



## **Reproductive Organ Activities of *Morinda lucida* Ethanol Root Extract on Male and Female Albino Rats Tramadol HCL Induced Infertility**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Infertility is a serious issue disturbing reproductive ages in all society now and calling for solution for continuity. Infertility were induced on groups of animals with separate sexes; M, M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub>; F, F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> with daily subcutaneous administration of 20 mg/kg body weight tramadol HCl for 42 days before respective ethanol root extract administrations of (500, 1000, 1500) mg/ kg body weight for 10 days. The animals were anaesthetized and sacrificed; uterus, ovaries, testes, epididymis were dissected out for histomorphological studies. There is evidence of dose treatment of infertility among treated groups. From the organ weight study, both the male and the female organ weight in the groups remained significantly unchanged. There are dose dependent treatments with the male and female tramadol treatment. The result is more pronounced in the increased dose of ethanol root extract (1000 mg/kg and 1500 mg/ kg) body weight treated testicular

cells. This study indicated that *Morinda lucida* has a prophylactic effect against tramadol-induced testicular damage.

**Keywords:** *Reproductive organ activities; Morinda lucida; ethanol root extract; male and female albino rats; tramadol HCL induced infertility.*

## 1. INTRODUCTION

This is a system of sex organ used to produce male and female sex cell; Female reproductive organ comprises of internal structures (cervix, uterus, fallopian tube and ovary) while male sex organ includes; testes, prostate [1]. The male sex organ is responsible for the production and storage of male gamete: the two testes are an oval shaped organ that produces male gamete and androgen, the during ejaculation prostate gland secretes sperm produced in the testes through ejaculatory duct which carries sperm and the fluid secreted by seminal vesicle to unit area in the urethra [2,3]. The female sex organ ovary prepares and nurture oocyte for ovulation process and release through the uterine tube of fallopian tube into the uterus which nourishes fertile eggs [4].

Drugs interfere with fertility by stimulating the body to impact changes on hormonal balance thereby affecting luteinizing hormone (LH) which secretion causes release of ovaries and follicle stimulating hormone (FSH) by the pituitary gland [5]. Effect on male sex organs involve inhibition in testosterone which is the main hormone that signal testicle in production thus leading to decrease in sperm production [6]. Alcohol consumption and drugs causes infertility, such as steroids, opiates and marijuana affects testosterone and interferes in disruption of ovulation and endometrial reception of sperm cells [7]. Interruption of reactive oxygen species (ROS) level and antioxidant defence system imbalance induced by oxidative stress lead to cellular changes causing damages on these organs [8].

## 2. MATERIALS AND METHODS

Studies on activities of ethanol root *Morinda lucida* extracts on tramadol HCl induced infertility were conducted according to Salah et al. [9] and El-Ghawet, [10] with modifications.

### 2.1 Animals

Sixty (60) adult male and female rats respectively, between 7-9 weeks of body weight 120-150g were used in this study.

### 2.2 Drugs and Chemicals

Tramacet (Tramadol HCL injection) 100 mg / 2 ml manufactured by Ciron drugs & pharmaceuticals Pvt. Ltd were purchased from Renox pharmaceutical shops Park Avenue GRA Enugu metropolis, Enugu State, Nigeria

### 2.3 Ethanol Root Extracts Preparation

#### 2.3.1 Crude extraction preparation procedure

The root of the plant was washed, air dried, finely ground into powder using a grinder and wrapped in nylon to avoid contamination. *Morinda lucida* root extracts were prepared by immersing 250 g of ground powder into to 1000 ml of 80% ethanol respectively before subjection to the following model:

The ethanol extractions were left for 72 hrs at room temperature.

1. Resultant crude extractions were obtained by first filtration through the muslin cloth; then further filtrations through Whatman No. 1 filter paper.
2. The filtrates were concentrated using an evaporator set at 40°C.

Each jelly concentrate obtained was placed in a well labelled plastic container and stored in a refrigerator at 4°C until required.

### 2.4 Experimental Treatment Design

Each sex of albino rats were grouped into 10 groups M, M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub>, F, F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, M<sub>0</sub> and F<sub>0</sub>.

1. Groups M, M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub> and F, F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub> (6 rats each) were treated with daily subcutaneous administration of 20 mg/kg b. wt daily tramadol HCl for 42 days.
2. On Day 43, respective groups M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub> and F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub> received ethanol root extract doses of (500, 1000 and 1500) mg/kg b. wt for 10 days respectively.
3. Group M and F were sacrificed as positive control.

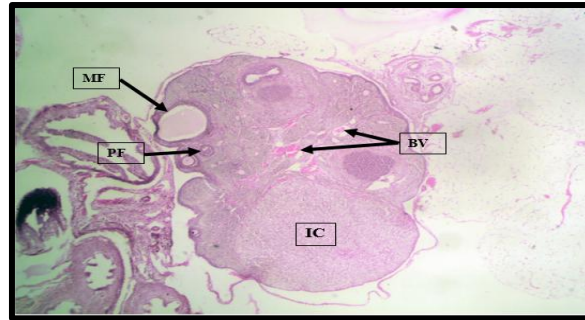
4. Group  $M_0$  and  $F_0$  served as male and female negative control respectively.
5. Group  $M_{01}$ ,  $M_{02}$ ,  $M_{03}$  and  $F_{01}$ ,  $F_{02}$ ,  $F_{03}$  received only ethanol root extract doses of (500, 1000 and 1500) mg/kg b. wt for 10 days respectively.

The rats were sacrificed under anesthesia on Day 43 and 53 target organs; uterus, ovaries, testes and epididymis were

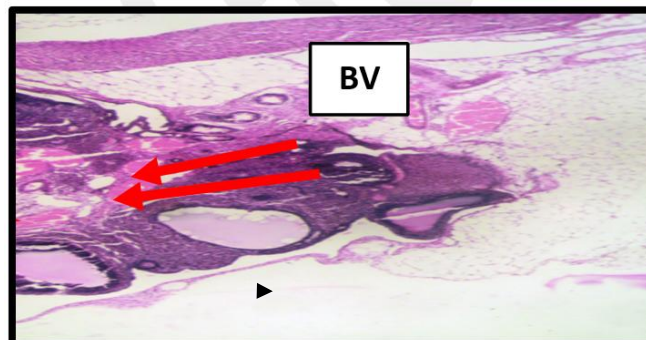
dissected out; weighed, examined macroscopically and processed for histological studies.

### 3. RESULTS

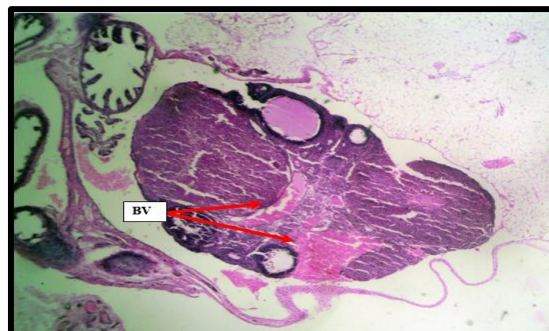
Effect on reproductive system following administration of tramadol female reproductive system ovaries.



Section of ovary from an animal in Group  $F_0$  showing normal features. IC – interstitial cells, MF – mature follicles, PF – primary follicles, BV – blood vessels. Stain: Haematoxylin and eosin. Magnification: X100



Section of ovary from an animal in Group F showing normal features. With dilated blood vessels (BV). Stain: Haematoxylin and eosin. Magnification: X100



Section of ovary from an animal in Group  $F_1$  showing normal features, but with the blood vessels appearing dilated (arrows). Stain: Haematoxylin and eosin. Magnification: X100

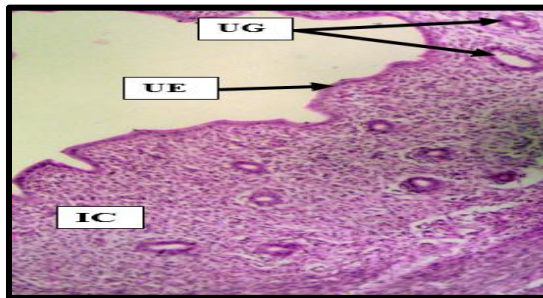


Section of ovary from an animal in Group F<sub>2</sub> showing normal features, but with the blood vessels appearing dilated (arrows). Stain: Haematoxylin and eosin. Magnification: X100

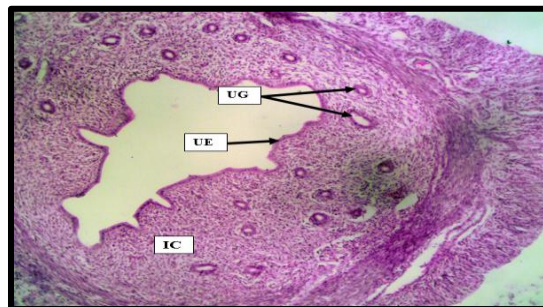


Section of ovary from an animal in Group F<sub>3</sub> showing normal features. IC – interstitial cells, MF – mature follicles, PF – primary follicles. Stain: Haematoxylin and eosin. Magnification: X100

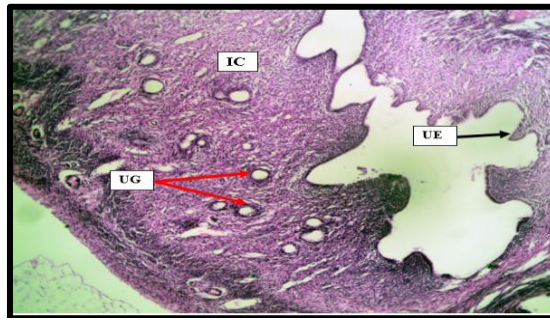
#### UTERUS



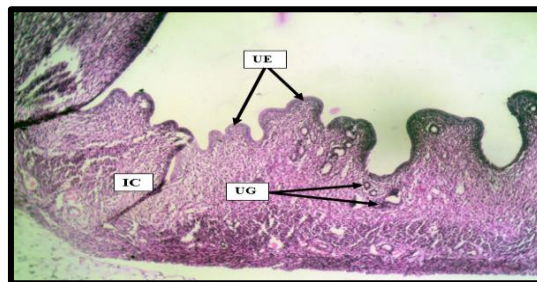
Section of the uterine wall from a rat in Group F<sub>0</sub> showing normal features. UE – uterine epithelial lining, UG – uterine glands, IC – interstitial connective tissue cells. Stain: Haematoxylin and eosin. Magnification: X100



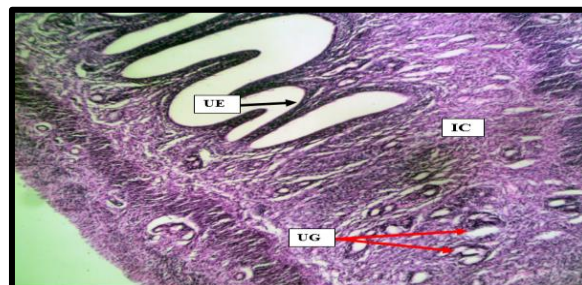
Section of the uterine wall from a rat in Group F showing normal features. UE – uterine epithelial lining, UG – uterine glands, IC – interstitial connective tissue cells. Stain: Haematoxylin and eosin. Magnification: X100



Section of the uterine wall from a rat in Group F<sub>1</sub> showing normal features. UE – uterine epithelial lining, UG – uterine glands, IC – interstitial connective tissue cells. Stain: Haematoxylin and eosin. Magnification: X100



Section of the uterine wall from a rat in Group F<sub>2</sub> showing normal features. UE – uterine epithelia lining, UG – uterine glands, IC – interstitial connective tissue cells. Stain: Haematoxylin and eosin. Magnification: X100



Section of the uterine wall from a rat in Group F<sub>3</sub> showing normal features. UE – uterine epithelia lining, UG – uterine glands, IC – interstitial connective tissue cells. Stain: Haematoxylin and eosin. Magnification: X100

### 3.1 Relative Organ Weight (ROW) Ovaries and Uterus

There were no significant changes in the relative ovaries and uterus organ weight among the treated groups as shown in Table 1.

Histopathology findings Ovaries.

The F<sub>0</sub>, F<sub>01</sub>, F<sub>02</sub> and F<sub>03</sub> group showed normal features in interstitial cells, mature follicles, primary follicles and blood vessels while the F (positive control) group presents normal features

with dilated blood vessels. Section of ovary from an animal in Group F<sub>1</sub> shows normal features, but with the blood vessels appearing dilated as shown in slide but F<sub>2</sub> and F<sub>3</sub> group show normal features without cellular alterations.

### 3.2 Uterus

The uteri among all the groups showed normal histoarchitecture without degenerative changes in the epithelia lining, uterine glands and intestinal connective tissue cell.

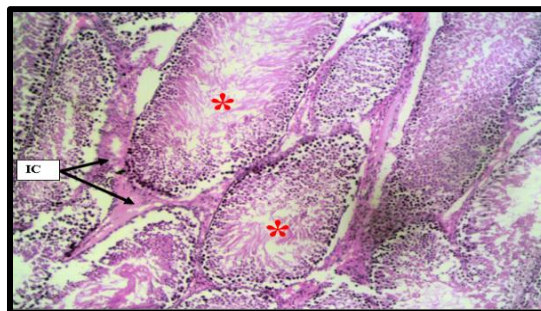
**Table 1. Relative organ weight (ROW) ovaries and uterus**

Group	R.O.W. of ovary and uterus
F (Negative control – Tramadol only)	0.32 ± 0.00
F1	0.34 ± 0.01
F2	0.33 ± 0.03
F3	0.32 ± 0.01
F <sub>0</sub> (baseline control - untreated)	0.32 ± 0.02

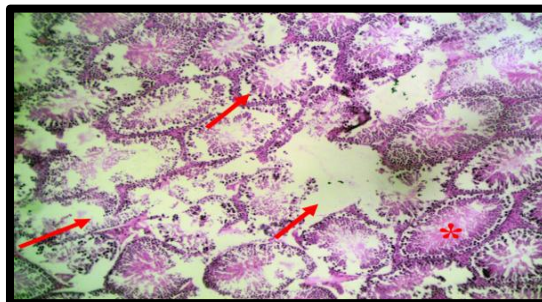
*Values for R.O.W. are expressed as mean ± S.E.M. Statistical significance was set a P < 0.05.*

### 3.3 Male Reproductive System

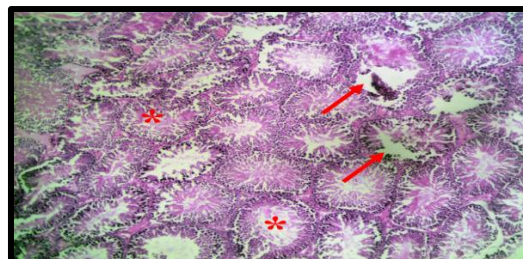
#### 3.3.1 Testes



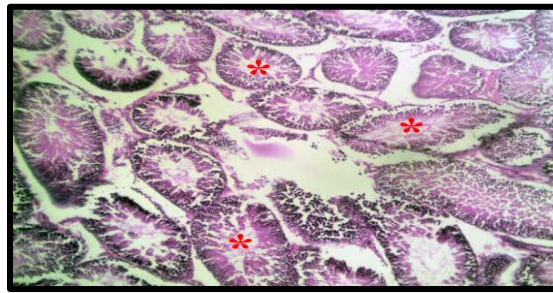
Section of testis of animal in group M<sub>0</sub> (baseline control) showing normal features. The seminiferous tubules (\*) and interstitial (leydig cells - IC) are normal. Stain: Haematoxylin and eosin. Magnification: X100



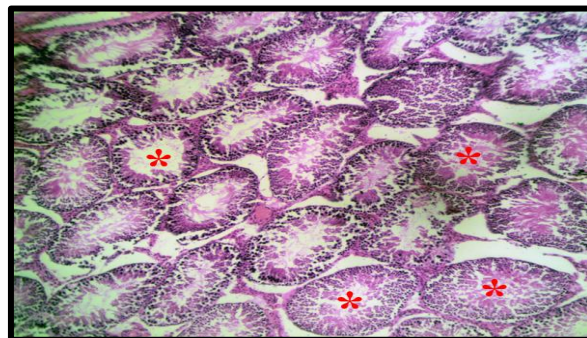
Section of testis of animal in group M. Some seminiferous tubules appear to have severely degenerated germinal epithelia (arrows) while others are normal (\*). Stain: Haematoxylin and eosin. Magnification: X100



Section of testis of animal in group M<sub>1</sub> showing normal features. Majority of the seminiferous tubules (\*) appear normal while few appear to have sloughed off germinal epithelia (arrow). Stain: Haematoxylin and eosin. Magnification: X100



Section of testis of animal in group M<sub>2</sub> showing normal features. The seminiferous tubules (\*) are normal. Stain: Haematoxylin and eosin. Magnification: X100



Section of testis of animal in group M<sub>3</sub> showing normal features. The seminiferous tubules (\*) are normal. Stain: Haematoxylin and eosin. Magnification: X100

Table 2. Relative organ weight (ROW) of testes and epididymis

Group	R.O.W. of testes and epididymis
M (positive control – tramadol only)	1.23 ± 0.00
M <sub>1</sub>	1.27 ± 0.01
M <sub>2</sub>	1.25 ± 0.02
M <sub>3</sub>	1.24 ± 0.01
M <sub>0</sub> (baseline control – untreated)	1.23 ± 0.01

Values for R.O.W. are expressed as mean ± S.E.M. Statistical significance was set at  $P < 0.05$

### 3.4 Relative Organ Weight (Row) of Testes and Epididymis

There were no relative organ weight significant changes observed among the groups as shown in Table 2 Histopathology findings Testes.

Group M<sub>0</sub> (negative control) and M<sub>01</sub>, M<sub>02</sub>, M<sub>03</sub> showed normal convoluted seminiferous tubules in a stroma with leydig cells while group M (positive control) appears to have severely degenerated germinal epithelium in seminiferous tubules while the cellular architectures are normal. M<sub>1</sub> (500mg/kg b. wt) group shows few sloughed off germinal epithelium in the seminiferous tubules while increased doses concentration (group M<sub>2</sub> and M<sub>3</sub>) shows normal

testicular histoarchitecture with intact seminiferous tubules and interstitial cell.

### 4. DISCUSSION

Tramadol is one of the most popular misused opiates used in the treatment of sexual premature ejaculation that can disrupt testosterone production [6,11]. Abuse of tramadol may accumulate into toxic metabolites that increase pharmacokinetic interaction, decrease elimination and induce oxidative stress by decreasing the antioxidant levels in the body thus resulting in toxicity that may accompany infertility [12]. Its effect may involve reduction in luteinizing hormone plasma level (LH), follicle stimulating hormone (FSH), increase in prolactin,

estradiol, nitric oxide, lipid peroxidation and decreased antioxidant enzyme activities [5,9].

From the organ weight study, both the male and the female organ weight in the groups remained significantly unchanged. There are dose dependent treatments with the male and female tramadol treatment. The result is more pronounced in the increased dose of ethanol root extract (1000mg/kg and 1500mg/ kg) body weight treated testicular cells. Plants with polyphenols and flavonoids have shown tolerate toxicity effect of tramadol [13]. Furthermore, the phytochemistry of *Morinda lucida* root extracts revealed a high content of total polyphenols and flavonoids; despite, the seminiferous tubules appear to have severely degenerated germinal epithelium in negative control group by free radical scavenging activity [14].

## 5. CONCLUSION

This study indicated that *Morinda lucida* has a prophylactic effect against tramadol-induced testicular damage.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT AND ETHICAL APPROVAL

As per university standard guideline, participant consent and ethical approval have been collected and preserved by the authors

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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