Oyedeji K.O. et al /J. Pharm. Sci. & Res. Vol. 13(9), 2021, 586-589

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

Effect of Nifedipine on Reproductive Function in Female Wistar Rats

Oyedeji K.O.*[1], Akaduchieme C. H.[1], Zachariah Richard[2]

[1]Department of Physiology, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Nigeria
[2]Department of Anatomy, College of Medical Sciences, Ahmadu Bello University, Zaria.

* sinaoyedeji@yahoo.com

Abstract

Aim: This study was designed to investigate the effect of nifedipine on reproductive function in female Wistar rats.
Methods: Sixteen female rats (120 – 160 g) were used for the estrous cycle and histopathological studies. Nifedipine (0.57 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 nifedipine (0.57 mg/kg) produced a significant ($p<0.05$) reduction in the estrous phase, but caused insignificant ($p>0.05$) changes in the proestrous, metestrous and diestrous 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 nifedipine probably has anti-fertility effect without deleterious effects on the ovaries and uteri at histological level in female Wistar rats.

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

INTRODUCTION

Nifedipine is a calcium channel blocker medication used to manage angina, high blood pressure, Raynaud's phenomenon, and premature labour. It is one of the treatments of choice for Prinzmetal angina. It may be used to treat severe high blood pressure in pregnancy. Its use in preterm labor may allow more time for steroids to improve the baby's lung function and provide time for transfer of the mother to a well qualified medical facility before delivery. It is a calcium channel blocker of the dihydropyridine type [1].

Nifedipine has been reported to have effect on carbohydrate metabolism [2]. Its effect on the contractile responses of isolated rat bladder has been reported [3]. Its effect on the contractile responses of the longitudinal and circular muscle of the isolated guinea-pig ileum to various agonists has been reported [4]. Its effects on normotensive rat placental blood flow, placental weight and fetal weight has been reported [5]. Nifedipine has been reported to prevent renal injury in rats with chronic nitric oxide inhibition [6]. Its effect on reproductive function in male rats has also been reported [7]. However, due to scanty information from literature on the effect of nifedipine 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

Nifedipine tablets (Pharmabase Nigeria Ltd.) were bought from Danax Pharmacy, Ibadan, Nigeria.

Nifedipine (20 mg) was dissolved in 10 ml of distilled water to give a concentration of 2.0 mg/ml.

The dosage of nifedipine 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 agent. The smears were classified into one of the phases of the estrous cycle using the Marcondes technique [8].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 [9, 10]. 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 0.57 mg/kg of nifedipine.

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 0.57 mg/kg of nifedipine.

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 nifedipine (0.57 mg/kg) produced a significant ($p<0.05$) decrease in the estrous phase, but caused insignificant ($p>0.05$) changes in the proestrous, metestrous and diestrous phases of the estrous cycle relative to their respective controls (Fig. 1).

Treatment of rats with nifedipine (0.57 mg/kg) for 50 days produced no pathological effect on the ovaries, which is similar to what was observed in the control rats (Plates 1 and 2).

Treatment of rats with nifedipine (0.57 mg/kg) for 50 days produced no visible lesion on the uteri, which is similar to what was observed in the control rats (Plates 3 and 4).

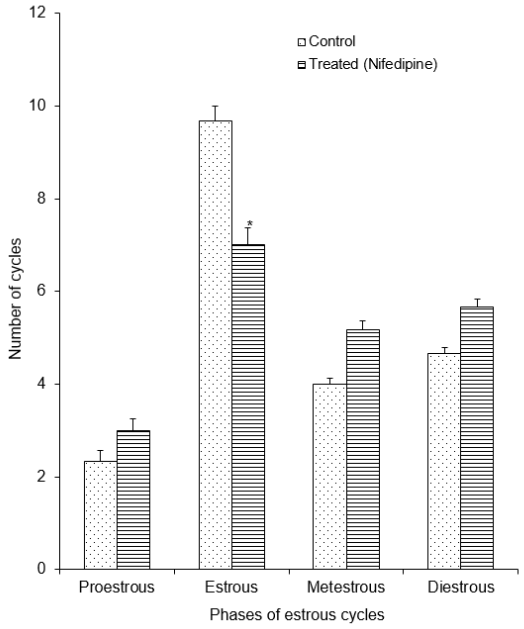

Fig. 1: Effect of 21 days treatment with nifedipine on estrous cycle (n = 6, *p<0.05)

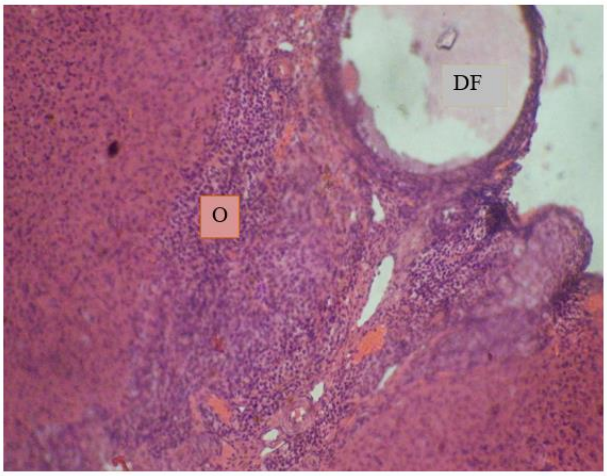

Plate 1: Effect of 0.5 ml distilled water (control) on the ovary at x100. Photomicrograph showing a normal ovary (O) with a developing follicle (DF).

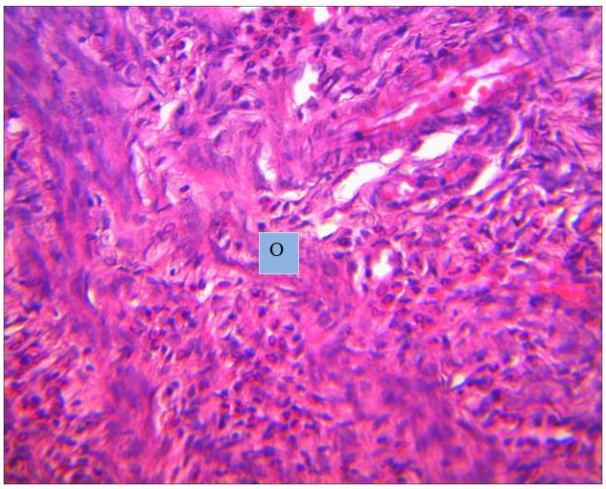

Plate 2: Effect of nifedipine (0.57 mg/kg) on the ovary at x100
Photomicrograph showing an ovary (O) with no pathologic lesion seen.

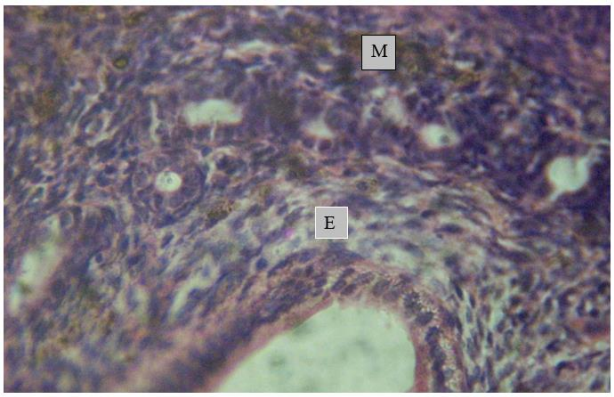

Plate 3: Effect of 0.5 ml distilled water (control) on the uterus at x100.
Photomicrograph showing normal endometria (E) and myometrium (M).

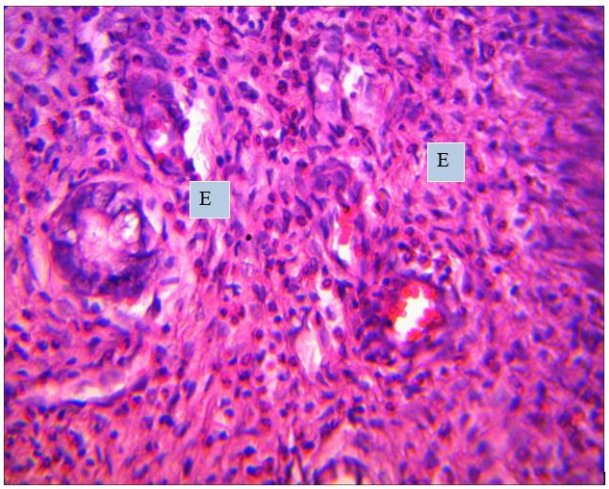

Plate 4: Effect of nifedipine (0.57 mg/kg) on the uterus at x100
Photomicrograph showing endometrial (E) layer with no pathologic lesion seen.

DISCUSSION

The estrous cycle study revealed that nifedipine caused a significant change in the duration of a phase of the estrous cycle. Contrary report was given by [11] 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 [12].

Treatment of rats with nifedipine 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 [13] in alcohol treated rats.

The ovarian photomicrographs of the nifedipine 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 [11] in *Portulaca oleracea* treated rats.

The uterine photomicrographs of the nifedipine treated rats revealed no pathologic lesion, which probably indicates the non-toxic effect of the drug on the uteri at histological level. Similar results were reported by [14] in *Allium sativum* extract treated rats.

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

REFERENCES

[1] The American Society of Health-System Pharmacists. Archived from the original on 8 August 2018. Retrieved 17 September 2019.
[2] Hörl WH, Haag M, Riegel W, Heidland A. Effect of nifedipine on carbohydrate metabolism in the rat. Res in Exptal Med 1987; 187: 203–210.
[3] Zar MA, Iravani MM, Luheshi GN. Effect of nifedipine on the contractile responses of the isolated rat bladder. The J Urol 1990; 143 (4): 835- 839.
[4] Grbovic L, Radmanovic B. The effect of nifedipine on the contractile responses of the longitudinal and circular muscle of the isolated guinea-pig ileum to the various agonists. Archives int de pharmacodyn et de therap ID 33914433.
[5] Furuhashi N, Tsujiei M, Kimura H, Yajima A. Effect of nifedipine on normotensive rat placental blood flow, placental weight and fetal weight. Gynecol Obstet Invest 1991; 32:1–3.
[6] Miriam OR, Edson A, Marcelo NM, Gilberto DN, Roberto Z. Nifedipine prevents renal injury in rats with chronic nitric oxide inhibition. Hypertension 1995; 26:150–155.
[7] Iranloye BO , Morakinyo AO , Uwah J, Daramola OA. Effect of nifedipine on reproductive functions in male rats. Nig Quart J Hospit Med 2009; 19:3.
[8] Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: some helpful considerations. Braz J Bio 2002; 62 (4a): 609 – 614.
[9] Long JA, Evans HM. The estrous cycle in the rat and its associated phenomena. Memo Uni Califor 1922; 6: 1-148.
[10] Mandl AM. The phases of the oestrous cycle in the adult white rat. J Exptal Bio 1951; 28: 576-584.

[11] Oyedeji KO, Bolarinwa AF. Effects of extracts of *Portulaca oleracea* on reproductive functions in female albino rats. Afr J Biomed Res 2010; 13: 213-218.
[12] Circosta C, Sanogo R, Occhiuto F. Effects of *Calotropis proceraon* estrous cycle and on estrogenic functionality in rats. Farmaco 2001; 56: 373-378.
[13] Oyedeji KO, Bolarinwa AF, Fashina AM (2013). Effect of alcohol consumption on hematological and reproductive parameters in female albino rats. IOSR J Dent Med Sci 2013; 3 (5):76-79.
[14] Raji LO, Fayemi OE, Ameen SA, Jagun AT. The effect of aqueous extract of *Allium sativum* (Garlic) on some aspects of reproduction in the female albino rats. Global Vet 2012; 8 (4): 414-420.