

Effect of Cyclophosphamide on Reproductive Function in Female Wistar Rats

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Abstract

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

Methods: Eighteen female rats (120 – 160 g) were used for the estrous cycle and histopathological studies. Cyclophosphamide (7.14 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 cyclophosphamide (7.14 mg/kg) produced significant ($p<0.05$) decrease and increase respectively in proestrous and metestrous phases of the estrous cycle relative to their respective controls. The histopathological study revealed that treatment of rats with cyclophosphamide (7.14 mg/kg) for 50 days presented with dense hemorrhage within the endometria and myometria.

Conclusion: It can therefore be concluded that cyclophosphamide probably has dominant pro-fertility effect, but also probably induced deleterious effect on the uteri in female Wistar rats.

Keywords: Cyclophosphamide, Proestrous, Metestrous, Endometria, Rats.

INTRODUCTION

Cyclophosphamide, also known as cytophosphane among other names [1], is a medication used as chemotherapy and to suppress the immune system [2]. As chemotherapy it is used to treat lymphoma, multiple myeloma, leukemia, ovarian cancer, breast cancer, small cell lung cancer, neuroblastoma, and sarcoma [2]. As an immune suppressor it is used in nephrotic syndrome, granulomatosis with polyangiitis, and following organ transplant, among other conditions [2, 3]. It is taken by mouth or injection into a vein [2].

The toxic effect of different doses of cyclophosphamide on liver and kidney tissue in Swiss albino mice has been reported [4]. Its variable effect in rodent models of experimental allergic encephalomyelitis has been reported [5]. Its toxicologic and hematologic effect in experimental animals have been reported [6]. Its effect on albino rats' liver has been reported [7]. Its effect on hippocampal cell proliferation and spatial working memory in rat has been documented [8]. Its immunomodulating on cytotoxic activity of rat and mouse splenocytes has been reported [9]. The effect of cyclophosphamide and its combination with metformin on the survival rate in mice have also been reported [10].

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

Cyclophosphamide (Naman Pharma Drugs, India) was bought from Danax Pharmacy, Ibadan, Nigeria.

Cyclophosphamide (500 mg) was dissolved in 10 ml of distilled water to give a concentration of 50 mg/ml.

The dosage of cyclophosphamide 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 alkylating agent. The smears were classified into one of the phases of the estrous cycle using the Marcondes technique [11]. Vaginal secretion was collected with a plastic pipette filled with 10 μ L 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 [12, 13]. 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 7.14 mg/kg of cyclophosphamide (i.p.).

Histopathological Study

In another set of experiment, twelve matured female rats divided into two equal groups (six animals per group) received the following treatment of the alkylating agent (i.p.) 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 7.14 mg/kg of cyclophosphamide (i.p.).

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 57°C, thereafter the tissues were embedded. Serial sections were cut using rotary microtome at 5 microns (5 µm). The satisfactory ribbons were picked up from a water bath (50 - 55°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 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 1 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 cyclophosphamide (7.14 mg/kg) produced significant ($p < 0.05$) decrease and increase respectively in proestrous and metestrous phases, but induced insignificant ($p > 0.05$) changes in the estrous and diestrous phases of the estrous cycle relative to their respective controls (Figure 1).

Treatment of rats with cyclophosphamide (7.14 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 cyclophosphamide (7.14 mg/kg) for 50 days presented with dense hemorrhage in the endometria and myometria (Plates 3 and 4).

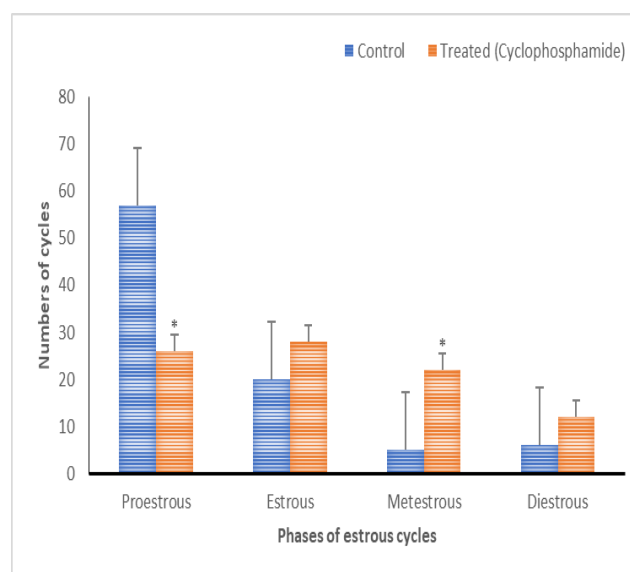


Figure 1: Effect of 21 days treatment with cyclophosphamide on estrous cycle (n = 6, * $p < 0.05$)

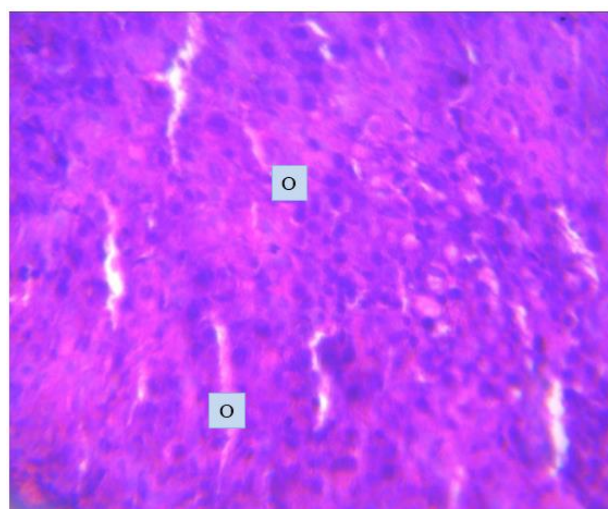


Plate 1: Effect of 0.5 ml/100 g distilled water (control) on the ovary at x100.

Photomicrograph showing a normal ovary (O) with no visible lesion seen.

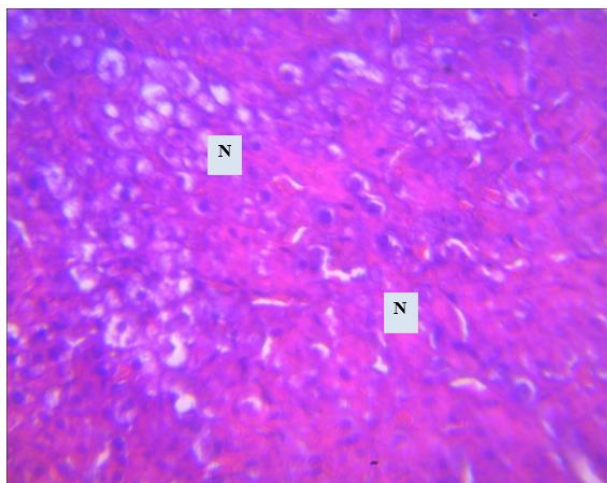


Plate 2: Effect of cyclophosphamide (7.14 mg/kg) on the ovary at x100
Photomicrograph showing an ovary with no pathological lesion (N).

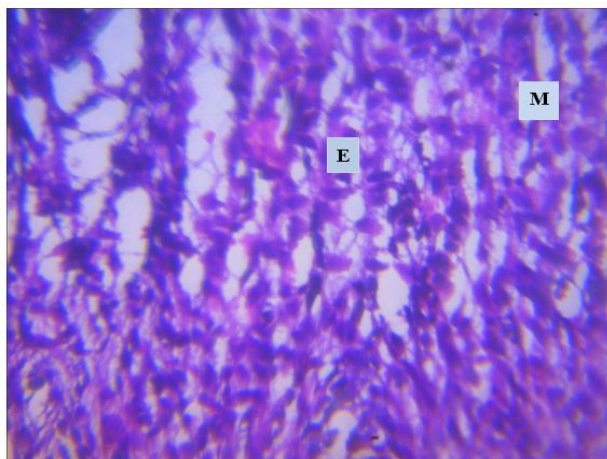


Plate 3: Effect of 0.5 ml/100 g distilled water (control) on the uterus at x100.
Photomicrograph showing normal endometria (E) and myometrium (M) with no visible lesion seen.

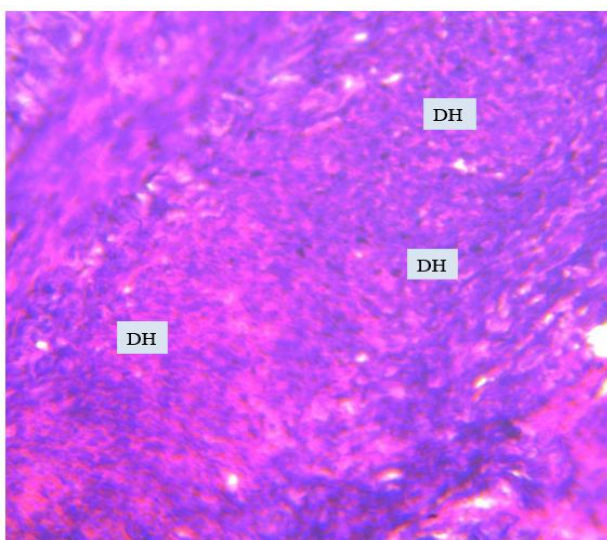


Plate 4: Effect of cyclophosphamide (7.14 mg/kg) on the uterus at x100
Photomicrograph showing dense hemorrhage (DH) within the endometrium and myometrium.

DISCUSSION

The estrous cycle study revealed that cyclophosphamide caused significant changes in the duration of different phases of the estrous cycle. Contrary report was given by [14] in *Portulaca oleracea* extracts treated rats. This suggests that the alkylating 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 [15].

Treatment of rats with cyclophosphamide caused a significant decrease in proestrous phase of the estrous cycle which suggests the maturation of the follicles in the preovulatory phase was hastened. Contrary result was reported by [16] in alcohol treated rats.

Treatment of rats with cyclophosphamide induced a significant increase in the metestrous phase of the estrous cycle which probably indicates the availability of matured Graafian follicles. Similar result was reported by [17] in tetracycline treated rats.

The ovarian photomicrographs of the cyclophosphamide 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 [14] in *Portulaca oleracea* treated rats.

The uterine photomicrographs of cyclophosphamide treated rats presented with dense hemorrhage within the endometria and myometria which could be due to deep venous thrombosis. Similar result was reported by [18] in their work on the morphometric evaluation of endometrial blood vessels.

In conclusion, this study has shown that cyclophosphamide probably has dominant pro-fertility effect in female Wistar rats. It also revealed that cyclophosphamide probably induced deleterious effect on the uteri at histological level in female Wistar rats. However, the effect of this anticancer 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 cyclophosphamide 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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