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Effect of Tetracycline on Haematological and Reproductive Parameters in Female Albino Rats

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Abstract: Tetracycline has been reported to be a broad-spectrum bacteriostatic autibiotics that inhibit protein synthesis but there is a dearth of information on its effect on blood chemistry and reproduction in female Albino rats. This study was designed to investigate the effect of this drug on haematological and reproductive parameters in female Albino rats. Tetracycline (5 mg kg^{-1} BW) was administered to the rats for 30 days for haematological and histopathological study and 21 days for estrous cycle study. Distilled water (0.5 mL) served as the control. Red Blood Cell (RBC) and Total White Blood Cell (TWBC) counts were determined using haemocytometer. Packed Cell Volume (PCV) was determine by micro-Haematocrit method. Differential leucocyte count was done using Schilling method. Vaginal smears were stained using the Papanicolaou's staining technique. Routine histological technique was used in preparing the histological sections of the ovaries and uteri. Data were analysed using student's t-test at $p<0.05$. Treatment of rats with tetracycline caused non-significant changes in RBC, TWBC, PCV and differential leucocyte values relative to their respective controls. Tetracycline caused significant decrease in the proestrous phase and a significant increase in the metestrous phase of the estrous cycle relative to their respective controls. However, it produced no pathological effect on the ovaries and uteri.

Key words: Tetracycline, Albino rats, red blood cell, ovaries, uteri

INTRODUCTION

Tetracyclines are broad-spectrum bacteriostatic antibiotics that inhibit protein synthesis. They are active against many gram-positive and gram-negative bacteria including anaerobes, rickettsiae, chlamydiae, mycoplasmas and against some protozoa (Katzung *et al*., 2009). They are a group of closely related compounds that, as the name implies, consist of four fused rings with a system of conjugated double bonds (Harvey *et al*., 1997).

Tetracycline has been reported to have antimicrobial effect with iron-chelating property (Grenier *et al*., 2000). Tetracycline has also been reported to probably has a role in reducing the duration and severity of cholera (Bhattacharya, 2003) and its effects on overall mortality is questioned (Parsi, 2001). Tetracycline has been reported to inhibit the replication of DNA on the cell membrane at high doses (Craig and Stitzel, 1982). Tetracycline has also been reported to be among the antibiotics with high teratogenic risk to humans (Friedman *et al*., 1990).

However, due to dearth of information from literature on the effect of tetracycline on haematological and reproductive parameters in female Albino rats, this study, therefore aims at investigating the effect of tetracycline on these aforementioned parameters.

MATERIALS AND METHODS

Experimental animals: Adult female Albino rats weighing between 160 and 180 g bred in the Animal House of Physiology Department, LAUTECH, Ogbomoso were used. They were housed under standard laboratory conditions with a 12 h daylight cycle and had free access to feed and water; they were acclimatized to laboratory conditions for 2 weeks before the commencement of the experiments. All experiments were carried out in compliance with the recommendations of Helsinki's declaration on guiding principles on care and use of animals.

Drug: Tetracycline hydrochloride capsules (Glaxo Smith Pharm. Ltd.) were bought from Jeopat Pharmacy, Ogbomoso, Nigeria.

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About 500 mg of tetracycline was dissolved in 1 L of distilled water to give a concentration of 0.5 mg mL^{-1}. The dosage of tetracycline administered in these study was in accordance with those reported by Isaksen and Mabley (2000).

Experimental design

Haematological and histopathological study: About 10 animals were randomly divided into two groups with each group consisting of 5 rats. The two groups of rats were subjected to the following oral treatments once a day for 30 days: Group I rats received 5 mg kg^{-1} BW of tetracycline; Group II rats received 0.5 mL of distilled water as the control group.

About 24 h (day 31) after the last dosing of the two groups, blood samples were collected and the animals were then euthenised by cervical dislocation. The ovaries and uteri were dissected out, cleaned of fat, blotted with filter papers and then fixed in Bouin's fluid. The tissues were then processed histological.

Estrous cycle study: Vaginal smears of female rats were examined microscopically every day at a constant interval of 9.00-10.00 a.m. for 21 days. The smears were stained using the Papanicolaou's staining technique and the recognized cells were classified into different phases of estrous cycle based on their relative proportions. The durations of the different phases of the estrous were determined. Then, 5 rats showing at least three regular 4-5 day cycles were given 5 mg kg^{-1} BW of tetracycline for 21 days and their vaginal smears were evaluated similarly during this period. In this study, the experimental animals also served as the control, vis-a-vis, the first 21 days served as the control days, while the last 21 days served as the treatment days.

Collection of blood samples: Blood samples were collected through the medical cantus into EDTA bottles for haematological analysis.

Determination of haematological parameters: The Red Blood Cells (RBC) and Total White Blood Cells (TWBC) counts were determined by the improved Neubauer haemocytometer method. The Haemoglobin (Hb) concentration was determined according to Jain (1986), using the Cyanomethaemoglobin method. The Packed Cell Volume (PCV) was determined by the micro-Haematocrit method according to Dacie and Lewis (1991). Schilling method of differential lecukocyte count was used to determine the distribution of the various white blood cells (Mitruka and Rawnsley, 1977). Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were computed according to Jain (1986).

Ovarian and uterine histology: After weighing the ovaries and uteri, they were immediately fixed in Bouin's fluid for 12 h and the Bouin's fixative was washed from the samples with 70% alcohol. The tissues were then cut in slabs of about 0.5 cm transversely and the tissues were dehydrated by passing through different grades of alcohol: 70% alcohol for 2 h, 95% alcohol for 2 h, 100% alcohol for 2 h, 100% alcohol for 2 h and finally 100% alcohol for 2 h. The tissues were then cleared to remove the alcohol, the clearing embedded. Serial sections were cut using rotary microtone at 5 μm. The satisfactory ribbons were picked up from a water bath (50-55°C) with microscope slides that had been coated on one side with egg albumin as an adhesive and the slides were dried in an oven. Each section was deparaffinized in xylene for 1 min before immersed in absolute alcohol for 1 min and later in descending grades of alcohol for about 30 sec each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 min. The slides were rinsed in water, then differentiated in 1% acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 sec and rinsed in water for a few sec, before being immersed in 70%, 90% and twice in absolute alcohol for 30 sec each to dehydrate the preparations. The preparations were cleared of alcohol by dipping them in xylene for 1 min. Each slide was then cleaned, blotted and mounted with DPX and cover slip and examined under the microscope. Photomicrographs were taken at x40, x100 and x400 magnifications.

Statistical analysis: The mean and Standard Error of Mean (SEM) were calculated for all values. Comparisons between the control and the treated groups were done using the student's t-test. Differences were considered statistically significant at $p<0.05$.

RESULTS

Effect on haematological parameters: Treatment of rats for 30 days with tetracycline (5 mg kg^{-1} BW) caused non-significant ($p>0.05$) changes in PCV, Hb, RBC, MCV, MCHC, MCH, TWBC, platelet, neutrophil, lymphocyte, eosinophil and monocyte values relative to their controls (Table 1).

Effect on estrous cycle: Treatment of rats for 21 days with tetracycline (5 mg kg^{-1} BW) caused significant ($p<0.05$) decreae in the proestrous phase and a significant ($p<0.05$) increase in the metestrous phase of the estrous cycle relative to their respective controls but their were non-significant ($p>0.05$) changes in the estrous and diestrous phases of the estrous cycle (Table 2).

Table 1: Effect of 30 days treatment of rats with tetracycline (5 mg kg^{-1} BW) on haematological parameters (n = 5, $p<0.05$)

Parameters	Control	Treatment
PCV (%)	34.40±2.18	36.80±1.16
Hb (g dL^{-1})	11.36±0.73	12.24±0.41
RBC ($\times 10^6$ μL^{-1})	5.59±0.35	6.16±0.26
MCV (FL)	61.61±0.97	59.96±1.43
MCHC (g dL^{-1})	32.98±0.23	32.26±0.21
MCH (Pg)	20.35±0.41	19.77±0.58
TWBC ($\times 10^3$ μL^{-1})	6.75±0.37	7.76±0.89
Platelets ($\times 10^5$ μL^{-1})	1.18±0.04	1.32±0.19
Neutrophils (%)	33.80±8.19	27.80±3.60
Lymphocutes (%)	62.80±8.68	69.40±3.06
Esoinophlis (%)	1.80±0.58	1.60±0.51
Monocytes (%)	1.60±0.68	1.20±0.49

Table 2: Effect of 21 days treatment with tetracycline on estrus cycle (n = 5, $^*p<0.05$)

Phases	Control	Treated
Proetrous	7.80±1.24	3.60±1.03*
Estrous	5.00±1.14	5.00±1.05
Metestous	3.80±0.74	7.20±1.16*
Diestrous	4.40±0.60	5.20±1.40

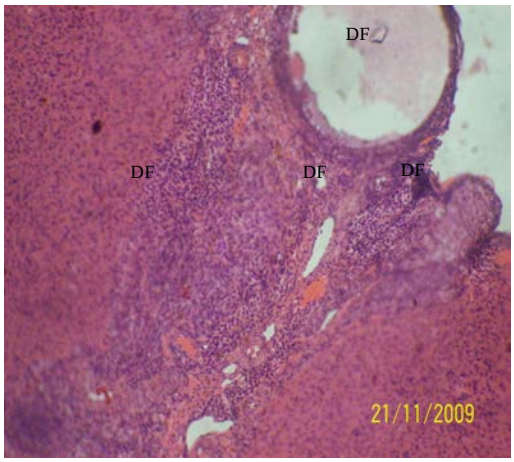

Fig. 1: Effect of 0.5 mL distilled water (control) on the ovary at x100; photomicrograph showing a normal ovary with few Developing Follicles (DF)

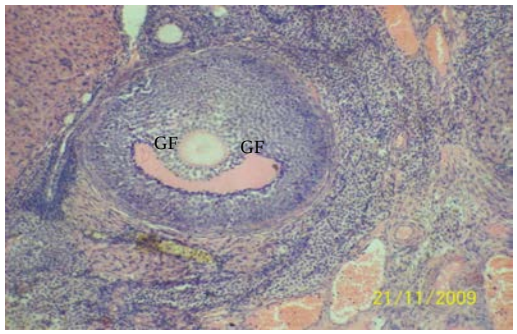

Fig. 2: Effect of 5 mg kg^{-1} BW tetracycline on the ovary at x100; photomicrograph showing an ovary with a matured Graffian Follicle (GF) with no pathologic lesion

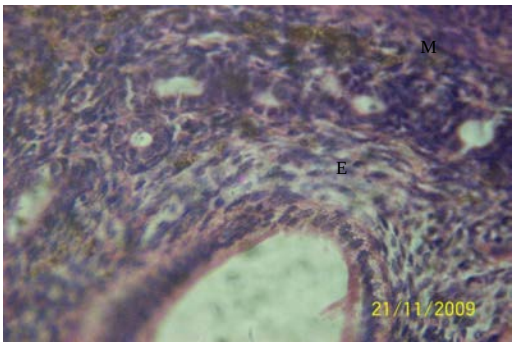

Fig. 3: Effect of 0.5 mL distilled water (control) on the uterus at x100; photomicrograph showing a normal Endometria (E) and Myometrium (M)

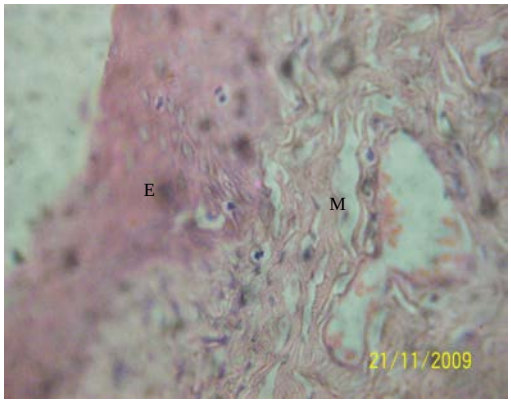

Fig. 4: Effect of 5 mg kg^{-1} BW tetracycline on the uterus at x100; photomicrograph showing a normal Endometrial (E) and Myometrial (M) layers with no pathologic lesion present

Histopathological observations: Rats treated for 30 days with tetracycline (5 mg kg^{-1} BW) presented with normal ovaries with matured graffian follicles with no pathological lesion which is similar to what was observed in the control. Likewise, the uteri of the treated rats presented with normal endometrial and myometrial layers with no visible lesion which is similar to what was observed in the control (Fig. 1-4).

DISCUSSION

The haematological study has shown that treatment of rats with tetracycline caused non-significant changes on the RBC count and indices relating to it (Hb, PCV, MCV, MCH and MCHC) which might indicate that there were no destruction of matured RBC and no change in rate of erythropoiesis. This could also indicate that the drug does not have the potential to stimulate erythropoietin release from the kidneys as well as being

unable to effect changes in the oxygen-carrying capacity of blood and the amount of oxygen delivered to the tissues, since RBC and Hb are known to be very important in transfereing respiratory gases. The drug caused non-significant changes in total WBC, neutrophil, eosinophil and lymphocyte counts which suggest that the immune systems have not been compromised. The drug caused non-significant change in platelet count which could indicate its inability to stimulate hemostasis.

Treatment of rats for 21 days with tetracycline caused significant decrease in the proestrous phase of the estrous cycle and this probably indicates that the maturation of the follicles in the preovulatory phase was hastened leading to maturation of graffian follicles. Similar result was given by Okoko *et al.* (2008) in *Abrus Precartorius* extract treat rats. The drug also caused significant increase in the metestrous phase which probably indicates the availability of matured graffian follicles. Similar result was given by Shibeshi *et al.* (2006) in *Achyranthes aspera* extract treated rats. It should be noted that changes in the duration of the proestrous and metestrous phases of the estrous cycle suggests that the drug caused an imbalance of the ovarian and extraovarian hormones, since it has been reported that imbalance in these hormones lead to irregularity in the ovarian functions and duration of the estrous cycle (Circosta *et al.*, 2001). However, there was non-significant change in the duration of the estrous phase which probably indicates that ovulation was not compromised. Similar results were reported by Oyedeji and Bolarinwa (2010) in *Portulaca oleracea* extracts treated rats.

Photomicrographs of ovaries and uteri of the tetracycline treated rats show matured graffian follicles and normal endometrial as well as myometrial layers with no pathologic lesions present which suggests the non-toxic effect of tetracycline on the ovaries and uteri. Similar results were reported by Oyedeji and Bolarinwa (2010) in *Portulaca oleracea* extracts treated rats.

CONCLUSION

The findings indicate that tetracycline has a pro-fertility effect with no deleterious effect on the blood chemistry and reproductive parameters in female Albino rats.

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