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SOME EFFECTS OF TETRACYCLINE ADMINISTRATION ON THE REPRODUCTIVE PARAMETERS OF THE TESTES IN ADULT WISTAR RATS

Ajibade A J[1]*, Fakunle P B[1] , Oyewo O O , Ashamu EA , Olagoke A D [1]

[1]Department of Anatomy, Ladoke Akintola University of Technology Ogbomoso, Oyo State, Nigeria

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A B S T R A C T

Some chronic effects of tetracycline that is commonly used for the treatment of urinary tract infections, stomach acne, gonorrhea and claymidia was investigated on the body weight and semen quality of testes of adult male wistar rats.

Twenty- four (24) adult male Wistar rats were separated into four groups each containing 6 wistar rats (n=6). Group A served as the control group while groups B, C, and D served as the experimental groups. Group received distilled water, while group B, C, and D received respectively 10.7, 21.4 and 28.6mg/kg/bw/day of tetracycline orally for 21 days. The rats were sacrificed on the 22nd day and their testes were harvested and fixed in Bouin's fluid for light microscopy.

The body weights of the treated rats did not increase significantly ($P>0.05$) compared with the controls. The sperm quality decreased significantly ($P<0.05$) in a dose-dependent manner in the treated rats compared with the controls. Conclusion: This study concluded that tetracycline has adverse effect on the sperm parameters of the testes in wistar rats.

INTRODUCTION

Tetracycline is a broad spectrum antibiotic employed clinically in the treatment of bacterial infections. It is known to cause or induce testicular damage and a number of biochemical dysfunctions and suspected to induce testicular damage to animals but there is paucity of data on its effect and mechanism of action on the male reproductive system (Farombi *et al*, 2008). Approximately 50% of known causes of primary infertility are attributed to male factor (Yales *et al*, 1989). However, the etiology of male factor infertility is not easy to define. Environmental pollutants as well as modern day social habits such as smoking, consumption of alcohol and drug taking have all been associated with male infertility (Marshburn *et al*, 1989). Antibiotics such as tetracycline are commonly prescribed for a multiple of everyday condition. Not surprisingly, a proportion of male patients attending infertility clinic may have been prescribed antibiotics by their general practitioners to treat their unrelated infection (Corinne *et al*, 1998). Studies in mammals have shown that antibiotics such as tetracycline may have significant adverse effect on spermatogenesis and sperm transport through the reproductive tract as well as affecting sperm function (Amacher *et al*, 1997). Tetracycline have important activities against mycoplasma, rickettsia, chlamydia (trachoma psittacosis, salpingitis, urethritis, pelvic inflammatory) yersinia bruculia (lyme disease) and actinomyces (Viera *et al*, 2007). Tetracycline is also effective in cholera, bronchitis due to heamophilis influenza and in chloroquine-resistant malaria. If available, doxycycline is preferred over tetracycline because of better absorption and lower dosage frequency (Olson *et al*, 2006). For many years now, antibiotic drugs has been found to be of high demand especially in the day to day activities in the treatment of bacterial infections, inflammation infections and even viral infections in some cases (DeRossi *et al*, 2002).

However, this antibiotic drug such as tetracycline has been found out by scientists to have various adverse effects especially when administered in high doses. One of the notable adverse effects of anti biotic drugs is its ability to cause infertility (DeRossi *et al*, 2002). Tetracycline is used to treat many bacterial infections such as urinary tract infections, acne, gonorrhea, claymidia and others (Bhattacharya *et al*, 2003). It is used as a marker of bone growth for biopsies in human and wild life to detect consumption of medicine and vaccine containing baits (Harvey *et al*, 2009). In genetic engineering, tetracycline is used in transcriptional activation (Olson *et al*, 2000). Tetracycline is also used to treat ulcers caused by bacterial infections (Mayton, 2004) and in cell biology as selective agent in cell culture system (William, 2000).

*Corresponding author: +91
Email: adeshinaajibade@yahoo.co.uk

The testes are the male reproductive organ or male sex glands which are located behind the penis in a pouch of skin called the scrotum. They are found to produce and store sperm; they also produce the male hormone-testosterone. The testes are usually two and are egg-shaped, it is called the 'testicle' in the singular form and 'testes' when regarding to both. It is one of the major and most significant organs in the male as it deals with the formation of male gametes (sperm) which in-turn fertilizes female egg. Despite, several reports on the toxicological effect of tetracycline, its adverse effect cannot be elucidated and since reproduction is one of the important characteristics of life, consequently, this study investigated the possible effect of this drug on sperm count, sperm motility and progressivity in relation to tetracycline – induced male infertility.

24 male albino rats of wistar strain, weighing between 150g and 190g were used for this research. The rats were fed daily with normal rat growers marsh purchased from Bova Jay, Ogbomoso, Nigeria and water was given to the animals *ad libitum.* All the rats were carefully assessed, screened and confirmed to be healthy during the period of acclimatization. The animals were treated in accordance with the ''Guide for the care and use of laboratory animals'' prepared by the national academy of sciences and published by the National Institute of Health.

The rats were randomly assigned into four groups. Each group contained six rats (n=6). Group A served as the control group and received distilled water orally while group B, C and D were the experimental groups and they were given tetracycline orally with the aid of an oral canula. Tetracycline capsules were bought from Radomak Pharmacy, Ogbomoso, Nigeria. Group B, C and D orally received low dose of tetracycline (10.7mg/kg/bw), normal dose (21.4mg/kg/bw) and high doses of tetracycline (28.6mg/kg/bw) respectively for 21 consecutive days.

The animals were sacrificed by cervical dislocation on the 22nd day and the epididymis and testis were harvested immediately for semen analysis. The epididymis sperm were collected and viewed under the microscope for analysis of sperm parameters i.e. sperm count, sperm motility and sperm morphology.

The epididymis and testis were then fixed in bouin's fluid for histological procedure following the methods of Carleton (1967).

Semen Analysis and Sperm Count

The semen was collected from the caudal epididymis and was temporarily preserved in 1ml of normal saline. The sperm cells were immobilized using a diluting fluid (sodium bicarbonate). The number of cells in the sample was determined by using the hand tally counter .The progressivity of the sperm cells was viewed under a binocular microscope with objective lense of x10.

Statistical Analysis

All data were presented as Mean ± S.E.M. Statistical analysis of the data of comparisons between the control and treated groups in this study were carried out using analysis of variance and significance of group data difference was tested for using student's t-test. P< 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The body weights of the treated rats did not increase significantly compared with the controls (Table 1 and Figure 1). The effect of tetracycline administered on the microscopic sperm count seemed to the dose- dependent as the sperm count decreased significantly (P< 0.05) as the dose increased, a dose – response relationship as shown in Table 2 and Figure 2. Similarly, the rapid progressive motility grading of the sperm decreased significantly in a dose- dependent manner, while the dead sperm cells increased in a dose-dependent relationship as indicated in Table 3 and Figure 3. Normal spermatozoa in morphology grading also decreased significantly in a dose- dependent manner as shown in Table 4. Conversely, the tail and middle- piece defects increased in a dose –dependent manner (Table4).

Table 1 Mean ± S.E.M of the body weights (g) of the rats before and after treatment

	CONTROL A (n=6)	GROUP B (n=6)	GROUP C (n=6)	GROUP D (n=6)
Initial weight(g)	154.47±1.10	153.03±1.42	155.23±2.30	165.93±9.19
Final weight(g)	163.05±1.98	155.80±1.92	157.83±2.42	168.92±9.07
Percentage of weight gain or loss (%)	+5.26	+1.78	+1.64	+1.77

The above table 1 shows the initial and final body weight of animals used for this research. This table shows an increase in the body weight of all the groups of animals used for the research.

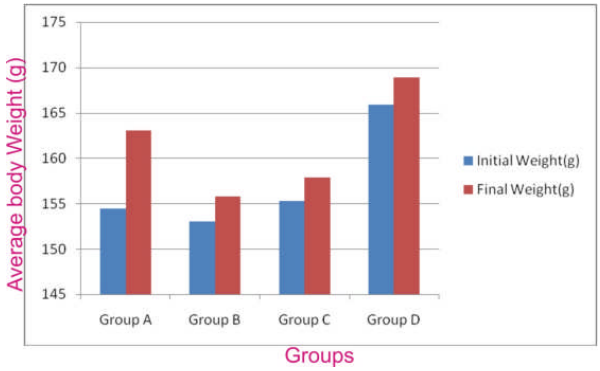

Fig. 1 Graphical representation showing initial and final body weight (g) of wistar rats

Table 2 Mean ± S.E.M of microscopic sperm count (10^6cell/mls) of testes

Groups	Group A (n=6)	Group B (n=6)	Group C (n=6)	Group D (n=6)
Microscopic count (10^6)cell/mls	77.82 ± 2.46^{abc}	41.94 ± 3.69^{ad}	36.42 ± 3.41^{b}	30.82 ± 1.74^{cd}

Mean with the same superscripts at the same row shows significant difference at level of p<0.05

The Table 2 above shows a significant difference (P<0.05) between Group A (control) and the treated groups. There

was also a significant difference (P<0.05) between Group B and Group D.

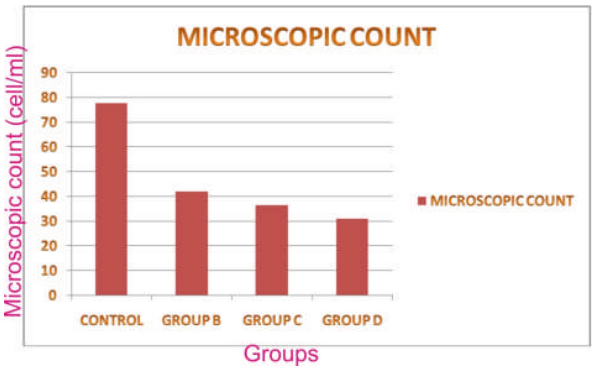

Fig. 2 Graphical representation showing the microscopic sperm count of the testes

Table 3 Shows the mean ± S.E.M of motility grading (10^6cell/mls) of testes

Groups	A (n=6)	B (n=6)	C (n=6)	D (n=6)
Rapid Progressive motility (%)	58.00±3.74[ab]	46.00±5.10	44.00±2.45[a]	40.00±4.47[b]
Slow Progressive motility (%)	28.00±3.74	22.00±2.00	30.00±3.16	28.00±3.74
Non Progressive motility (%)	8.00±3.00	18.00±3.74	16.00±2.45	14.00±2.45
Dead Sperm Cells (%)	6.00±1. 0[a]	14.00±2.45	9.00±1.00	18.00±3.74[a]

Mean with the same superscripts at the same row shows significant difference at level of p<0.05

The Table 3 shows that there was a significant difference (P<0.05) between the sperm motility of Group A (Control) animals and Group C animals. There was also a significant difference between the Group A animals and the Group D animals. There was a significant decrease in the sperm motility grading of tetracycline-treated rats.

Table 4 shows the Mean ± S.E.M of the Morphology Grading of the Testes.

Groups/Grading	A (n=6)	B (n=6)	C (n=6)	D (n=6)
Normal spermatozoa (%)	58.00±3.74[ab]	54.00±2.45[c]	46.00±4.00[a]	40.00±4.47a[bc]
Head defect (%)	32.00±3.74	34.00±2.45	30.00±3.16	28.00±4.47
Middle-piece defect (%)	5.00±0.00[a]	6.00±0.00	15.00±3.16	18.00±2.00[a]
Tail defect (%)	**5.00±0.00[a]**	**6.00±0.00**	**9.00±1.00**	**14.00±4.00[a]**

Mean with the same superscripts at the same row shows significant difference at level of p<0.05

The table above shows a significant difference (decrease) of (P<0.05) between Group A and Group C treated animals. There was a significant difference of (P<0.05) between Group A and Group D. Group B and Group D also show a significant difference of (P<0.05).

DISCUSSION

There was no significant difference in the body weight of animals when the initial and final body weights of control and treated groups were compared, although the weight gain in controls was more prominent than in treated animals (as shown in Table 1 and Fig 1 above). This result agrees with the findings of Farombi et al (2008) in

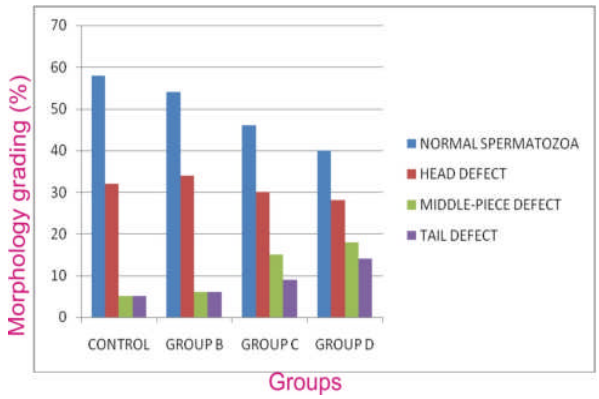

Fig 4 Graphical Representation of Morphology Grading of the Testes

"Tetracycline-induced reproductive toxicity in male rats which showed that tetracycline did not have a significant effect on the body weight of animals but tetracycline administration caused significant decrease in the relative weight of testes, epididymis and seminal vesicles. Eteng et al, (2008) reported that Pefloxacin produced a significant (<0.05) decrease in body weight gain and growth rate in both male and female treated animals

Previous research findings have shown that antibiotics adversely affect male reproductive functions (Timmermans, 1974; Schlegel *et al*., 1991; Hargreaves *et al*., 1998). Administration of Metronidazole and tetracycline significantly (p<0.05) reduced the weight of the epididymis, sperm count, motility and serum testosterone levels (Raji et al, 2007).Sunny et al (2008) had similarly reported that Atrazine exposure has a dose-dependent adverse effect on the testicular and epididymal sperm numbers, motility, viability, morphology, and daily sperm production.). Previous studies by Raji *et al* (2006) and Awobajo *et al* (2006) have revealed that ampicillin, cloxacillin and tetracycline significantly reduced sperm count, motility, viability and morphologically normal spermatozoa and testosterone secretion. Many extrinsic and environmental factors including the increased use of antibiotics have been implicated as potential causes of male infertility (Nelson and Bunge, 1974; Schelegel *et al*., 1991).edit this PLS

Microscopic Count

Approximately 50% of known causes of primary infertility have implicated male factor (Yales et al, 1989).With recent research, it has been proposed that tetracycline intake has adverse effects on sperm production quality (Farombi et al, 2008).Comparing the control group with the treated groups, as shown in table 2 above, there was a significant decrease in the sperm microscopic count in the treated groups (B, C and D) when compared with the control group. There was also a significant difference (p<0.05) between group B and group D. This reduction in microscopic sperm count following tetracycline administration may be due to pathological effects of the drug on the sperm cells that resulted in significantly decreased sperm count in the treated rats (Table 2 and figure 2). The microscopic count of sperm cells decreased in a dose dependent manner. This shows that

increasing dosage of tetracycline caused a decline in microscopic count which may adversely affect spermatogenesis in tetracycline –treated rats.

Sperm Motility Grading

Comparing the control and the treated group, the sperm motility significantly decreased in the treated group. In the rapid progressive motility, there was significant difference ($p<0.05$) between control group and Groups C and D, there was no significant difference between Group B and other groups (Table 3 and Fig. 3). Administration of tetracycline caused a reduction in the epididymal sperm motility, percentage of live spermatozoa, sperm count, and an increase in abnormal sperm morphology, as well as induction of adverse histopathologic changes in the testes (Farombi *et al*, 2008). The reduction of sperm motility of the treated rats is consistent with the result of Farombi et al. (2008) in "Tetracycline-induced reproductive toxicity in male rats. The decrease in the sperm motility of the treated rats may probably be due to toxic effect of the drug on sperm activity. This result signifies that the higher the dose, the lower the sperm motility, which means a slower spermatogenic process.

Sperm Morphology Grading

In table 3 above, the morphology grading of the sperm cells in the treated groups decreased significantly when compared with the control group. There was a significant decrease in the normal spermatozoa between control group and treated group C and D. The findings indicated that high doses of tetracycline adversely affected the sperm morphology in the treated rats. There was a significant difference ($P<0.05$) between groups B and D. The significant decrease in the morphology grading in the treated rats may have resulted from sperm pathology which were evident in some of the parameters of the morphology grading assessed. The decrease in sperm morphology grading might be due to toxic effect of the drug on sperm morphology. Therefore morphology grading in the treated rats decreased with an increase in the dosage which may consequently impair spermatogenesis. Farombi et al(2008), further stated in their results that the administration of tetracycline caused a reduction in the epididymal sperm motility, percentage of live spermatozoa, sperm count, and an increase in abnormal sperm morphology, as well as induction of adverse histopathologic changes in the testes. While Vitamin C and NAC significantly mitigated the toxic effect of tetracycline on sperm parameters, the antioxidants did not improve the adverse histopathologic changes induced by antibiotic.

It also stipulated that Pefloxacin had a significant negative effect on the reproductive function of the animals. Corinnie *et al*, (1998), had reported that tetracycline, chloroquine, erythromycin and Co-trimoxazole considerably impaired sperm movement characteristics and significantly reduced sperm viability *in vitro*. Amoxycillin had no significant effect on either of these parameters over the concentration range tested. Chloroquine had a dual effect, enhancing rapid motility at low concentration, but inhibiting it at higher concentration. Unlike the tetracyclines, co-trimoxazole treatment of rats resulted in a significant impairment of spermatogenesis (Corinnie *et al*, 1998). They further stated in the research that at 100µg/ml erythromycin, there was a significant decline in rapid moving spermatozoa which was enhanced at higher concentrations. This result agrees with the findings of this research that tetracycline cause a decline in motility of sperm as shown on Table 3 and Fig. 3 above.

Research work by Corinne et al, (1998) on sperm function following in vitro' administration of tetracycline at concentration as low as 2.5ug/ml, has shown a significant dose-dependent inhibition in percent rapid-moving spermatozoa, mean velocity (VAP), straight line velocity (VSL) and curvilinear velocity (VCL), but at 50ug/ml tetracycline, all spermatozoa were static.

CONCLUSION

The findings from parameters considered in this research show that tetracycline had adverse effect on the testes of the wistar rats investigated. This implies that the administration of high doses of tetracycline may predispose to infertility in male. Some previous research findings on tetracycline all support the findings of this research. Tetracycline is therefore toxic on the testes and caution should be exercised when administration is required during reproductive age as tetracycline has tendency to raise the risk of infertility. This study recommends against the consumption of tetracycline for a long period of time (especially male individual) in treatment of any form of diseases unless strongly recommended and monitored by medical personnel. We recommend that further studies aim at corroborating these findings should be conducted.

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