



Effect of Artesunate on Reproductive Function in Female Wistar Rats

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ABSTRACT

This study was designed to investigate the effect of artesunate on reproductive function in female Wistar rats. Fifteen female rats (120 – 160 g) were used for the estrous cycle and histopathological studies. Artesunate (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 $p=0.05$. Treatment of rats for 21 days with artesunate (1.43 mg/kg) produced significant ($p<0.05$) increments in the estrous and metestrous phases as well as a significant ($p<0.05$) reduction in the proestrous phase of the estrous cycle relative to their respective controls. The histopathological study presented with ovarian medullar that is severely congested (hemorrhagic) including expanded lumen of the endometrial glands. It can therefore be concluded that artesunate probably has pro-fertility effect with deleterious effect on the ovaries at histological level in female Wistar rats.

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

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INTRODUCTION

Artesunate, an artemisinin-derivative, has been used for decades in humans for the treatment of severe *falciparum* malaria, which is a uniformly lethal disease if not treated promptly with potent antimalarial drugs. The WHO recommends the use of parenteral artesunate for the treatment of severe *falciparum* malaria since 2005¹. Although the mechanism of action remains to be completely elucidated, various data prove the efficacy of artesunate *in vitro*, *in vivo* in rodent and monkey challenge models, and in clinical and field isolates, by the number of successfully cured patients. Adsorption, distribution and elimination data are known for several routes of administration in commonly used laboratory species, including pregnant animals².

The toxicity profile of artesunate has been reported in rats and dogs². Its oral administrative effect on the histology of the kidney in rats has been reported³. The haematological and biochemical effects of its subchronic exposure to rats have been reported⁴. Its long term administration has been reported to induce reproductive toxicity in male rats⁵. Its teratogenic effect on the central nervous system of Wistar rat foetus has been reported⁶. Artesunate has also been reported to attenuate traumatic brain injury-induced impairments in rats⁷. However, due to scanty

information from literature on the effect of artesunate on reproductive parameters in female rats, this study therefore aims at investigating the effect of this antimalarial 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

Artesunate tablets (CUSNAT PHARMACEUTICALS LTD.) were bought from Danax Pharmacy, Ibadan, Nigeria.

Artesunate (50 mg) was dissolved in 10 ml of distilled water to give a concentration of 5.0 mg/ml.

The dosage of artesunate used in this study was in accordance with that reported by the manufacturer.

Experimental Design

Study on Estrous Cycle

Five 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 4.30 – 5.30 p.m. for 21 days before and after treatments with the antimalarial drug. The smears were classified into one of the phases of the estrous cycle using the Marcondes technique⁸. 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 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 5 rats for this estrous cycle study received 1.43 mg/kg of artesunate.

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 antimalarial 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 artesunate.

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 μ m). 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 artesunate (1.43 mg/kg) produced significant ($p < 0.05$) increments in the estrous and metestrous phases as well as a significant ($p < 0.05$) reduction in the proestrous phase of the estrous cycle relative to their respective controls (Figure 1).

Treatment of rats with artesunate (1.43 mg/kg) for 50 days presented with ovarian medullar that is severely congested (hemorrhagic) and most of the follicles seen are matured, contrary to what was observed in the control rats (Plates 1 and 2).

Treatment of rats with artesunate (1.43 mg/kg) for 50 days presented with endometrial glands whose lumen appeared expanded, contrary to what was observed in the control rats (Plates 3 and 4).

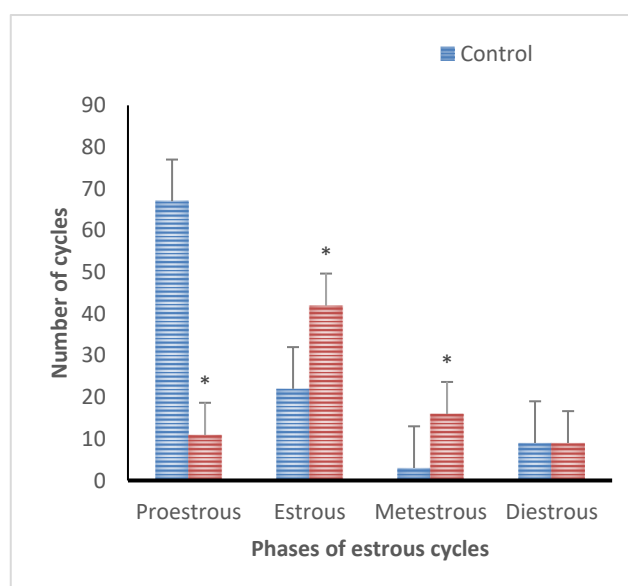


Figure 1: Effect of 21 days treatment with artesunate on estrous cycle (n = 5, * $p < 0.05$)

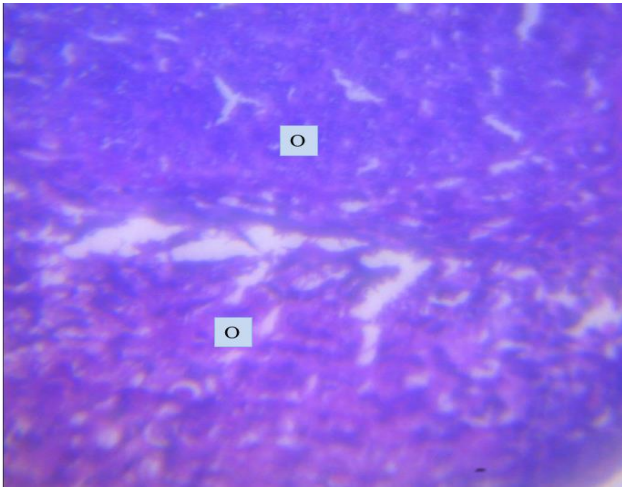


Plate 1: Effect of 0.5 ml/100 g distilled water (control) on the ovary at x400. Photomicrograph showing a normal ovary (O) with no visible lesions seen.

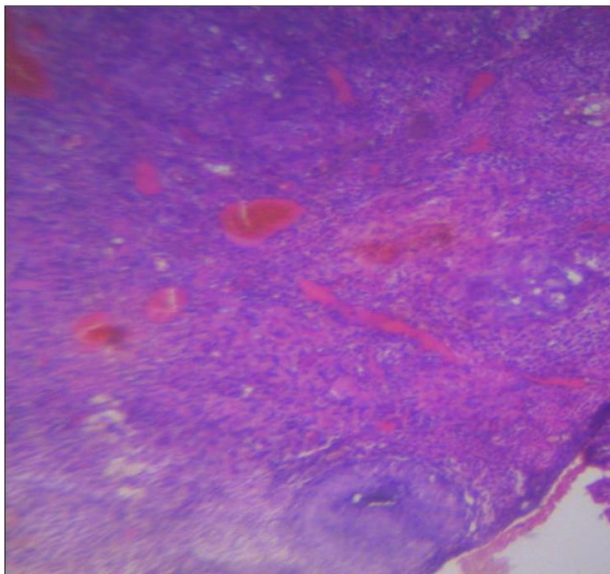


Plate 2: Effect of artesunate (1.43 mg/kg) on the ovary at x400. Photomicrograph showing an ovary with a severely congested medulla (CM).

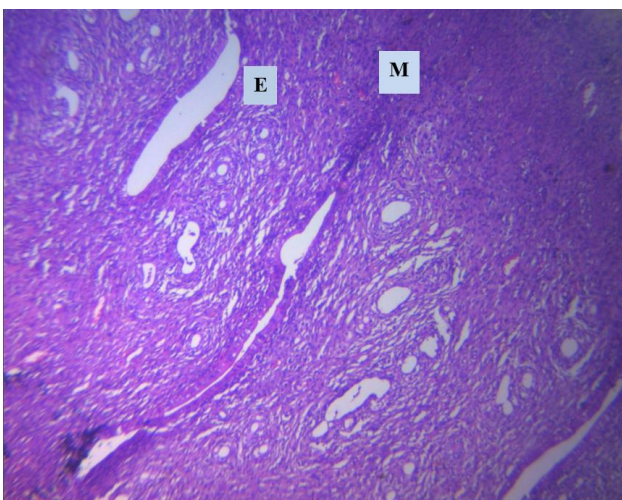


Plate 3: Effect of 0.5 ml/100 g distilled water (control) on the uterus at x400. Photomicrograph showing normal

endometria (E) and myometrium (M) with no visible lesions seen.

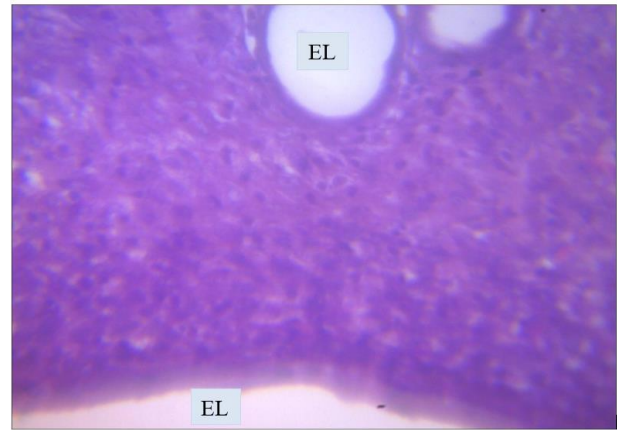


Plate 4: Effect of artesunate (1.43 mg/kg) on the uterus at x400. Photomicrograph showing expanded lumen (EL) of the endometrial glands.

DISCUSSION

The estrous cycle study revealed that artesunate 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 antimalarial 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 artesunate caused significant decrease in proestrous phase of the estrous cycle which probably indicates that the maturation of the follicles in the preovulatory phase was hastened leading to maturation of the Graafian follicles. Contrary result was reported by ¹³ in alcohol treated rats. Treatment of rats with artesunate caused significant increase in estrous phase of the estrous cycle which suggests the availability of matured Graafian follicles and would lead to ovulation. Similar result was reported by ¹⁴ in alcohol treated rats. Also, treatment of rats with artesunate caused significant increase in metestrous phase of the estrous cycle which probably indicates the availability of matured Graafian follicles. Similar result was given by ¹⁵ in tetracycline treated rats.

The ovarian photomicrographs of the artesunate treated rats presented with severely congested (hemorrhagic) ovarian medulla which could be due to deep venous thrombosis. This is similar to the result obtained by ¹⁶ in Sumithion treated rats.

The uterine photomicrographs of the artesunate treated rats presented with expanded lumen of the endometrial glands which could be due to increase in blood flow to the uterine arteries as a result of increased progesterone secretion (from the corpus luteum).

It can therefore be concluded that artesunate probably has pro-fertility effect with deleterious effect on the ovaries at

histological level in female Wistar rats. However, the effect of this drug on human reproductive function is unknown; nevertheless, considering these findings in animal model, it is recommended that women with infertility problems could take artesunate for infertility therapeutic purpose.

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