Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2013, 3(2):154-160

ISSN : 2249-7412
CODEN (USA): AJPSKY

Ameliorative roles of ethanolic leaf extract of *Senna fistula* on destructive effects of alloxan on the rat testis

Onanuga O. Ismail[1], Ibrahim B. Ridwan[1], Amin Abdulbasit[2], Bolaji O. Akeem[3], Folarin O. Roehan[1], Safiriyu A. Abass[2] and Ibrahim O. Saheed[4]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria
[2]Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria
[3]Department of Medicine and Surgery, Faculty of Clinical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria
[4]Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ibadan, Ibadan, Nigeria

---

ABSTRACT

In this study, we investigated the effects of ethanolic leaf extract of Senna fistula on the histopathology of the testicles of alloxan-induced diabetic rats. Adult male Wistar rats were randomly assigned into 5 groups (n=7); Groups A and B served as Normal control [normal saline] and diabetic control (untreated). Groups C, D, and E served as the Diabetic treated groups receiving 5mg/Kg glibenclamide, 100mg/Kg Senna fistula and 200mg/Kg Senna fistula respectively. Diabetes was induced by intraperitoneal injection of 100 mg/kg Alloxan monohydrate. Treatment lasted for 28 days after which animals were sacrificed. Haematoxylin and eosin were employed for the histological examination of the testes, while tissue homogenates were assayed for testosterone, superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels. The examination of the testicles of diabetic rats revealed reduction in diameters, thickening in the wall, and degenerative changes in the seminiferous tubules in addition to reduction in the number of spermatogenic cells which were ameliorated in the treated groups. Activities of testosterone, SOD and GPx were significantly increased in the treated groups when compared with the diabetic untreated group. Extract of Senna fistula ameliorates the oxidative stress-induced degenerative changes in the testis of alloxan-induced diabetic rats.

Key words: Diabetes, *Senna fistula*, histopathology, oxidative stress, testosterone

---

INTRODUCTION

Diabetes mellitus (DM) is recognized as one of the leading causes of death in developing countries. The high prevalence of the disease can be attributed to improved nutritional condition coupled with shortage of modern facilities necessary for early diagnosis of the disease [1]. Diabetes mellitus (DM) is a syndrome, with features such as hyperglycemia, lipoprotein abnormalities, elevate basal metabolic rate, alteration in enzyme activity and high oxidative stress-induced damage to pancreatic beta cells [2]. Diabetes are shown to alter testosterone levels and impair spermatogenesis through damaging testicular functions in experimental animals and in humans [3, 4, 5, 6, 7]. It has been reported that diabetes-induced pathological changes in testicular tissues are encountered in tunica albuginea, seminiferous tubules and interstitial connective tissue of the testicles and in Leydig cells [3, 8]. Steger and Rabe show that diabetes frequently spoils reproductive functions of males and females and plunder hormonal functions, particularly the secretion of hypothalamic Luteinizing –Hormone-Releasing-Hormone [3].

The management of diabetes mellitus without any side effects is still a challenge to the medical system. There is an increasing demand by patients to use natural products with anti-diabetic activity, as insulin and oral hypoglycemic drugs though effective are not devoid of undesirable side effects [9]. *Senna fistula* is a flowering plant that belongs to the family of *Fabaceae*, subfamily *caesalpinioideae*. It is also called *Aidan-tooroo* by the Yorubas in Nigeria. The plant is used traditionally in the management of diabetes [reffered to as *Ito suga* by the Yorubas]. The present study was undertaken to evaluate the protective activity of *Senna fistula* against diabetes-induced testicular toxicity injury in experimental rats.

MATERIALS AND METHODS

PLANT MATERIAL

Fresh leaves of *Senna fistula* were collected from University of Ilorin biological garden. Identification was done at the Herbarium of the Department of Plant Biology, University of Ilorin. The plant material was washed, air-dried, and blended.

PREPARATION OF EXTRACT

150 g of the blended plant material was dissolved in 800 ml of 70% ethanol for 24 hrs. The mixture was filtered through filter paper. The filtrate was evaporated at a regulated temperature of 40^{o}C in an oven. After evaporation, 20 g of the dried concentrated extract was dissolved in 100 ml of distilled water to make a 200 mg/ml solution of *Senna fistula*.

EXPERIMENTAL ANIMALS

Laboratory investigations on the animals were carried out in accordance with the ethical guidelines stipulated by the ethical committee of the College of Health Sciences, University of Ilorin, Nigeria. These guidelines were in accordance with the internationally accepted principles for laboratory animal use and care. Thirty-five adult male Wistar rats [weighed between 200-230 g] were used for this experiment. The animals were kept and housed in the animal holdings of the Department of Anatomy, University of Ilorin, Nigeria, under standard laboratory conditions of temperature, light and humidity and were feed with rat pellets and water *Ad libitum*. The rats were randomly divided into five groups [A, B, C, D and E] of seven animals each. Standard drug-glibenclamide and *S. fistula* were administered orally.

EXPERIMENTAL DESIGN

The rats were divided into five groups of 7 rats each.
Group A – Normal control rats
Group B– Diabetes control [alloxan monohydrate 100 mg/kg i.p]
Group C– Alloxan induced diabetic rats + standard drug Glibenclamide [5 mg/kg]
Group D– Alloxan induced diabetic rats + ethanolic extract of *Senna fistula* [100 mg/kg]
Group E– Alloxan induced diabetic rats + ethanolic extract of *Senna fistula* [200 mg/kg]

INDUCTION OF DIABETES

The animals were fasted overnight. Diabetes was induced by single intraperitoneal [i.p] injection of alloxan monohydrate [100 mg/kg] in sterile normal saline [0.9%]. The diabetic state was determined after 72 hrs of alloxination through the tail using the one touch ultra-glucometer. Blood glucose level was estimated by one touch glucometer weekly.

ANIMAL SACRIFICE

After 28 days of treatment, rats were sacrificed by cervical dislocation on the 29th day. Testes were removed and washed with ice cold normal saline [0.9%] to remove the blood. Right testicular tissue samples were placed in 0.25 M chilled sucrose solution for homogenization. Tissue homogenate was collected in a 5 ml plain serum bottle and was centrifuged [5000 rpm] for 10mins. The supernatants were immediately stored in the freezer [-20°C] and assayed for testosterone, GPx and SOD within 48hrs. Quantitative estimation of testosterone was assayed using method described by Lashansky, [10]; Tietz, [11] with Accu-Bind ELISA Kit. GPx assay was carried out as described by Pagila and Valentine [12] while SOD assay was done following the procedure described by Marklund and Marklund [13].

HISTOPATHOLOGY

Testicular tissues from each group were fixed in Bouin's solution for 24 hours and were embedded in paraffin blocks. Four micrometer thick sections were stained with Hematoxylin Eosin (HE) for histopathologic examination. The slides were then examined at magnifications of X 400 with 5.0 Mega Pixel eyepiece under a light microscope.

STATISTICAL ANALYSIS

The distributions of the data were analyzed using the paired Student's t-test. $p<0.05$ was considered to be statistically significant [14].

RESULTS

Hypoglycemic effect of *S. fistula* in alloxan-induced diabetic rats

Fasting Blood Glucose Levels (FBGL) in healthy (control) rats and FBGL in alloxan-induced diabetic rats are shown in figure 1. The administration of Glibenclamide, oral anti-diabetic agent, markedly reduced FBGL in alloxan-induced diabetic rats as expected. After four weeks of *S. fistula* and standard drug (glibenclamide) administration, blood glucose level of animals receiving *S. fistula* [at both doses] and glibenclamide lowered significantly (*P<0.05) when compared with the diabetic control group. The hypoglycemic activity of *S. fistula* was dose dependent [more effective at the higher dose of 200mg/Kg]. Significant lowering of blood glucose level was observed from the second week and third week of *S. fistula* (200mg/Kg) and *S. fistula* (100mg/Kg) administration through to the fourth week respectively.

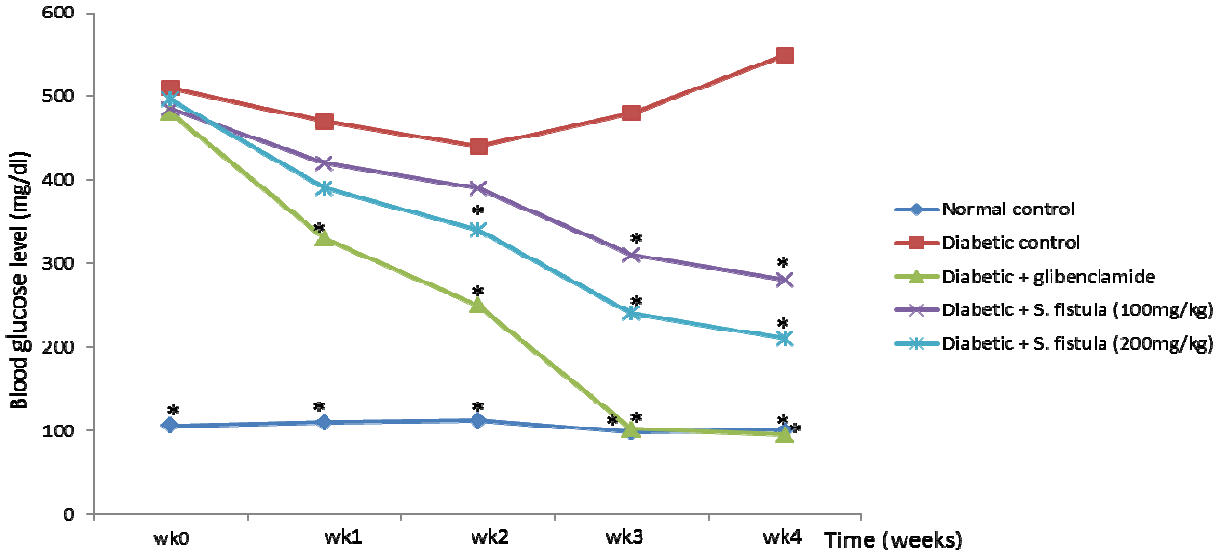

Fig 1: Hypoglycemic effect of *S. fistula* in alloxan-induced diabetic rats.
*P< 0.05 significantly different from diabetic control

Histopathological Examination

Control animals (group A) showed seminiferous tubules with adequate germ cells with all stages of maturation, it's wall revealed 4-5 cells thick representing all stages of spermatogenesis (i.e. functional spermatogonia till sperm formation). The lumen of the seminiferous tubules was filled with spermatozoa (Fig. 5A).

Regarding diabetic rats (group B) elicited obvious reduction in the number of seminiferous tubules (Fig 5B) which are widely separated from each other, no interstitial tissue could be elicited, spermatogenesis cells irregularly arranged. The cells elicited pyknotic nuclei with ill-defined membrane, the lumen showed complete absence of spermatozoa.

In contrary, group C (Alloxan + Glabeclamide), groups D & E (alloxan + ethanolic extract of *S. fistula* both at 100mg/kg and 200mg/kg respectively) revealed the same pictures as that of control group (Fig 5C, D & E).

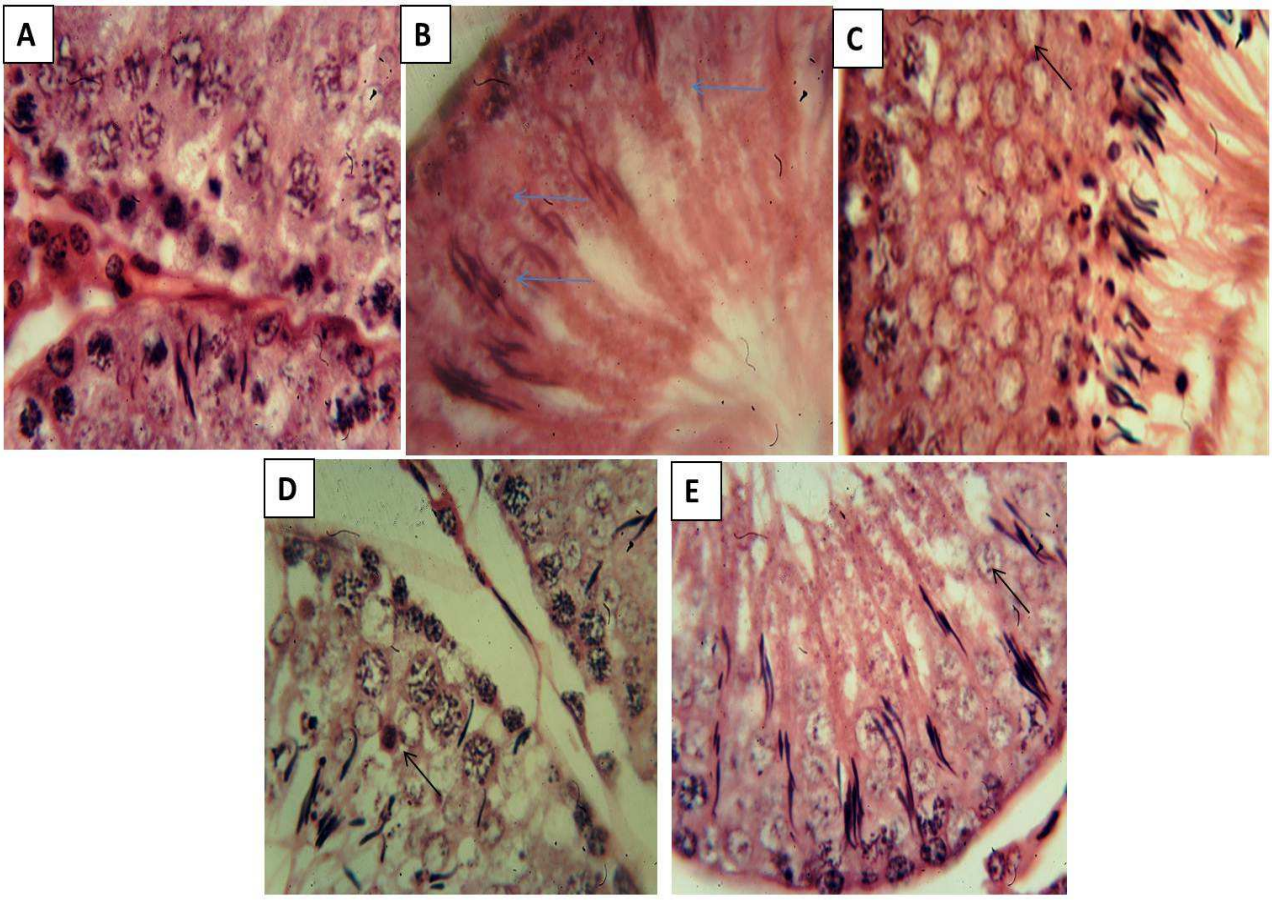

Fig. 5. A) Representative sample of testicular section of control group filled with spermatogenic series. No giant cells are present B) Representative sample of testicular section of diabetic group showing giant cells, degeneration of seminiferous tubules. →giant cell C, D & E) Representative sample of testicular section of treated groups showing decreased number of giant cells in the seminiferous tubules and relative increase in spermatogenic series (Hematoxylin-eosin stain ×400)

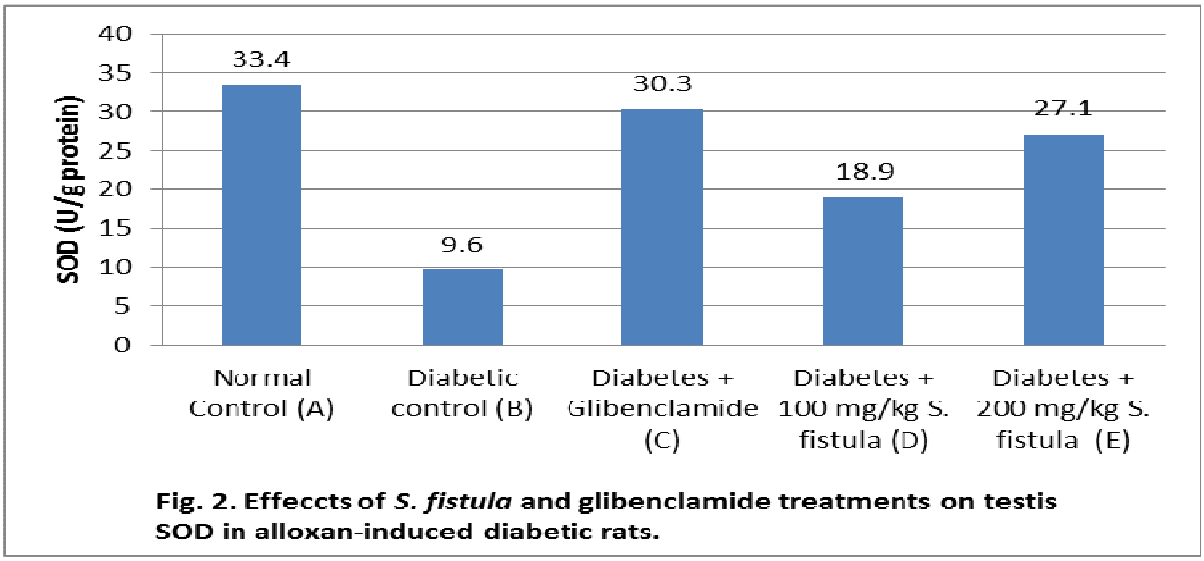

Fig. 2. Effeccts of *S. fistula* and glibenclamide treatments on testis SOD in alloxan-induced diabetic rats.

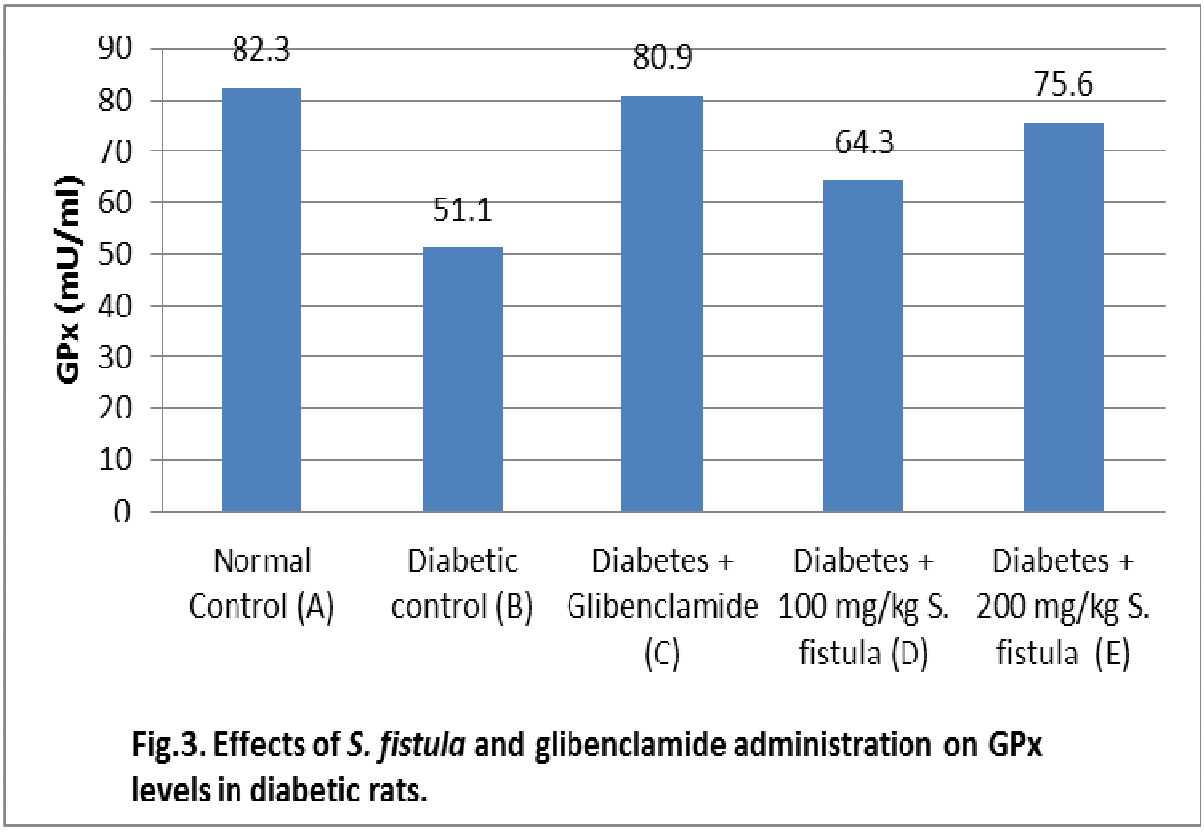

Fig.3. Effects of *S. fistula* and glibenclamide administration on GPx levels in diabetic rats.

Amelioration of oxidative stress markers in both *S. fistula* and glibenclamide treated diabetic rats.

Figures 2 & 3 show the levels of oxidative stress markers (SOD and GPx) in the experimental groups. The intraperitoneal injection of alloxan caused significant decrease ($P \leq 0.05$) in testis SOD and GPx activities when compared to the normal control group. The ethanolic extract of *S. fistula* [both at 100mg/kg and 200mg/kg] significantly ($P \leq 0.05$) elevated both SOD and GPx levels when compared with the diabetic group in a dose dependent manner. Glibenclamide also significantly ($P \leq 0.05$) elevated both SOD and GPx activities (Figs. 2 &3).

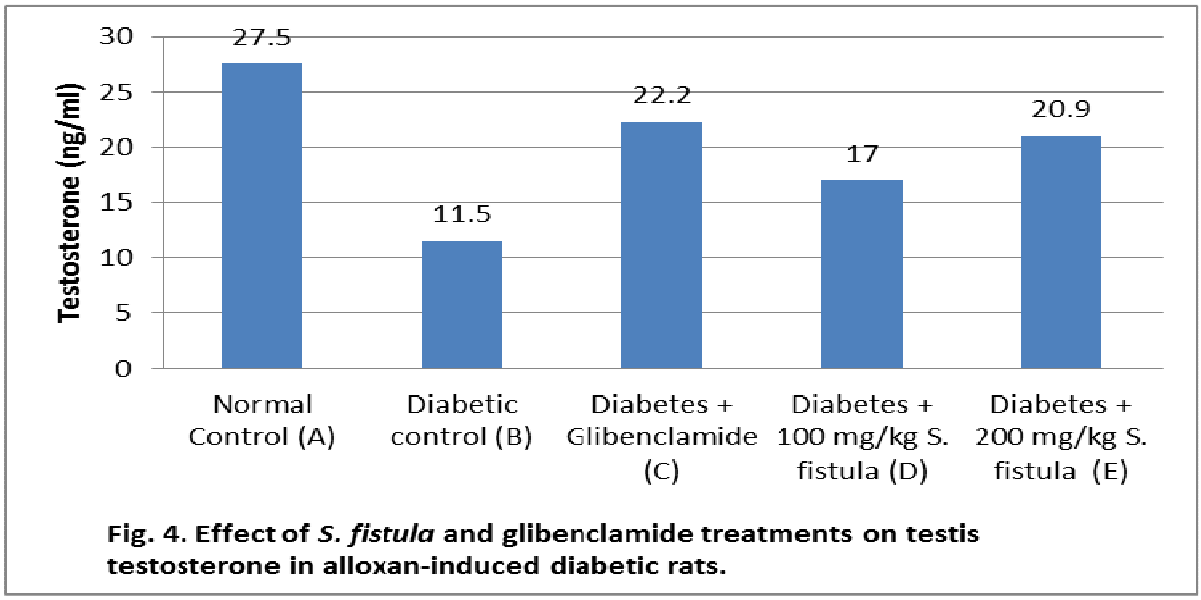

Fig. 4. Effect of *S. fistula* and glibenclamide treatments on testis testosterone in alloxan-induced diabetic rats.

Changes in the testosterone level in the testis of both *S. fistula* and glibenclamide treated diabetic rats.

Figure 4 shows the levels of testosterone in the experimental groups. The intraperitoneal injection of alloxan caused significant decrease ($P \leq 0.05$) in testis testosterone activities when compared to the normal control group. The ethanolic extract of *S. fistula* (both at 100mg/kg and 200mg/kg) significantly ($P \leq 0.05$) elevated testosterone levels

when compared with the diabetic group in a dose dependent manner. Glibenclamide also significantly ($P \leq 0.05$) elevated testosterone activities (Fig. 4).

DISCUSSION

In the present study, the seminiferous tubules and interstitium of testis in control groups were normal and the complete spermatogenic cells in the seminiferous tubules were healthy and uniformly arranged (Fig. 1). By contrast, we showed both normal and damaged seminiferous tubules in the testis of alloxan-induced diabetic rats.

Various plants are reported to be used in the treatment of diabetes [15]. Recently, several researches have assessed the hypoglycemic effect of a variety of plants as well [15, 16, 17, 18]. Several studies show tubular atrophies in testicles of diabetic humans [5] and in alloxan-induced diabetic rats [4, 17, 18]. Ethanolic extract of *S. fistula* showed meaningful hypoglycemic activity at 200 mg/kg dose; however, the changes occurring in time in the values of FBGL obtained were standardized according to the study of Onturk and Ozbek [14].

Histological findings are descriptive for understanding diabetes-associated pathological changes in the testicles. Spermatogenic cells are completely shed in some tubules while, Sertoli cells are preserved in some tubules, indicating that Sertoli cells are more resistant to diabetes than the spermatogenic cells [16]. Cameron [5] reveal the thickening of the basal membranes in diabetic patients. They state that the basal membrane thickening further hinders the nourishment of the already impaired tubules; thereby, adversely affects spermatogenesis. Similarly in the current study, we found thickening in the basal membranes in our diabetic rat. Moreover, we also found that sertoli cells were more sensitive to acute ischemia than spermatogenic cell series. These observations are similar to earlier findings by Ozturk [16]. Germ cell degeneration, vacuolation or more severely affected seminiferous tubules had sloughing of germ cell and giant cells in to lumen (Fig. 2 and 3). Even though, the destruction in some of the seminiferous tubules was not intense, giant cells were present in their lumens (Fig. 4). Another new finding that could be elicited in this study is the protection of testis by the ethanolic extract of *S. fistula* from the destruction role of alloxan. This could be explained either by the direct toxic effect on many organ as pancreas [19], kidney [20] and so on testis as well, or could be due to indirect effect through diabetic effect on metabolic changes on seminiferous tubules [21].

Testosterone level was also elevated in the *S. fistula* and glibenclamide treated groups. A relation between the secretion of testosterone and inhibino hormone has been established. In humans and experimental animals, decreased blood glucose level is shown to yield lowered testosterone levels [16, 22, 23, 24]. Insufficient insulin is thought to lower testosterone levels through suppressing LH and FSH secretions [4]. Testosterone is synthesized and secreted by Leydig cells; this secretion is further stimulated through binding of LH to the receptors on Leydig cells. Testosterone is required for spermatogenesis and the functions of Sertoli cells, which secrete androgen binding hormone, retaining testosterone in seminiferous tubules, upon FSH stimulation.

SOD and GPx are antioxidant enzymes which establish the defensive system against reactive oxygen species [ROS] [25]. Reduction in SOD activity is an important index for injuries caused by ROS. SOD eliminates superoxide anions by converting them to hydrogen peroxide [H_2O_2] hence reducing their toxic effects [26]. Glutathione peroxidase [GPx] is a selenium containing enzyme present in significant concentrations that helps detoxifies H_2O_2 to H_2O through the oxidation of reduced glutathione [27]. In the present study, both SOD and GPx activities are significantly reduced in the alloxan-induced diabetic group when compared to the control group due to the continual production of superoxide anions.

In this study the oral administration of the ethanolic leave extract of *S. fistula* elevated the activities of SOD and GPx when compared with the diabetic group indicating an antioxidant enhancing potential of this extract which may be due to the components it contains. In accordance with our study, Zeinab [28] reported that this reduced antioxidant production may be due to increase in oxygen metabolite that causes a decrease in the activity of the antioxidant defense system.

CONCLUSION

The present study shows that *Senna fistula* ameliorates the stress-induced morphological and biochemical changes characteristic of the testes in diabetes.

REFERENCES

[1] Ogbonnia SO, Adekunle AA, Olagbende–Dada SO, Anyika EN, Enwuru VN, Orolepe M, *Afr J Biotech*, **2008**, 7(17), 2998-3003.
[2] Kameswara RB, Appa RCH, *Phytomed*, **2001**, 8, 88-93.
[3] Steger RW, Rabe MB, *Proc Soc Exp Biol Med*, **1997**, 214, 1-11.
[4] Cai L, Chen S, Evans T, Deng DX, Mukherjee K, Chakrabarti S, *Urol Res*, **2000**, 28, 342-347.
[5] Cameron DF, Murray FT, Drylie DD, *Anat Rec*, **1985**, 213, 53-62.
[6] Alexopoulou OJ, Jamart D, Maiter MP, Hermans R, de Hertough, de Nayer Buysschaert M, *Diabetes Metab*, **2001,** 27, 329-336.
[7] Sanguinetti RE, Ogawa K, Kurohmaru M, Hayashi I, *Exp. Anim*, **2004**, 44, 71-73.
[8] Anderson J.E. Thliveris J.A. Morphometry and cytochemistry of Leydig cells in experimental diabetes. Am. J. Anat., 1987; 180: 41-48.
[9] Kameswara RB, Appa RCH, *Phytomed*, **2001**, 8, 88-93.
[10] Lashansky G, *J Clin Endocrinol Metab*, **1991**, 58, 674.
[11] Tietz NW, Pruden EL, Siggaard-Andersen O, In: Tietz textbook of Clinical chemistry, W.B. Saunders Company London, **1994,** 1354.
[12] Pagila DE, Valentine WN, *Journal of Laboratory and Clinical medicine*, **1967,** 70, 158-169.
[13] Marklund S, Marklund G, *Eur J Biochem* **1974**, 47, 469-74.
[14] Onturk H, Ozbek H, *Genel Tip Derg*. **2007**, 17, 231-236.
[15] Ozbek H, *Van Tip Derg*, **2002**, 9, 98-103.
[16] Ozbek H, Ozgokce F, Ceylan E, Tas A, Tuncturk M, *Van Tip Derg*, **2002**, 9, 73-77.
[17] Guneli E, Tugyan K, Ozturk H, Gumustekin M, Cilaker S, Uysal N, *Eur Surg Res*, **2008**, 40, 354-360.
[18] Anderson JE, Thliveris JA, *Anat Rec*, **1986**; 214: 378-382.
[19] Ananthan R, Latha M, Ramkumar K, Baskar C, Narmatha V, *Nutrition*, **2004,** 20, 280 – 285.
[20] Usenmez T, Yildiz O, Oktenli C, Ozgurtas T, Kutlu M, *Journal of Diabetes and Its Complications*, **2000,** (14), 53 – 59.
[21] Gyton AC, Hall JE, Textbook of medical Physiology, WB Saunders Company. 9th ed., **1996**; 1003 – 1016.
[22] Stellato RK, Feldman HA, Hamdy O, Horton ES, McKinlay JB, *Diabetes Care*, **2000**, 23, 490-494.
[23] Bucholtz DC, Chiesa A, Pappano WN, Nagatani S, Tsukamura H, Maeda KI, Foster DL, *Biol Reprod*, **2000,** 62, 1248-1255.
[24] Sudha S, Valli G, Julie PM, Arunakaran J, Govindarajulu P, Balasubramanian K, *Exp Clin Endocrinol Diabetes*, **2000**, 108, 14-20.
[25] Lil JL, Stantman FW, Lardy HA, *Arch Biochem Biophys*, **1988**, 263(1), 150-160.
[26] Curtis SJ, Mortiz M, Sondgrass PJ, *Gastroentrol*, **1972,** 62(1), 84-92.
[27] Bruce A, Freeman D, James C, Lab Invest, **1982,** 47, 412-426.
[28] Zeinab HK, Iman D, Mohamed M, *Australian Journal of Basic and Applied Sciences*, **2011**, 5(7), 387-396.