Research Article

Effect of Methotrexate on Reproductive Function in Female Wistar Rats

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Received: 14-09-2021; **Revised:** 18-11-2021; **Accepted:** 27-11-2021; **Published on:** 20-12-2021.

ABSTRACT

This study was designed to investigate the effect of methotrexate on reproductive function in female Wistar rats. Eighteen female rats (120 – 160 g) were used for the estrous cycle and histopathological studies. Methotrexate (0.071 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. Treatment of rats for 21 days with methotrexate (0.071 mg/kg) produced significant ($p<0.05$) reductions in the proestrous and estrous phase of the estrous cycle as well as significant ($p<0.05$) increments in the metestrous and diestrous phases of the estrous cycle relative to their respective controls. The histopathological study revealed that treatment of rats with methotrexate (0.071 mg/kg) for 50 days presented with ovarian medullary part that is congested and oedematous as well as with multiple foci of hemorrhage within the endometria. It can therefore be concluded that that methotrexate probably has dominant pro-fertility effect, but also probably induced deleterious effects on the ovaries and uteri in female Wistar rats.

Keywords: Methotrexate, Proestrous, Estrous, Endometria, Rats.

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DOI:
10.47583/ijpsrr.2021.v71i02.008

DOI link: http://dx.doi.org/10.47583/ijpsrr.2021.v71i02.008

INTRODUCTION

Methotrexate, formerly known as amethopterin, is a chemotherapy agent and immune-system suppressant. It is used to treat cancer, autoimmune diseases, and ectopic pregnancy and for medical abortions [1]. Types of cancers it is used for include breast cancer, leukemia, lung cancer, lymphoma, gestational trophoblastic disease, and osteosarcoma. Types of autoimmune diseases it is used for include psoriasis, rheumatoid arthritis, and Crohn's disease. It can be given by mouth or by injection [1].

The effect of methotrexate on some parameters of kidney in newborn mice has been reported [2]. Its cytotoxic and genotoxic effects in germ cells of male Swiss mice has been reported [3]; its effect on the labeling index of the tongue and palate epithelium in mice has been reported [4]. Its low-dose greater antirheumatic effect in collagen induced-arthritis rats has been reported [5]. The mechanism of its low-dose inflammatory suppressive effect has been reported [6]. Methotrexate has also been reported to prevent renal injury in experimental diabetic rats via anti-inflammatory actions [7].

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

Methotrexate (Samarth Life Sciencies PVT. Ltd., India) was bought from Danax Pharmacy, Ibadan, Nigeria.

Methotrexate (2.5 mg) was dissolved in l0 ml of distilled water to give a concentration of 0.25 mg/ml.

The dosage of methotrexate 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 antimetabolite. 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 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 [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 0.071 mg/kg of methotrexate orally.

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 antimetabolite 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 0.071 mg/kg of methotrexate.

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 microtone 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 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 methotrexate (0.071 mg/kg) produced significant ($p<0.05$) reductions in the proestrous and estrous phase as well as significant ($p<0.05$) increments in the metestrous and diestrous phases relative to their respective controls (Figure 1).

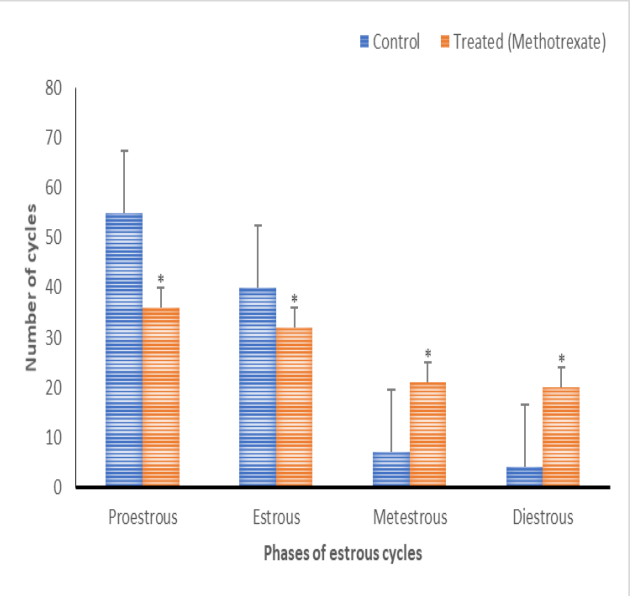

Figure 1: Effect of 21 days treatment with methotrexate on estrous cycle (n = 6, *$p<0.05$)

Treatment of rats with methotrexate (0.071 mg/kg) for 50 days presented with ovarian medullary part that is congested and oedematous, which is contrary to what was observed in the control rats (Plates 1 and 2).

Treatment of rats with methotrexate (0.071 mg/kg) for 50 days presented with multiple foci of hemorrhage within the endometria (Plates 3 and 4).

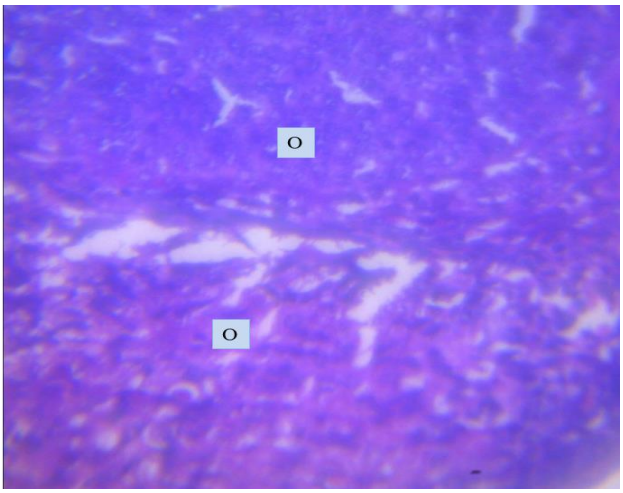

Plate 1: Effect of 0.5 ml/100 g distilled water (control) on the ovary at x100. Photomicrograph showing a normal ovary (O) with no visible lesion seen.

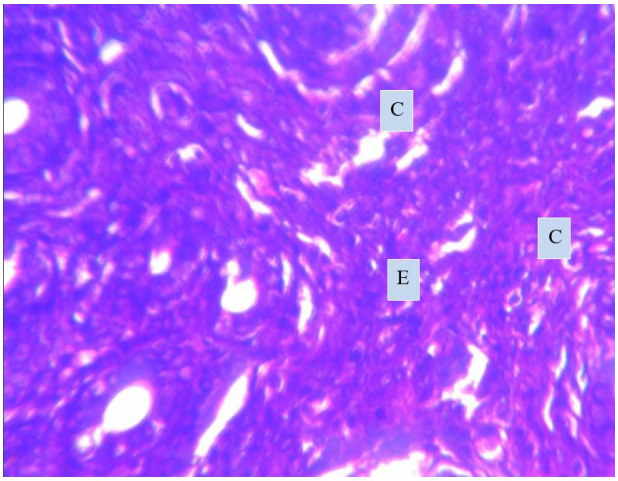

Plate 2: Effect of methotrexate (0.071 mg/kg) on the ovary at x100. Photomicrograph showing an ovary with congested (C) and oedematous (E) in the medullary part.

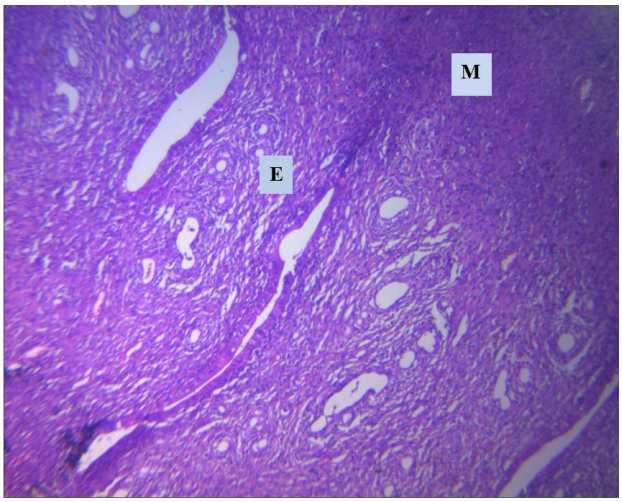

Plate 3: Effect of 0.5 ml/100 g distilled water (control) on the uterus at x100. Photomicrograph showing normal endometria (E) and myometrium (M) with no visible lesion seen.

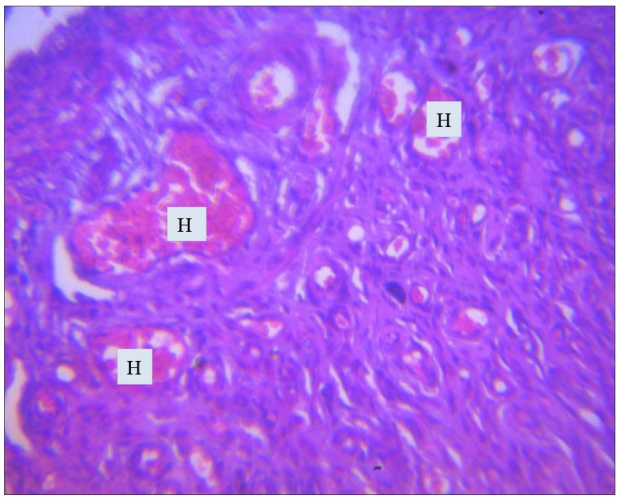

Plate 4: Effect of methotrexate (0.071 mg/kg) on the uterus at x100. Photomicrograph showing multiple foci of hemorrhage (H) within the endometrium.

DISCUSSION

The estrous cycle study revealed that methotrexate caused significant changes in the duration of different phases of the estrous cycle. Contrary report was given by [11] in *Portulaca oleracea* extracts treated rats. This suggests that the antimetabolite 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 methotrexate caused significant decrease in proestrus phase of the estrous cycle which suggests that the maturation of the follicles in the preovulatory phase was hastened *vis-à-vis*, leading to maturation of the Graafian follicles. Contrary result was reported by [13] in alcohol treated rats.

Treatment of rats with methotrexate caused significant decrease in the estrous phase of the estrous cycle which suggests the availability of matured Graafian follicles. Contrary result was reported by [13] in alcohol treated rats.

Treatment of rats with methotrexate caused significant increase in the metestrous phase of the estrous cycle which probably indicates the availability of matured Graafian follicles. Similar result was reported by [14] in tetracycline treated rats.

Treatment of rats with methotrexate caused significant increase in the diestrous phase of the estrous cycle which probably indicates a decrease in the frequency of ovulation. Contrary result was reported by [15] in amlodipine treated rats.

The ovarian photomicrographs of the methotrexate treated rats presented with congested (hemorrhagic) ovarian medullar which could be due to venous thrombosis. This is similar to the result obtained by [16] in Sumithion treated rats. It also presented with oedematous ovarian medulla. It has been reported that there were five pathophysiological causes of oedema which can be due to: (i) increased hydrostatic pressure (ii) reduced oncotic

pressure (iii) lymphatic obstruction (iv) sodium retention or (v) inflammation [17]. Hence, the oedema induced by this drug might be caused by any of the aforementioned causes. Similar result was reported by [18] in mercury vapour treated rats.

The uterine photomicrographs of the methotrexate treated rats presented with multiple foci of hemorrhage within the endometria which could be due to venous thrombosis. Similar result was reported by[19] in their work on the morphometric evaluation of endometrial blood vessels.

In conclusion, this study has shown that methotrexate probably has dominant pro-fertility effect in female Wistar rats. It also revealed that methotrexate probably induced deleterious effect on the ovaries and uteri at histologic 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 methotrexate for infertility therapeutic purpose.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.