**Gross and Histological Changes of Female Reproductive Organ Following Chronic Tramadol Hydrochloride Administration in** **Albino Wistar Rat**

**ABSTRACT**

This study was aimed to study the gross and histological changes associated with oral administration of tramadol hydrochloride on the ovaries and uterus of female wistar albino rat. Tramadol is a centrally active synthetic opioid analgesic that is used extensively for pain management, and has been associated with significant physiological and behavioral side effects, which include dependence and reproductive toxicity. A total of 20 mature female wistar rats were used. They were divided into two groups: a control group (n=10) were given feed and water only, and the treatment group (n=10) were administered with 50mg/kg/day of tramadol hydrochloride orally for 28 days. The body weight of the laboratory animals was measured using sensitive weighing balance, and is done at the beginning and termination of the study. At the beginning of the experiment the mean weight of the control group was 96.2grams and at the end of the experiment was 101.8grams, also the mean weight of the treated group before drug administration was 111.9grams and after drug administration was 105.7grams. The results showed gradual decrease in body weight in tramadol-treated group, this could be due to reduced appetite and gastrointestinal effects of the drug. At the termination of the study animals were humanely stunned using chloroform and their ovary and uterus were obtained. The organs were fixed in 10% buffered formalin, and stained using Hematoxylin and Eosin and the slides were examined using light microscope at x40 magnification. Histological findings in the control group revealed normal architecture of the uterus and ovaries, while the treatment group showed ovarian wall degeneration, atrophy of the ovarian follicles, and congestion of the ovarian blood vessels. In the uterus there was atrophy of the endometrial glands, vacuolation of the uterine cell. In conclusion, tramadol hydrochloride administration has a significant adverse effect on the female reproductive system of albino rats.

**Key words: Tramadol Hydrochloride, Albino Rats, Histology, Morphometry, Ovary, Uterus.**



**INRTODUCTION**

The rat (*Rattus norvegicus*) in 1850 was the first mammalian specie to be domesticated for the purpose of research. *The* Sprague-Dawley, Wistar and Fischer 344 are the mostly used strains of the laboratory rats and are all sub-strain derivation of the Norway rat, *Rattus norvegicus* (Kohn and Barhold, 2013). The Wistar strain of the albino rats was developed at the Wistar Institute in Philadelphia. Albino and hooded rats are one of the most commonly used laboratory animal for the past 150 years (Kuramoto *et al*., 2012). For over a century rats have a leading role in most research fields. Most species of albino rats are found in the tropical and sub-tropical areas world-wide. The species seems to have originated in central Asia, and has spread almost all over the continent during the last two centuries (Hedrich, 2000). The Albino strain of the brown rat (Rattus norvegicus), are used widely as experimental animals in research due to their docile nature, short generational interval, and genetic uniformity (Baker *et* *al*., 2023). They have the ability to survive in various habitats including, urban environments, and agricultural regions. and also adapt to extreme weather conditions Albino rats are omnivores in nature, they have the ability to consume wide variety of food, ranging from grains, seeds, fruits, vegetables, and protein sources like meats (Turner *et* *al*., 2023). The albino rats are commonly used for research in the laboratory due to their numerous advantages as a model organism for experiments. They are similar to humans with regards to physiological and genetic similarities, making them an ideal model for studying human diseases and developing new treatments (Rosenmund *et* *al*., 2020). Rats have been used as research tools in various fields, including drug development, toxicology, immunology, and neuroscience (Barnett, 2002).

Tramadol hydrochloride is an opioid analgesic that is widely sold worldwide (Shipton 2000). It is the second opioid analgesic that has been reported to be a substance of abuse among medical practitioners (Adams *et al.*, 2006). In some parts of the world tramadol is considered as a control drug and therefore requires prescription before use. However, it is readily available by remote prescription including internet pharmacies with relative ease (Solarino *et al.*, 2010). Tramadol hydrochloride dependence became an increasing trend and at the same time alarming problem in some part of the world including Africa (Roussin *et al.,* 2012). Easy and wide availability of tramadol as a pain killer for intermediate pain and many other forms of chronic pains could be the basic factors facilitating its spread and abuse (Hussein and Elguindy, 2017). Tramadol hydrochloride has been reported to have deleterious effects on both male and female albino rats. Studies by Paulis and Abbas, (2015) reported that administration of Tramadol in female rats reduced both pituitary sex hormones (LH and FSH) and gonadal hormones progressive weight loss in female wistar albino rats. The aim of this study is to determine the effect of Tramadol hydrochloride on gross and histology of the ovary and uterus in female wistar albino rats.

**MATERIALS AND METHODS**

**Study Area**

The study was conducted in Maiduguri, Nigeria, situated at an altitude of 354m above sea level, between latitude 10.2oN and 13.4oN and longitudes 9.8oE and 14.4oE. Maiduguri is located in North Eastern Nigeria.

**Experimental Animals**

A total of 20 adult female wistar albino rats was used for this study. They were purchased from the College of Medical Sciences, University of Maiduguri.

**Housing and Acclimatization**

The rats were kept for two weeks in order to adapt to the new environment before the commencement of the experiment. They were kept in a cage, the floor was tiled and roofed with Asbestos. It was well ventilated with temperature of about 350-370C. The laboratory animals

were kept in the laboratory animal’s house of the Faculty of Veterinary Medicine University of Maiduguri

**Source of the Drug**

The drug (Tramadol hydrochloride 50mg/kg) was obtained from the Faculty of Pharmaceutical Sciences, University of Maiduguri.

**Grouping**

The rats were divided into two groups of A, (control) and B (Experimental). with a total of 10 animals per experimental group.

**Feeding Regimen**

All animals used in this experiment were fed with poultry feed obtained from Ultima feed. The feed is made up of essential nutrients including 20% protein, 4.5% fats and major amino acids, with 5% fiber, fortified minerals and vitamins and energy of 3050kcal/kg. The experimental animals were fed daily and given drinking water ad libitum. The experiment lasted for 28 days including acclimatization period. Both the treatment and control group were fed and given water accordingly.

**Drug Administration**

**Dosage**

Tramadol was administered orally at a daily dose 50mg/kg for a treatment duration of 28 days as follows;  
**Monitoring**

The rats were monitored for general health, behavioral changes, and any side effects throughout the treatment period.

**Body Weight Determination**

The experimental animals were weighed on a sensitive weighing balance before and after the experiment

At end termination of the study, the experimental animals were stunned humanely euthanized using chloroform.

**Histology**

About 0.5 cm2 cut sections of the uterine caruncle were fixed in 10% phosphate-buffered formalin for 72 hours. The sections were trimmed and embedded in paraffin wax as described by Baker *et al*. (1985). The paraffin blocks were then sectioned at 4µm thickness and stained with hematoxylin and eosin (H&E). The stained slides were observed using light microscopy at ×10 magnifications, and photomicrographs were taken.

RESULTS

**Table (1): Changes in mean total body weight of adult female albino rats, control and tramadol-treated groups (28 days)**

Before drug administration (g) after drug administration (g)

Control group 96.2 101.8

Treated group 11.9 105.7

B

A

ZZZZ

OOOO

Figure 1A: Photomicrograph of the ovary of the control showing the normal architecture of the ovary and intact wall.

Figure 1B: Photomicrograph of the ovary of the treated group showing atrophy of the ovarian follicles (blue arrow) there are occlusions of the lumen of the ovarian blood vessel, degenerative changes in the ovarian wall looking thinner (yellow arrow) (H&E X40)

**B**

**A**

Figure 2A: Photomicrograph of the uterus of the control group showing normal architecture of the uterine wall (H&E X40)

Figure 2B: Photomicrograph of the uterus of the treatment group showing vacuolation of the uterine cells (blue arrow), atrophy of the endometrial glands and hyperemic uterine tissue (yellow arrow) (H&E X40)

**DISCUSSION**

Tramadol an opioid analgesic is associated with toxicity to those who take overdose of the drug as pain killer (Shadnia *et al*., 2008). This study is aimed at determining the effect of chronic administration of tramadol hydrochloride on female Wistar albino rats. Our findings reveal that administration of tramadol hydrochloride at 50mg/kg/ day showed a decrease in body weight in the treatment group when compared to the control group. This is in line with the findings made by (El-Mottaleb *et al.,*2019). Shuey *et al.,* and Paulis and Abbas who also reported that there was a decrease in body weight of wistar albino rats treated with tramadol hydrochloride at 40mg/kg and 80mg/kg. Tramadol has been reported to have a gastrointestinal side effect. This could the reason for the decrease in weight in the treated group. This is in consonance with the findings of (Bayrak *et* *al*., 2016) who also reported that change in body weight can be attributed to tramadol’s adverse effects on metabolism and overall health. This weight loss in the treatment group may be due to the effect of tramadol on the satiety center located in the hypothalamus, which is involved in regulating hunger and energy homeostasis. Opioid use, including tramadol, has been associated with alterations in appetite and metabolism, often leading to either increased or decreased food intake (Harrison *et* *al*., 2008). However, this finding does not agree with that of Ahmed and Kurkar who reported that tramadol did not affect the body weight of male wistar albino rats.

Our present study reveals that chronic administration of tramadol in wistar albino rats resulted in atrophy of the ovarian follicles, there are occlusions of the lumen of the ovarian blood vessel, degenerative changes in the ovarian wall looking thinner in the treatment as compared to the control group whose architecture is normal. The uterus shows vacuolation of the uterine cells, atrophy of the endometrial glands and hyperemic uterine tissue while the treatment group had normal uterine wall architecture. Paulis and Abbas reported that chronic treatment with tramadol hydrochloride resulted in decrease pituitary hormone (LH and FSH). Opioids have the ability to the decrease the release of GnRH from the hypothalamus. El-Mottaleb *et al.,*2019 reported that tramadol hydrochloride in treatment group shows atrophy of glands and stroma with increase of cellularity and hypertrophy of the endometrial glands. This agrees with our study which shows vacuolation of the uterine cells, atrophy of the endometrial glands and hyperemic uterine tissue, and also agrees with that of El-Ghawet *et al.,*2015 who reported atrophic glands and stroma with appearance of inflammatory cells in the stroma with increase of cellularity and hypertrophy of the endometrial glands.

**CONCLUSION**

The findings of this study indicate tramadol administered at 50mg/kg/day has adverse effect on body weight and histology of the reproductive organs of female wistar rats, thereby causing decrease in body weight, causing atrophy of the ovarian follicles, occlusion of the lumen of the ovarian blood vessels and thinning of the ovarian wall. Also, in the uterus it causes vacuolation of the uterine cells and atrophy of the endometrial glands. It is crucial for healthcare practitioners to consider these risks when prescribing tramadol, particularly to individuals concerned about fertility.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

**REFERENCES**

Adams,A., Joseph,B., and Williams, C.(2006). Tramadol dependence and abuse trends among medical practitioners

Baker F., Silverton, R. and Kilshaw, D. (1985). Introduction to histology. In: Introduction of Medical Laboratory Technology, 6,161. London, UK: Butterworths London.

Barnett, R. (2002). Opioids and male reproductive health. Reproductive Biology Review, 23(4), 987-1004.

Bayrak, R., Yilmaz, M., & Cakmak, B. (2016). The effects of tramadol on body weight and f ertility in male rats. Fertility & Sterility, 106(3), e60.

El-Ghawet HA (2015): Effects of tramadol on the reproductive function of wistar albino rats. Euro J Exp Bio., 5(1):56-64.

El-Mottaleb, A., Ahmed, H. A., Mahmoud, S. F., Hassan, A. I., Tealeb, A. S. M. I., & Almorsy, G. Z. (2019). Effects of Chronic Use of Tramadol on Uterus and Ovary of Albino Rats. *The Egyptian Journal of Hospital Medicine*, *76*(1), 3184-3190.

Harris, R. B. S., et al. (2008). Critical role of the medullary leptin receptor in the regulation of food intake and body weight. \*Endocrinology, 149(9), 4432-4440.

Hedrich,H.(2000).Geographical distribution and ecological adaptations of albino rats.

Hussien, R., & Elguindy, M. (2017). Assessment of the role of naloxone in the prognosis of tramadol intoxicated patients. *J Clin Toxicol*, *7*(366), 21610495.

Kohn, D. F., & Barthold, S. W. (2013). Biology and diseases of rats. *Laboratory animal medicine*, 91.

Kuramoto,T.,Kurosawa,M.,and Serizawa,A.(2012).Genetics of albino mutations in laboratory rat strains.

Paulis MG, Abbas MF (2015): Tramadol subchronic toxicity on pituitary-gonadal axis and ovarian functions in adult female rats. First international forensic medicine and clinical Available toxicology Conference, Minia University,

Rosenmund, C (2020). "The utility of rats in preclinical research."Nature Reviews Neuroscience, 21(3), 112-124.

Roussin, A., Doazan-d’Ouince, O., Géniaux, H., & Halberer, C. (2015). Evaluation of abuse and dependence in addiction monitoring systems: tramadol as an example. *Therapies*, *70*(2), 213-221.

Shadnia, S., Soltaninejad, K., Heydari, K., Sasanian, G., & Abdollahi, M. (2008). Tramadol intoxication: a review of 114 cases. *Human & experimental toxicology*, *27*(3), 201-205.

Shipton,E.A.(2000).Global trends in tramadol use and abuse:A comprehensive review.

Shuey,D.L.,Stump,D.G., and Carliss,R.D.(2008). Effects of the opioid analgesic oxymorphonehydrochloride on reproductive function in male and female rats. Birth Defects Research Part B:Developmental and Reproductive Toxicology,83(1),12–18

Solarino,M.,Rodriguez,A.,and Bonito,V.(2010).The role of internet pharmacies intramadol availability and misuse.

Turner, A., Patel, H., & Kim, Y. (2023). Omnivorous Behavior in Rodents: Feeding Patterns and Preferences of Albino Rats. Journal of Cognitive Behavior in Animals, 9(3), 215-224.