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ANTIFERTILITY PROPERTIES OF DEXTROMETHORPHAN ON THE FEMALE REPRODUCTIVE SYSTEM

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ABSTRACT

Female infertility is a well-known health issue over the world including Africa; it presents a particularly vexing clinical problem and contributes to infertility among female through the use and or abuse of medications. Dextromethorphan (DM) is a dextro-rotatory isomer of levophanol and a major constituent of over 125 over the counter (OTC) cough syrups. There is a wide spread abuse of Cough syrups in Nigeria particularly amongst adolescent females, which have been reported to have anti-fertility properties. The availability of OTC drugs is facilitated by pharmacies and increasing number of convenience stores and most recently the internet. The aim of this study is to determine the effect of (DM) on female reproductive system using Sprague-Dawley rats as models Twenty rats were used and grouped into four, designated as A-D (155±20g; N=20, A-D). Group A served as control that received 0ml of DM, Groups B-D received 20, 40 and 80 mg/kg respectively for 28 days. At the end of treatment period, the animals were euthanized, the organs (Ovaries and Uteri) were harvested for histology and oxidative stress markers. Blood was collected and stored for hormonal milieu. Results showed increase in MDA and decrease in SOD, CAT and GSH values of both uteri and ovaries when treatment groups were compared to control. Sections showed sloughed epithelial cells with moderate infiltration of the endometrial stroma by inflammatory cells in the uterine and there was decrease in hormonal parameters checked among the treatment groups This study suggests that DM has a deleterious effect on the reproductive hormones, as well as cause damages to ovaries and uterus in females and in-turn lead to infecundity.

Key words: Dextromethorphan, Hormonal milieu, Oxidative stress, Uteri, Ovaries

Introduction

Infertility in humans is defined as the inability to become pregnant after one year of frequent intercourse (3-5 times per week) between a male and female partner without the use of contraception (Chowdhury *et al*., 2017). About 40% of cases are caused by female factors, 30% are caused by male causes, 20% are caused by a combination of both, and 10% are caused by unknown variables (Cedars and Jaffe, 2005). The medicalization of infertility has unknowingly led to a disregard for couples' emotional reactions, which include distress, loss of control, and stigmatization. In addition, in many cultures, failure to conceive is viewed as a form of rejection, which can lead to a great deal of worry and depression (Domar and Cousineau, 2007).

Dextromethorphan (DM) is a dextro-rotatory isomer of levophanol and a major constituent of over 125 over the counter (OTC) cough syrups (Biose *et al.,* 2013). *In-vivo* and *ex-vivo* studies have shown that DM has pain suppressive properties comparable to ketamine via its N-methyl-D-Aspartate (NMDA) receptor antagonism. There is a wide spread abuse of Cough syrups in Nigeria particularly amongst adolescent females, which have been reported to have anti-fertility properties (Biose *et al.,* 2013). The availability of OTC drugs in many countries is facilitated by pharmacies and increasing number of outlets such as supermarkets, convenience stores and most recently the internet (Fittler *et al.,* 2022; Kumar, 2022; Hoek *et al.,* 2023). Reports have shown episodic and sporadic abuse of DM in several countries particularly, the United States of America, Canada, Denmark and most part of Asia (Murray and Breweton, 1993; Wolfe and Caravati, 1995; Darboe *et al.,* 1996; Shek, 2012). The incidence of infertility is a problem affecting couples. Childlessness is like a chronic illness that uses up a large amount of a couple’s resources emotional and financial and involves the expenditure of a considerable amount of time, money and physical and emotional energy. Childlessness may bring our feelings of resentment, of guilt, and of despair. Almost all the couples expect to have their own babies, once they get

married. But getting pregnant is like a game of odds, or game of luck. It is impossible to predict when an individual couple will succeed in achieving pregnancy.

Approximately 15% of couples are infertile. Of this 15%, male infertility counts for approximately 20% of the cases. Female infertility accounts for up to 70% of these cases, largely due to the very complex processes involved in the female reproductive system (Sharma *et al.,* 2010). Female infertility is a common contributor to difficulties in producing children. At least half of all couples consulting for infertility will involve a female partner with a "problem". In the old days, the female partner used to bear the brunt of blame and only about 5% of couples seeking help with having a baby were thought to be due to a male infertility (Sharma *et al.,* 2010).

It has been recorded that drug abuse is a common phenomenon in the average society in Nigeria. It has also been noted that parents, peer groups, and society at large contribute to the alarming rate of drug abuse in the society. Sequel to the increase in the number of female infertility and most especially the high percentage of unknown causes of female infertility over the years in Nigeria, there is the need to investigate the causes of infertility in females. Therefore, this study attempts to investigate the effect of Dextromethorphan on the female reproductive structures and functions using Sprague-Dawley rat as the experimental model.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

A total of twenty (20) adult female Sprague-Dawley rats were used for this study, weighing 155±20g. They were procured and kept in the animal house of Anatomy program of Bowen University, Iwo campus, Nigeria, and were identified by a Zoologist. They were kept in industrial cages at room temperature and allowed to acclimatize to their new environment before

the experiment commenced. They had access to feed and water *ad libitum* throughout the experimentation. Moderate constant environmental conditions were maintained with proper aeration and a good source of light. The weighing and observations were done before and after the administration of the extract, respectively. The weights of the animals were estimated at acquisition, during acclimatization and at the final part of the experiment using an electronic analytical weight balance.

PROCUREMENT AND ADMINISTRATION OF DEXTROMETHORPHAN

The pure substance of Dextromethorphan was purchased from Fairy Pharmaceuticals Ltd, China with batch number 1031404902, manufactured date March 2022, expiry date, March 2025 with the specification monograph number SPEC-3004-2.04. Upon administration, the dissolved Dextromethorphan was administered orally to the experimental animals through an oral cannula.

ANIMAL GROUPING

The twenty animals were randomly divided into four groups designated as A-D of five rats each. Group A served as control, and they received 1 mL of distilled water. Group B received 20 mg/kg of DM, group C received 40 mg/kg of DM and group D received 80 mg/kg of dextromethorphan (DM) for duration of 4 weeks. At the end of treatment period, the animals from each group were euthanized. The organs (Ovaries and Uteri) were harvested for histology and oxidative stress markers. Blood was collected and stored for hormonal milieu.

ETHICAL APPROVAL

College of Medicine's Health Ethics Committee, Bowen University Teaching Hospital, Ogbomosho, Oyo State Nigeria, approved all experimental methods and procedures as long we strictly adhered to the university's guidelines for conducting research using animals (BUREC/24/16/142).

Blood Sampling and Hormonal Assay

Blood was collected from ocular sinuses of the eye using capillary tubes and left to clot for separating the serum after centrifugation at 3000 rpm for 10 minutes. The sera were kept in a freezer at -80◦C for hormonal assay was performed. Estrogen, Progesterone, Luteinising (LH), and Follicle-Stimulating Hormones (FSH) were measured as described by our previous study (Adebajo *et al.,* 2022)

Organ homogenate processes for antioxidant parameters

The ovaries and uteri were washed in ice cold 1.15 % KCl solution, blotted and weighed. They were then homogenized with 0.1 M phosphate buffer (pH 7.2). The tissues were introduced into mortar and laboratory sand was then added. This was crushed using a pestle. The resulting homogenate was centrifuged at 2500 rpm speed for 15 mins. Thereafter, it was removed from the centrifuge and the supernatant was decanted and stored at -20 °C until analysis. Superoxide Dismutase (SOD) was assayed by its ability to inhibit the autooxidation of epinephrine, determined by the increase in absorbance at 480 nm. The enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min. Catalase (CAT) Catalase was assayed colorimetrically at 620 nm and expressed as μmoles of H2O2 consumed/min/mg/protein. Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Buege and Aust. The

supernatant was removed, and the absorbance was read at 532 nm. MDA was calculated using the molar extinction coefficient for MDA thiobarbituric acid (TBA) - complex of 1.56 × 105 M-1cm-1.

STATISTICAL ANALYSIS

Statistical analysis was done using GraphPad software version 9.5 for windows; numerical data obtained from the experiment were expressed as mean ± SEM (standard error of mean). The differences were compared for statistical significance using One Way Analysis of Variance (ANOVA) and Post Hoc Tukey's tests. Differences were considered significant at $p < 0.05$.

RESULTS

EFFECT OF ORALLY ADMINISTERED DEXTROMETHORPHAN ON THE BODY WEIGHT OF ADULT FEMALE SPRAGUE-DAWLEY RATS

A dose dependent decrease in body weight was recorded in the percentage body weight among the treatment groups (Table 1)

Table 1: **EFFECT OF ORALLY ADMINISTERED DEXTROMETHORPHAN ON THE BODY WEIGHT OF ADULT FEMALE SPRAGUE-DAWLEY RATS**

GROUP	BEFORE ADMINISTRATION	AFTER ADMINISTRATION	% WEIGHT DIFFERENCE
CONTROL (0 mg/kg)	139.2±4.641	154.2±5.463*	10.77%
LOW DOSE (20 mg/kg)	169.6±3.203	159.8±3.308	5.77%
MEDIUM DOSE (40 mg/kg)	163.6±3.311	158.4±3.444	3.17%
HIGH DOSE (100 mg/kg)	168.6±4.308	160.8±4.954	4.63%

Values are mean ± standard error of mean; n=5, *p<0.05.

EFFECT OF ORALLY ADMINISTERED DEXTROMETHORPHAN ON HORMONAL MILIEU OF ADULT FEMALE SPRAGUE-DAWLEY RATS

A dose-dependent decrease was recorded when treatment was compared to control in the values of hormonal parameters checked. When medium and high doses were compared to low dose, significant decrease in values was recorded. Similar significant decrease was noted when high dose was compared to medium dose (Figure 1a-d).

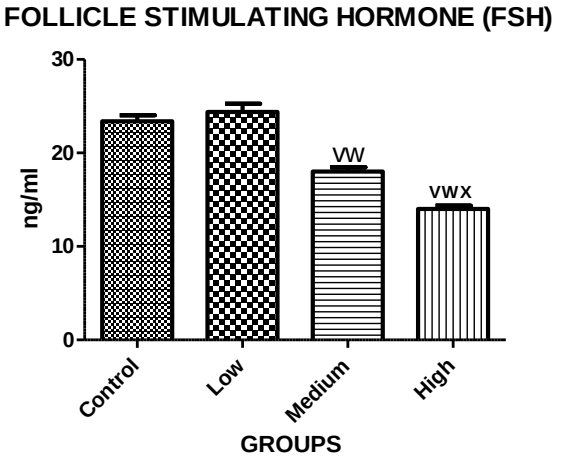


Figure 1a

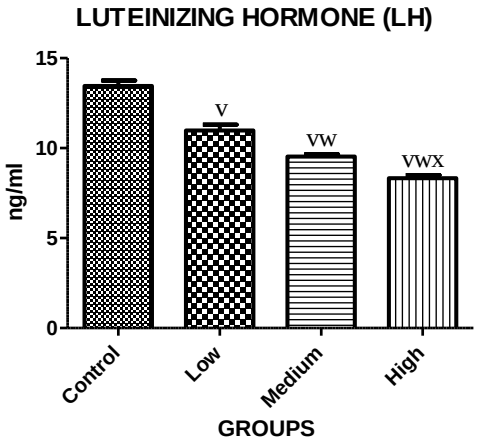


Figure 1b

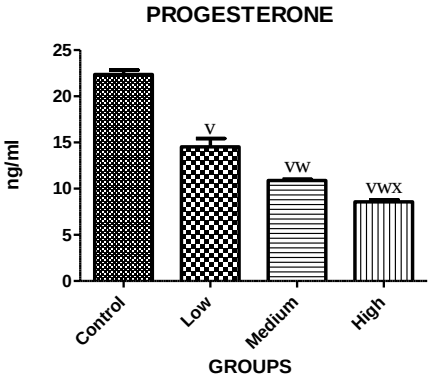


Figure 1c

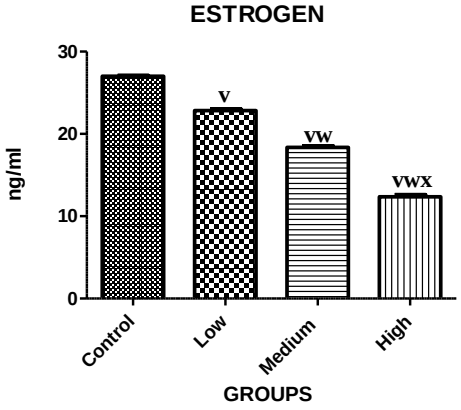


Figure 1d

Values are expressed on Mean ± Standard Error of Mean (SEM). $^{V}P<0.05$ significant compared to control, $^{W}P<0.05$ significant compared to Low dose, $^{X}P<0.05$ significant compared to medium dose.

EFFECT OF ORALLY ADMINISTERED DEXTROMETHORPHAN ON OVARIAN OXIDATIVE STRESS MARKERS IN ADULT FEMALE SPRAGUE-DAWLEY RATS

In the value of MDA, significant decrease was recorded when treatment groups were compared control, similar decrease was noted when medium and high doses were compared to low dose and when high dose was compared to medium (Table 2a). Dose-dependent decrease was noted when treatment groups were compared to control and similar pattern of decrease was seen when medium and high doses were compared to low dose and when high dose was compared to medium (Table 2b-d).

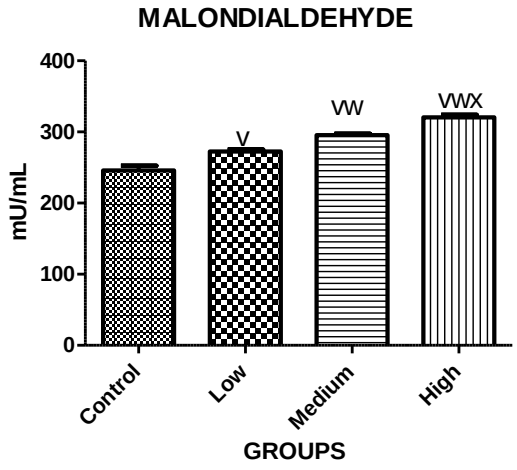


Figure 2a

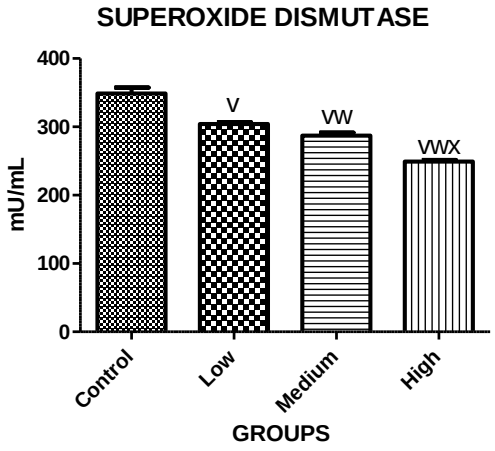
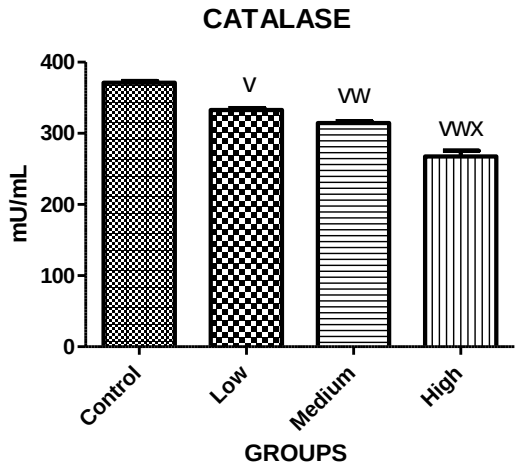
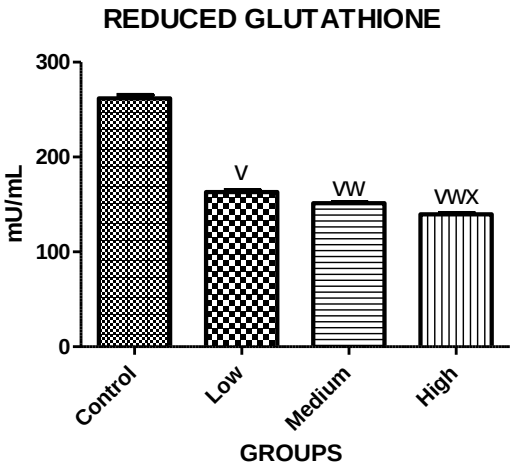
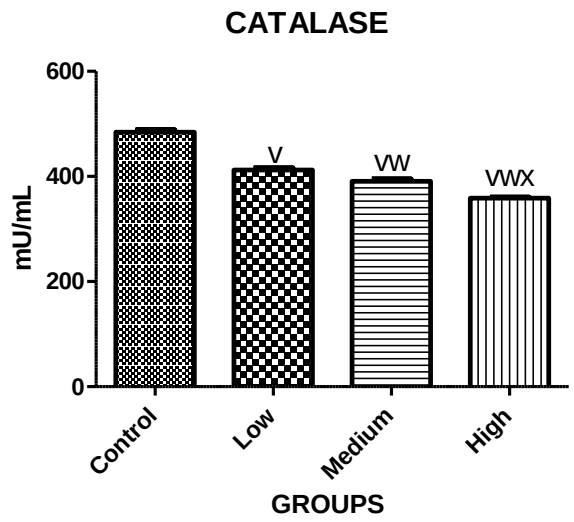


Figure 2b







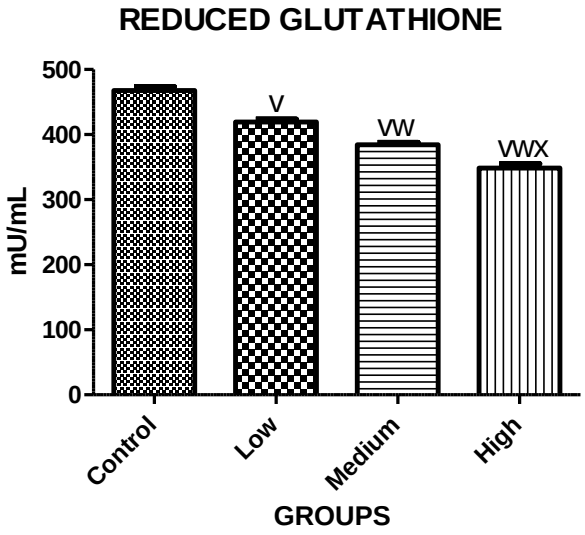


Figure 2c

Figure 2d

Values are expressed on Mean ± Standard Error of Mean (SEM). $^{V}P<0.05$ significant compared to control, $^{W}P<0.05$ significant compared to Low dose, $^{X}P<0.05$ significant compared to medium dose.

EFFECT OF ORALLY ADMINISTERED DEXTROMETHORPHAN ON UTERINE OXIDATIVE STRESS MARKERS IN ADULT FEMALE SPRAGUE-DAWLEY RATS

In the value of MDA, significant decrease was recorded when treatment groups were compared control, similar decrease was noted when medium and high doses were compared to low dose and when high dose was compared to medium (Table 3a). Dose-dependent decrease was noted when treatment groups were compared to control and similar pattern of decrease was seen when medium and high doses were compared to low dose and when high dose was compared to medium (Table 3b-d).

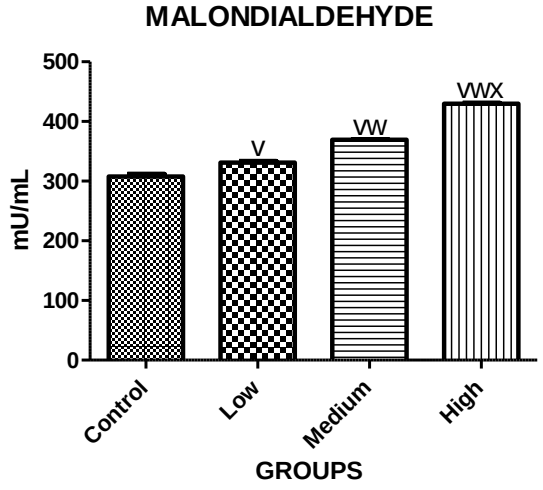


Figure 3a

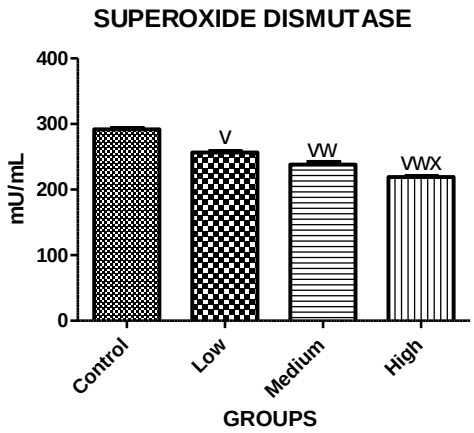


Figure 3b

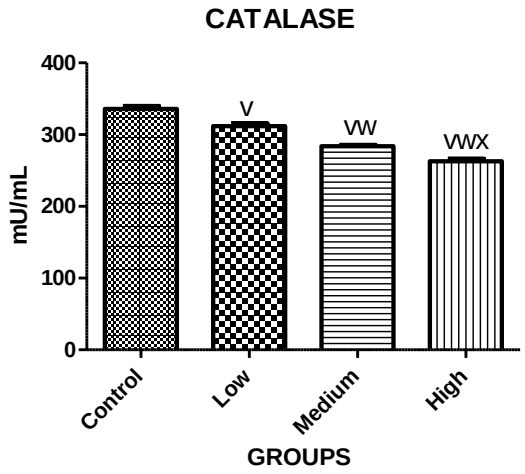


Figure 3c

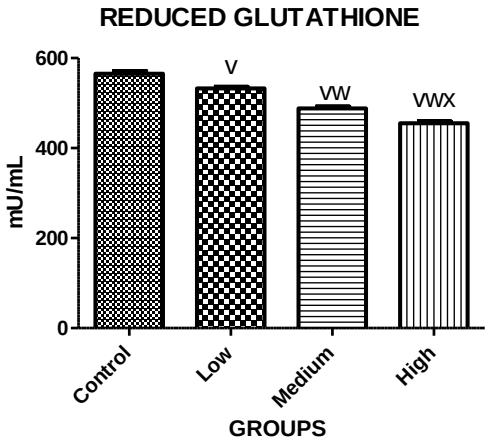
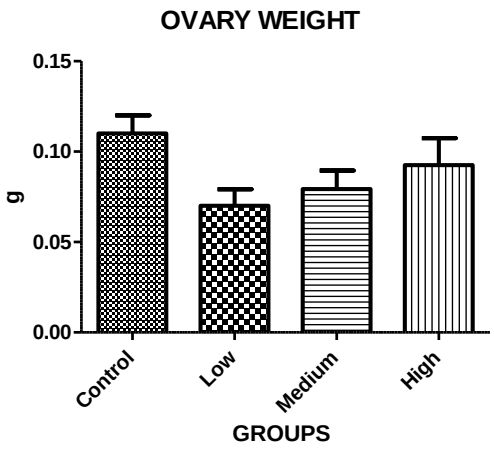


Figure 3d

Values are expressed on Mean ± Standard Error of Mean (SEM). $^{V}P<0.05$ significant compared to control, $^{W}P<0.05$ significant compared to Low dose, $^{X}P<0.05$ significant compared to medium dose.

EFFECT OF ORALLY ADMINISTERED DEXTROMETHORPHAN ON OVARIAN AND UTERINE WEIGHTS IN ADULT SPRAGUE-DAWLEY RATS

In the value of ovarian and uterine weight, dose-dependent decrease was noted when treatment groups were compared to control (Figure 4a-b)



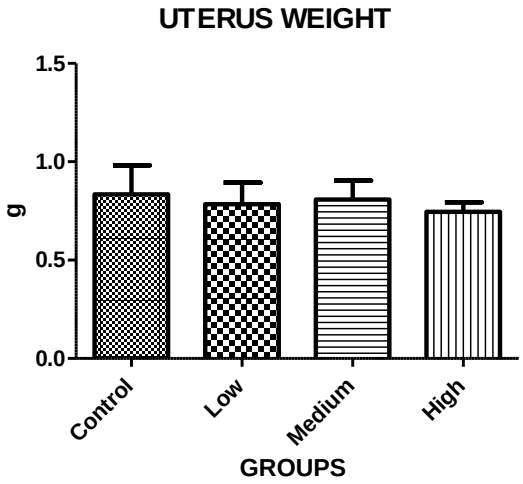
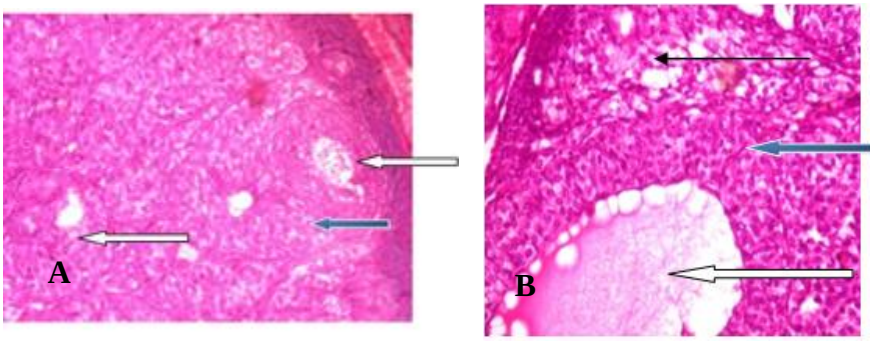
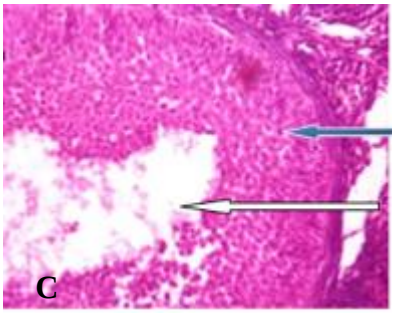


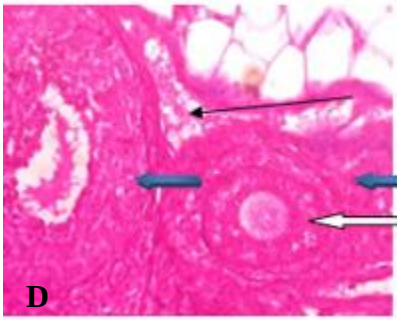
Figure 4a Figure 4b

Values are expressed on Mean ± Standard Error of Mean (SEM). $^{V}P<0.05$ significant compared to control, $^{W}P<0.05$ significant compared to Low dose, $^{X}P<0.05$ significant compared to medium dose.

EFFECT OF ORALLY ADMINISTERED DEXTROMETHORPHAN ON OVARIAN CYTOARCHITECTURE IN ADULT SPRAGUE-DAWLEY RATS

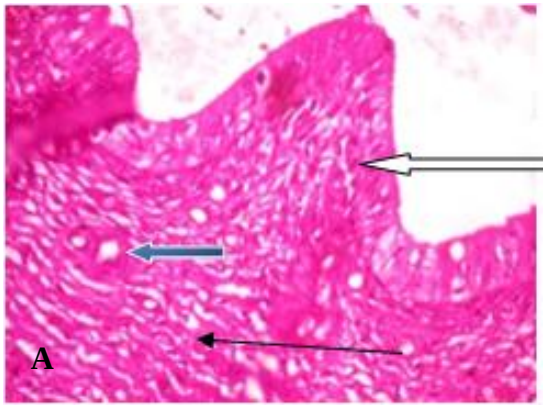


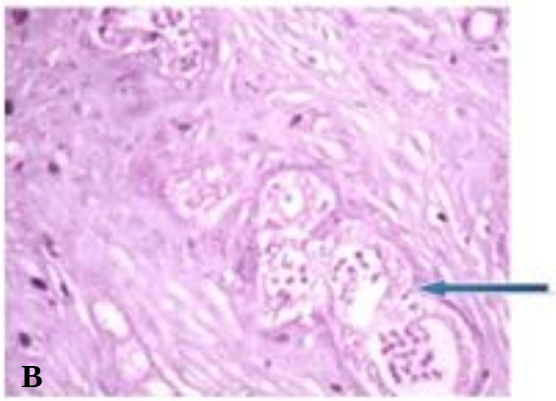


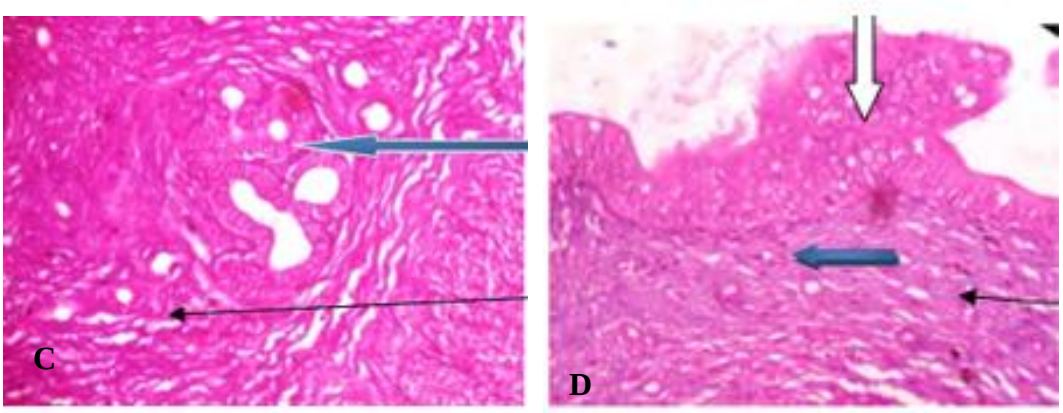


Photomicrographs of A: showing normal antral follicle (white arrow) with normal theca cells (blue arrow) within the ovarian cortex.The ovarian stroma show normal connective tissues and Luteinized stromal cells; B: showing normal antral follicle and graafian follicle (white arrow) with normal theca cells (blue arrow) within the ovarian cortex.The ovarian stroma show normal connective tissues and Luteinized stromal cells (slender arrow); C: showing some normal antral follicle (white arrow) with normal theca cells (blue arrow) within the ovarian cortex; D: showing normal antral follicle (white arrow) with normal theca cells (blue arrow) within the ovarian cortex, the ovarian stroma show normal connective tissues and Luteinized stromal cells (slender arrow).

EFFECT OF ORALLY ADMINISTERED DEXTROMETHORPHAN ON UTERINE CYTOARCHITECTURE IN ADULT SPRAGUE-DAWLEY RATS







Photomicrographs of A: showing normal showing normal endometrium epithelial layer (white arrow), there are normal endometrial gland (blue arrow); B: showing normal endometrium epithelial layer, there is mild proliferation of endometrial gland seen (blue arrow) with sloughed epithelial cells, there is moderate infiltration of the endometrial stroma by inflammatory cells; C: showing mildly thickened endometrium epithelial layer, there is mild proliferation of endometrial gland seen (blue arrow), there is moderate infiltration of the endometrial stroma by inflammatory cells (slender arrow); D: showing thickened endometrium epithelial layer (white arrow), there are normal endometrial gland seen (blue arrow), there is moderate infiltration of the endometrial stroma by inflammatory cells (slender arrow).

DISCUSSION

Certain drugs have been observed to disrupt the normal level of hormones, malfunction organs, affect pregnancy and fetal structure especially when overdosed. Infertility caused by drug-induced damage, whether permanent or transitory, is a significant clinical issue. Many commonly used medications can be harmful to the gonads as studied by Fody *et al.,* (1985). This study investigated the possible effect of Dextromethorphan on the ovaries and uteruses of adult Sprague-Dawley rats, as well as, hormonal and oxidative stress markers level. Non-opioid antitussive drugs like Dextromethorphan can temporarily relieve cough caused by the common cold, the flu, or other conditions (Kaci, 2021). However, depending on the amount or the

components of the exact formulation that was ingested, the medicine might cause a variety of side effects (Boyer *et al.*,2004).

Dextromethorphan showed a significant decrease in the four hormones (Follicle stimulating hormone (FSH), luteinizing hormone (LH), Estrogen and Progesterone) when treatment groups were compared to the control group in experimental rats. The significant increase observed in the level of MDA in the ovaries and uteruses of treatment groups both the pregnant and non-pregnant Wistar rats, with significant reduction in levels of SOD, GSH and CAT may be a clear indication that Dextromethorphan induced oxidative stress on the ovaries and uterus (Sharma and Agarwal, 1996; Sikka, 2001; Dandekar *et al*., 2002; Armagen *et al*., 2006). A decrease in CAT activity could be attributed to an increase in Malondialdehyde, a lipid peroxidation product that can form cross linkages and inactivate numerous membrane-bound enzymes (Brigelius, 1999). The decrease in cellular enzymes SOD and non- enzymatic antioxidant GSH observed in this study for both the pregnant and non-pregnant Wistar rats of the ovaries and uteruses is clearly indicative of oxidative stress (Kapoor *et al.,* 2011). Similar observations were made by Aly *et al*., 2009; Khan and Dawood, 2009, and Grosicka-Macia *et al*., 2011. It is self-evident that CAT and SOD form a mutually beneficial defense team. Dextromethorphan generated significant oxidative stress, which may fail the body's inbuilt mechanisms and cause ovarian damage, according to the current study's antioxidant enzyme activity and decreased GSH level in the ovary. Reduced SOD, CAT, and GSH activities in the current study could potentially be related (Kapoor *et al.,* 2011).

The result of this study concurs with the study of (Parlaktas *et al*., 2007), that NMDA receptor antagonists enhanced oxidative stress levels in rats, according to the authors. However, the current study contradicts that of (Topsakal *et al*., 2002), who found that Dextromethorphan has immediate antioxidant action in rat erythrocytes following spinal cord tissue injury. Histological

plates in this study may also revealed harmful effects of Dextromethorphan in causing congestion in the lumen of the uterus, as well as vascular congestion in the ovaries. This may indicate pelvic congestion syndrome as stated in a study by Ignacio *et al.,* (2008).

CONCLUSION

This study suggests that Dextromethorphan has a deleterious effect on the reproductive hormones, as well as cause damages to ovaries and uterus in females and in-turn lead to infecundity.

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