

# Effect of Ibuprofen on Reproductive Function in Female Wistar Rats

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## Abstract

**Aim:** This study was designed to investigate the effect of ibuprofen on reproductive function in female Wistar rats.

**Methods:** Fifteen female rats (120 – 160 g) were used for the estrous cycle and histopathological studies. Ibuprofen (5.71 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 ibuprofen (5.71 mg/kg) produced significant ( $p<0.05$ ) reduction in the proestrous phase as well as a significant ( $p<0.05$ ) increment in the estrous phase of the estrous cycle relative to their respective controls. The histopathological study presented with expanded glandular structures of the endometria, few are necrotic, there is moderate to severe congestion of the endometria.

**Conclusion:** It can therefore be concluded that ibuprofen probably has pro-fertility as well as deleterious effect on the uteri at histological level in female Wistar rats

**Keywords:** Ibuprofen, Proestrous, Estrous, Ovaries, Rats.

## INTRODUCTION

Ibuprofen is a medication in the nonsteroidal anti-inflammatory drug (NSAID) class that is used for treating pain, fever, and inflammation. This includes painful menstrual periods, migraines, and rheumatoid arthritis. It may also be used to close a patent ductus arteriosus in a premature baby. It can be used by mouth or intravenously. It typically begins working within an hour [1].

The gastrointestinal toxicity of ibuprofen has been reported using surrogate markers in rats [2]. Its regular use has been reported to reduce rat penile prostaglandins and induces cavernosal fibrosis [3]. The adverse effect induced by ibuprofen toxicity on immunological and biochemical parameters in Swiss albino mice has been reported [4]. It has been reported that ibuprofen antagonizes selective actions of estrogen and tamoxifen on rat bone [5]. It has been reported to increase the serum Omentin level in rats after abdominal surgery [6]. Ibuprofen has also been reported to attenuate nephropathy in streptozotocin-induced diabetic rats [7]. However, due to scanty information from literature on the effect of ibuprofen on reproductive parameters in female rats, this study therefore aims at investigating the effect of this NSAID 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

Ibuprofen tablets (KrishatPharma Ind. Ltd., Nigeria) were bought from Danax Pharmacy, Ibadan, Nigeria.

Ibuprofen (200 mg) was dissolved in 10 ml of distilled water to give a concentration of 20 mg/ml.

The dosage of Ibuprofen 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 NSAID. 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  $\mu$ 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 5.71 mg/kg of ibuprofen.

### 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 NSAID 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 5.71 mg/kg of ibuprofen.

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 (5µm). 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 ibuprofen (5.71 mg/kg) produced significant ( $p < 0.05$ ) reduction in the proestrous phase as well as a significant ( $p < 0.05$ ) increment in the estrous phase of the estrous cycle relative to their respective controls (Figure. 1).

Treatment of rats with ibuprofen (5.71 mg/kg) for 50 days produced no visible lesions on the ovaries, which is similar to what was observed in the control rats (Plates 1 and 2).

Treatment of rats with ibuprofen (5.71 mg/kg) for 50 presented with expanded glandular structures of the endometria, few are necrotic, there is moderate to severe congestion, which is contrary to what was observed in the control rats (Plates 3 and 4).

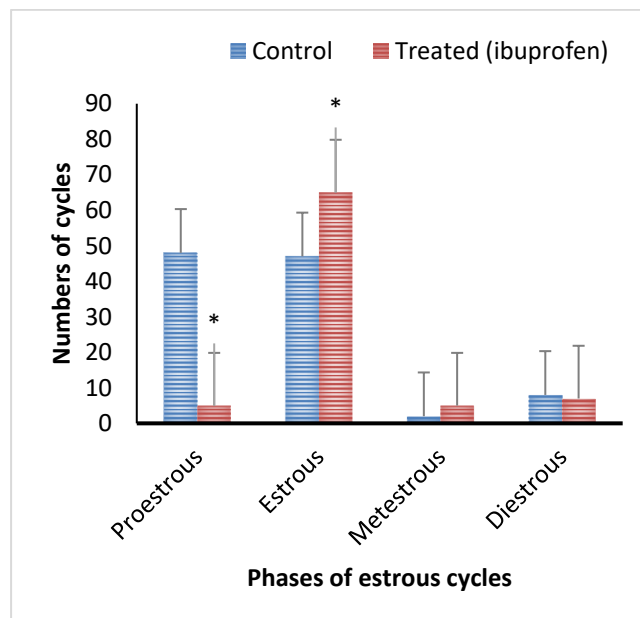


Figure 1: Effect of 21 days treatment with ibuprofen on estrous cycle (n = 5, \* $p < 0.05$ )

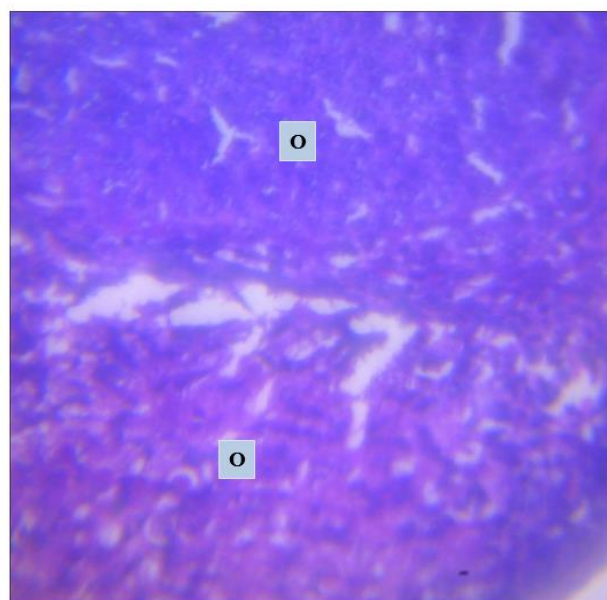


Plate 1: Effect of 0.5 ml/100 g distilled water (control) on the ovary at x400.

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

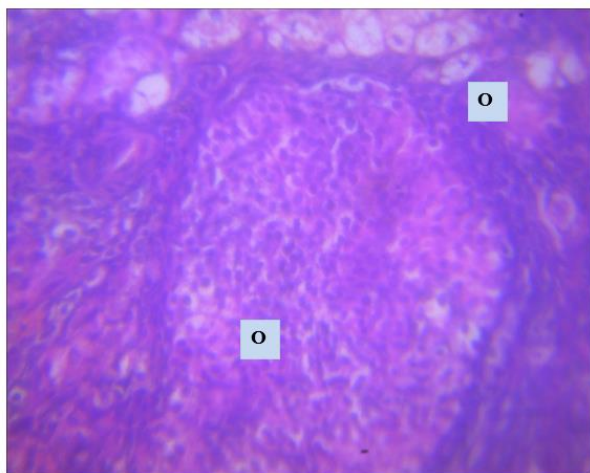


Plate 2: Effect of ibuprofen (5.71 mg/kg) on the ovary at x400

Photomicrograph showing an ovary (O) with no pathologic lesions seen.

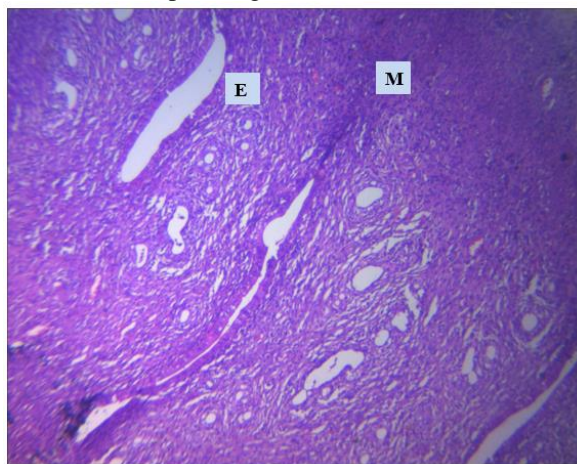


Plate 3: Effect of 0.5 ml/100 g distilled water (control) on the uterus at x400.

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

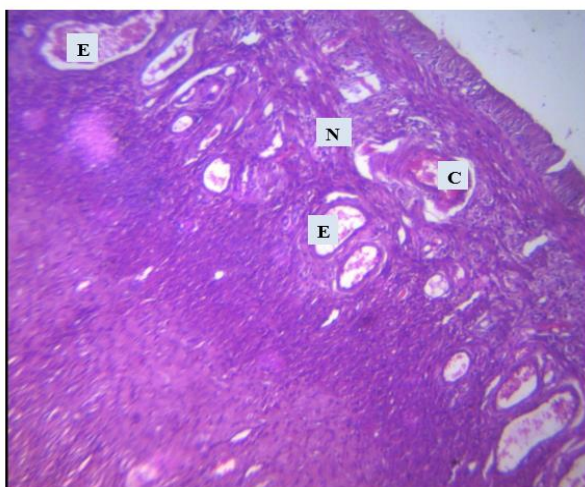


Plate 4: Effect of ibuprofen (5.71 mg/kg) on the uterus at x400

Photomicrograph showing expanded glandular structures of the endometrial (E) and necrotic structures (N) and moderate to severe congestion (C).

## DISCUSSION

The estrous cycle study revealed that ibuprofen 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 NSAID 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 ibuprofen 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 ibuprofen 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.

The ovarian photomicrographs of the ibuprofen treated rats presented with no visible lesions, 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 ibuprofen treated rats presented with expanded glandular structures of the endometrial 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 also presented with few necrotic structures of the endometrial which could be due to disease, injury, or failure of the blood supply. Similar result was reported by [15] in *Rumex steudelii* methanolic root extract treated female rats. Furthermore, it presented with moderate to severe congestion of the endometrial which could be due to moderate to severe venous thrombosis. Similar result was reported by [16] in their work on the morphometric evaluation of endometrial blood vessels.

It can therefore be concluded that ibuprofen probably has pro-fertility as well as deleterious effect on the uteri at histological level in female Wistar rats. However, the effect of this NSAID on human reproductive function is unknown; nevertheless, considering these findings in animal model, it is recommended that women with infertility problems could take ibuprofen 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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