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Full Length Research Paper

Effects of tetracycline on testis and testosterone level in adult male Wistar rats

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Tetracycline is a class of broad spectrum antibiotic drug effective against strains of streptococci, gram negative, bacilli, rickettsias and spirochetes etc. They are used to treat urogenital tract infections (UTIs) and bronchitis. This study accesses the effect of the drug on the testis (connective tissue) and testosterone level in adult male wistar rats. Fifteen adult wistar rats weighing between 165 and 195 g were divided equally into three groups. Group A served as the control that received no treatment, while the experimental groups B and C received 0.02 g/kg b.w (low dose) and 0.04 g/kg b.w (high dose) of tetracycline respectively for fourteen (14) days. The result showed that the staining intensity was reduced in groups B and C as compared to the control group. Dystrophy of the testis connective tissues were noticed, with a corresponding significant diminution in the number of Leydig cells were as well observed in experimental groups B and C. In addition to the aforementioned changes, degeneration of the spermatogonial cells was also observed in group C. Furthermore, hormonal assay shows a great reduction in level of testosterone in groups B and C rats. The overall appearance of the subjects however resembles that of Froelich's syndrome. This result revealed that tetracycline caused a disruption of the normal environment in the testis and a reduction in testosterone level. However, these changes which may result in male infertility might be dose and time dependent.

Key words: Tetracycline, testis, testosterone level, wistar rats.

INTRODUCTION

Testes are the primary reproductive organs or gonads in the male. They are ovoid reproductive and endocrine organs responsible for sperm production and are suspended in the scrotum by scrotal tissues including the non-striated dartos muscle and the spermatic cords.

Average testicular dimensions are 4 to 5 cm in length, 2.5 cm in breadth and 3 cm in anteroposterior diameter; their weight varies from 10.5 to 14 g. The left testis usually lies lower than the right testis. Each testis lies obliquely within the scrotum, its upper pole tilted anterolaterally and the lower posteromedially. The anterior aspect is convex, the posterior nearly straight, with the spermatic cord attached to it.

Anterior, medial and lateral surfaces and both poles are convex, smooth and covered by the visceral layer of the serosal tunica vaginalis, which separates them from the parietal layer and the scrotal tissues external to this. Between these two layers, there is always a very fine film of fluid. This fluid layer can increase on occasions, creating a hydrocele. The posterior aspect is only partly covered by tunica serosa; the epididymis adjoins its lateral part (Gray's anatomy, year).

Each testis is surrounded by a thick capsule of dense connective tissue, the tunica albuginea. The tunica albuginea is thickened on the posterior surface of the testes to form the mediastinum testis, from which fibrous septa penetrate the gland, dividing it into about 250 pyramidal compartments called the testicular lobules. These septa are incomplete, and there is frequent intercommunication between the lobules. Each lobule is occupied by one to four seminiferous tubules enmeshed in a web of loose connective tissue that is rich in blood and lymphatic vessels, nerves, and interstitial cells, also known as Leydig cells. Seminiferous tubules produce male reproductive cells, the spermatozoa, whereas interstitial cells secrete testicular androgens (Basic

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Histology, year).

Testes follow the "path of descent" from high in the posterior fetal abdomen to the inguinal ring and beyond to the inguinal canal and into the scrotum. In most cases (97% full-term and 70% preterm), both testes have descended by birth. In most other cases, only one testis fails to descend (cryptorchidism) and that will probably express itself within a year.

The testes grow in response to the start of spermatogenesis. Size depends on lytic function, sperm production (amount of spermatogenesis present in testis), interstitial fluid, and sertoli cell fluid production. After puberty, the volume of the testes can be increased by over 500% as compared to the pre-pubertal size. Before one reaches puberty testicles are fully descended. Testicular size as a proportion of body weight varies widely (Crane and Scott, 2002).

Testes may shrink or atrophy during hormone replacement therapy or through chemical castration. In all cases, the loss in testes volume corresponds with a loss of spermatogenesis (Shackelford and Goetz, 2007). Testosterone is a steroid hormone from the androgen group and is found in mammals, reptiles, (Cox and John-Alder, 2005) birds (Reed et al., 2006) and other vertebrates.

In mammals, testosterone is primarily secreted in the testicles of males and ovaries of females, although, small amounts are also secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid. In men, testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate as well as, promoting secondary sexual characteristics such as increased muscle, bone mass, and the growth of body hair (Mooradian et al., 1987).

Testosterone effects can be classified as virilizing and anabolic, though, the distinction is somewhat artificial, as many of the effects can be considered both. Anabolic effects include growth of muscle mass and strength, increased bone density and strength, and stimulation of linear growth and bone maturation. Androgenic effects include maturation of the sex organs, particularly, the penis and the formation of the scrotum in the fetus, and after birth (usually at puberty) a deepening of the voice, growth of the beard and axillary hair. Many of these fall into the category of male secondary sex characteristics (Wu et al., 1976).

Tetracyclines have proved to be an extremely important group of drugs because of their wide range of antibiotic activity (Weinstein, 1968; Dowling, 1955; Kucers, 1972). They are a family of compounds, basically polycyclic napthacene carboxamides (Boyne, 1968). The gross structure of oxytetracycline was first determined by Woodward and collaborators (Hochstein et al., 1952) using chemical degradation of the compound and ultraviolet and infrared spectral and analytical techniques.

The appropriate configuration of substituent on the A-ring is critical for antibiotic activity (Stephens et al., 1956). The OH on C_6 (C-ring) is highly labile, responding to both acid and base degradations, this will lead to a relative loss ofactivity, as do any substitutions on the C_1, or C_{12} atoms (Boyne, 1968).

Investigations into the tetracycline molecule (Von et al., 1971) and its biosynthesis (Money and Scott, 1968) have shown that the hydroxyl on C_{12a} is present early in the pathway and required for antibiotic function. In general, it has been demonstrated that structural variations at the C_{11}, C_{12}, C_{12a}, C_6, C_5, and C_4 sites may result in a loss of *in vitro* antibiotic activity of the molecule. No specific protein binding site has been designated on the molecule, but tetracyclines complex with aliphatic/carboxylic acids or urea derivatives in the molecular ratio of two tetracyclines to one (Inouye and Iitaka, 1964).

Furthermore, the sites enclosed in boxes in Figure 1 have been implicated in cation binding, which is of importance to *in vivo* antibiotic activity (Weinberg, 1957; Baker et al., 1966). They are used in the treatment of susceptible bacterial infections of both gram-positive and gram-negative organisms; also indicated for acne, exacerbations of chronic bronchitis, and treatment of gonorrhea and syphilis in patients that are allergic to penicillin and as part of a multidrug regimen for eradication to reduce the risk of duodenal ulcer recurrence (American Academy of Pediatrics Committee on Drugs, 2001).

However, use of tetracyclines during tooth development may cause permanent discoloration of the teeth and enamel, hypoplasia and retardation of skeletal development and bone growth with risk being the greatest for children <4 years and those receiving high doses; use with caution in patients with renal or hepatic impairment (for example, elderly) (Cuddihy, 1994).

Its adverse effects may include; pericarditis, increase in intracranial pressure, bulging fontanels in infants, pseudotumor cerebri, paresthesia, photosensitivity, pruritus, pigmentation of nails, exfoliative dermatitis, diabetes insipidus syndrome, discoloration of teeth and enamel hypoplasia (young children), nausea, diarrhea, vomiting, esophagitis, anorexia, abdominal cramps, antibiotic-associated pseudo membranous colitis, staphylococcal enterocolitis, pancreatitis, thrombophlebitis, hepatotoxicity, acute renal failure, azotemia, renal damage, anaphylaxis, hypersensitivity reactions and candida super infection (Gardner et al., 1995; Wandstrat and Phillips, 1995; Yoshikawa, 1990; Seymour and Heasman, 1995) etc.

MATERIALS AND METHODS

Management

A total number of 15 adult male wistar rats weighing between 165 and 195 g were used in this study, with the

Table 1. Testosterone assay.

Experimental groups	Number of rats	Mean ± S.D
Group A (Control)	5	34.00±0.79
Group B (Low dose)	5	$25.00\pm0.79^{\alpha}$
Group C (High dose)	5	$18.00\pm1.27^{\beta,\pi}$

The means of the groups were compared using T-test at $P < 0.05$. α- there is a statistical significant difference between groups A and B; β- there is a statistical significant difference between groups A and C and π- there is a statistical significant difference between groups B and C.

Table 2. Testes weight per 100 g body weight of the groups.

Experimental groups	Number of rats	Mean ± S.D
Group A (Control)	5	1.10 ± 0.01
Group B (Low dose)	5	$1.12 \pm 0.05^{\gamma}$
Group C (High dose)	5	$1.09 \pm 0.02^{\infty,\mu}$

The means of the groups were compared using T-test at $P < 0.05$. γ - there is no statistical significant difference between group A and group B; ∞ - there is no statistical significant difference between groups A and C and μ - there is a statistical significant difference between groups B and C.

experiment lasting for a period of four weeks. The animals were procured from the breeding stock of the Department of Anatomy, Ladoke Akintola University, Ogbomoso and observed to be all physically healthy.

Upon procurement, the rats were kept at the animal house provided by the Department of Anatomy, Olabisi Onabanjo University and divided into three groups of 5 animals each (groups A, B and C) for a period of two weeks acclimatization and two weeks of induction (orally). They were fed with standard commercial rat pellet. Food, water and air were given *ad libitum.* The animal room was well ventilated within a temperature range of 25 to 27°C.

Drug induction

Tetracycline was obtained from a general pharmacy store in Lagos. The drug was made in China under the trade name LIFLIN® by YANGZHOU PHARMACY COMPANY LTD China, composing of 250 g tetracycline hydrochloride. Rats in groups B and C received tetracycline treatment for 14 days continually at a dosage following reference. Solutions of different doses of the drugs were made by dissolving the content of the capsules in normal saline and administered orally. Group A (control group) animals were not induced; group B (low dose group) were induced orally with- 0.02 g/kg body weight of tetracycline; and group C (high dose) were also induced orally with 0.04 g/kg body weight of tetracycline. The dosages were both dissolved in 1 ml of normal saline. The weight of the tetracycline was obtained using an electronic sensitive balance.

Histology

All the animals were sacrificed by cervical dislocation after 14 days of induction. The thoracic vertebrate was opened under aseptic condition; the same procedure was performed throughout with testes removed (and weighed), prepared for histological section and blood samples taken for biochemical (testosterone) assays. Testes were fixed in 10% formal saline, blocks embedded in paraffin and sections cut at 5 micron which was then stained with H and E and mounted in Canada balsam. Microscopic examination of the sections was then carried out under a light microscope

All results were expressed as Mean ± standard deviation (S.D) for each group. All grouped data were statistically evaluated using SPSS 15.0 software. Hypothesis testing methods included the independent - samples t- test. Statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

From the values obtainable from Table 1 and pattern of data distribution in Figure 2; one would detect an apparent significant effect (at $P<0.05$) caused by the induced drug, upon the testosterone level in the subjects (both in low and high dosages respectively). This is further appreciated in Figures 5, 6 and 7 which showed diminished Leydig cells in the testicles, hence, suggesting diminished level of testosterone in the rats. In addition to that, there is drastic depreciation in spermatogenesis as the leydig cell that helps in

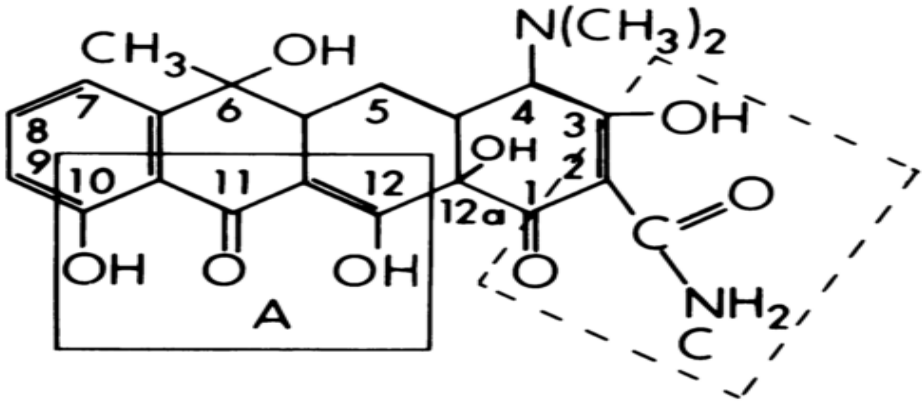

Figure 1. Tetracycline molecule with areas of potential metal cation chelation in boxes.

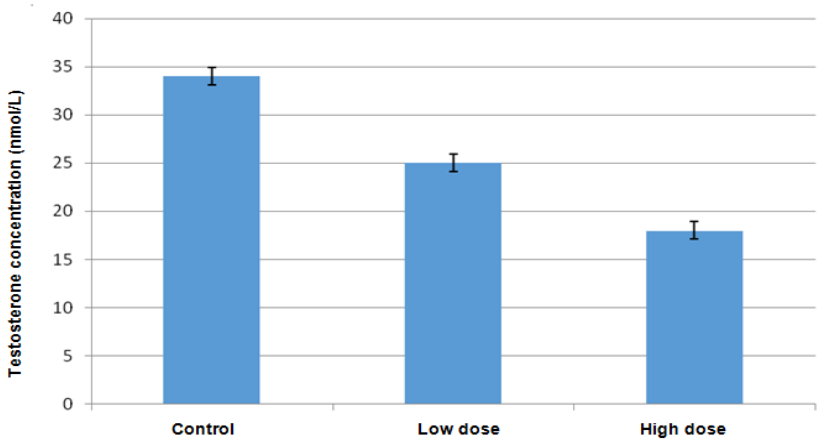

Figure 2. Testosterone assay graph.

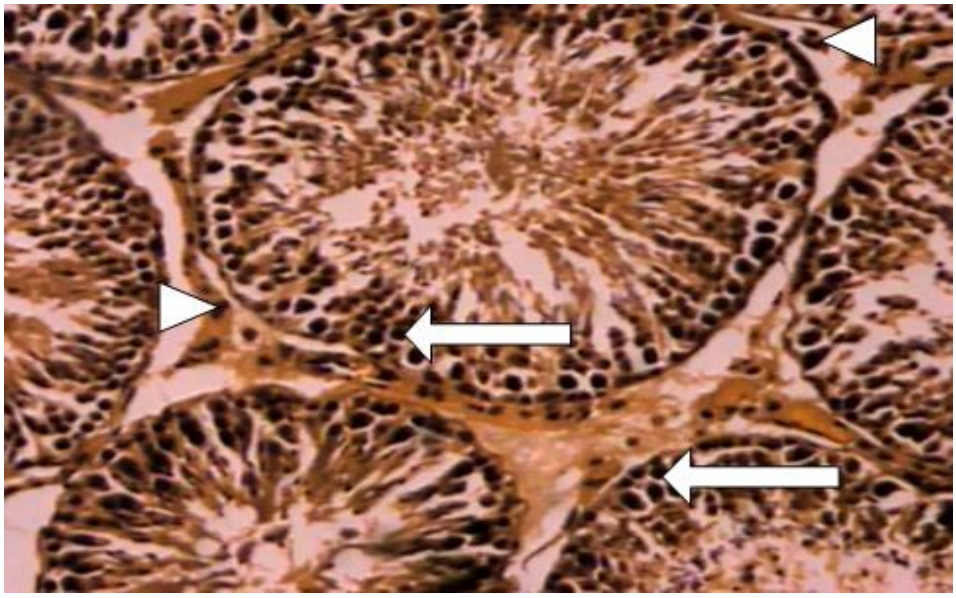

Figure 3. Photomicrograph showing the cross section of H and E staining of a control rat. Labeled are seminiferous tubules (white arrow), arrow head points at the interstitial (Leydig) cells in the connective tissue (Magnification × 1800).

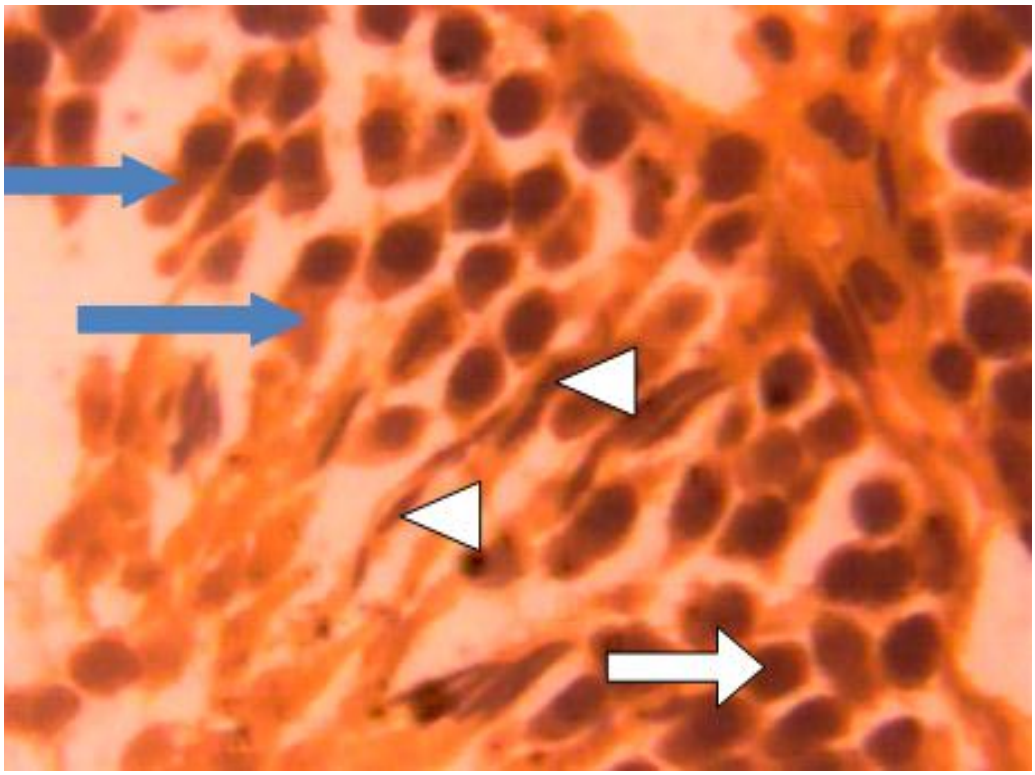

Figure 4. Photomicrograph showing the cross section of H and E staining of the testis of a control rat with the blue arrow pointing at spermatids, white arrow showing spermatogonia cell and the arrowheads pointing at elongated spermatids (Magnification × 1800).

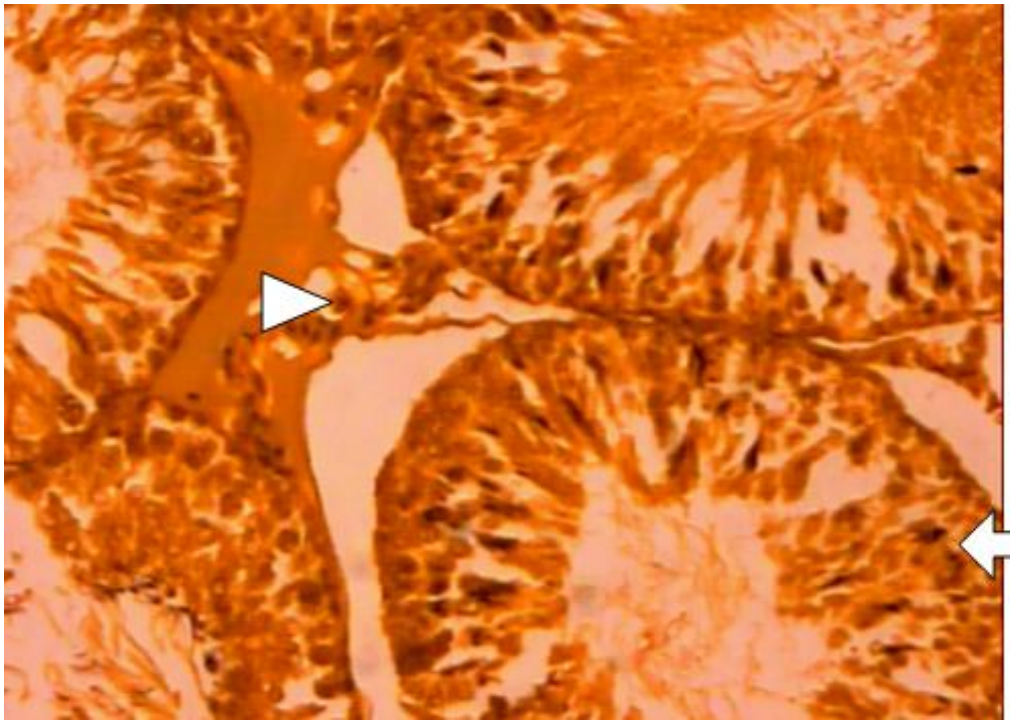

Figure 5. Photomicrograph showing the cross section of H and E staining of the testis of rat induced with low-dose of tetracycline with the nuclei (arrowed) of the spermatogonia cells and the Leydig cells (arrowhead) not well defined compared to the control rat above (Magnification × 1800).

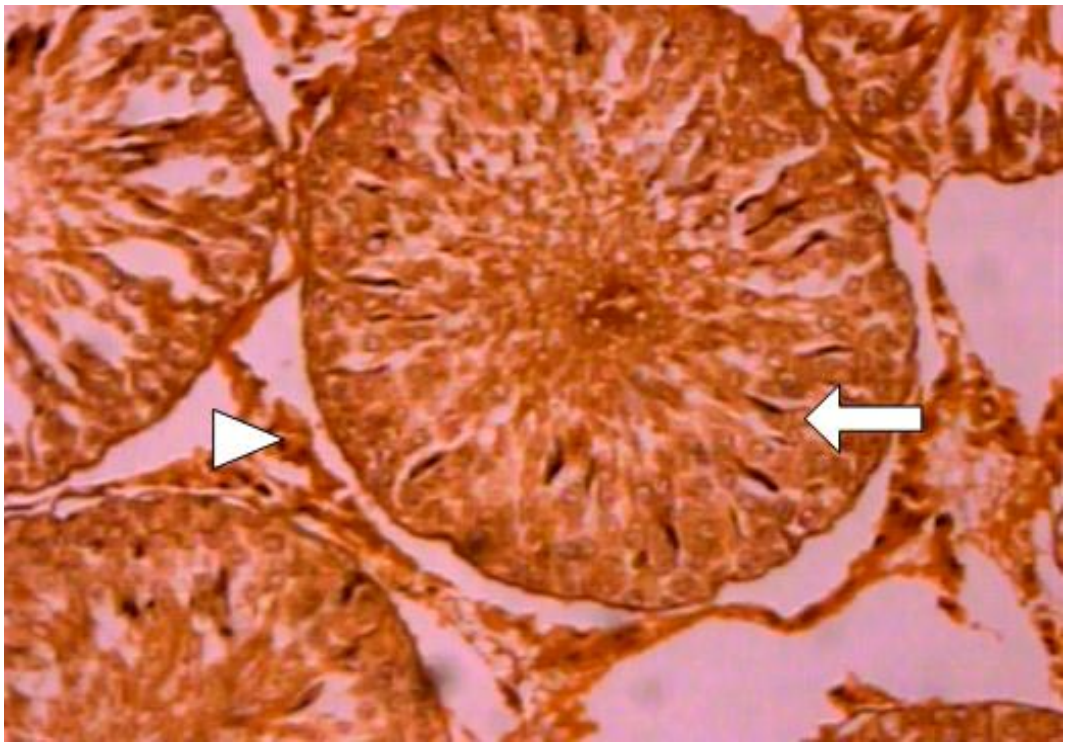

Figure 6. Photomicrograph showing the cross section of H and E staining of the testis of rat induced with high-dose of tetracycline with the nuclei (arrowed) of the spermatogonia cells and the Leydig cells (arrowhead) mostly disappeared (Magnification × 1800).

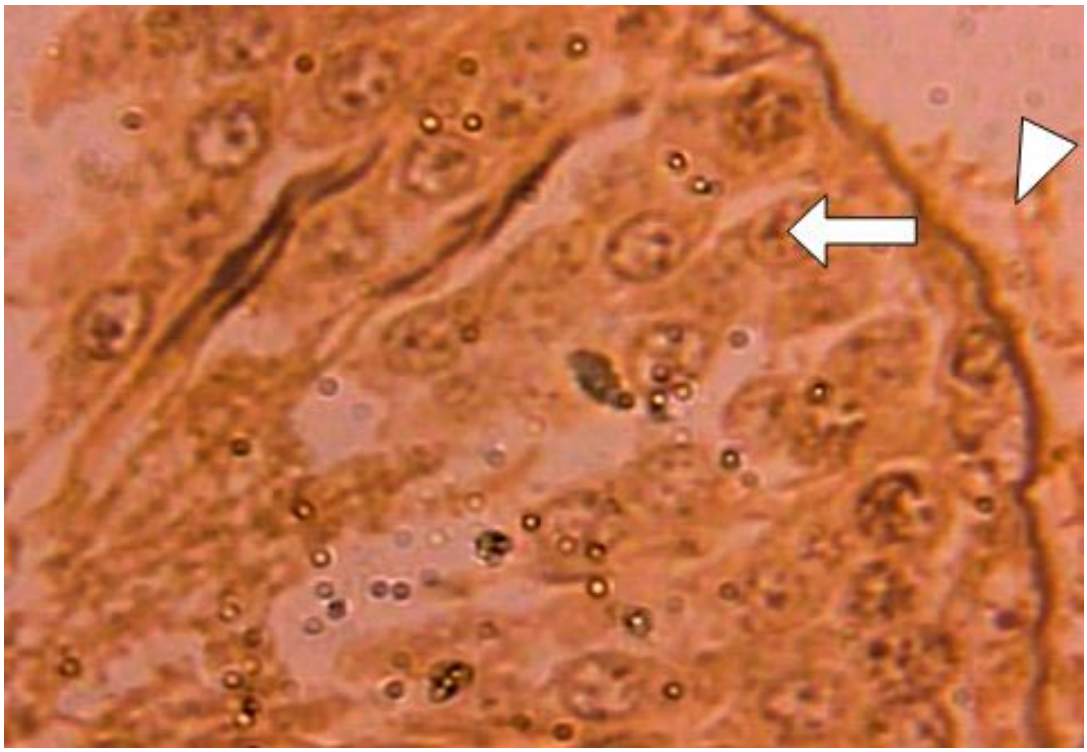

Figure 7. Photomicrograph showing cross section of H and E staining of the testis of a rat treated with high-dose of tetracycline with the nuclei (arrowed) of the spermatogonia cells mostly disappeared and the interstitial connective tissue (arrow head) devoid of the Leydig cells (magnification × 1800).

testosterone production has been affected.

Hence, there is little or no spermatogenesis due to the shortage or lack of testosterone. However, values observed in Table 2 proved no significant effect in the weight of testes in the three groups. These then suggest that the drug has no significant role in the hypertrophy neither does it cause any form of shrinkage of the testis, although, it has been proven to be one of the drugs inducing micro-vesicular steatosis (Hu-Quan, 2006).

Conclusion

Tetracycline causes a drastic reduction in the level of testosterone leading to decreased spermatogenesis; it is steatogenic and also destroys cells of the seminiferous epithelium in wistar rats and hypothetically, in humans as well since wistar rats and man are quite similar analogously in physiological make up.

RECOMMENDATION

We recommend that more awareness should be made by health workers and various organizations concerned on the harmful effects of self-medication of tetracycline on male fertility. Also, medical scientists should make more research work to produce an alternative to the use of tetracycline by male subjects in the society.

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