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Effect of Hydrochlorothiazide (Esidrex) on Reproductive Function in Female Wistar Rats

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Abstract

Aim: This study was designed to investigate the effect of hydrochlorothiazide on reproductive function in female Wistar rats.

Methods: Sixteen female rats (120 – 160 g) were used for the estrous cycle and histopathological studies. Hydrochlorothiazide (0.71 mg/kg) was administered orally on daily basis for 21 and 50 days respectively for the estrous cycle and histological studies. Estrous cycle was carried out using the technique of Marcondes *et al*., histologies of the ovaries and uteri were also carried out. Data were analysed using descriptive statistics and student's t-test at p=0.05.

Results: Treatment of rats for 21 days with hydrochlorothiazide (0.71 mg/kg) produced significant ($p<0.05$) increase and decrease respectively in proestrous and estrous phases of the estrous cycle relative to their respective controls. The histopathological study presented with no deleterious effects on the ovarian and uterine tissues in the rats.

Conclusion: It can therefore be concluded that hydrochlorothiazide probably has anti-fertility effect without deleterious effects on the ovaries and uteri at histological level in female Wistar rats.

Keywords: Hydrochlorothiazide, Proestrous, Estrous, Ovaries, Rats.

INTRODUCTION

Hydrochlorothiazide is a diuretic medication often used to treat high blood pressure and swelling due to fluid build-up [1]. Other uses include diabetes insipidus, renal tubular acidosis, and to decrease the risk of kidney stones in those with high calcium level in the urine. For high blood pressure it is often recommended as a first line treatment [2].

Hydrochlorothiazide has been reported to induce developmental toxicity in mice and rats [3]. Its potential interaction with green tea extract on diuretic activity in rats has been reported [4]. Hydrochlorothiazide has been reported to potentiate contractile activity of mouse cavernosal smooth muscle [5]. Its proconvulsive effect in an *in vitro* rat seizure model [6] as well as pathologic effects of its chronic administration to F344 rats have been reported [7]. Chronic hydrochlorothiazide administration has been reported to increase H+-ATPase B1 subunit abundance in rat kidney [8]. Its effect on reproductive parameters in male Wistar rats has also been reported [9]. However, due to scanty information from literature on the effect of hydrochlorothiazide on reproductive parameters in female rats, this study therefore aims at investigating the effect of this antihypertensive agent on these aforementioned parameters in female rats.

MATERIALS AND METHODS

Experimental Animals

Adult female rats weighing between 120 g – 160 g bred in the Pre-Clinical Animal House of the College of Medicine and Health Sciences, Afe Babalola University were used. They were housed under standard laboratory conditions and had free access to feed and water; they were acclimatized for two weeks to laboratory conditions before the commencement of the experiments. All experiments were carried out in compliance with the recommendations of Afe Babalola University Ethics Committee on guiding principles on care and use of animals.

Drug

Hydrochlorothiazide (esidrex) tablets (Norvatis Pharma Ltd.) were bought from Danax Pharmacy, Ibadan, Nigeria. Hydrochlorothiazide (25 mg) was dissolved in 10 ml of distilled water to give a concentration of 2.5 mg/ml.

The dosage of hydrochlorothiazide used in this study was in accordance with that reported by the manufacturer.

Experimental Design

Study on Estrous Cycle

Six matured female rats showing at least three regular 4 – 5 day cycles were used for this study. Vaginal lavages (smears) were examined microscopically every day at a constant interval of 6.30 – 7.30 a.m. for 21 days before and after treatments with the antihypertensive drug. The smears were classified into one of the phases of the estrous cycle using the Marcondes technique [10]. Vaginal secretion was collected with a plastic pipette filled with 10 µL of normal saline (NaCl 0.9 %) by inserting the tip into the rat's vagina, but not deeply. Vaginal fluid was placed on glass slide. One drop was collected with a clean tip from each rat. Unstained material was observed under a light microscope, without the use of condenser lens, with 10 and 40 x objective lenses. Three types of cells could be recognized: round and nucleated ones are epithelial cells; irregular ones without nucleus are the cornified cells; and the little round ones are the leucocytes. The proportion (preponderance) among them was used for the determination of estrous cycle phases [11, 12]. The duration of the estrous cycle was determined. In this study, the experimental animals also served as the control. The first 21 days served as the control days, while the last 21 days served as the treatment days. Each of the 6 rats for

this estrous cycle study received 0.71 mg/kg of hydrochlorothiazide.

Histopathological Study

In another set of experiment, ten matured female rats divided into two equal groups (five animals per group) received the following treatment of the antihypertensive agent and control orally per day for fifty days as follows:

Group I rats received 0.5 ml/100 g of distilled water as the control group.

Group II rats received 0.71 mg/kg of hydrochlorothiazide.

On the 51st day, all the rats were sacrificed by an overdose of diethyl ether. The ovaries and uteri were dissected out, cleaned of fat and immediately fixed in Bouin's fluid.

Histological preparation of tissues

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, 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 57oC, thereafter the tissues were embedded. Serial sections were cut using rotary microtone at 5 microns (5µm). The satisfactory ribbons were picked up from a water bath (50 -55oC) with microscope slides that had been coated on one slide 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 alcohols for about 30 seconds each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solutions of hematoxylin for about 18 minutes. The slides were rinsed in water, and then differentiated in 1 % acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 seconds and rinsed in water for a few seconds, before being immersed in 70 %, 90 % and twice in absolute alcohol for 30 seconds each to dehydrate the preparations. The preparations were cleared of alcohol by dripping them in xylene for l minute. Each slide was then cleaned, blotted and mounted with DPX and cover slip, and examined under the microscope. Photomicrographs were taken at x40 and x100 magnifications.

Statistical Analysis

The mean and standard error of mean (S.E.M.) were calculated for all values. Comparison between the control and the treated group was done using student's t-test. Differences were considered statistically significant at $p<0.05$.

RESULTS

Treatment of rats for 21 days with hydrochlorothiazide (0.71 mg/kg) produced significant ($p<0.05$) increase and decrease respectively in proestrous and estrous phases of the estrous cycle relative to their respective controls (Fig. 1).

Treatment of rats with hydrochlorothiazide (0.71 mg/kg) for 50 days produced no pathological effects on the ovaries (with few developing follicles and a matured follicle seen), which is similar to what was observed in the control rats (Plates 1 and 2).

Treatment of rats with hydrochlorothiazide (0.71 mg/kg) for 50 days produced no visible lesions on the uteri, which is similar to what was observed in the control rats (Plates 3 and 4)

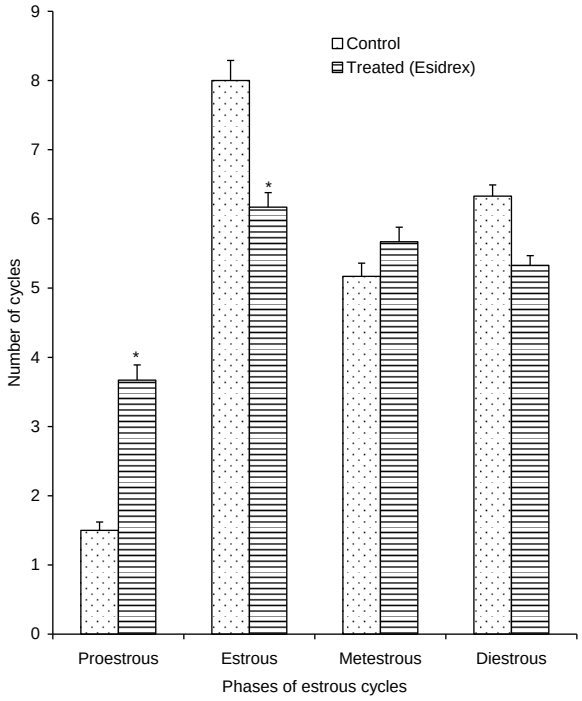

Fig. 1: Effect of 21 days treatment with hydrochlorothiazide (Esidrex) on estrous cycle (n = 6, *p<0.05)

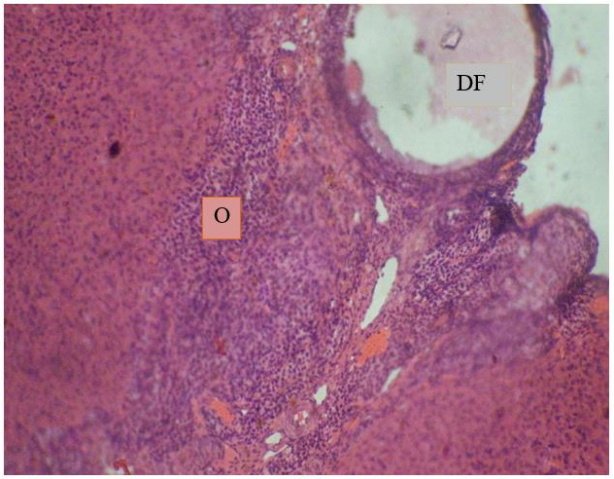

Plate 1: Effect of 0.5 ml distilled water (control) on the ovary at x100.

Photomicrograph showing a normal ovary (O) with a developing follicle (DF).

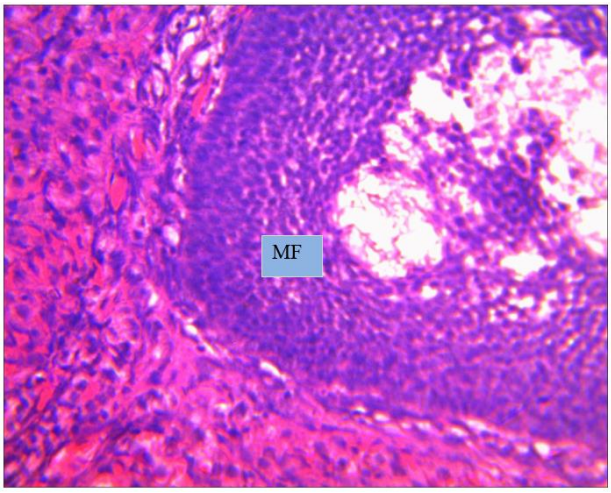

Plate 2: Effect of hydrochlorothiazide (0.71 mg/kg) on the ovary at x100

Photomicrograph showing an ovary with a matured follicle (MF) with no pathologic lesion seen.

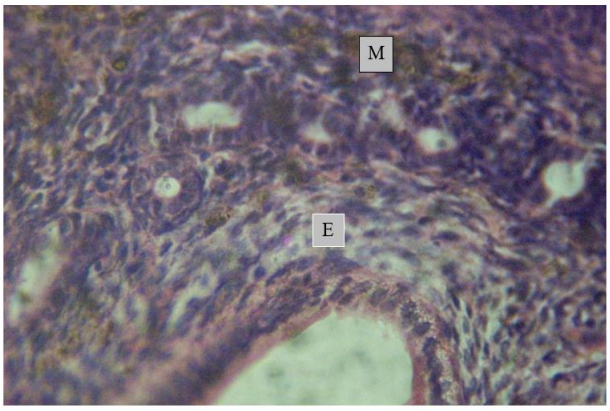

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Plate 3: Effect of 0.5 ml distilled water (control) on the uterus at x100.

Photomicrograph showing normal endometria (E) and myometrium (M).

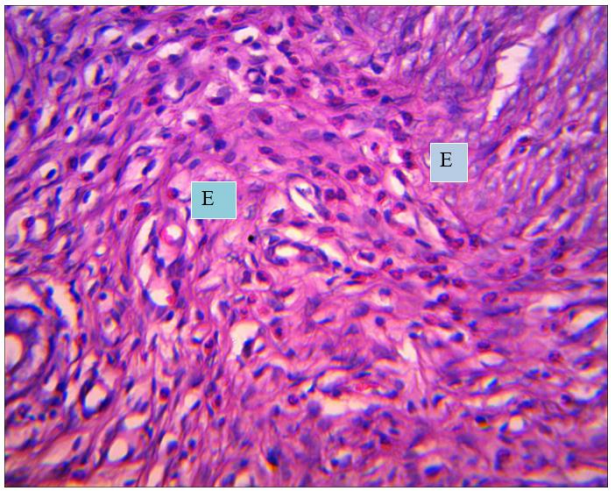

Plate 4: Effect of hydrochlorothiazide (0.71 mg/kg) on the uterus at x100

Photomicrograph showing endometrial (E) layer with no pathologic lesion present.

DISCUSSION

The estrous cycle study revealed that hydrochlorothiazide caused significant changes in the duration of two phases of the estrous cycle. Contrary report was given by [13] in *Portulaca oleracea* extracts treated rats. This suggests that the antihypertensive drug caused imbalances 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 [14].

Treatment of rats with hydrochlorothiazide caused significant increase in proestrous phase of the estrous cycle which probably indicates that the maturation of the follicles in the pre-ovulatory phase was delayed *vis-a-vis* leading to non-maturation of the Graafian follicles. Similar result was reported by [15] in alcohol treated rats. Also hydrochlorothiazide caused significant reduction in estrous phase of the estrous cycle which suggests the non-availability of matured Graafian follicles and would not lead to ovulation. Contrary result was reported by [15] in alcohol treated rats.

The ovarian photomicrographs of the hydrochlorothiazide treated rats presented with no pathologic lesion which suggests the non-toxic effect of the drug on the ovaries at histological level. Similar results were reported by [13] in *Portulaca oleracea* treated rats.

The uterine photomicrographs of the hydrochlorothiazide treated rats showed no pathologic lesion which probably indicates the non-toxic effect of the drug on the uteri at histological level. Similar results were reported by [16] in *Allium sativum* extract treated rats.

It can therefore be concluded that hydrochlorothiazide probably has anti-fertility effect without deleterious effects on the ovaries and uteri at histological level in female Wistar rats. However, the effect of hydrochlorothiazide on human reproductive function is unknown; nevertheless, considering these findings in animal model, it is recommended that women should be cautious about taking this antihypertensive agent because of its likely anti-fertility effect.

Conflict of Interest

We vehemently declare that there is no conflict of interests in this research work.

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