EFFECT OF TETRACYCLINE ON HAEMATOLOGICAL AND REPRODUCTIVE PARAMETERS IN FEMALE ALBINO RATS

OYEDEJI K.O1, BOLARINWA A.F2, AFOLABI O.A.1

1Department of Physiology, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomosho, Nigeria, 2Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria

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ABSTRACT

Tetracycline has been reported to be a broad-spectrum bacteriostatic antibiotic 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 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-haematoctit 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.

Tetracycline caused significant decrease in the proestrus phase and a significant increase in the metestrus phase showing at least three phases of the estrous cycle based on their relative proportions. The durations of the different phases of the estrous were determined. Then, five rats showing at least three regular 4 - 5 day cycles were given 5 mg/kg 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-à-vis, the first 21 days served as the control days, while the last 21 days served as the treatment days.

KEYWORDS: 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, mycoplasm as 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 g and 180 g bred in the Animal House of Physiology Department, LAUTECH, Ogbomosho were used. They were housed under standard laboratory conditions with a 12 hours daylight cycle and had free access to feed and water; they were acclimatized to laboratory conditions for two 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.

Five hundred milligrain (500 mg) of tetracycline was dissolved in 1 litre of distilled water to give a concentration of 0.5 mg/ml. The dosage of tetracycline administered in this study was in accordance with those reported by Isaksen and Mabley (2000).

Experimental Design

Haematological and Histopathological Study

Ten animals were randomly divided into two groups with each group consisting of five 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 BW of tetracycline.

Group II rats received 0.5 ml of distilled water as the control group.

Twenty-four hours (day 21) 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 as described below.

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, five rats showing at least three regular 4 - 5 day cycles were given 5 mg/kg 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-à-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 canthus 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 microhaematocrit method according to Dacie and Lewis (1991). Schilling method of differential leucocyte count was used to determine the distribution of the various white blood cells (Mitruka and Rawnksley, 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 hours 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 hours, 95% alcohol for 2 hours, 100% alcohol for 2 hours, 100% alcohol for 2 hours and finally 100% alcohol for 2 hours. The tissues were then cleared to remove the alcohol, the clearing was done for 6 hours using xylene. The tissues were then infiltrated in molten Paraffin wax for 2 hours in an oven at 57°C, thereafter the tissues were embedded. Serial sections were cut using rotary microtome at 5 microns (5μm). The satisfactory ribbons were picked up from a water bath (50°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 minute before immersed in absolute alcohol for 1 minute and later in descending grades of alcohol for about 30 seconds each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 minutes. The slides were rinsed in water, then differentiated in 1% acid alcohol and then put inside a running tap water to blu...}

Effect on Estrous Cycle
Treatment of rats for 21 days with tetracycline (5 mg/kg BW) caused significant (p<0.05) decrease 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 there were non-significant (p >0.05) changes in the estrous and diestrous phases of the estrous cycle.

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

<table>
<thead>
<tr>
<th>Phases</th>
<th>Control</th>
<th>Treated</th>
</tr>
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<tbody>
<tr>
<td>Proestrous</td>
<td>7.80±1.24</td>
<td>3.60±1.05*</td>
</tr>
<tr>
<td>Estrous</td>
<td>5.00±1.14</td>
<td>5.00±1.05</td>
</tr>
<tr>
<td>Metestrous</td>
<td>3.80±0.74</td>
<td>7.20±1.16*</td>
</tr>
<tr>
<td>Diestrous</td>
<td>4.40±0.60</td>
<td>5.20±1.40</td>
</tr>
</tbody>
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Histopathological Observations
Rats treated for 30 days with tetracycline (5 mg/kg 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.

Plate 1: Effect of 0.5 ml distilled water (control) on the ovary at X100. Photomicrograph showing a normal ovary with few developing follicles (DF).

Plate 2: Effect of 5 mg/kg BW tetracycline on the ovary at X100. Photomicrograph showing an ovary with a matured Graffian follicle (GF) with no pathologic lesion.
Plate 3: Effect of 0.5 ml distilled water (control) on the uterus at X100. Photomicrograph showing a normal endometria (E) and myometrium (M).

Plate 4: Effect of 5mg/kg BW tetracycline on the uterus at X100. Photomicrograph showing a normal endometrial (E) and myometrial (M) layers with no pathologic lesion present.

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 transferring 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 Precatorius extract treated 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 Actinobacillus actinomycetemcomitans extracts 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 (Girosta 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.

It can be concluded that tetracycline probably has pro-fertility effect with no deleterious effect on the blood chemistry and reproductive parameters in female albino rats.

REFERENCES