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Morphological Evaluation of the Effects of Ethanolic Leaf- Extract of *Newbouldia Laevis* (P. Beauv.) on Streptozotocin-Induced Gonadotoxicity in Adult Male Wistar Rats

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ABSTRACT

The study was designed to assess the effects of *Newbouldia laevis* on diabetes-induced testicular damage. Forty-two Wistar rats (200 - 250 g) were categorized into six groups- control, saline, diabetic, pre-diabetic, post-diabetic and extract only, each comprising of seven rats per group, to assess the effects of ethanolic leaf extract of *Newbouldia laevis* on streptozotocin-induced testicular damage. The control group and others received feed mash and water *ad libitum,* the saline group had saline intraperitoneally. Diabetes was induced in the treatment groups following overnight fasting, through intraperitoneal (i.p.) single dose of streptozotocin (55 mg/kg). The pre-diabetic extract treated group received 100 mg/kg of extract in the first twelve weeks before they were induced with diabetes. The diabetic group was induced with diabetes on the twelfth week but without extract treatment. The post-diabetic group was induced on the eighth week and had twelve weeks of extract at 100 mg/kg body weight post-induction. The extract-only treated group received only the 100mg/kg of ethanolic leaf extract of *Newbouldia laevis* only for twelve weeks. All the groups were sacrificed at the end of the twenty-fourth week. The effects of these treatments on the hormonal profile, testicular and epididymal cytoarchitecture of the Wistar rats were assessed. There were significant ($p<0.05$) changes in oestrogen, luteinizing hormone, prolactin, testosterone values and oestrogen-testosterone ratio mainly in the diabetic and post-diabetic groups but insignificant ($p>0.05$) follicle-stimulating hormone and progesterone values compared to control. The sections of the testes and epididymis from the diabetic group had various cytoarchitectural lesions, inflamed testicular interstitium, luminal depletion of spermatozoa and arrested spermatogenesis. The post-diabetic group suffered less severe lesions. The pre-diabetic group had testicular lumen containing matured spermatozoa from normal spermatogenesis. The findings of this investigation confirm protective effects of ethanolic leaf-extract of *Newbouldia laevis* on streptozotocin-induced gonadotoxicity in male Wistar rats.

Key words: Morphological, *Newbouldia laevis*, Streptozotocin, Gonadotoxicity, Male Wistar Rats

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1. INTRODUCTION

Diabetes mellitus, a well-known metabolic disorder has been reported to be associated with reproductive malfunction in both sexes (American Diabetes Association, 2009). Two major types of diabetes known are type I and type II. Type I results from absolute lack of insulin due to reduction in the beta cell mass (Anderson, 1980; American Diabetes Association, 2009) and type II diabetes mellitus is either due to derangement in insulin secretion caused by delay or insufficiency (Anderson, 1980; Meir and Bonadonna, 2013) or the inability of peripheral tissues to respond to the secreted insulin (Anderson, 1980; Keen et al., 1982; Khan et al., 2006). The prevalence of diabetes mellitus has been on the rise worldwide (Shaw et al., 2010). While effective management modalities exist to address the absolute or relative insulin deficiency in the vulnerable; ignorance, poverty and inadequate expertise gives room to emergence of diabetic complications, one of which is abnormalities in sexual function such as erectile dysfunction, diminished libido and infertility (Feng et al., 2001) in the males. Streptozotocin, a cytotoxic agent which had been widely used in inducing type I experimental diabetes (Shrilatha et al., 2007) has its toxic effects not directly related to the actions of the drug but by its induced diabetes (Oksanen, 1975) which alters serum insulin level by destroying the beta cells of the pancreas. The resultant

infertility as a possible long-term complication of diabetes has been attributed to the alteration of spermatogenesis process (Guo-Lian Ding et al., 2015). Certain regulatory factors of spermatogenesis such as hormones and oxidative stress are known to affect this process (Aitken and Roman, 2008; Yoganathan et al., 1989; Shetty et al., 2000). Agents that can therefore modulate gonadal hormone pattern and free-radical damage will protect against gonadal damage from diabetes mellitus (Fang et al., 2002; Tsai et al., 2005). Spermatogenesis is influenced by the serum levels of several gonadal hormones, follicle-stimulating hormone and luteinizing hormone inclusive, which promote spermatogenesis by facilitating the role of the seminiferous epithelium on the developing germ cells and enhancing testosterone production, respectively (Fink, 1988; Ramaswamy and Weinbauer, 2014)). The secretion of these hormones are regulated by the hypothalamic-pituitary-gonadal axis (Ganong, 2001). The role of anti-oxidants in counteracting free-radical damage has also been stressed (Fujii et al., 2003). The search for remedies to up-regulate insulin deficiency or moderate hormonal inbalance and free-radical damage is still a matter for continuous emphasis (Mohasseb et al*.,* 2011; Ataman and Osinubi, 2014).

Newbouldia laevis is an angiosperm, well distributed across the West African coast (Burkill, 1985) and its leaves had been indicated useful in addressing male infertility on account of its phytochemical and nutrient values (Odugbemi, 2008; Ogunlesi et al., 2009; Ataman et al*,* 2015,). The phytochemical properties and acute toxicity of this plant has been previously evaluated and reported (Ataman et al., 2015) with saponins, steroids, flavonoids, tannins and cardiac glycosides as the major constituents. The acute toxicity study revealed that the drug is safer on oral route compared to the intraperitoneal route with the median lethal dose (LD_{50}) as 5 g/kg for the intraperitoneally-treated, but could not be evaluated in the group treated via the oral route due to its high margin of safety. The plant contains carbohydrate, protein, fat, vitamin C, selenium, zinc, magnessium, calcium, sodium, potassium and chloride on proximate and mineral analyses (Ataman et al., 2015). This study investigates the morphological effects of ethanolic leaf-extract of *Newbouldia laevis* on streptozotocin-induced testicular damage in Wistar rats.

2. METHODOLOGY

2.1 Preparation of Extract and Drug Procurement

Fresh leaves of *Newbouldia laevis* were harvested from cultivated farmlands and premises at the University of Benin, Ugbowo, Benin City between April and June, 2010. The identity of this medicinal plant was authenticated at the Forest Research Institute of Nigeria (FRIN), Ibadan by Mr. Ekundayo A. Adewale and a herbarium specimen of the plant with herbarium no. FHI-109521 was prepared and deposited at the Institute.

The leaves of *Newbouldia laevis* were air-dried at room temperature for two months and further oven-dried at 40^0C before grinding to powder. Three grammes of the powdered sample were subjected to ethanolic extraction by means of Soxhlet apparatus and the extract evaporated in-vacuo (Sofowora, 1992; Trease and Evans, 2002). Yield of 18.4 % was obtained and fresh solution of the extract was prepared in saline for use, with their concentrations determined. The ethanolic extract of *Newbouldia laevis* was administered by gastric gavage to assess its effects on streptozotocin-induced testicular damage in Wistar rats.

2.2 Drugs and Chemicals

All chemicals/reagents were of analytical grade.

2.3 Animals and Intervention

Experimental procedures involving the animals and their care were conducted in conformity with International and Institutional guidelines for the care of laboratory animals in Biomedical Research, as promulgated by Canadian Council of Animal Care (1985). Further, the animal experimental models used were in conformity to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the care and use of Animals (American Physiological Society, 2002; Saalu et al., 2009).

Forty-two Wistar rats weighing between 200 - 250 g were bred and housed in well ventilated wire wooden cages in the Animal facility of the Department of Anatomy, University of Benin, Benin City. An approval to conduct the research was sought and obtained from the Post Graduate Committee of the Department. The rats were maintained under standard natural photoperiodic condition of 12 hr of light alternating with 12 hr of darkness (i.e. L:D;12:12) with room temperature of between 25 to 26^0C and humidity of 65$\pm$5 %. They were given water *ad libitum* and unrestricted access to feeds obtained from Bendel feeds and flour mill, Ewu; in Edo State, Nigeria. They were allowed to acclimatize for three weeks (21 days) before the commencement of the experiments. The weights of the animals were noted at procurement, during acclimatization, at commencement of the experiments and twice within a week, and at the end of the experiment, using an electronic analytical and precision balance (Mettler Pm 4800 Delta Range^R). The experimental animals were categorized into the following treatment groups, each comprising of seven rats:

- **Control Group:** Received feed-mash and water only, *ad libitum* throughout the duration of treatment before sacrifice at the end of the twenty-fourth week.
- **Saline Group:** This group served as negative control. They received equivalent volume of physiological saline before sacrifice at the end of the twenty-fourth week.
- **Diabetic Treatment Groups:**

Diabetes was induced in these groups of rats following overnight fasting, by intraperitoneal (i.p.) injection of a single dose of streptozotocin (55 mg/kg) product of China by Sigma Aldrich Co., which induced diabetes by rapid depletion of beta cell mass causing reduction in insulin release and hyperglycaemia. Streptozotocin was dissolved in a freshly prepared 0.01 M citrate buffer at pH 4.5. The streptozotocin-injected animals were given 1 ml of 50 % dextrose in 1:1 dilution with distilled water to prevent initial hypoglycaemia and mortality observable within the first 12-24 hours post-induction. Diabetes was confirmed using the 'Fine test' blood glucose monitoring kit (glucometer), with strips manufactured by infopia Co. Ltd; Korea and rats with blood glucose levels of 250 mg % or more were considered diabetic and used in the experiment. The range of blood glucose of all the diabetic rats was maintained between 300- 450 mg % using titrated dosage of insulin and proper rehydration where necessary, throughout the period of experiment before their sacrifice.

- Pre-diabetic Extract Treated: This group received 100 mg/kg of extract treatment in the first twelve weeks before they were induced with diabetes. The rats were sacrificed at the end of the twenty-fourth week.
- Diabetic Group: This group was induced with diabetes on the twelfth week and without any extract treatment, the rats were sacrificed at the end of the twenty-fourth week.
- Post-diabetic Extract Treated: This group was induced with diabetes on the eighth week and had twelve weeks treatment of extract at 100 mg/kg body weight post-induction.

The animals were sacrificed at the end of the twenty-fourth week.

- Extract Only Group: This group had twelve weeks treatment on 100 mg/kg of ethanolic leaf-extract of *Newbouldia laevis* orally (week 8-20). They were sacrificed at the end of the twenty-fourth week.

2.4 Animal sacrifice and Routine Histological Preparation:

The rats at the time of sacrifice were weighed and then anaesthesized by placing them in a closed jar containing cotton wool soaked with chloroform anaesthetic. The abdominal cavity was opened up through a midline abdominal incision to expose the reproductive organs. Blood samples were obtained from the abdominal aorta for serum hormonal assay. Total serum Testosterone, LH and FSH, Progesterone, Prolactin and Oestrogen in treatments and control groups were evaluated in the Department of Chemical Pathology, University of Benin, using the respective kits, products of DRG Instruments GmbH, Germany. The procedures involved in the various tests are as contained in the guiding principles of DRG Diagnostics User's Manual for oestrogen, (Hall, 1988; Ratcliff, 1988), LH (Harris and Waftolin, 1970; Pierce and Parsons, 1981, Tietz, 1999), FSH, Prolactin (Engvall 1980, Tietz 1999), Progesterone (Filicori et al., 1984, Tietz, 1999) and testosterone (Tietz, 1999). The testes and epididymis excised and trimmed of all fat were harvested and prepared for microscopy. The organs were fixed in bouin's fluid for 24 hours, after which it was processed using the automated tissue processor and stained with haematoxylin and eosin staining techniques (Drury and Wallington, 1980). Photomicrographs were obtained at magnification x40, x100 and x400.

2.5 Statistical Analysis:

This was done using the statistical package Genstat Release 8.1 (PC/Windows XP, 2005 and Microsoft excel. All data were expressed as Mean + SEM of number of experiments. Means separation was done using the Duncan multiple range test (Duncan, 1957; Snedecor and Cochran, 1980). Significant differences between the mean of each group and the control were determined using the student's t- test ***($p<0.001$)[a], ($p<0.005$)[b], ($p<0.05$)[c].***

3. RESULTS

3.1 Hormonal Assay

The data on the table below shows the values of oestrogen, follicle-stimulating hormone, luteinizing hormone, prolactin, progesterone and testosterone of each experimental animal in the various groups. This data were analyzed and the summary is shown in the Table. From the results shown in the Table, the mean value of oestradiol (oestrogen) and the standard error of mean in: the control group was 21.71±0.4ng/ml, saline group was 21.73±0.61ng/ml, diabetic group was 15.71±1.93ng/ml, pre-diabetic group was 25.50±1.49ng/ml, post-diabetic group was 20.47±1.61ng/ml, while the extract only treated group had mean oestrogen value of 18.26±1.83ng/ml. The mean value of oestrogen was significant in the diabetic groups *($p<0.05$)* and insignificant in the other groups *($p>0.05$),* compared to control.
Analysis of FSH value (IU/ml) for the various groups is also shown. The mean value for the: control group was 0.10±0.00, saline group was 0.10±0.00, diabetic group was 0.07±0.02, pre-diabetic group was 0.10±0.00, post-diabetic group was 0.10±0.00 and 0.14±0.03 was for the extract group. The mean value of FSH was insignificant in the all the groups (*$p>0.05$),* compared to control.
The mean value of LH (IU/ml) for the control was 1.80±0.04 and for the saline group was 1.77±0.06. The diabetic group had mean LH

value of 0.23±0.10, 0.79±0.26 for pre-diabetic group and for the post-diabetic group, it was 0.37±0.12. The extract group had a mean LH value of 1.64±0.20 IU/ml. The mean value of LH was significant in the diabetic group *(p<0.001),* pre-diabetic and post-diabetic groups *(p<0.005)* and insignificant in the other groups *(p>0.05),* compared to control.

The mean value of prolactin (ng/ml) in the control group was 2.04±0.09 and saline group had a value of 1.84±0.10. For the diabetic group, the mean value of prolactin was 0.31±0.10, for the pre-diabetic group was 0.57±0.09 and for the post-diabetic group was 0.43±0.08. The extract group had mean prolactin value of 1.26±0.27. The mean value of prolactin was significant in the diabetic, pre-diabetic, post-diabetic groups *(p<0.001)* and extract only group *(p<0.05))* but insignificant in the other groups *(p>0.05),* compared to control.

The mean value of progesterone for: the control group was 3.44±0.59, the saline group was 2.47±0.22 and the diabetic group was 3.20±0.93, 5.10±1.05 for pre-diabetic and 3.69±0.71 for the post-diabetic group. The progesterone value for the extract only treated group was 2.41±0.50. The mean value of progesterone was insignificant in all the treatment groups *(p>0.05),* compared to control.

The mean testosterone assay for the control group was 9.50±1.80 and for saline group was 6.26±2.15. For the diabetic group, the mean value of testosterone was 0.21±0.05, for pre-diabetic group was 1.84±0.97, for the post-diabetic group was 0.37±0.10 and for the extract treated group was 10.03±1.90. The mean value of testosterone was significant in the diabetic and post-diabetic groups *(p<0.005),* pre-diabetic groups *(p<0.05)* and insignificant in the saline group *(p>0.05)*, compared to control. Also, the oestrogen: testosterone ratio for the control group was 2.28 and 3.47 for the saline group. For the diabetic group, the ratio was 74.81, but 13.85 and 55.32 for the pre-diabetic and post-diabetic groups, respectively. The extract only group had oestrogen: testosterone ratio of 1.82. The ratio was significant *(p<0.005)* in the diabetic group, *(p<0.05)* in the post-diabetic group but insignificant *(p>0.05),* in the pre-diabetic and saline groups, compared to control.

Table showing Mean hormonal profile and standard error of mean of control and treatment animals

Groups	Oestrogen (ng/ml)	FSH (IU/ml)	LH (IU/ml)	Prolactin (ng/ml)	Progesterone (ng/ml)	Testosterone (ng/ml)
Control	21.71±0.43	0.10±0.00	1.80±0.04	2.04±0.09	3.44±0.59	9.50±1.80
Saline	21.73±0.61	0.10±0.00	1.77±0.06	1.84±0.10	2.47±0.22	6.26±2.15
Diabetic	15.71±1.93[c]	0.07±0.02	0.23±0.10[a]	0.31±0.10[a]	3.20±0.93	0.21±0.05[b]
Pre-diabetic	25.50±1.49	0.10±0.00	0.79±0.26[b]	0.57±0.09[a]	5.10±1.05	1.84±0.97[c]
Post-diabetic	20.47±1.61	0.10±0.00	0.37±0.12[b]	0.43±0.08[a]	3.69±0.71	0.37±0.10[b]
Extract	18.26±1.83	0.14±0.03	1.64±0.20	1.26±0.27[c]	2.41±0.50	10.03±1.90

*** Values are Mean ± SEM. Means with alphabetic remarks are significantly different from control: *(p<0.001)[a], (p<0.005)[b], (p<0.05)[c]*.**
Vertical comparisons only

Control: Received feed mash and water only

Saline: Received equivalent volume of normal saline

Diabetic: Induced with diabetes and received no extract treatment

FSH: Follicle stimulating hormone

Pre-diabetic: Received extract before diabetes induction

Post-diabetic: Received extract post induction with diabetes

Extract: Treated with extract of *Newbouldia laevis* only

LH: Luteinizing Hormone, Ng/ml: nanogram/milliliters

3.2 Histology:

The control sections of the testes essentially showed the lumen of the seminiferous tubules with spermatogenic cell series in progression from the basement membrane towards the adluminal compactment (Figs.1,2). The testicular interstitium were essentially normal containing the interstitial cells (of Leydig). The sections of the epididymis contained matured spermatozoa stored in the lumen, and the epididymal linning comprised of tall columnal cells (Figs. 13,14). The testicular sections of the saline treatments were normal, with normal progression of the germ cell series and normal testicular interstitium (Figs. 3,4). The epididymis of saline treatment, just as the control section, had normal lumen containing stored spermatozoa and the epididymal lining comprised of tall columnal cells with no abnormality seen (Figs. 15,16). The sections of the testes and epididymis from the diabetes treatment group which

had no extract treatment had various testicular lesions such as seminiferous tubules with atrophic changes, necrosis, detachment of the basement membrane from the seminiferous epithelium, paucity of cells in the tubular lumen, disrupted seminiferous epithelium and arrested spermatogenesis. Congested interstitial blood vessel as well as inflamed testicular interstitium with few interstitial cells (of Leydig) were noticed (Figs. 5,6). The epididymis showed luminal depletion of spermatozoa, discontinuity of epithelial lining, focal necrosis and fibrosis (Figs. 17,18). The experimental group that received leaf extract of *Newbouldia laevis* as pre-treatment before the induction of diabetes had testicular and epididymal lumen containing matured spermatozoa from normal spermatogenesis. Without disruption, the seminiferous tubules contained well-differentiated germ cell series beneath the basement membrane. The interstitial cells of Leydig were present. Thus, normal testicular cytoarchitecture was well preserved (Figs. 7,8,19,20). In the post-diabetic treatment group which was treated with same extract of *Newbouldia laevis* after previous testicular damage by streptozotocin, showed evidence of seminiferous tubules which lumen contained abnormally differentiated germ cell line, disrupted seminiferous epithelium and oedema, but no complete arrest of spermatogenesis was observed (Figs. 9,10). The epididymis of the post-diabetic group had lumen with hypocellularity, containing few spermatozoa (Figs. 21,22). The treatment group that received the leaf extract of *Newbouldia laevis* exclusively, without being subjected to any other form of treatment had testicular sections with normal seminiferous tubules. The lumen were enriched with spermatozoa that had differentiated normally through the germ line, undisrupted. The testicular interstitium contained Leydig cells (Figs. 11,12). Also, the epididymis of this treatment group were well filled with matured spermatozoa (Figs. 23,24). The cytoarchitectural pattern of the group was not significantly different from that of control.

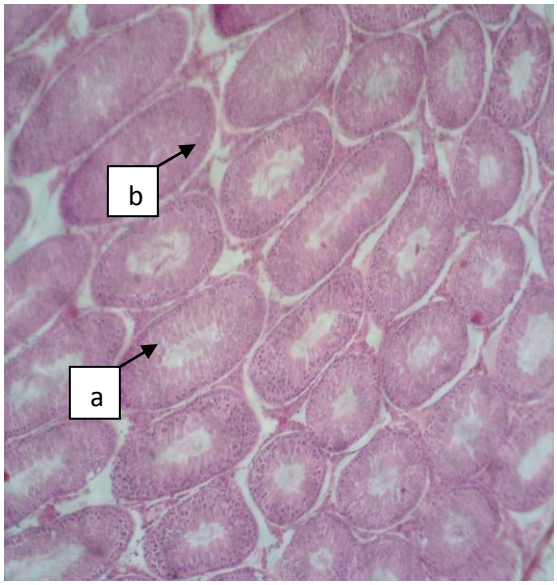

Figure 1. Section of the control C1 testis (a) lumen of the seminiferous tubule showing clusters of spermatozoa and cells of the spermatogenic series (b) normal testicular interstitium with Leydig cell [H&E x40]

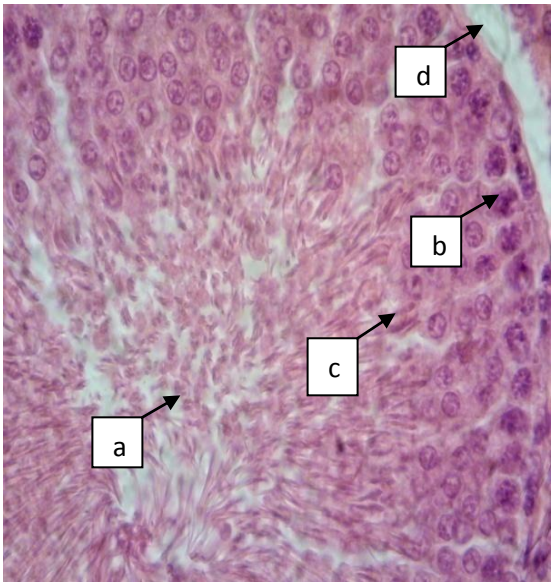

Figure 2. Section of the control C3 testis (a) normal seminiferous tubule with spermatozoa in the lumen, (b) early germ cells of the spermatogenic series, (c) Late germ cells of the spermatogenic series forming spermatids (d) basement membrane [H&E x400]

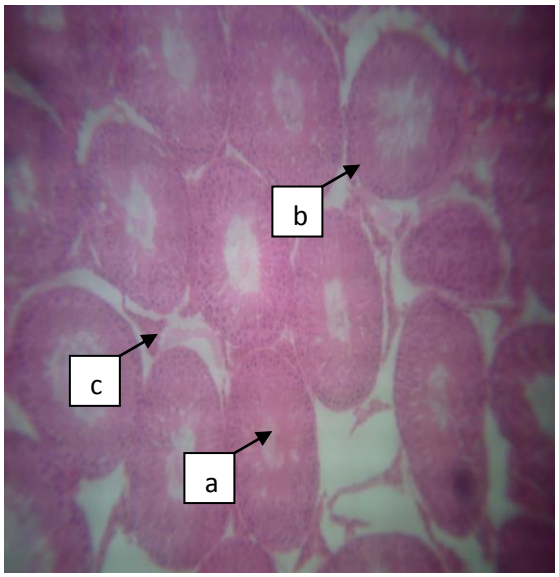

Figure 3. Section of the saline group S7 testis (a) lumen of the seminiferous tubule containing spermatozoa (b) early germ cells of the spermatogenic series (c) testicular interstitium with Leydig cell [H&E x100]

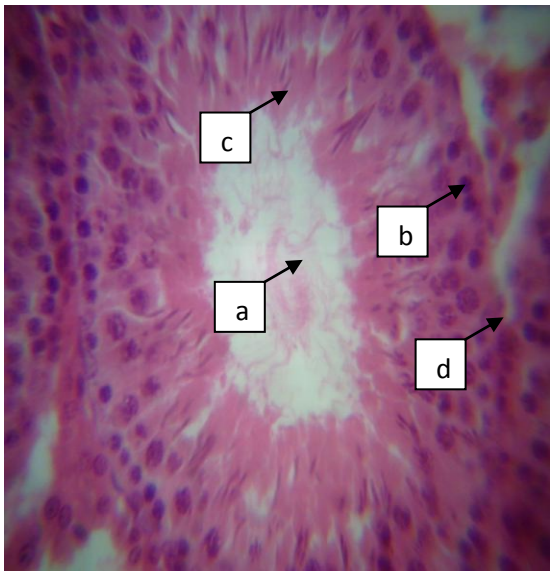

Figure 4. Section of the testis S7 (a) lumen of the seminiferous tubule containing spermatozoa (b) early germ cells of the spermatogenic series (c) late germ cells of the spermatogenic series (d) basement membrane [H&E x400]

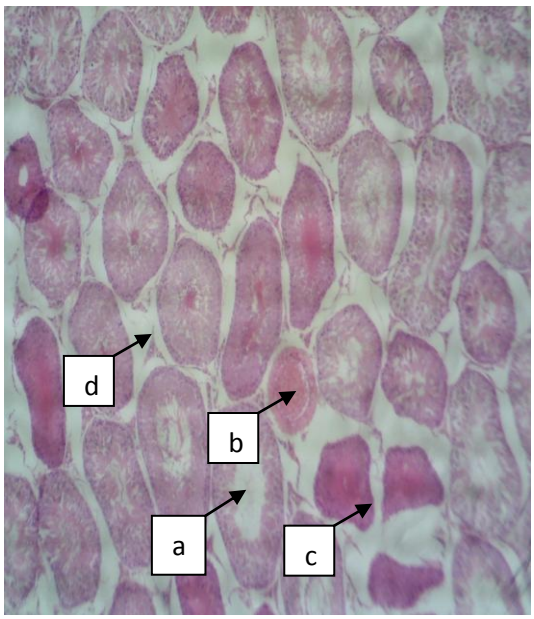

Figure 5. Section of diabetic group DM6 testis (a) seminiferous tubule with hypocellularity in the lumen (b) congested interstitial blood vessel (c) atrophic and irregular seminiferous tubules (d) testicular interstitium with inflammatory changes. Leydig cells are present [H&E x40]

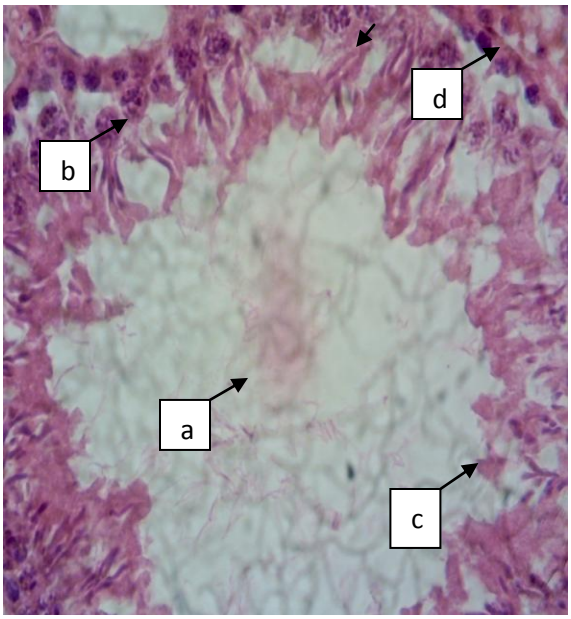

Figure 6. Section of diabetic group DM6 testis (a) seminiferous tubule with disrupted and empty lumen due to necrosis, (b) arrested and distorted distribution of germ cells of the spermatogenic series (c) distorted seminiferous epithelium (d) basement membrane [H&E x400]

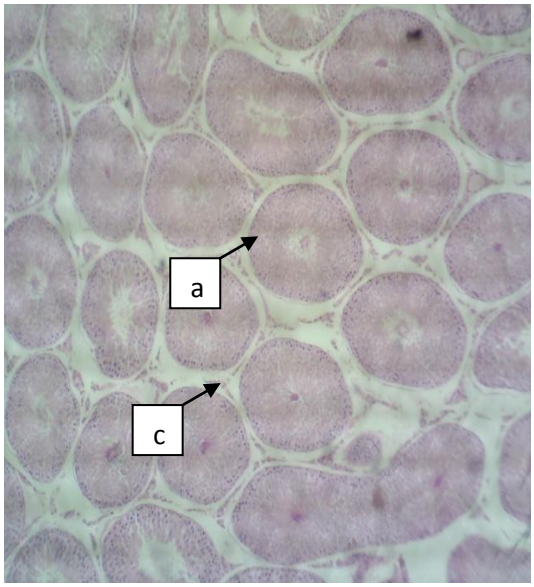

Figure 7. Section of Pre-diabetic group PrDm3 testis (a) seminiferous tubule with developing germ cell series maturing into the lumen (b) testicular interstitium with Leydig cells. [H&E x40]

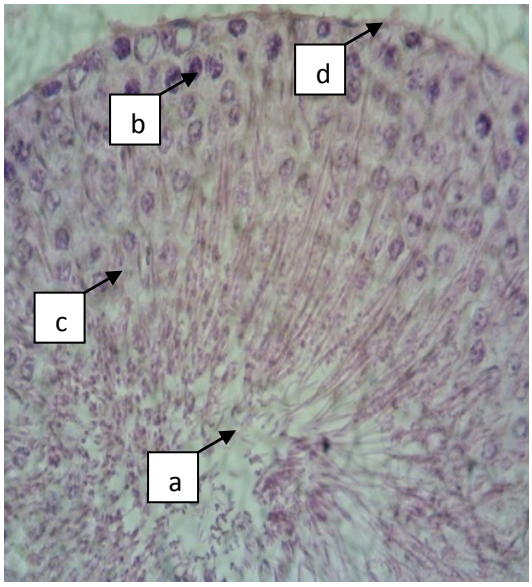

Figure 8. Section of Pre-diabetic group PrDm3 testis (a) seminiferous tubule with lumen containing matured spermatozoa from well differentiated early and late germ cell series (b and c) beneath the basement membrane (d) developing in the seminiferous epithelium towards the adluminal compactment. No abnormality is seen. [H&E x400]

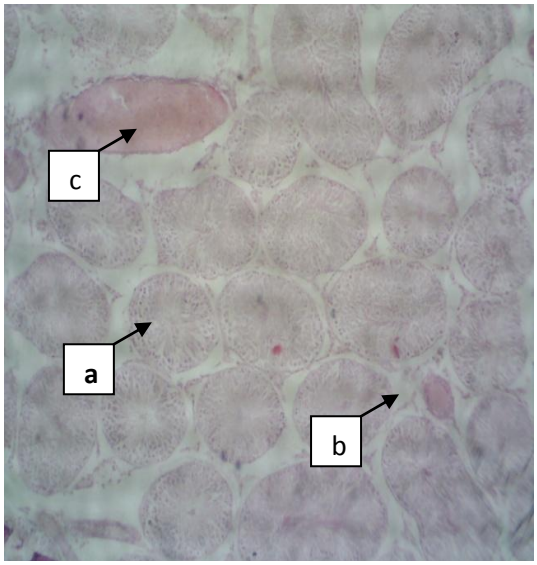

Figure 9. Section of post-diabetic PoDm5 testis (a) seminiferous tubule (b) testicular interstitium with Leydig cells (c) congested interstitial blood vessels [H&E x40]

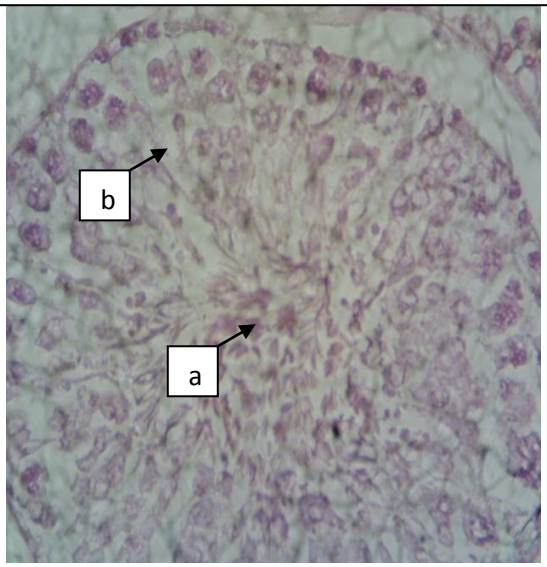

Figure 10. Section of Post-diabetic PoDm7 testis (a) lumen of seminiferous tubule with altered distribution of spermatids and spermatozoa (b) distorted seminiferous epithelium and fluid retention. [H&E x400]

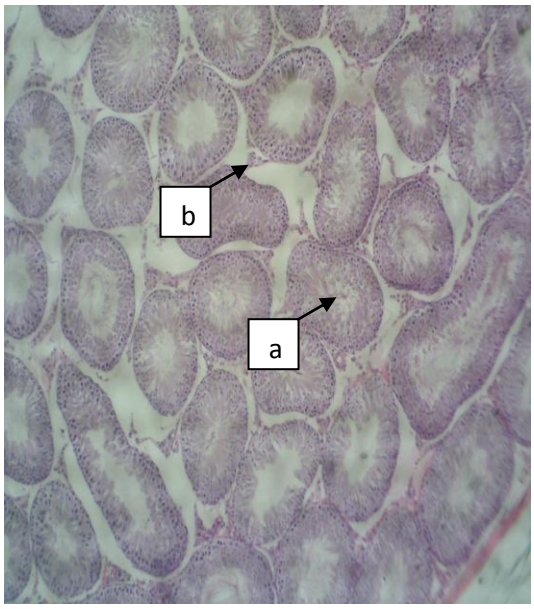

Figure 11. Section of extract only group EX3 testis (a) lumen of the seminiferous tubule and maturation germ cells (b) Leydig cell in interstitium [H&E x40]

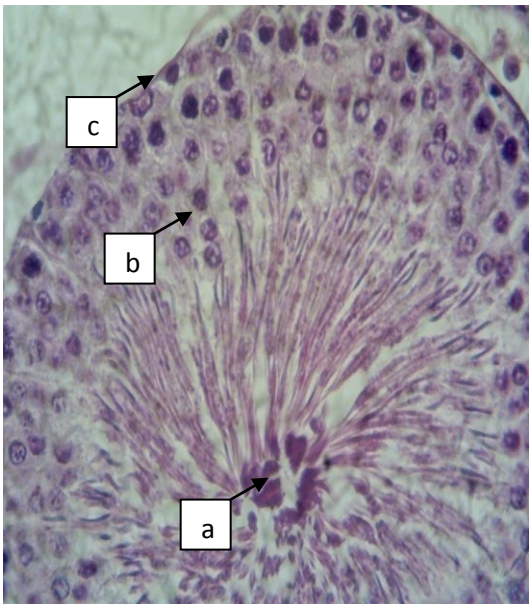

Figure 12. Section of extract only group Ex3 testis (a) lumen of the seminiferous tubule richly containing mature spermatozoa (b) maturation germ cells of the spermatogenic series in normal seminiferous epithelium (c) normal basement membrane. [H&E x400]

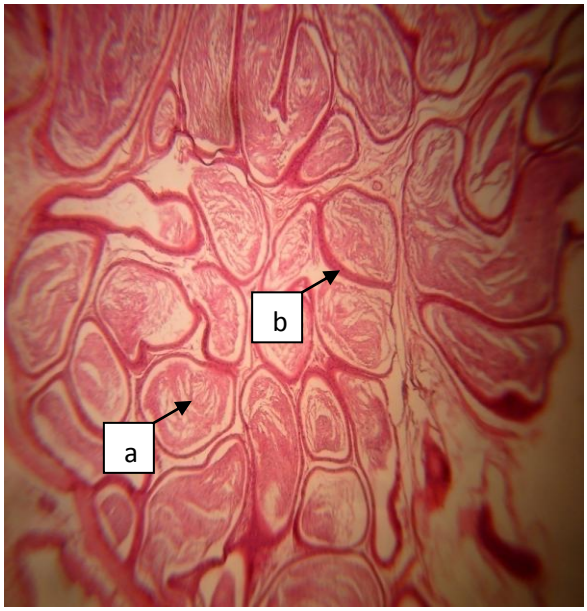

Figure 13. Section of control C4 epididymis (a) lumen containing spermatozoa (b) normal epididymal lining [H&E x40]

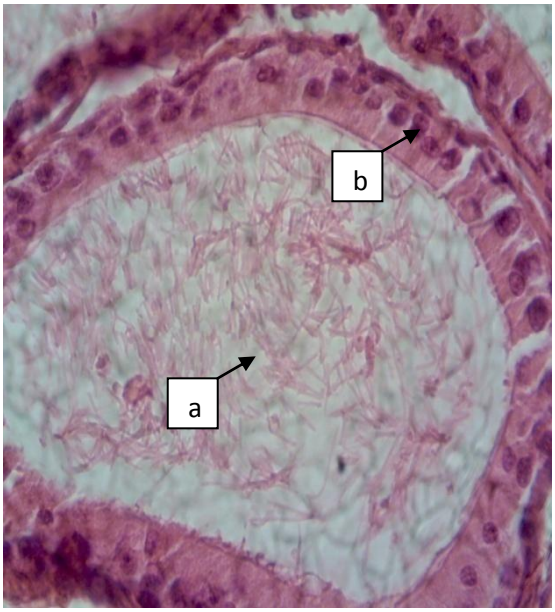

Figure 14. Section of the control C1 epididymis (a) clusters of spermatozoa in the lumen (b) normal epithelial lining of the epididymis [H&E x400]

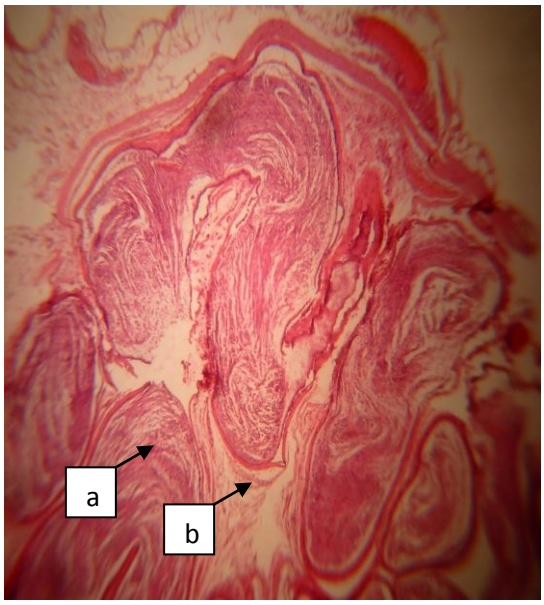

Figure15. Section of epididymis from saline group S7 (a) lumen containing spermatozoa (b) normal epididymal lining with mild interstitial oedema [H&E x40]

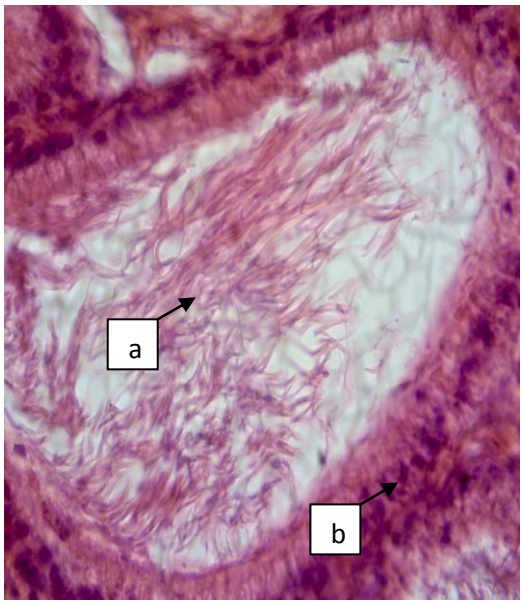

Figure 16. Section of epididymis from saline group S7 (a) lumen containing spermatozoa (b) normal epididymal lining [H&E x400]

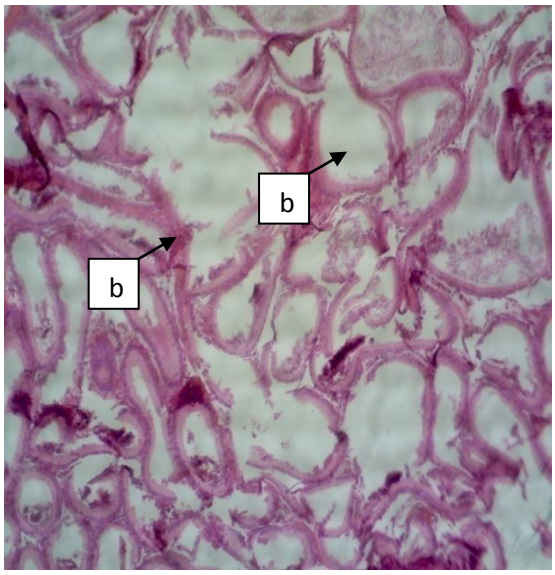

Figure 17. Section of diabetic group DM6 epididymis (a) empty lumen (b) necrosis and disruption of normal epithelial lining with fibrotic changes. [H&E x40]

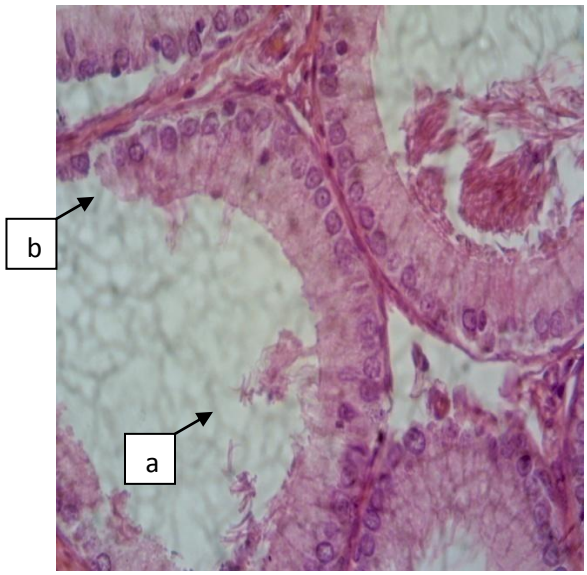

Figure 18. Section of diabetic group DM6 epididymis (a) empty lumen with little or no spermatozoa (b) disruption of epithelial lining of the epididymis [H&E x400]

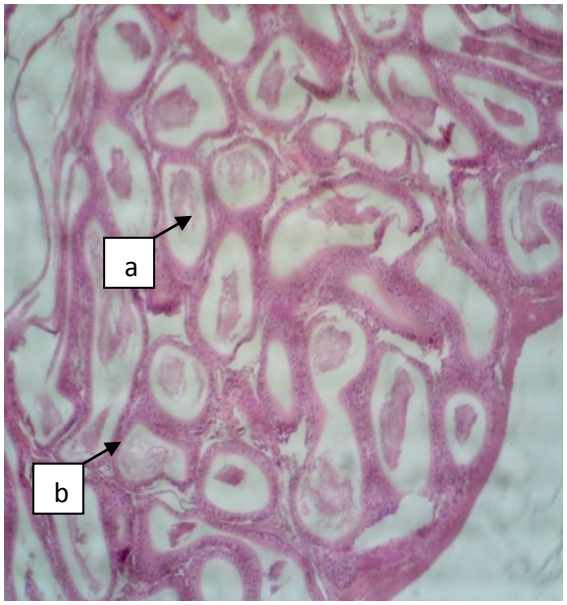

Figure 19. Section of Pre-diabetic group PrDm3 epididymis (a) moderate clusters of spermatozoa in the lumen (b) epithelial lining with no remarkable abnormality. [H&E x40]

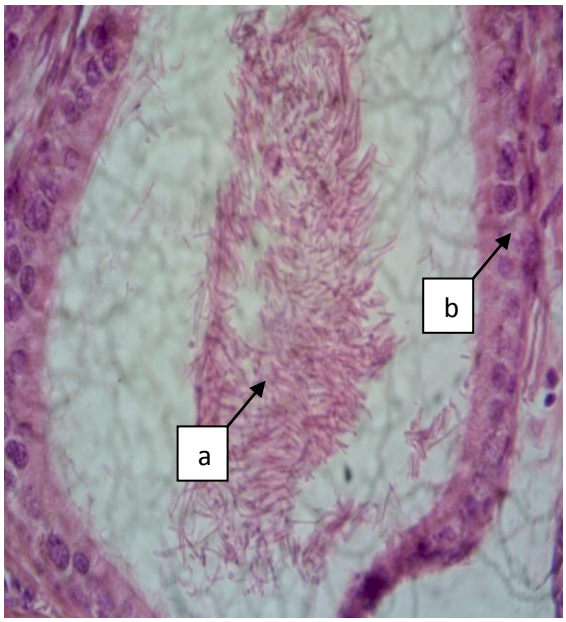

Figure 20. Section of pre-diabetic PrDm3 epididymis (a) moderate clusters of spermatozoa in the lumen (b) normal epithelial lining of the epididymis. [H&E x400]

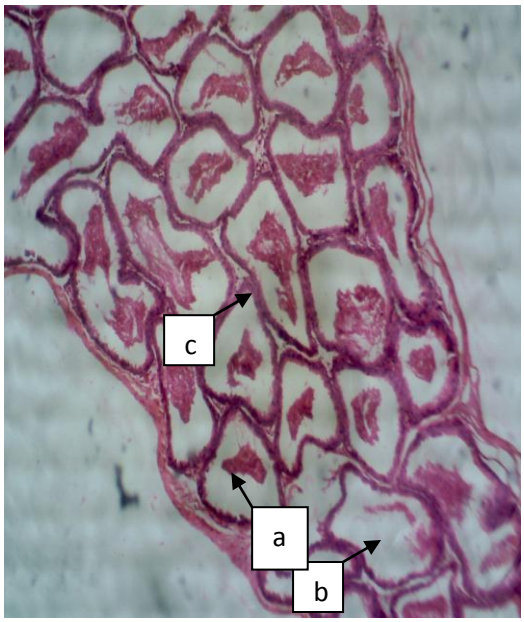

Figure 21. Section of post-diabetic PoDM7 epididymis (a) lumen with moderate spermatozoa (b) areas of hypocellularity in the lumen (c) epithelial lining of the epididymis appears normal [H&E x400]

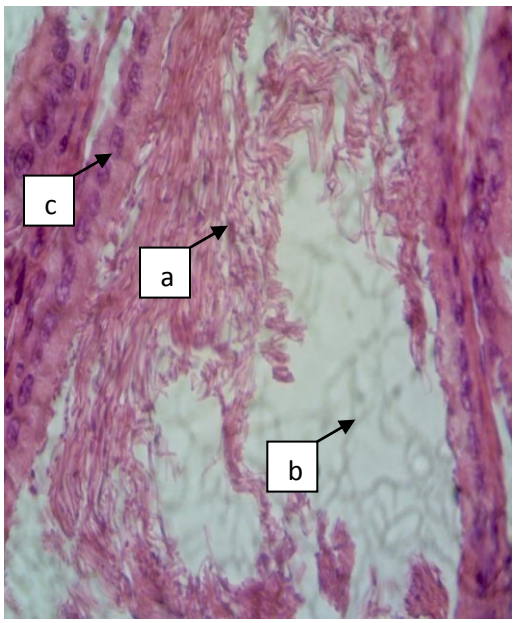

Figure 22. Section of post-diabetic PoDM7 epididymis (a) lumen with scanty spermatozoa (b) areas of hypocellularity in the lumen (c) epithelial lining of the epididymis appears normal [H&E x400]

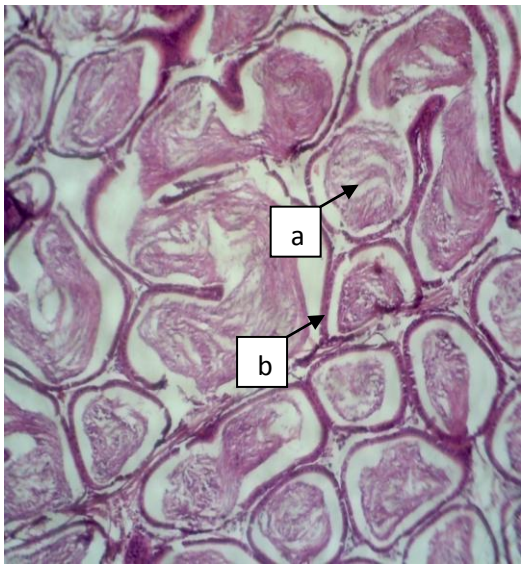

Figure 23. Section of extract only group EX1 epididymis (a) clusters of spermatozoa in the lumen (b) normal epithelial lining of the epididymis. [H&E x40

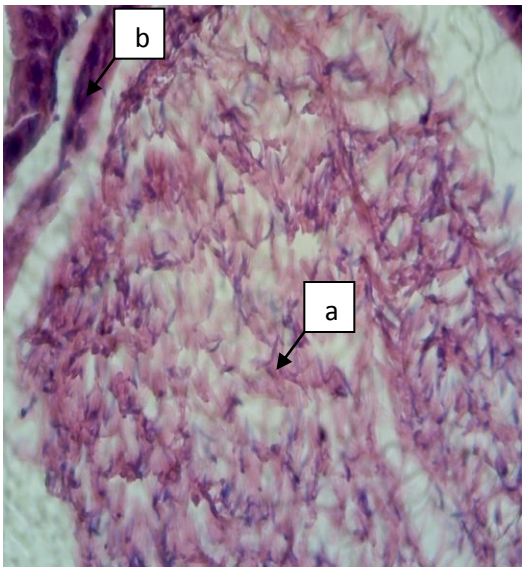

Figure 24. Section of extract only group EX1 epididymis (a) clusters of spermatozoa in the lumen (b) normal epithelial lining of the epididymis. [H&E x400]

4. DISCUSSION

The study revealed altered gonadal hormone pattern with significantly *(p<0.05)* affected serum levels of oestradiol, LH, prolactin and testosterone levels and mildly altered FSH value in the diabetic rats compared to the control. These findings correlate with the histological observations in the testes and epididymis of the diabetic rats which showed the various degrees of cytoarchitectural lesions in these tissues, which were however less severe in the post-diabetic treatment group. These findings are consistent with previous reports of smaller testes due to loss of interstitial tissue, decreased number of Leydig cells in STZ-diabetic rats (Ballester et al., 2004), damaged tubules, hypertrophic interstitial compactments, abnormally distributed Leydig cells (Ricci et al., 2009); significant reduction in spermatogenesis, testosterone, LH and FSH levels (Khaneshi et al., 2013). The possible mechanisms by which diabetes exerts these effects are by reducing the number of spermatogenic cells through altered Sertoli cell function, decreasing the tubular diameter by cell apoptosis and through atrophy of the seminiferous tubules (Richburg et al., 2000; Guneli et al., 2008). Kiyanifard et al. (2010), had already synchronised the relational interplay between serum levels of insulin, LH and FSH as variables determining the functional roles of the interstitial cells (of Leydig) and the Sertoli cells. The experimental group that received leaf-extract of *Newbouldia laevis* as pre-treatment before the induction of diabetes had testicular lumen containing matured spermatozoa from normal spermatogenesis. Thus, normal testicular and epididymal cytoarchitecture was well preserved. Diabetes is associated with increased oxidative stress (ROS), a condition which causes cellular injury by several mechanisms including lipid peroxidation, oxidative damage of proteins and DNAs (Buege and Aust, 1978; Hunt, 1992; Buttke and Sandstrom, 1994; Andy et al., 2009). This can cause significant reduction in sperm parameters and diabetic complications (Buttke and Sandstrom, 1994; Sexton and Jarrow, 1997; Baynes and

Thorpe, 1999; Brownlee, 2001). Indeed, in diabetic state, lipid peroxidation can be induced by protein glycation and glucose oxidation that further leads to the formation of free radicals (Mullarkey et al., 1990). If the generated free radicals are not scavenged by the anti-oxidant system, inbalance is said to ensue (Fang et al., 2002; Roy et al., 2011). Altered gonadal hormone profile had been reported as one of the complications in diabetes mellitus with decreases in FSH, LH and prolactin levels (Hutson et al., 1983; Benitez and Perez Diaz, 1985). The regulation of testicular function in diabetes mellitus would require a substance with anti-oxidant potential as well as ability to modify the serum level of insulin, FSH and LH essentially. Ascorbic acid, selenium and zinc were observed significant components of *Newbouldia laevis* (Ataman et al., 2015). These have been known to aid sperm parameters, protect against DNA damage and thus promote fertility (Wilson, 1973; Song et al., 2006; Glenville, 2008). It is therefore suggestive that these components of *Newbouldia laevis* might have provided protective effects especially in the pre-diabetic treatment group. Furthermore, the findings in the post-diabetic treatment did not quite reveal same protective effects, although palpable tendency to gradual full or partial recovery could be envisaged with time. The observations suggest that the protective mechanisms of the leaf extract of *Newbouldia laevis* might be multi-factorial. Apart from modulating gonadal hormones and preventing free radical damage, its well-known anti-hyperglycaemic action (Owolabi et al., 2011; Osigwe et al., 2015) which had been as well attributed to its hepatic enzyme modulation role (Kolawole and Akanji, 2014) is called to mind.

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