Effects of Zinc on Fertility Parameters in Tramadol-Treated Male Wistar Rats

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Abstract- ***Tramadol dependence is a growing public health concern with established deleterious effects on reproductive health, particularly in males. This study investigated the potential ameliorative effects of zinc on fertility parameters in male Wistar rats treated with tramadol. Twenty-five male Wistar rats were divided into five groups: control, two tramadol-treated groups (30 mg/kg and 60 mg/kg), two co-administration groups (tramadol + 3.5 mg/kg zinc), and a zinc-only control. All administrations were oral for 49 days, followed by a mating study and fertility assessment. Results consistently showed that zinc significantly mitigated the adverse effects of tramadol on male fertility parameters. These findings suggest zinc supplementation could be a beneficial intervention against tramadol-induced reproductive toxicity.***

Indexed Terms- ***Zinc, Tramadol, Fertility, Antioxidants, Wistar Rats, Male Reproduction***

I. INTRODUCTION

Tramadol dependence and misuse have emerged as a significant and escalating global public health problem [10], with a notable prevalence of misuse reported in countries like Nigeria [8]. While acknowledged as an effective analgesic [1], prolonged tramadol use is associated with addiction and severe systemic side effects, including cardiopulmonary arrest and hepatic failure in overdose cases [3] [6].

Crucially, studies have consistently demonstrated tramadol's detrimental effects on reproductive health [12] [14]. Specifically, chronic tramadol exposure has been linked to decreased semen quality and abnormalities in testicular tissues, largely attributed to induced oxidative stress [9].

In the context of male reproductive health, oxidative stress is a substantial contributor to subfertility [7]. Zinc, an essential micronutrient, plays a vital role in male fertility, supporting testicular development, sperm physiological functions, and maintaining seminal plasma quality [2] [5]. Furthermore, zinc possesses extensive antioxidant properties, acting to inhibit oxidative damage, regulate key enzymes (e.g., glutathione peroxidase 5), and mitigate DNA damage and apoptosis [4] [11] [13].

Despite the growing body of evidence highlighting tramadol's reproductive toxicity and the established benefits of zinc in male fertility, the specific ameliorative effects of zinc supplementation against tramadol-induced reproductive damage remain underexplored. Therefore, this study aimed to evaluate the effect of zinc on fertility parameters in male Wistar rats treated with tramadol. Our findings will contribute valuable insights into potential therapeutic strategies to combat tramadol-induced reproductive impairments.

II. METHODOLOGY

A. Animals and Ethical Approval

Twenty-five adult male Wistar rats (190-220g) were procured from the Animal Research Building, Faculty of Basic Medical Sciences, University of Port Harcourt. An additional twenty-five healthy, non-pregnant adult female Wistar rats were used for the mating study. All animals were housed in clean cages and acclimatized for two weeks under standard laboratory conditions (25°C, 55-65% humidity, natural light/dark cycles), with *ad libitum* access to commercially available rat chow and distilled water. All experimental procedures were approved by the relevant ethics body and adhered to ethical guidelines for animal care and use.

B. Reagents and Preparation

Tramadol hydrochloride (30 mg/kg and 60 mg/kg body weight) and zinc sulphate (3.5 mg/kg body weight) were prepared daily in distilled water to ensure accurate dosing. The tramadol doses were selected based on previous literature, aiming to

simulate therapeutic (30 mg/kg) and toxic (60 mg/kg) human equivalent doses in rats, while the zinc concentration was within the reported therapeutic range.

C. Experimental Design

After acclimatization, the male Wistar rats were randomly assigned into five groups of five animals each. Oral administration (using an orogastric cannula) was performed once daily (between 7:00 am and 10:00 am) for 49 consecutive days. The experimental groups were:

a. Group 1 (Control): Administered 1 mL distilled water.
b. Group 2 (Tramadol Low): Administered 30 mg/kg body weight tramadol hydrochloride.
c. Group 3 (Tramadol Low + Zinc): Administered 30 mg/kg body weight tramadol hydrochloride + 3.5 mg/kg body weight zinc sulphate.
d. Group 4 (Tramadol High + Zinc): Administered 60 mg/kg body weight tramadol hydrochloride + 3.5 mg/kg body weight zinc sulphate.
e. Group 5 (Zinc Control): Administered 3.5 mg/kg body weight zinc sulphate.

D. Fertility Assessment

On the 39th day of administration, the male rats were cohabited with the 25 healthy non-pregnant adult female Wistar rats in a 1:1 mating scheme for a period of 10 days. On the 56th day of the experiment (seven days after cohabitation), all 25 female rats were fasted overnight and then humanely sacrificed using diethyl ether. The uterus and fallopian tubes were carefully harvested and observed. The number of pregnant females, viable foetuses, and resorption sites (if present) were meticulously recorded. Fertility index and pregnancy success rates were calculated based on the number of pregnant females and viable foetuses relative to the total number of mated females in each group.

E. Statistical Analysis

All quantitative data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) software (version 25.0). Results are presented as mean ± standard error of the mean (M ± SEM). A P-value of less than 0.05 ($P < 0.05$) was considered statistically significant, with a 95% confidence level.

III. RESULTS AND DISCUSSION

The following results were obtained after administration of zinc on tramadol-induced male Wistar rats, in comparison with the control group (that received only water and animal feed) and the group that received tramadol only.

A. Analysis for Number of Pregnant Females

In comparison with the control group, the tramadol-only group (group 2) had a 66.67% decrease in the number of pregnant females, while test groups 3 (30mg/kg tramadol + 3.5mg/kg zinc) and 4 (60mg/kg tramadol + 3.5mg/kg zinc) both had 33.33% reduction. Test group 5 (zinc only) had a 33.33% increase in the number of pregnant females.

On the other hand, in comparison with the tramadol-only group (group 2), there was a 100% increase in the number of pregnant females for test groups 3 and 4, while test group 5 had a 300% increase in the number of pregnant females.

B. Analysis for Number of Foetuses

In comparison with the control group, the tramadol-only group (group 2) had a 46.15% decrease in the number of foetuses, while test groups 3 (30mg/kg tramadol + 3.5mg/kg zinc) and 4 (60mg/kg tramadol + 3.5mg/kg zinc) both had 15.38% reduction. Test group 5 (zinc only) had a 53.85% increase in the number of foetuses.

On the other hand, in comparison with the tramadol-only group (group 2), there was a 57.14% increase in the number of foetuses for test groups 3 and 4, while test group 5 had a 185.71% increase in the number of foetuses.

C. Analysis for Pregnancy Success (%)

In comparison with the control group, the tramadol-only group (group 2) had a 66.67% decrease in pregnancy success, while test groups 3 (30mg/kg tramadol + 3.5mg/kg zinc) and 4 (60mg/kg tramadol + 3.5mg/kg zinc) both had 33.33% reduction. Test group 5 (zinc only) had a 33.33% increase in pregnancy success.

On the other hand, in comparison with the tramadol-only group (group 2), there was a 100% increase in pregnancy success for test groups 3 and 4, while test group 5 had a 300% increase in the pregnancy success.

D. Analysis for Fertility Index (%)

In comparison with the control group, the tramadol-only group (group 2) had a 46.15% decrease in fertility index, while test groups 3 (30mg/kg tramadol + 3.5mg/kg zinc) and 4 (60mg/kg tramadol + 3.5mg/kg zinc) both had 15.38% reduction. Test group 5 (zinc only) had a 33.33% increase in fertility index.

On the other hand, in comparison with the tramadol-only group (group 2), there was a 57.14% increase in fertility index for test groups 3 and 4, while test group 5 had a 185.71% increase.

IV. CONCLUSION AND RECOMMENDATIONS

In conclusion, our research provides clear evidence that zinc alters, and specifically improves, the adverse effects of tramadol on male reproductive health parameters**.** This highlights the critical need to prevent tramadol misuse and underscores the potential therapeutic role of zinc in safeguarding fertility against drug-induced toxicity.

It is also important to note that while this study provides compelling evidence, it is not without limitations. Future research should explore the underlying molecular mechanisms through which zinc exerts its protective effects against tramadol toxicity, perhaps investigating specific oxidative stress markers, hormonal profiles, and histological changes in greater detail. Further studies could also explore different doses of zinc or longer treatment durations to optimize therapeutic strategies and assess the long-term impacts.

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