabetes mellitus (DM) is a degenerative disease with alteration in carbohydrate metabolism that affects male reproductive function at multiple levels particularly the endocrine control of spermatogenesis, or by impairing penile erection and ejaculation (Sexton and Jarow, 1997). About 90% of diabetic patients generally experience sexual abnormalities such as sexual dysfunction, impotence and infertility (Amaral *et al*., 2006). Male infertility is a common threat now a days and it has increased rapidly because of hyperglycaemia (Andy *et al*., 2009) which results in the generation of reactive oxygen species (ROS) leading to oxidative stress in a variety of tissues (Brownlee, 2001). There is evidence that hyperglycaemia causes oxidative stress, stimulating macrophages and the over expression of pro-inflammatory cytokines as IL- 1β in rat testis (Abeer, 2010) leading to damage of testicular tissue.

A recent study has shown increased apoptosis in the seminiferous tubule of streptozotocin (STZ) induced diabetic testis of mice and rats (Cai *et al*., 2000; Roy S *et al*., 2013). Furthermore, oxidative stress has been recognized as a strong mediator of apoptosis and the mechanisms related to this are still unclear (Leon *et al*., 2005). The balance between ROS and antioxidants is a major mechanism in preventing damage by oxidative stress. Antioxidants may also be useful in the treatment of male infertility (Park *et al*., 2003).

One of the most potent methods to induce experimental diabetes mellitus is chemical induction by alloxan (Etuk *et al*., 2010; Mythili *et al*., 2004). Alloxan is a cyclic urea derivative causes selective necrosis of the β-cells of pancreatic islets through the generation of cytotoxic reactive oxygen species (ROS) (Lenzen *et al*., 2008). The objective of the present study was to examine the histopathology of rat testis after treatment of alloxan.

MATERIALS AND METHODS:

Animals and housing

The laboratory experiments were performed using twenty adult male albino rats weighing 80-100 g were housed in polypropylene cages and were acclimatized in laboratory condition for two weeks with natural light and dark schedules prior to experimentation. The animals were fed standard rodent diet and water was provided *ad libitum* (Guria S *et al*., 2012 and 2014).

Rats were divided into two main groups:

Group I (Control group): rats were received normal rodent diet and water.

Group II (Diabetic group): Diabetes was induced by single intra-peritoneal injection of alloxan monohydrate (Sigma) in a dose of 60 mg/kg body weight (BW) (after Hassen NS *et al*., 2007). Thirty minutes after alloxan administration, food and water were offered to the animals. The presence of diabetes was confirmed by estimation of serum glucose

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level by portable glucose analyzer and using tail blood samples.

Glucose tolerance test (GTT)

For glucose tolerance test (GTT) blood was collected first from the tail veins of control and alloxan treated rats after 18 hr of fasting followed by challenge with glucose (25 mg glucose/100 g body weight) and at the following time point after glucose infusion: 1.5 hr, 2.5 hr and 24 hr. blood glucose was measured using a blood glucose monitoring system (glucometer) (Guria S *et al*., 2012 and 2014).

Histological analysis

3 weeks after the experiment, animals from each group were randomly selected, and a portion of the testis was excised from the ether anaesthetized rat, fixed in Bouin's fixative. After routine processing the tissues were embedded in paraffin wax. The sections of testis were stained with Haematoxyline-Eosin (H-E) stain for histological analysis (Guria S *et al*., 2012 and 2014).

NBT (Nitroblue Tetrazolium) staining of testicular cells

Testis cells were treated with NBT for superoxide. The superoxide anion production was evaluated using NBT reduction test.

RESULT:

Blood glucose level

In the control rats the blood glucose level returned to the normal level after 24 hr of glucose feeding. Like control rats, in diabetic rats glucose level increased after 1.5 hr of glucose challenge but the elevated glucose didn't return to control level even after 24 hr of glucose challenge (Table1).

Group	0hr	1.5hr	2.5hr	24hr
Control rats	39.28±1.62	71.22±1.79	50.39±2.13	40.67±1.97
Alloxan treated rats	37.69±2.03	64.31±2.47	51.22±1.20	50.03±1.96

Table1. Blood glucose level (mg/dl) during glucose tolerance test in control and alloxan treated rats. Values are the blood sugar level (mg/dl) expressed as mean ± SE. P-value < 0.05 is considered to be statistically significant.

Microscopic examination

Control animals (group I) showed seminiferous tubules with adequate germ cells and all stages of maturation (i.e. functional spermatogonia till sperm formation). The lumen of the control seminiferous tubules was filled with spermatozoa (Fig 1a and 2a). Diabetic rats (group II) showed obvious reduction in the number of seminiferous tubules (SFT) which were widely separated from each other and spermatogenesis cells were irregularly arranged. The cells showed pyknotic nuclei with ill defined membrane, the lumen showed complete absence of spermatozoa or less sperm (Fig 1b and 2b). Cell loss and spermatogenic arrest were evident in some tubules. Degeneration of germ cells was obvious. The tubules showed vacuolation and distorted appearance. There was separation of the spermatomgenic cells from the basement membrane in many tubules (Fig 1b and 2b).

The seminiferous tubule structure in the diabetic rats was found to be disrupted, and there was a considerable decrease in the

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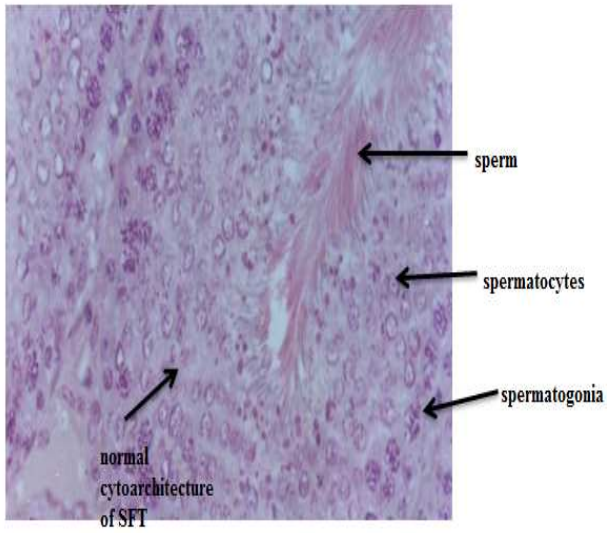
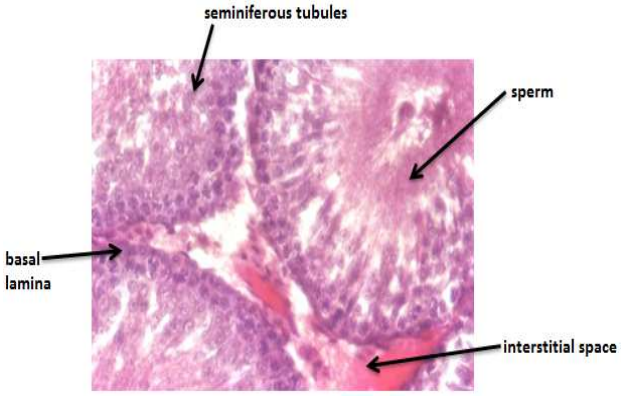
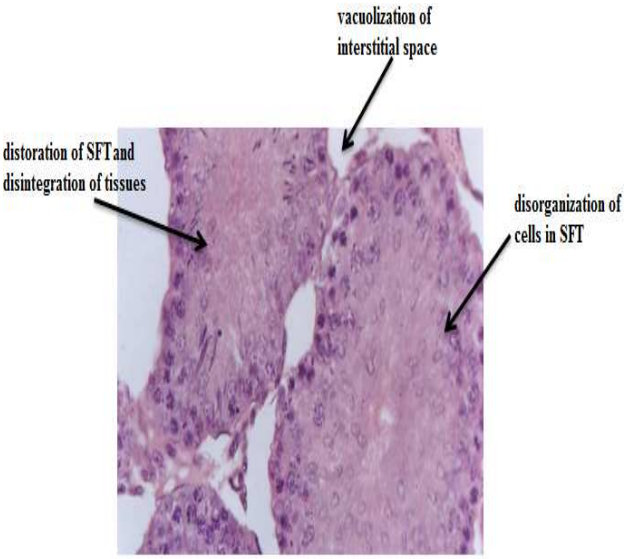
number of spermatogenic cell series like spermatogonia, sertoli cells, primary spermatocytes, spermatids (Fig 1b and 2b). Examination of sections of testis of treated rat showed that testis had lost its characteristic architecture compared with the control group. There is marked deformation of seminiferous tubules with almost complete degeneration of connective tissue between them. Cells in seminiferous tubules showed nuclear pyknosis. Sertoli cells have lost their integrity.



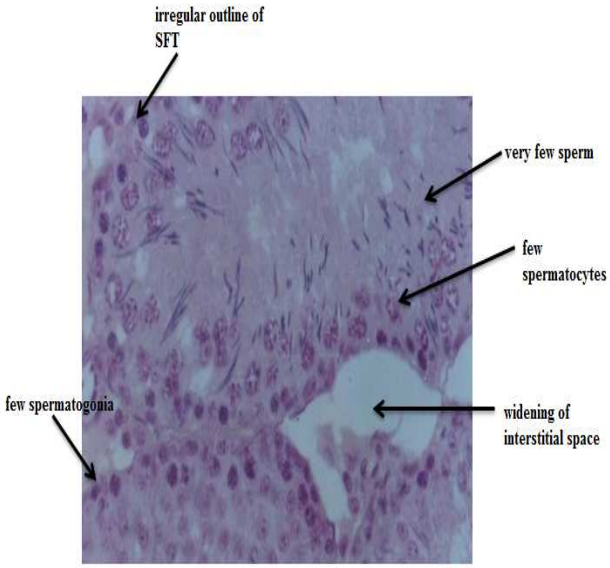
(Fig 2a)



(Fig 1a)



(Fig 1b)



(Fig 2b)

Figure 1 and 2: Histological profile of testis tissue section in experimental animals. Fig 1a and 2a represents normal rat testis (group I) showing varying stages of sperm development, spermatogonia and sertoli cells resting on the basement membrane. Primary spermatocytes, spermatids and late spermatids were also observed (H & E ×100). Treated group (Fig 1b and 2b) showing irregular seminiferous tubules, irregular basement membrane with few spermatogenic cell series (H & E ×100)

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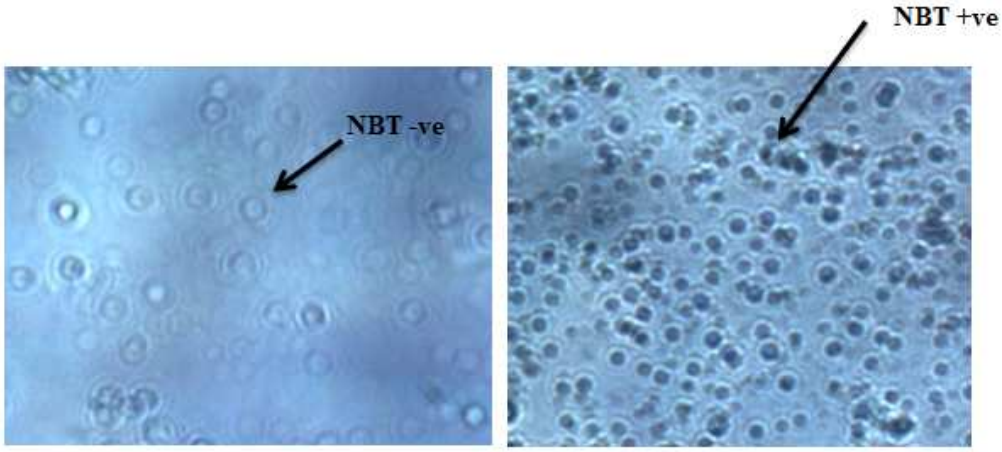
NBT (Nitro blue Tetrazolium) staining of testicular cells

Field of control testicular cells showed very much lesser number of NBT positive cells (Fig 3a) whereas field of treated testis had a greater number of NBT positive cells (Fig 3b).



(Fig 3a) **(Fig 3b)**

Figure 3: Field of control testicular cells possess very much lesser number of NBT positive cells (a) whereas field of treated testis has a greater number of NBT positive cells (b).

DISCUSSION:

Type I diabetes mellitus may affect endocrine function and spermatogenesis (Sexton and Jarow, 1997). In diabetes mellitus, hyperglycaemia increases oxidative stress and causes DNA damage in testis and significant reduction in sperm parameters like sperm motility, sperm count and sperm viability (Amaral *et al*., 2006). Oxidative stress plays a role in the development of diabetic complications (Sexton and Jarow, 1997).

It is well known that alloxan is a beta – cytotoxic agent of pancreas, so used widely to induce diabetes in laboratory animals. It is clear that diabetes could cause destruction of testis (Mahera N Al - Shaikh *et al*., 2006). This could be explained either by the direct toxic effect on many organ as pancreas, kidney and so on testis as well, or could be due to indirect effect through diabetic effect on metabolic changes on seminiferous tubules (Hassan A *et al*., 1993; Anderso J and Thliveries J, 1986; Ananthan R *et al*., 2004, Usenmez T *et al*., 2000 ; Mahera N Al - Shaikh *et al*., 2006). In alloxan treated diabetic rat increments of blood glucose levels were observed after GTT and the hyperglycemia persisted even 24 h after glucose load (Guria S *et al*., 2012 and 2014). It is believed that insulin response to a glucose load is relatively decreased in diabetes (Lenzen *et al*., 2008). The results of this study revealed that the alloxan had a destructive effect on seminiferous tubule cells in the testis of rats. In alloxan diabetic rats, these tubules were dilated and the spermatogenic cells irregularly arranged, spermatogenesis was arrested with highly reduction in the number of spermatides and spermatozoa.

Significant number of treated testis cells showed greater NBT positive response. Increased production of ROS occurs in treated condition. Accumulation of ROS is damaging

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to various cellular components and macromolecules including plasma membrane, nucleic acids, and proteins and eventually leads to cell death. NBT reacts with O_2^- to form a dark blue colour whereas the superoxide negative cells did not retain the stain. The present findings may provide a novel approach to elucidate the mechanisms of action of alloxan on testis in diabetes. But further experiments are in progress to solve this paradox.

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