Oyedeji K.O. et al /J. Pharm. Sci. & Res. Vol. 11(12), 2019, 3745-3748

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Effect of Atenolol (Beta Blocker) on Reproductive Function in Female Wistar Rats

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Abstract

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

Methods: Sixteen female rats (120 – 160 g) were used for the estrous cycle and histopathological studies. Atenolol (1.43 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 atenolol (1.43 mg/kg) produced significant ($p<0.05$) increase and decrease in the proestrous and diestrous phases of estrous cycle respectively with no significant ($p>0.05$) changes in the estrous and metestrous phases of the estrous cycle relative to their respective controls, as well as induced no pathological effects on the ovarian and uterine tissues in the rats.

Conclusion: It can therefore be concluded that atenolol probably have both anti-fertility and pro-fertility effect without deleterious effect at histological level in female Wistar rats.

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

INTRODUCTION

Atenolol has been widely used in humans during the last 3–4 decades to treat various cardiovascular pathologies and has been classically considered a safe drug without significant side effects [1].

Atenolol is a cardioselective beta adrenoreceptor blocking agent without intrinsic sympathomimetic activity. It has a markedly greater effect on cardiac than bronchial or vascular adrenoreceptors [2, 3] and reduces blood pressure mainly by reducing cardiac output, in contrast to the nonselective 3-blockers that reduce blood pressure mainly by decreasing the peripheral vascular resistance [4]. Atenolol mainly causes its hypotensive effect by decreasing heart rate and cardiac contractility in both humans and experimental animals [5].

Atenolol has been reported to have anxiolytic effect in human subjects [6] as well as having antidepressant effect in mice [7]. Atenolol has been reported to cause decreased membrane fatty acid unsaturation and oxidative stress in heart and skeletal muscle mitochondria and improves immunity and behavior [8] in mice. It has also been reported that pretreatment of rats with atenolol ameliorates the acute hemodynamic changes and prevented ZD6126-induced increases in both troponin T and myocardial necrosis [9].

However, due to dearth of information from literature on the effect of atenolol on reproductive parameter in female rats, this study therefore aims at investigating the effect of this antihypertensive agent on this parameter.

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

Atenolol tablets (Bristol Laboratories Ltd) were bought from Danax Pharmacy, Ibadan, Nigeria.

Atenolol (100 mg) was dissolved in 10 ml of distilled water to give a concentration of 10.0 mg/ml.

The dosage of atenolol 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 9.30 – 10.30 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. A different glass slide was used for each cage of animals. 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 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 1.43 mg/kg of atenolol.

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 hypertensive 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 1.43 mg/kg of atenolol.
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 57^{o}C, thereafter the tissues were embedded. Serial sections were cut using rotary microtone at 5 microns (5 μm). The satisfactory ribbons were picked up from a water bath (50 -55^{o}C) 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 7 0%, 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, x100 and x400 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 atenolol (1.43 mg/kg) produced a significant ($p<0.05$) increase and decrease in the proestrous and diestrous phases of estrous cycle respectively, but induced no significant ($p>0.05$) changes in the estrous and metestrous phases of the estrous cycle relative to their respective controls (Fig. 1).
Treatment of rats with atenolol (1.43 mg/kg) for 50 days produced no pathological effects on the ovaries, except follicles that are seen at different developmental follicular stages, which is similar to what was observed in the control rats (Plates 1 and 2).
Treatment of rats with atenolol (1.43 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).

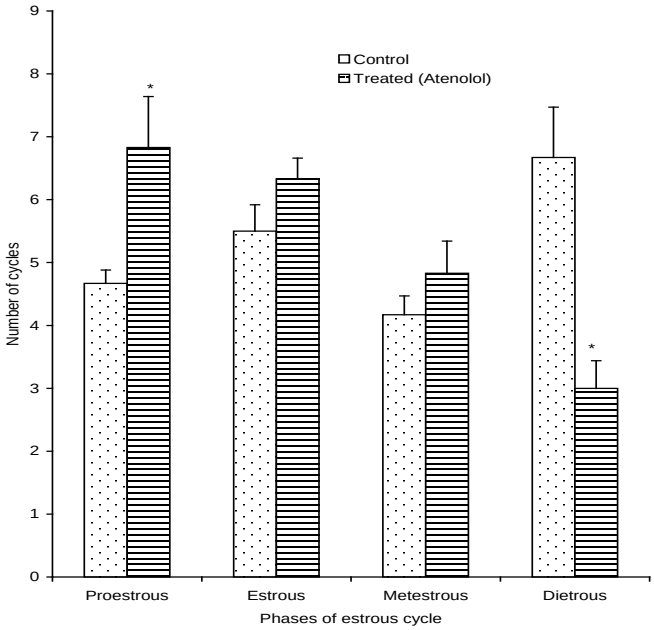

Fig. 1: Effect of 21 days treatment with atenolol on estrous cycle (n = 6, *$p<0.05$).

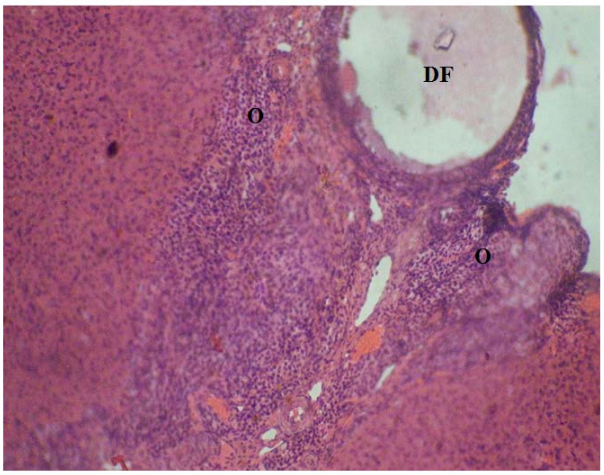

Plate 1: Effect of 0.5ml distilled water (control) on the ovary at x100.
Photomicrograph showing a normal ovary (O) with a developing follicle (DF).

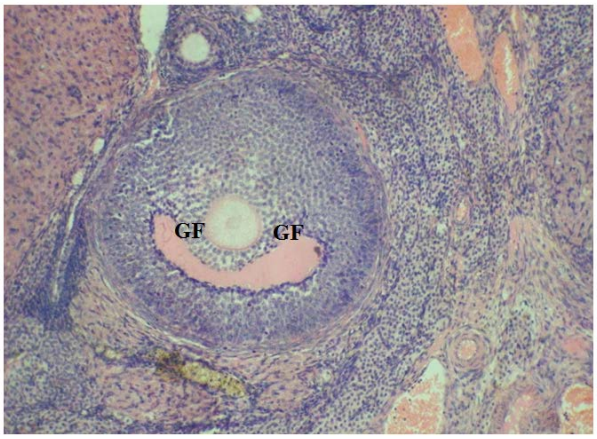

Plate 2: Effect of atenolol (1.43 mg/kg) on the ovary at x100
Photomicrograph showing an ovary with a matured Graffian follicle (GF) with no pathologic lesions.

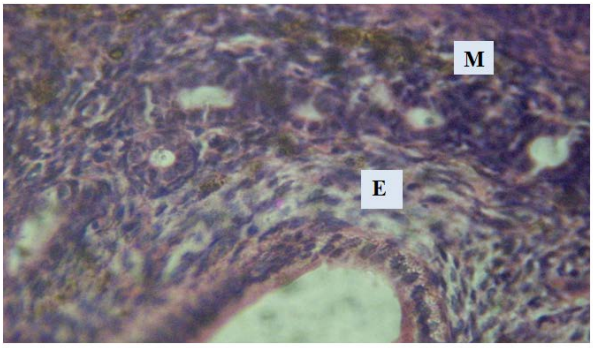

Plate 3: Effect of 0.5ml distilled water (control) on the uterus at x100.
Photomicrograph showing normal endometria (E) and myometrium (M).

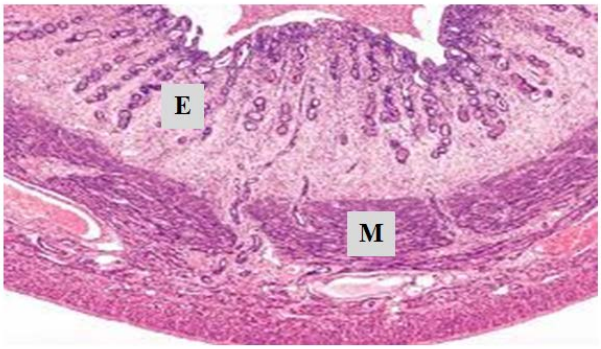

Plate 4: Effect of atenolol (1.43 mg/kg) on the uterus at x100
Photomicrograph showing normal endometrial (E) and myometrial (M) layers with no pathologic lesion present.

DISCUSSION

The estrous cycle study revealed that atenolol caused significant changes in the duration of 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 atenolol caused significant increase in proestrous phase of the estrous cycle which probably indicates that the maturation of the follicles in the preovulatory 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, atenolol caused significant reduction in diestrous phase of the estrous cycle which probably indicates an increase in the frequency of ovulation. Similar result was reported by [16] in wood ash extract treated *Mus musculus*.

The ovarian photomicrographs of the control and atenolol treated rats showed developing follicles and Graffian follicle respectively with no pathologic lesions present which suggests the non-toxic effect of the drug on the ovaries at histologic level. Similar results were reported by [13] in *Portulaca oleracea* treated rats.

The uterine photomicrographs of the control and atenolol treated rats showed normal endometrial and myometrial layers without pathologic lesions which probably indicate the non-toxic effect of the drug on the uteri at histologic level. Similar results were reported by [17] in *Allium sativum* extract treated rats.

In conclusion, this study has shown that atenolol probably has anti-fertility and pro-fertility effect without deleterious effect on the ovaries and uteri at histological level in female rats.

However, the effect of this antihypertensive agent on human reproductive function is unknown; nevertheless, considering these findings in animal model, it is recommended that women with infertility problems could take this antihypertensive drug (atenolol) with caution for infertility therapeutic purpose.

Conflict Of Interest

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

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