Deleterious Morphological Changes and Hormonal Profile Induced by Tetracycline on the Male Accessory Organs of Adult Male Wistar Rats

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ABSTRACT---- ***Tetracycline (TET) is a popular broad spectrum antibiotics known to man for treating infections mainly bacterial for many years now. However, due to paucity of knowledge; we aim to study its impact and mechanism on the male reproductive organs of treated Wister rats using histomorphometric and hormonal analysis methods.***

Twenty-one adult male Wister rats with average weight of 135g were divided into three groups. Two treated groups received a daily oral dose of TET at 2.0 mg/kg bwt and 4.0mg /kg bwt respectively via gastric gavage, while equal volume of normal saline was administered to the control group for five weeks. The result showed that TET administration caused a significant reduction in the testosterone level, as well as induction of adverse histo-pathologic changes in the testes. However, there was a significant increase (P<0.05) in the weights of the male accessory sex glands-(prostate and seminal vesicles) as compared to the control animals in a dose- dependent manner. Hence, we concluded that TET administration is deleterious on male accessory organs with mechanisms associated with Testosterone imbalance probably at HPT-axis level.

Keywords--- Tetracycline, Testosterone, Testis, Prostate, Seminal vesicle

1. INTRODUCTION

Tetracycline (TET) is a broad spectrum polypeptide antibiotic produced by the streptomyces genus of antinobacteria medically indicated for treating infections mainly bacterial infections in the hospitals and other health settings. It is commonly used to treat acnes today and played a historical role in stamping out cholera in developed world [1].

TET may be used in the treatment of infection involving the respiratory tract, sinuses, middle ear, urinary tract and intestines. Moreover, it is used in the treatment of gonorrhea, especially in patients allergic to β – lactams, and macrolides. However, their use is also becoming less popular due to wide spread resistance bacterial strain development [2].

In addition, breakdown products of TET when expired are shown to be toxic and can cause Fanconi's syndrome, a potentially fatal disease affecting proximal tubular function in the nephrons of the kidney. Hence, it is advised that expired drugs especially TET should be discarded for obvious reason. It was thought that antibiotics especially TET could impair the effectiveness of many types of hormonal contraception in the body as reflected in some recent research works. Consequently, patients on TET are given barrier contraceptives by their physicians to prevent unwanted pregnancies [3].

Testosterone (T) appears to be major circulating androgens in man and adult mammalian species including rats that are produced mainly by the Leydig cells of the testis (~95%) and the adrenal glands (~5%) [4] [5]. In addition,

stimulation of these androgens will lead to the anatomical (growth) and physiological changes in these organs especially maintaining spermatogenesis and secondary sexual characteristics in the male in general [5].

Studies have shown that T is irreversibly converted to a dihydrotestosterone (DHT), a more potent androgen by 5α-reductase enzyme activity important in testicles, skin, intestines, brain, bones, adipose tissues and other tissues the prostate [6][7].

In addition, most androgenic activities are either through their direct stimulatory increase in intratesticular testosterone level that in turn enhances spermatogenesis and positively influences sperm transport and maturation through its action on epididymis, ductus deferens and seminal vesicles [8], or by their rebound effect through the suppression of both spermatogenesis and gonadotrophins (follicle stimulating hormone and luteinizing hormone) secretion [8][9][10].

Other studies have shown that TET can cause biochemical dysfunction suspected to induce testicular damage to animals [1]. However, due to paucity of knowledge; we aim to study its impact and mechanism on the male reproductive organs of treated Wister rats using histomorphometric and hormonal analysis methods.

2. MATERIALS AND METHODS

2.1. Procurement of Initial Care of Research Animals

Twenty-one (21) adult Wistar rats, approximately 90 days old and average weight of 135g procured from the central animal house, University of Ibadan, Oyo State (Nigeria), were used for this study. The animals were maintained in a well-kept wooden cage in the animal holding laboratory of Department of Anatomy, Olabisi Onabanjo University, Ikenne Campus, at a temperature of 27^0C. They were allowed to acclimatize in the new environment for a period of two weeks during which they were fed with rat pellets and tap water *ad-libitum*. The rat pellet was obtained from Sesco Feed, Nigeria Ltd, Ikenne-Remo, Ogun State. The diet consists of 71% carbohydrate, 18% protein, 7% fat, 4% salt mixture and adequate minerals and animal studies were conducted according to the Institute of Animal Ethics Committee regulations of the Faculty of Basic Medical Sciences of the University.

2.2. Tetracycline Purchase and Animals Treatment

Tetracycline (TET) was obtained from Mex pharmacy in Sagamu, Ogun State, Nigeria. The drug was a product of Yangzhou No. 3 Pharmaceutical Co. Ltd. China, NAFDAC REG NO; 04-1685 under the trade name LIFLIN®. Rats in groups A & B receive tetracycline treatment dissolved in normal saline for five weeks via calibrated oro-gastric tube continually. The normal control animal (Group C) received 1ml of normal saline.

Rats in group A were given an oral dose of TET via a calibrated oro-gastric tube in a low dose of 2mg/kg b.wt of TET., group B receive oral tetracycline treatment at high dose of 4mg/kg b.w of tetracycline in 1ml of normal saline for five weeks. Group C rat serving as the control received the vehicle 1ml normal saline by oral means. The animal weights were monitored on daily basis as the study progressed.

2.3. Sacrificing of the Animals

At the end of the five weeks, all the animals were sacrificed by cervical dislocation after which the testis, and accessory sex glands like prostate gland and seminal vesicle were excised and weighed and fixed immediately and blood were collected in EDTA bottle and centrifuge at 5000g rev/min for 5-mins, then the supernatant were taken using pipette into a plain bottle for testosterone analysis. The tissues were then processed variously for H & E staining, special staining, and hormonal Analysis.

2.4. Tissues Processing for H & E Staining

The testis, prostate gland and seminal vesicle were excised and immediately fixed in 10% formal saline for 24hours at 4^0C for preservation [11]. We transferred the fixed tissues into graded alcohol and then processed for 17.5 hours in an automated Shandon processor after which they passed through a mixture of equal concentration of xylene. Following clearance in xylene the sections were then infiltrated and embedded in molten paraffin wax. However, prior to embedding, the mounted sections were orientated perpendicular to the long axes of the testes before we ensure cutting with the aid of the rotary microtone. These sections were designated as vertical sections Serial sections of 5 m thickness were cut, floated onto clean slides coated with Mayer `s egg albumin for proper cementing of the sections to the slides and were then stained with Haematoxylin and Eosin stains as described in a previous study [12].

2.5 Serum Hormonal Assay.

Using the procedure enclosed with the kit purchased from Amersham International Plc. (UK), serum testosterone concentration was estimated by ELISA technique as previously described by Shittu et al., [12]. The inter- and intra-assay coefficients of variation for the Testosterone were <15%.

2.6 Statistical Analysis

All results were expressed as Mean ± 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 $P < 0.05$.

3. RESULTS

There was a significant ($P<0.05$) decreased in the testicular weight of the treated animals compared to the control animal group. Conversely, there is a significant ($P<0.05$) increase in the weights of the male accessory organs especially with prostate been more pronounced than the seminal vesicle in the treated groups as compared to the control animals in a dose dependent manner as reflected in table 1.

There was a significant reduction in the level of the T both at high and low dose TET compared to the control animal group as reflected in table 2.

Histo-morphometric analysis of the testicular photomicrographs at X40 magnification showed slight reductions in the number of spermatogenic activities in the treated animal (B and C) testes especially in high dose as compared to control A. In addition, more of the seminiferous tubules in the treated (B and C) appeared empty as compared to the control-A testis. However, the shapes of the seminiferous tubules remains intact that is, the histo-architecture of TET treated animals (both at low and high dose) is not different from that of control animal testis.

In addition, at X100 magnification, photomicrographs of control testis showed different stages of spermatogenesis. While the TET-treated animals showed the presence of vacuoles and exfoliation of germ cells especially in the high dose TET-treated group.

4. DISCUSSION

There were no differential changes in the actual raw weights of treated animals except for the significant ($P<0.05$) decreased observed in the relative testicular weights of TET-treated animals compared to the control and similar to other previos study [1]. Conversely, there were significant ($P<0.05$) increased in the relative weights of the accessory organs, with more pronounced effects on the prostate than the seminal vesicle and the difference may be due to lower dose of TET used in the present study (4mg/kg) as compared to 28.6mg/kg in other study [1].

Spermatogenesis is a process that involved a complex hormonal interplay that is androgen and FSH-dependant as found in matured males [12]. Hence, incidental withdrawal of either hormone is also found to have toxic effects on the progression of germ cells through the process of spermatogenesis including sertoli cells functions [13].

Similarly, the resultant reduction of intratesticular androgen (T) in the TET-treated animals induced at either local or central level as found in the present study is responsible for inhibition of spermatogenesis associated with the low sperm quality as reflected in the photomicrographic plates of treated groups (figure II-III).

In addition, the low T-levels in the treated animals could be due to local biochemical dysfunction associated with enzymatic interference in the conversion of cholesterol to testosterone (a substrate essential for testosterone production in the animal system) [14] or increase oxidative stress induced by TET with overall resultant lower relative testicular weight and lower spermatogenic activity in testes of TET-treated animals compared to control as further corroborated by previous study [1].

However, DHT has the tendency to amplify the effects of T through its binding on AR [13] [14], which may also accounted for some degree of residual spermatogenesis and spermiogenesis found within some of the seminiferous tubules of the TET-treated groups in Figure II-III [13][15][16].

In addition, seminal vesicle and prostate are both androgenic organs; the prostate is reported to contribute to the circulating levels of DHT especially in hypertrophic prostate subjects [17][18]. Moreover, both T and DHT bind to the same androgen receptor (AR) with different affinities and different transcriptional activities [19]. Hence, the significant ($P<0.05$) increase in relative weights of both prostate and seminal vesicles in the TET-treated animals may have accounted for increase circulating DHT, which may further resulted to some of the residual spermatogenic activities as seen in some testicular tubules of TET-treated animals as reflected in figure II-III.

It showed that TET-treated effects in the present study may be as a result of a complex hormonal interplay at the level of hypothalamic-pituitary-testicular axis (HPT), while other local factors may play a role such as increase oxidative stress induced by the drug as corroborated by other previous study [1].

5. CONCLUSION

Tetracycline as an antibiotic is beneficial but may have deleterious effect on the male accessory organs especially testicular functions of male rats if abused. However, the effects are reversible if the drug is discontinued as the case may be.

6. REFERENCES

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Table 1: Effects Of Tetracycline On Relative Weights Of Testis And Accessory Sex Gland(g/Kg bwt)

Organs	**Control-A**	**Low Dose TET-B** (2.0 mg/kg bwt)	**High Dose TET-C** (4.0 mg/kg bwt)
Testis	6.50 ± 0.70	6.80 ± 0.30a	5.80 ± 0.50a
Prostate	0.12 ± 0.05	1.40 ± 0.40a	2.00 ± 0.30a
Seminal vesicle	0.80 ± 0.10	0.80 ± 0.30a	0.80 ± 0.40a

Value are recorded as Mean ± S.D, a- significant increase P<0.05

There was a significant increase in weight of prostate and seminal vesicle (P<0.05).

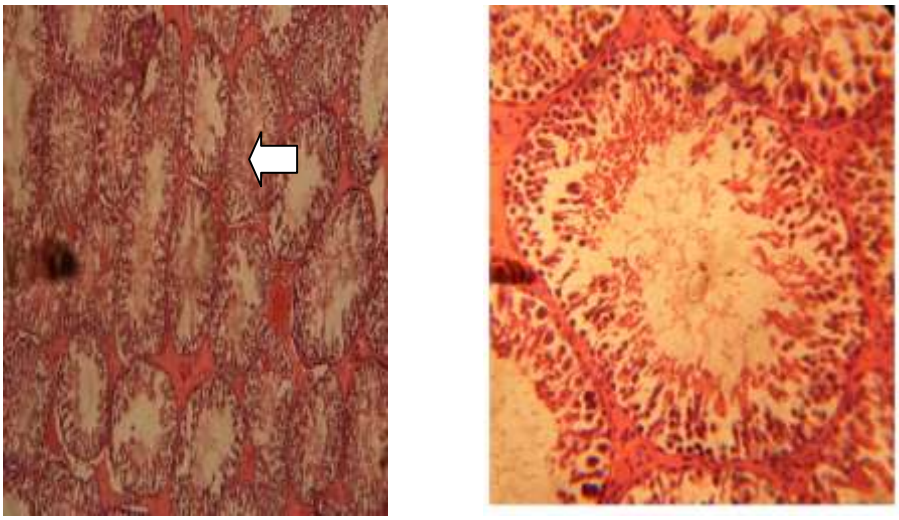
Table 2: Effects Of Tetracycline On Testosterone Level

Serum Assay	**Control-A**	**Low Dose TET-B** (2.0 mg/kg bwt)	**High Dose TET-C** (4.0 mg/kg bwt)
Testosterone level (ng/ml)	31 ± 2.41	22.4 ± 2.07[a]	17.8 ± 1.92[a]

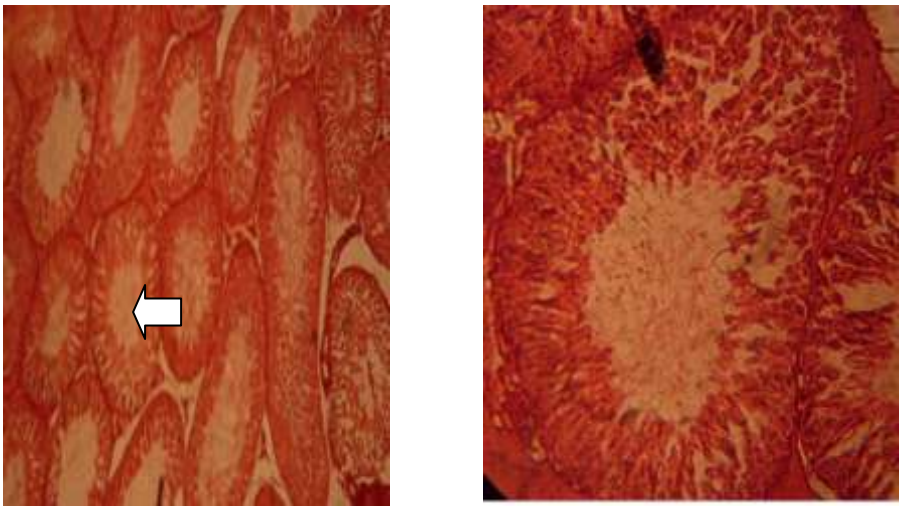
Value are recorded as Mean ± S.D, a- significant increase P<0.05

There was significant (P<0.05) reduction in Testosterone in treated animals compared to control.

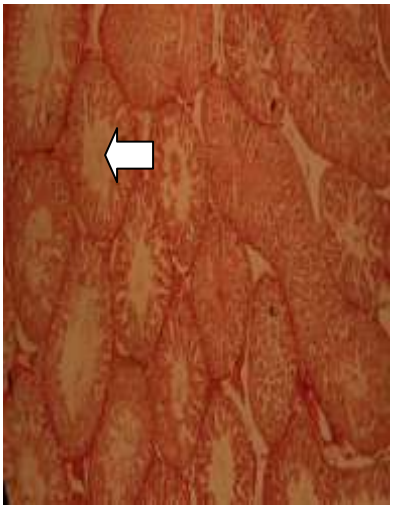
Figure 1: Histology Of The Testis Of Treated Rats At Varying Doses.

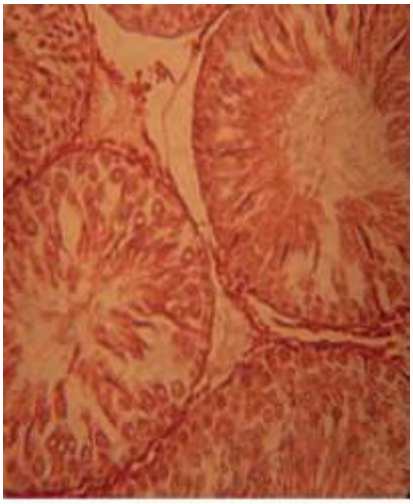


A- Control (Mag. X 40, X 100)
Arrow points to the seminiferous tubules containing the spermatogenic series.



B- Low dose (Mag X40, X100)
C- Arrow points to the seminiferous tubules containing the spermatogenic series.





C- High dose (Mag. X40, X 100)

X40- showed reduction in the number of spermatogenic activities in the treated animal (B and C) compared to control A. At X100 magnification, control group treated testis showed the different stages of spermatogenesis. While the Low Dose and High Dose TET showed the exfoliation of germ cells and appearance of vacuoles.