

Effects of tramadol on the reproductive function of wistar albino rats

Heba Atef El-Ghawet

Zoology Dept, Faculty of Science, Mansoura University, Egypt

ABSTRACT

Tramadol is a centrally active synthetic opioid analgesic that is used extensively. There is no lot of information about the reproductive effects of the drug in both male and females. The present work conducted to outline the histopathological changes in ovaries and uterus of females and testis of male Wistar rats. Forty rats (*Rattus norvegicus*) weighing approximately 150 g were used in the present study. Animals were divided into two groups; control male and females (n=20) and tramadol-treatment male and female group (n=20). Applied tramadol doses (40 mg /Kg body weight) were daily orally administered for 30 days. Doses calculated for animals using Paget and Barnes (1964) species introversion table of dosage. At the end of treatment, the animals were sacrificed and their ovary, uterus and testis were incised and processed for histological investigation and flow cytometric analysis of apoptosis. The present work revealed ovarian failure and vanishment of majority of the follicles which become replaced by atretic and cystic ones. There is a massive deterioration of endometrial gland with the uterine tube. The testis possessed disorganization of the seminiferous tubules with almost missing of sperm and comparatively decreased spermatogenic cells. Flowcytometric analysis showed apparent increase of apoptotic M1 spermatogenic and ovarian cells. The author concluded that tramadol reduced the fertility of both male and females.

Key words: Tramadol, Testis, Ovary, Flowcytometry. There is no conflict interest.

INTRODUCTION

Tramadol is a centrally active synthetic opioid analgesic that is used extensively. Its mode of action is not completely understood, but two acceptable complementary mechanisms are binding to mu opioid receptors (MOR) and inhibition of reuptake of noradrenaline and serotonin [1]. The drug exerted hypoglycaemia in users [2] which influence growth hormone (GH) secretion suggesting that their GH deficiency has a hypothalamic rather than pituitary origin[3]. From literature, multiple cases of toxicity and abuse of tramadol have been reported. The main symptoms of tramadol toxicity include central nervous system depression, nausea and vomiting, tachycardia, and seizures [4,5]. Fatal cases have been reported as a result of tramadol overdose. In those instances, death has been attributed to cardiopulmonary arrest and hepatic failure [6-8] as well as hypoglycemia [9].

The tramadol absorption is more than morphine reached to about 95-100% and absorbed rapidly in the small intestine. and reached highest peak after 5 hours [10]. It is widely distributed throughout the body, especially liver and kidneys [11]. There is no work concerning the reproduction function.

The present work was conducted to assess the reproductive toxicities of the drug on ovaries, uterus and testis.

MATERIALS AND METHODS

Drug and applied dose-treatment:

Tramal (Tramadol HCl), 50 mg capsules, was obtained from Mina- Pharm, Egypt. Its chemical name is (+) cis-2-[(dimethylamino)methyl]-1-(3-m ethoxyph-enyl) cyclohexanol hydrochloride. According to Matthiesen et al. [12], the LD₅₀ values of oral administration were estimated to be around 300–350 mg/kg body weight for (rat and mouse).The therapeutic dose (40 mg/kg body weight) of this drug for rat was calculated according to Paget and Barnes [13]. The chosen dose was nearly comparable to the human effective therapeutic dose (ETD).

Experimental work:

Forty rats (*Rattus norvegicus*) weighing approximately 150 g were used in the present study. All rats were kept under good ventilation and aerated room. Excess standard diet was supplied *ad libitum* during the experimental period. They were allowed free access to water. Animals were divided into four groups. Animals were divided into two groups; control male and females (n=20) and tramadol-treatment male and female group (n=20). Applied tramadol HCl doses (40 mg /Kg body weight) were suspended in saline solution and daily orally administered for 30 days. HCl suspended in saline solution equal to 40 mg /Kg body weight for 30 days. At the end of treatment, the animals was sacrificed and their ovaries, uterus and testis were incised. Part of the tissues were kept in refrigerator and the other ones were fixed in 10% phosphate buffered formalin (pH 7.4)for routine histological investigation.

Histological investigations: Testis and ovaries of both control and experimental groups were incised immediately, fixed in 10% phosphate buffered formalin (pH 7.4), dehydrated in ascending grades of ethyl alcohol, cleared in xylene and mounted in molten pararplast 58–62 °C. Serial 5 µm thick sections were cut and stained with Haematoxylin and eosin (H&E), examined under bright field light microscopy, and photographed.

Flow cytometric analysis of apoptosis :

DNA ploidy and apoptosis were analyzed using fluorescence activated cell sorting (FACS) flow cytometer (Becton Dickinson, Sunnyvale, CA) equipped with a 15 mW air-cooled 488 nm argon-ion laser. FL1 (FITC) signals were detected through a 530/30 nm band-pass filter; FL2 (PI) signals were detected through a 585/42 nm band-pass filter. A total of 20 000 events were recorded in list mode and analyzed using the Cell Quest Pro software (Becton Dickinson) at Mansoura University Hospital. The cell populations were gated assuming the linear forward scatter (FSC) and side scatter (SSC) properties. Biopsies from testis and ovaries of studied animals were taken, and cell suspension was prepared with Tris-EDTA buffer (pH 74) (Sigma-Aldrich Co.). Cell suspension was fixed in ice-cold 96–100% ethanol (Sigma) at 4 °C overnight, centrifuged at 1500 rpm for 10 min, and then resuspended in PBS containing 50 µg/mL propidium iodide (PI) (Sigma-Aldrich Co.). The cells were incubated at 37 °C for 30 min before analysis by flow cytometry. PI fluorescence excitation at 512 nm, with a relatively large Stokes shift,emits at a maximum wavelength of 617 nm. Apoptosis was indicated by the percentage of cells in G₀/G₁, S, and G₂/M phases of the cell cycle.

RESULTS

1. Histological Observations:

Ovary: The control ovary is a pearl-shaped-structure, covered by a thick connective tissue capsule - the tunica albuginea ensheathed by a simple squamous mesothelium called the germinal epithelium. The ovary has a cortex containing the developing follicles, and a highly vascular medulla with closely-packed, spindle-shaped fibroblasts. The cortex exhibited developing oocytes, including primordial, primary, secondary and mature ova. The growing follicles are characterized by their surrounding of granulosa cells. The mature follicles are ensheathed with multilayered granulosa cells and antral cavity (Fig.1 A-D).

In experimental tramadol-treatment, there was a detected damage of the growing follicles. Many of the mature follicles appeared atretic in the form of cystic-like structure. Atretic follicles possessed granulosa cells with the apparent increase of pyknosis and deformed antral cavity. The stroma showed the abundant increase of fibroblast activity (Fig. 2A-D).

Uterus:

In control, the uterus is composed of two main regions; the outer myometrium and inner one is the endometrium. The uterus outlined by a simple columnar epithelium (ciliated cells and secretory cells) and an underlying thick connective tissue stroma containing tortuous secretory endometrial glands. The glands extend through the entire thickness of the stroma. The stromal cells of the endometrium are embedded in a network of reticular fibers (Fig.3 A-B).

Testis:

The control testis reveals the typical features of normal structure. The seminiferous tubules showed typical arrangement of the spermatogenic cells, including spermatogonia, spermatocytes and spermatids as well as grouping spermatozoa. Sertoli cells are relatively few and distributed at fairly regular intervals. Thin connective tissue was seen in between the tubules (Fig.4A-B).

In tramadol-treatment, the testis showed focal disorganization of seminiferous tubules with marked depletion of the spermatogenic cell populations. Many of the seminiferous tubules lacked sperm, spermatids and secondary spermatocytes. Exfoliation of the damaged spermatocytes and spermatids were detected within the tubular lumina of many seminiferous tubules. The intertubular connective tissue become hyalinized and showed comparative reduction of interstitial cells (Fig. 5 A-D).

3. Cell cycle of ovary and testis:

From Table (1) and Fig (6), there was a considerable increase of M1 (subG1 apoptosis) and a decrease in the other cell cycle phases (M2,M3, and M4).

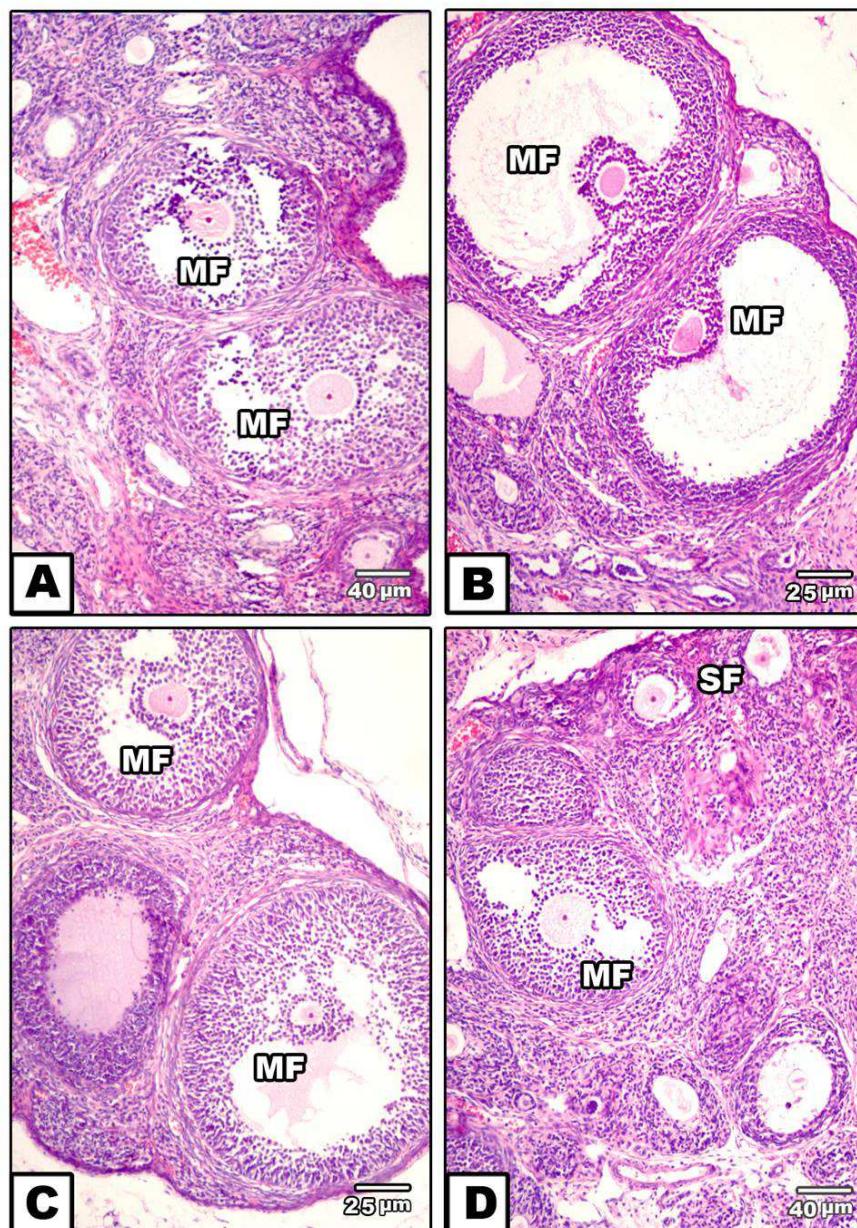


Fig.1(A-D). photomicrographs of histological sections of control ovary of rat showing different phases of growing secondary (SF) and mature (MF) ovarian follicles. HX-E

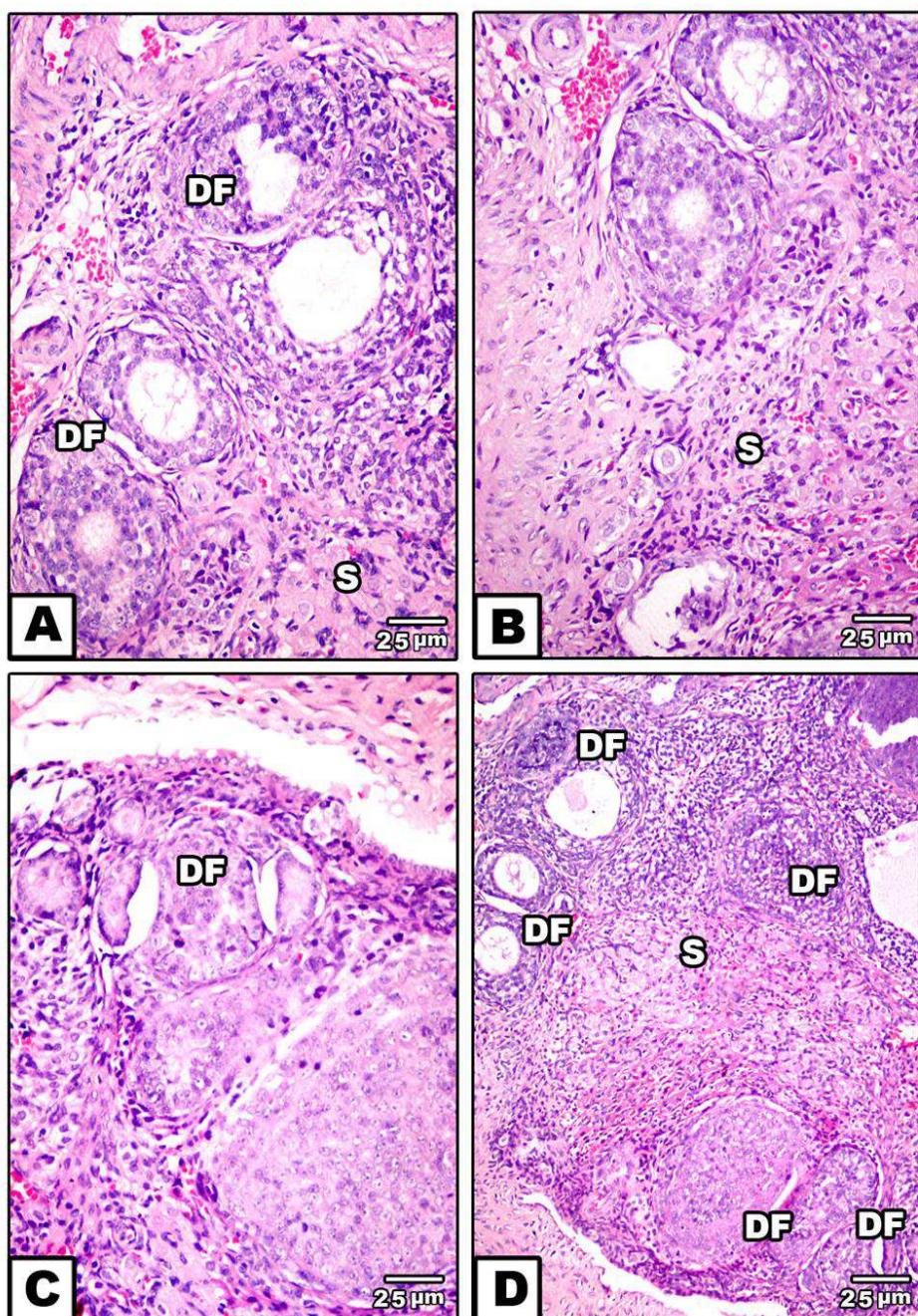


Fig.2(A-D). photomicrographs of histiological sections of tramadol-treated ovary of rat showing degenerated ovarian follicles and increased cellularity of ovarian stroma. Abbreviation; DF, degenerated follicles, S, stroma. H&E

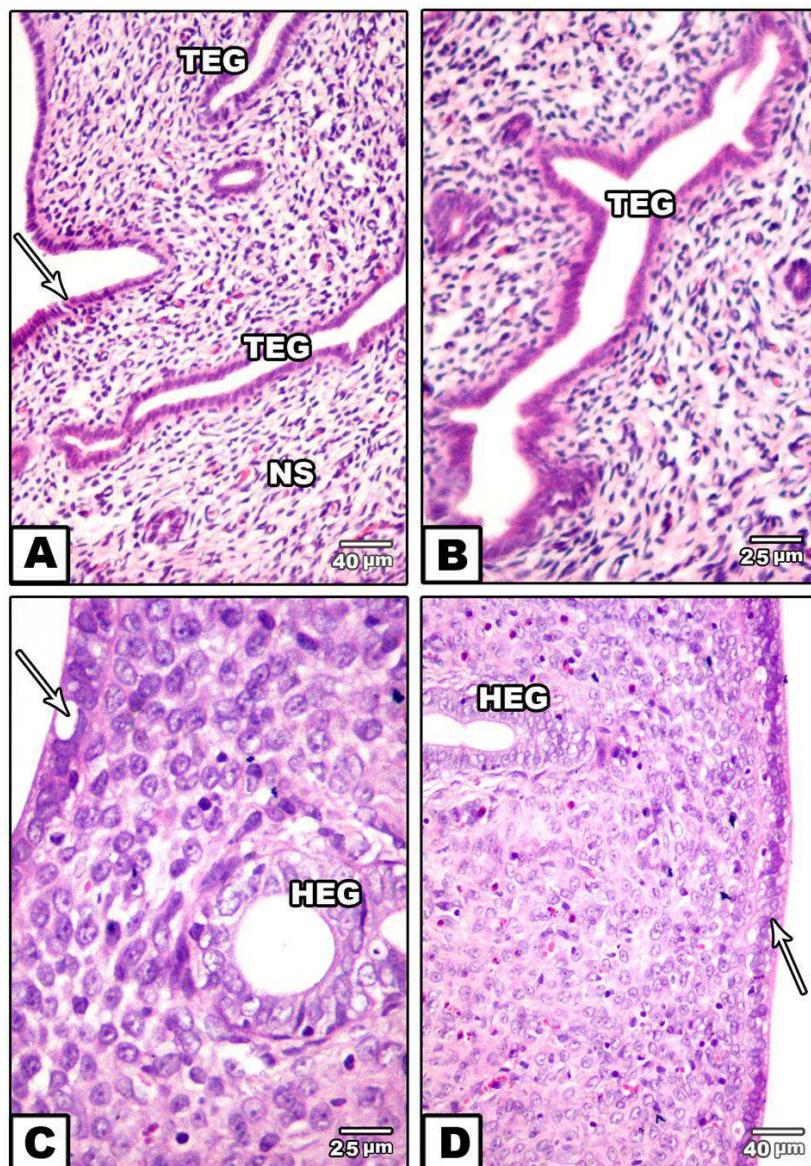


Fig.3(A-D). photomicrographs of histiological sections of uterus. A&B. Control uterus showing normal lining endometrial lining cell, tortuous endometrial glands, and normal stroma. C&D. Tramadol-treatment showing atrophied endometrial lining layer and hypertrophied endometrial glands (HEG). Note increased cellularity of endometrium. HX-E

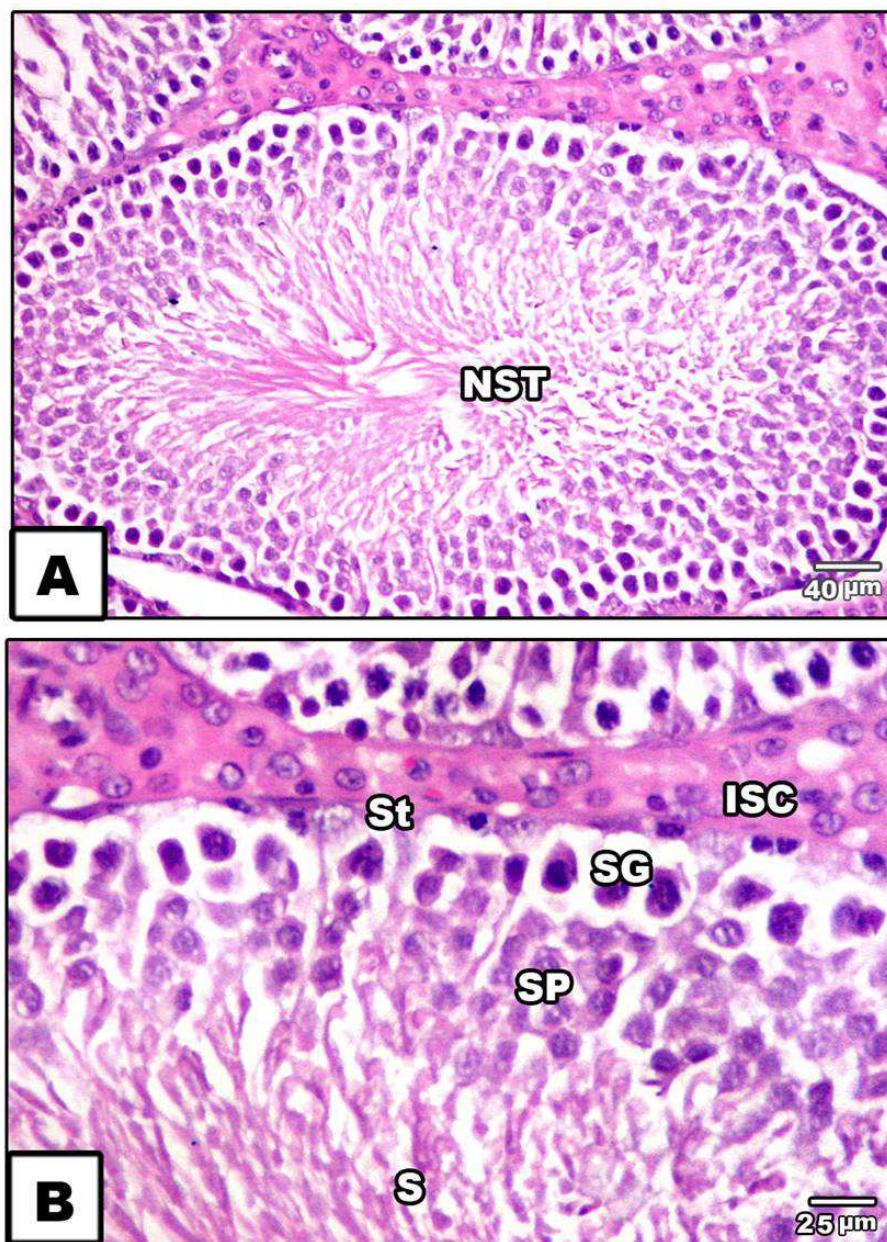


Fig.4(A-B). photomicrographs of histological sections of control testis showing normal seminiferous tubules (NST) with normal arrangement pattern of spermatogenic cells (Sertoli cell, St; Spermatogonia, SG, Spermatocytes, SP and sperm, S) . The intertubular tissue appears rich of interstitial cells (ISC). HX-E

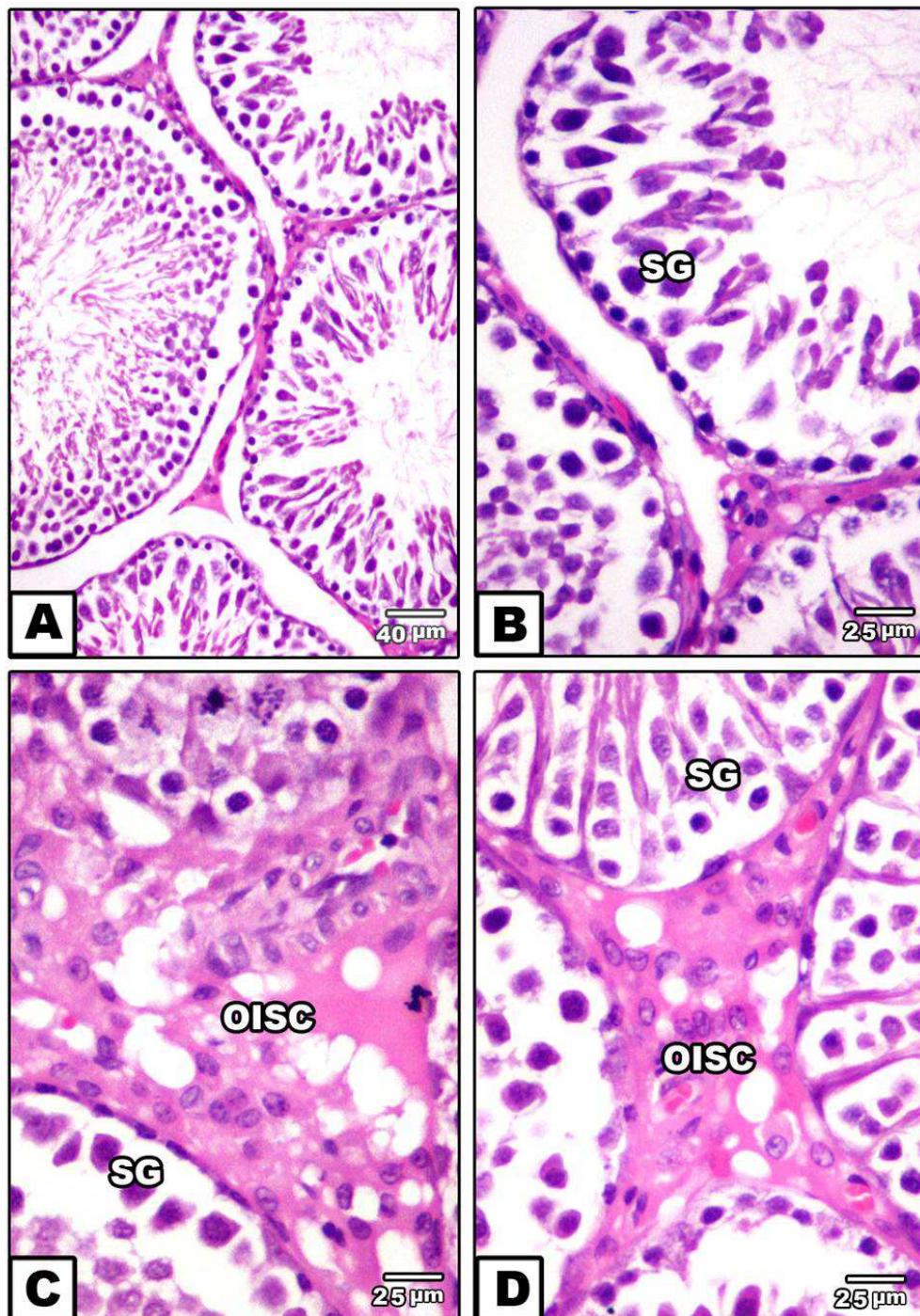


Fig.5(A-D). photomicrographs of histological sections of tramadol-treated testis . A&B. Showing inactive seminiferous tubules with comparative reduction of spermatogenic cells(SG) and decreased germ cell height. C&D. Showing edematous intertubular tissue containing degenerated interstitial cells (OISC, edematous interstitial cells). HX-E

Table (1). Flowcytometry of cell cycle of tramadol-treated testis and ovary of rat
Each replicate represent the $M \pm SE$. $n=5$. Significance at $*P < 0.05$.

| | Testis | | Ovary | |
|--|-----------------|---------------------|-----------------|---------------------|
| | Control | Tramadol -Treatment | Control | Tramadol -Treatment |
| M₁ (sub-G₀/G₁ apoptosis) | 10.6±0.8 | 37.7±4.2 | 1.7±0.1 | 4.3±0.4 |
| M₂ (G₀/1 phase) | 34.1±5.6 | 21.5±2.8 | 76.7±8.7 | 80.4±9.4 |
| M₃ (S phase) | 36.7±2.8 | 23.6±2.1 | 13.4±2.6 | 11.9±0.8 |
| M₄ (G₂/M phase) | 7.13±0.9 | 6.1±0.8 | 4.3±0.2 | 2.7±0.2 |

Each replicate represent the $M \pm SE$. $n=5$. Significance at $*P < 0.05$.

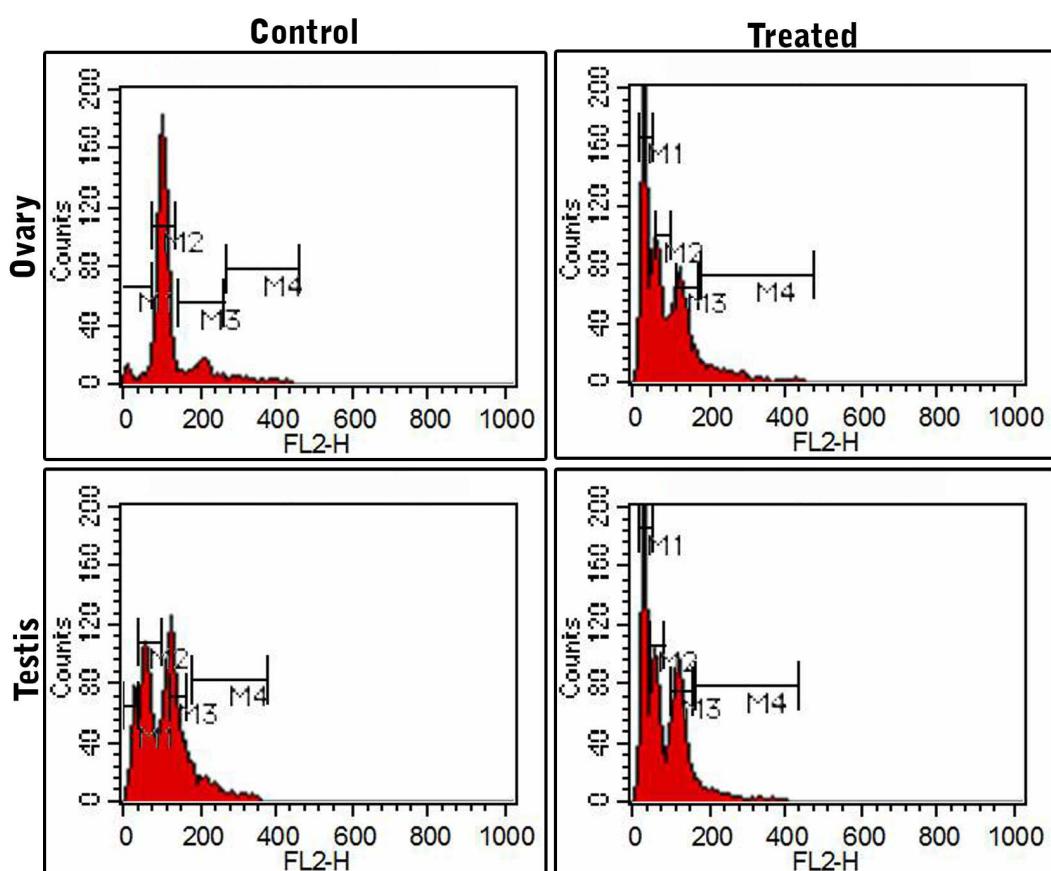


Fig. 6. Flowcytometry of tramadol-treated ovary and testis showing increase incidence of apoptosis of M1 phase

DISCUSSION

The wide use of narcotic drugs among young people's especially tramadol led me to run out this work to illustrate the dramatic effects in the male and female reproductive organs. The observed findings revealed marked deterioration of ovarian follicles and increased average of atretic and cystic follicles as well as of corpora like structure. The ovarian stroma showed marked increase of hypercellularity. This depletion was found to be associated with impaired ovarian function as measured by the number of oocytes ovulated and the duration of the subsequent estrous cycle. The ovarian dysfunction was confirmed by massive atrophy and decreased secretory function of the endometrial glands. As a result of least information about tramadol on ovarian and uterine function. The observed ovarian damage was supported by flowcytometric analysis which indicated increased average of apoptosis.

The present work agree with recent studies carried out by Ahmed and Kurkar [14] who reported that rats received subcutaneous injections of tramadol (40mg/kg body weight) three time per week for 8 weeks was found to reduced plasma levels of luteinizing hormone, follicle-stimulating hormone. Similar drugs such as administration of opioid antagonist naloxone to female rats was found to cause reproductive failure [15]. Clonidine and yohimbine drugs were found to decrease estradiol and increase the bulk of cysts and corpus lutea [16]. Thienorphine was a partial opioid agonist with long-lasting antinociceptive was found to induce a concentration-dependent decrease in the frequency and amplitude of the contraction on the isolated oestrus and pregnant uterine strips [17]. Tramadol caused a concentration-dependent inhibition of potassium chloride -induced myometrial contractility [18]. Estradiol was found to increase cell density and stimulate of cell proliferation. However, the opioid-induced inhibition of uterine cell proliferation which was mediated mainly by the mu opiate receptor [19].

In addition, tramadol-treatment caused focal disorganization of seminiferous tubules with marked depletion of the spermatogenic cell populations. Exfoliation of the damaged spermatocytes and spermatids were detected within the tubular lumina of many of the seminiferous tubules. The intertubular connective tissue become hyalinized and showed comparative reduction of interstitial cells. Following flowcytometric analysis , there was a marked increase of apoptic spermatogenic cells.

The observed findings supported the recent work carried out following tramadol-treatment by Ahmed and Kurkar [14] and Abdellatif et al.[120]. The authors revealed that tramadol-treatment led to a decrease of testosterone and total cholesterol and increased level of the testicular levels of nitric oxide and lipid peroxidation, and decreased the anti-oxidant enzymes activities significantly compared with the control group. These may facilitate the damage of spermatogenic cells via increase of reactive species. Also similar Cannabis is the most commonly abused drug in the world. Its administration led to disruption of the spermatogenic cells from the membranes [21].

Finally, the author concluded that chronic administration of tramadol lead reproduction dysfunction and increased average of infertility.

REFERENCES

- [1] Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL, *J Pharmacol Exp Ther* **1992**,260,275.
- [2] Grandvillain A, Jolimoy G, Authier F, Dautriche A, Duhoux F, Sgro C, *Presse Med.* **2006**,35(12, pt 1), 1842.
- [3] Tennese AA , Wevrick R, *Endocrinology*.2011, 152(3), 967.
- [4] Spiller HA, Gorman SE, Villalobos D, Benson BE, Ruskosky DR, Stancavage MM, Anderson DL, *J Toxicol Clin Toxicol.* **1997**,35,361.
- [5] Shadnia S, Soltaninejad K, Heydari K, Sasanian G, Abdollahi M, *Hum Exp Toxicol.* **2008**,27,201.
- [6] Loughrey MB, Loughrey CM, Johnston S, O'Rourke D, Forensic Sci Int. **2003**,134,332.
- [7] Garrett PM , *Anaesth Intensive Care*.**2004** ,32(4),575.
- [8] Daubin C, Quentin C, Goullié JP, Guillotin D, Lehoux P, Lepage O, Charbonneau P, *Clin Toxicol (Phila)*.**2007** ,45,961.
- [9] Mugunthan N , Davoren P, *Endocr Pract.* **2012**,18,e151.
- [10] Lintz, W, Erlaçin S, Frankus E , Uragg H, *Drug Res.*, **1981**,31, 1932.
- [11] Jellinek H, Haumer H, Grubhofer G, Klappacher, G, Jenny T , Weindlmayr-Goettel M, *Anaesthetic* **1990**, 39, 513.
- [12] Matthiesen T , Wöhrmann T, Coogan TP, Uragg H, (1998). *Toxicol. Lett.* **1998**, 95(1), 63.
- [13] Paget GE , Barnes JM, Evaluation of drug activities and pharmacometrics. Laurence D.R. and A. L. Bacharach, Eds., Academic Press, London, **1964** , .1, 135.
- [14] Ahmed MA , Kurkar A., *Clin Exp Pharmacol Physiol*.**2014**:41(4),317.
- [15] Du Toit L, Bennett NC, Gutjahr GH, Coen CW, *Physiol Behav*.**2006**,87(5),:89.
- [16] Zangeneh FZ, Mohammadi A, Ejtemaeimehr Sh, Naghizadeh MM, Fatemeh A, *Arch Gynecol Obstet*.**2011**,283(4),885.
- [17] Zhou P, Yan L, Yong Z, Yu G, Dong H, Yan H, Su R, Gong Z., *Eur J Pharmacol.* **2013**,703(1-3),83.
- [18] Shah NH, Thomas E, Jose R, Peedicayil J, *Auton Autacoid Pharmacol*.**2013**,33(1-2),1.
- [19] Környei JL, Vértes Z, Oszter A, Kovács S, Vértes M, *Eur J Pharmacol.* **1997**,336(1),65.
- [20] Abdellatif RB, Elgamal DA, Mohamed EE, *Andrologia*.,**2014**, doi: 10.1111/and.12316.
- [21] Yassa HA, Dawood Ael W, Shehata MM, Abdel-Hady RH, Aal KM, *Hum Exp Toxicol*.**2010**,29(1),37.