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Effect of Phenytoin on Reproductive Function in Female Wistar Rats

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Abstract

Introduction: This study was designed to investigate the effect of phenytoin on reproductive function in female Wistar rats.
Methods: Eighteen female rats (120 – 160 g) were used for the estrous cycle and histopathological studies. Phenytoin (2.8 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 phenytoin (2.8 mg/kg) produced a significant ($p<0.05$) decrease in proestrous phase of the estrous cycle relative to the respective control. The histopathological study revealed that treatment of rats with phenytoin (2.8 mg/kg) for 50 days presented with multiple foci of haemorrhage within the myometria, which is contrary to what was observed in the control rats.
Conclusion: It can therefore be concluded that phenytoin probably has pro-fertility effect, but probably induced deleterious effect on the uteri in female Wistar rats.

Keywords: Phenytoin, Proestrous, Diestrous, Endometria, Rats.

INTRODUCTION

Phenytoin is an anti-seizure medication. It is useful for the prevention of tonic-clonic seizures (also known as Grand Mal seizures) and focal seizures, but not absence seizures [1]. The intravenous form, fosphenytoin, is used for status epilepticus that does not improve with benzodiazepines. It may also be used for certain heart arrhythmias or neuropathic pain. It can be taken intravenously or by mouth [1].

The effect of intraperitoneal administration of the phenytoin on the skeletal system of rat fetus has been reported [2]. Its inductive effect of cerebral thrombosis in rats has been reported [3]. The alterations in tissue trace element and ascorbic acid metabolism in phenytoin-fed rats and mice have been reported [4]. Its effect on sodium conductances in rat hippocampal CA1 pyramidal neurons has been documented [5]. The effect of route of administration on phenytoin teratogenicity in A/J mice has been reported [6]. Its effect on *Satb2* and *Hoxa2* gene expressions in mouse embryonic craniofacial tissue has been reported [7]. Its effect on the polarization of immunity responses in experimental autoimmune encephalomyelitis has also been reported [8].

However, due to scanty information from literature on the effect of phenytoin on reproductive parameters in female rats, this study therefore aims at investigating the effect of this anticonvulsant 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

Phenytoin (Merit Pharm, Ltd, India.) was bought from Danax Pharmacy, Ibadan, Nigeria.

Phenytoin (100 mg) was dissolved in l0 ml of distilled water to give a concentration of 10 mg/ml.

The dosage of phenytoin 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 everyday at a constant interval of 4.30 – 5.30 p.m. for 21 days before and after treatments with the anticonvulsant agent. The smears were classified into one of the phases of the estrous cycle using the Marcondes technique [9]. 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 x10 and x40 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 [10, 11]. 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 2.8 mg/kg of phenytoin (orally).

Histopathological Study

In another set of experiment, twelve matured female rats divided into two equal groups (six animals per group) received the following treatments of the anticonvulsant 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 2.8 mg/kg of phenytoin.
On the 51st day, all the rats were sacrificed by an overdose of chloroform. 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 (5 µm). 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 phenytoin (2.8 mg/kg) produced a significant ($p<0.05$) decrease in proestrous phase, but caused insignificant ($p>0.05$) changes in the estrous, metestrous and diestrous phases of the estrous cycle relative to their respective controls (Figure 1).
Treatment of rats with phenytoin (2.8 mg/kg) for 50 days produced no visible lesion on the ovaries which is similar to what was observed in the control rats (Plates 1 and 2).
Treatment of rats with phenytoin (2.8 mg/kg) for 50 days presented with multiple foci of hemorrhage within the myometria, which is contrary to what was observed in the control rats (Plates 3 and 4).

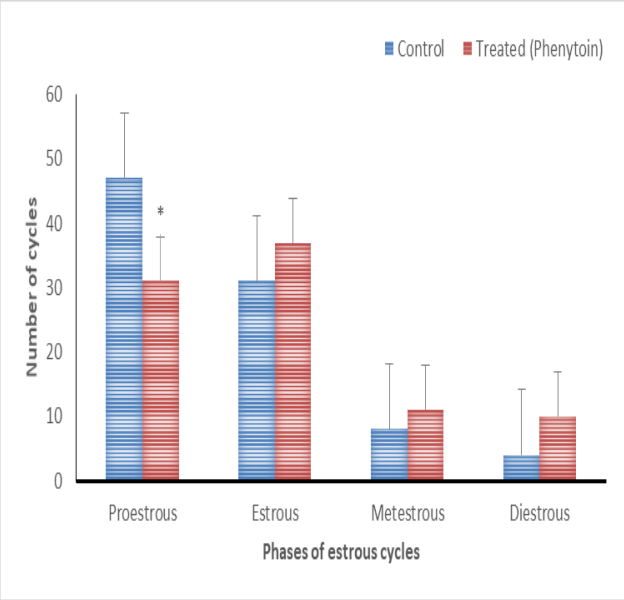

Figure 1: Effect of 21 days treatment with phenytoin on estrous cycle (n = 6, *p<0.05)

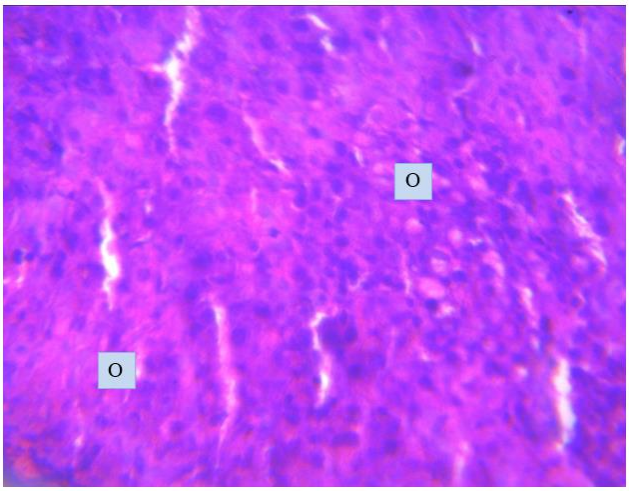

Plate 1: Effect of 0.5 ml/100 g distilled water (control) on the ovary at x100.
Photomicrograph showing a normal ovary (O) with no visible lesion seen.

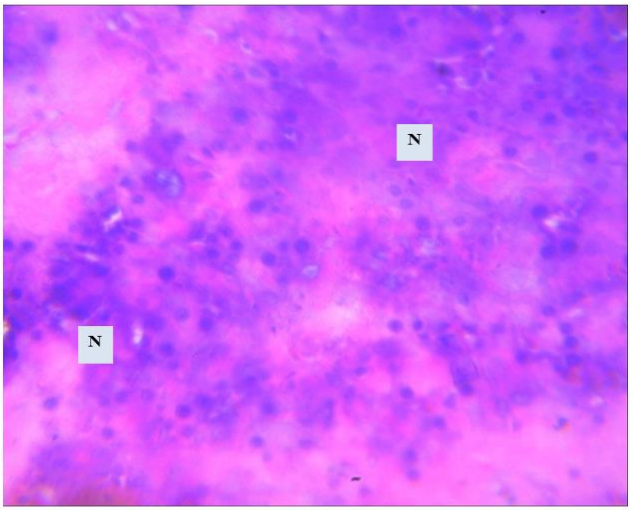

Plate 2: Effect of phenytoin (2.8 mg/kg) on the ovary at x100.
Photomicrograph showing an ovary with no pathological lesion (N).

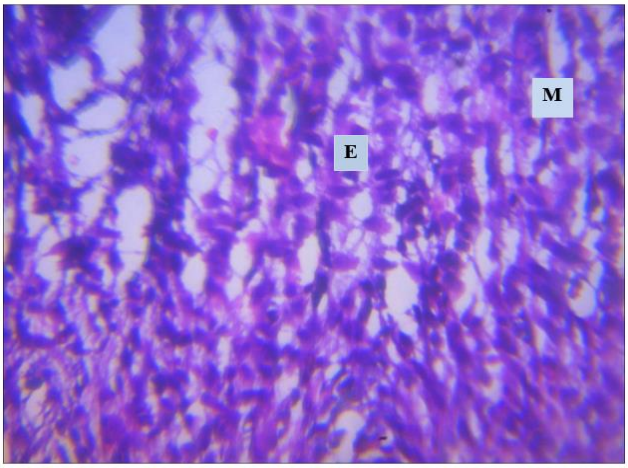

Plate 3: Effect of 0.5 ml/100 g distilled water (control) on the uterus at x100.
Photomicrograph showing normal endometria (E) and myometrium (M) with no visible lesion seen.

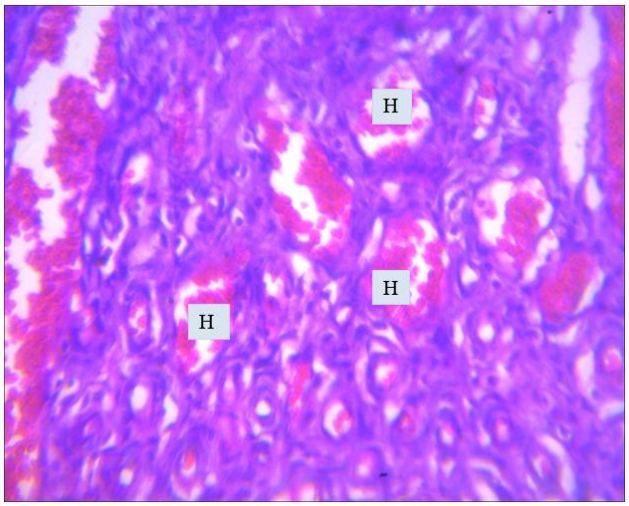

Plate 4: Effect of phenytoin (2.8 mg/kg) on the uterus at x100
Photomicrograph showing multiple foci of hemorrhage (H) within the myometrium.

DISCUSSION

The estrous cycle study revealed that phenytoin caused significant a change in the duration of a phase of the estrous cycle. Contrary report was given by [12] in *Portulaca oleracea* extracts treated rats. This suggests that the anticonvulsant agent 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 [13].

Treatment of rats with phenytoin caused significant decrease in proestrous phase of the estrous cycle which suggests that the maturation of the follicles in the pre-ovulatory phase was hastened *vis-à-vis*, leading to maturation of the Graafian follicles. Contrary result was reported by [14] in alcohol treated rats.

The ovarian photomicrographs of the phenytoin treated rats presented with no pathologic lesion which suggests the non-toxic effect of the drug on the ovaries at histologic level. Similar results were reported by [12] in *Portulaca oleracea* treated rats.

The uterine photomicrographs of the phenytoin treated rats presented with multiple foci of hemorrhage within the myometria which could be due to venous thrombosis. Similar result was reported by [15] in their work on the morphometric evaluation of endometrial blood vessels.

In conclusion, this study has shown that phenytoin probably has pro-fertility effects in female Wistar rats. It also revealed that phenytoin probably induced deleterious effect on the uteri but has no deleterious effect on the ovaries at histological level in female Wistar rats. However, the effect of this anticonvulsant agent on human reproductive function is unknown; nevertheless, considering these findings in animal model, it is recommended that women with infertility problems should exercise caution in the use of diazepam 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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