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Effect of Tramadol on Reproductive Function in Female Wistar Rats

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Abstract

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

Methods: Sixteen female rats (120 – 160 g) were used for the estrous cycle and histopathological studies. Tramadol (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.

Results: Treatment of rats for 21 days with tramadol (1.43 mg/kg) produced no significant (p>0.05) changes in all the different phases of estrous cycle relative to their respective controls, as well as induced no pathological effects on the ovarian and uterine tissues in the rats.

Conclusion: It can therefore be concluded that tramadol probably has no effect on fertility as well as exhibited non-deleterious effect on the reproductive tissues of female Wistar rats.

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

INTRODUCTION

Tramadol is a synthetic opioid drug that has a dual mechanism of analgesic action as a serotonin and norepinephrine reuptake inhibitor and a mu-opioid receptor agonist [1]. It is widely used as an analgesic for the treatment of postoperative, cancer, or chronic neuropathic pain [2]. Nowadays, this opioid drug has become one of the most widely prescribed drugs in the world [3].

However; studies have shown that long-term use of the opioids is associated with addiction, physical and psychological dependence [4]. Despite its therapeutic effects, tramadol also has harmful effects on various organs. This opioid drug has hepatotoxicity and nephrotoxicity effects and can cause liver and kidney damages [5]. Tramadol is a lipophilic opioid which, due to freely crossing the placenta, causes withdrawal syndrome in neonatal [6]. The effects of its neurotoxicity have been proven and continuous administration of tramadol resulted in weight loss of rats' brain [3]. Some studies have also demonstrated that tramadol administration impairs memory function in rodent models by activation of μ-opioid receptors [7, 8]. On the other hand, chronic administration of tramadol caused histological abnormalities such as increasing apoptosis in rat cerebral cortex associated with oxidative stress [4]. It has been reported that tramadol can induce seizures in patients [9]. Other studies have shown that tramadol leads to increasing oxidative stress in various tissues such as the brain [10]. Tramadol has been observed to decrease the expression of some genes in the hippocampus [11]. Its effect on reproductive parameters in male Wistar rats has also been reported [12]. However, due to scanty information from literature on the effect of tramadol on reproductive parameters in female rats, this study therefore aims at investigating the effect of this synthetic opioid 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

Tramadol tablets (Laider Int. Ltd., Nigeria) were bought from Danax Pharmacy, Ibadan, Nigeria.

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

The dosage of tramadol 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 every day at a constant interval of 6.30 – 7.30 a.m. for 21 days before and after treatments with the synthetic opioid. The smears were classified into one of the phases of the estrous cycle using the Marcondes technique [13].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 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 [14, 15]. 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 1.43 mg/kg of tramadol.

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 synthetic opioid 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 tramadol.

On the 51st day, all the rats were sacrificed by an overdose of diethyl ether. 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 (5µm). 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 tramadol (1.43 mg/kg) produced no significant ($p>0.05$) changes in all the different phases of estrous cycle (Proestrous, estrous, metestrous and diestrous) relative to their respective controls (Fig. 1).

Treatment of rats with tramadol (1.43 mg/kg) for 50 days produced no pathological effects on the ovaries, which is similar to what was observed in the control rats (Plates 1 and 2).

Treatment of rats with tramadol (1.43 mg/kg) for 50 days produced no pathological effects on the uteri, which is similar to what was observed in the control rats (Plates 3 and 4).

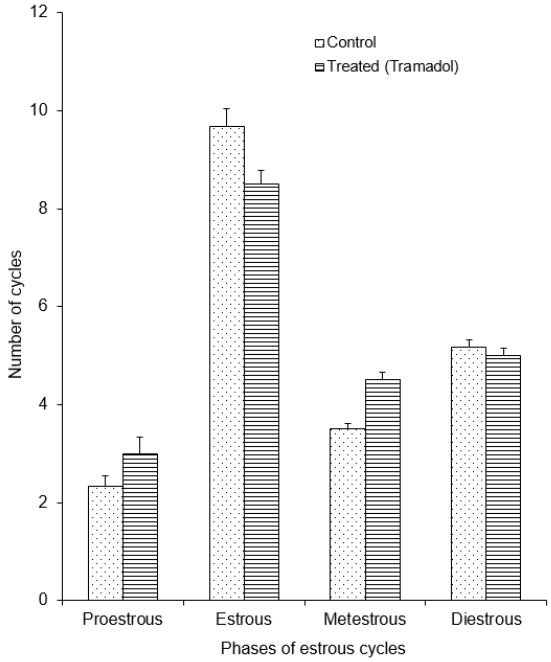

Fig. 1: Effect of 21 days treatment with tramadol on estrous cycle (n = 6, *p<0.05)

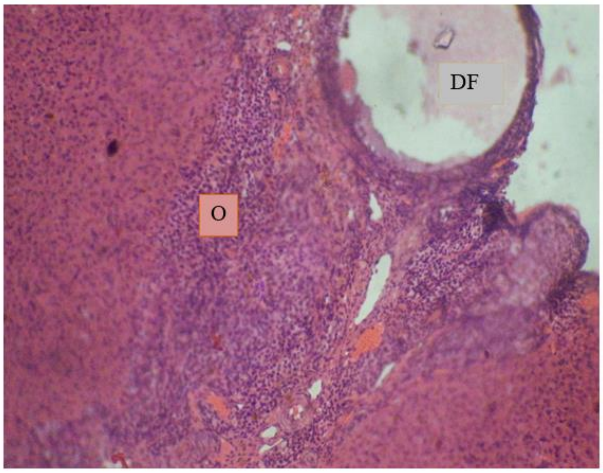

Plate 1: Effect of 0.5 ml distilled water (control) on the ovary at x100.

Photomicrograph showing a normal ovary (O) with a developing follicle (DF).

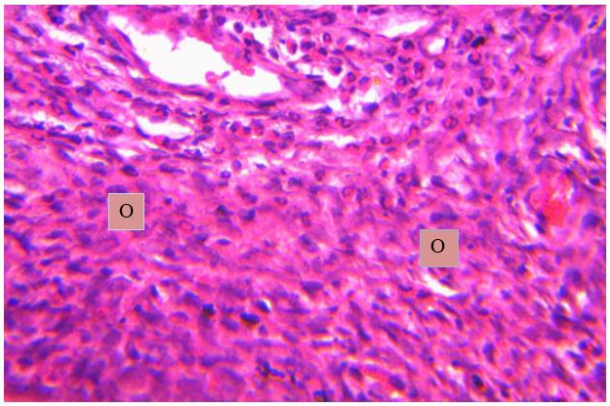

Plate 2: Effect of tramadol (1.43 mg/kg) on the ovary at x100
Photomicrograph showing an ovary (O) with no pathologic lesion seen.

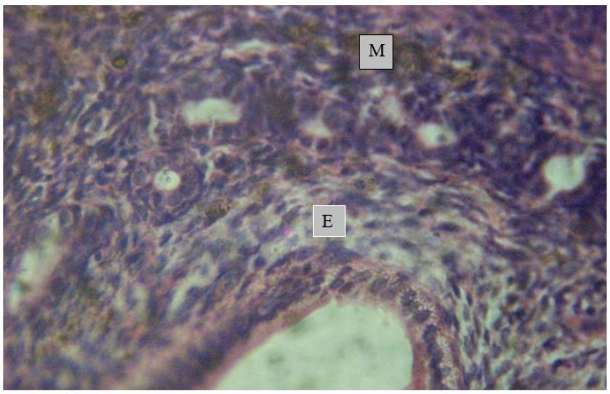

Plate 3: Effect of 0.5 ml distilled water (control) on the uterus at x100.
Photomicrograph showing normal endometria (E) and myometrium (M).

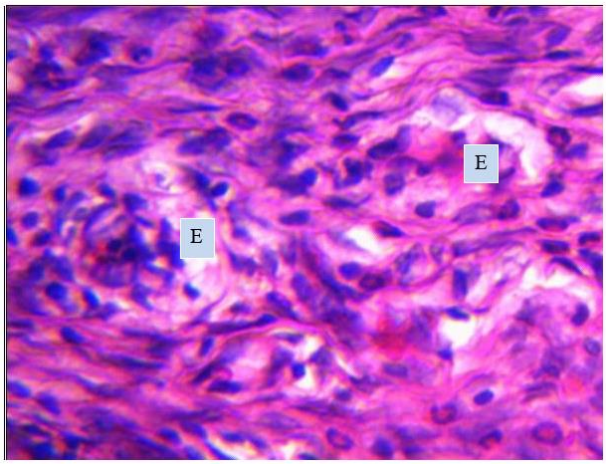

Plate 4: Effect of tramadol (1.43 mg/kg) on the uterus at x100
Photomicrograph showing endometrial (E) layer with no pathologic lesion present.

DISCUSSION

The estrous cycle study revealed that tramadol produced no significant changes in the duration of all the phases of the estrous cycle. Similar report was given by [16] in *Portulaca oleracea* extracts treated rats. This suggests that the synthetic opioid did not cause imbalance 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 [17]

The ovarian photomicrographs of the tramadol treated rats showed no pathologic lesion present which suggests the non-toxic effect of the drug on the ovaries at histological level. Similar results were reported by [16] in *Portulaca oleracea* treated rats.

The uterine photomicrographs of the tramadol treated rats presented with no pathologic lesion which probably indicate the non-toxic effect of the drug on the uteri at histological level. Similar results were reported by [18] in *Allium sativum* extract treated rats.

It can therefore be concluded that tramadol probably has no effect on fertility as well as exhibited non-deleterious effect on the reproductive tissues of female Wistar rats.

Conflict of Interest

We vehemently declare that there is no conflict of interests in this research work.

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