ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation The Toxicity Of Accumulated Dose Of Tramadol On Gonads Of Albino Rat.

Rana A. Ali[1], Mouchira M. Mohi-Eldin[2], and Heba A. Mohammed[1].

[1]Department of Zoology, Faculty of Science, South Valley University, Qena, Egypt.
[2] Department of Pathology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

ABSTRACT

In this experiment we focus on studying some changes on testicles and ovaries in albino rats, after exposure to high and low doses of Tramadol. Rats were divided into 6 groups (3 male groups and 3 female groups). Group (1) control male group, received (saline), Group (2) and Group (3) received oral doses of tramadol (high dose 90 mg / kg b.w. / day) and (low dose 45 mg / kg b.w. / day)respectively for two months, Group (4) control female group, received (saline), Group (5) and Group (6) received oral doses of tramadol (high dose 90 mg / kg b.w. / day) and (low dose 45 mg / kg b.w. / day)respectively for two months, hormonal and histopathological examination were carried out. significant decrease in sperm count of high dose, low dose groups. Testesteron, LH and FSH hormones exhibited significant decrease in its level of groups (2&3) when compared with control group at $P<0.05$. Revealed significant decrease in level of LH and FSH hormones of the female rats (groups5&6) when compared with female control group at $P<0.05$. Progesterone hormone revealed a significant increase in low dose when compared with control female. tramadol has a toxic effect on gonad functions, these findings may provide a possible explanation for delayed fertility and psychological changes associated with tramadol abuse.

Keywords: Tramadol, toxicity, gonads and Rats.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Nowadays, addiction is an expanding social and medical issue worldwide despite of all endeavors to prevent and control it. Analgesics are among the most prevalent medications which are being mishandled (**Rafati et al., 2012**); since it is more popular among teens in most countries worldwide; especially between males. Tramadol is promoted as a racemic blend of both R-and S-stereoisomers this is on the grounds that the two isomers supplement each other's pain relieving movement. Usually joined with paracetamol (acetaminophen) as this is known to enhance the viability of tramadol in calming torment ,tramadol/paracetamol is a quickly acting, longer-term, multimodal pain relieving, which is compelling and by and large all around endured in patients with direct to serious agony (**Dhillon S, 2010**)**.** Tramadol is metabolized to O-desmethyltramadol, which is a more potent opioid **(Raffa et al., 2012**) It is of the benzenoid class.

Its IUPAC Name is Tramadol but it has more than one hundred trade name for example, Tamadol, Tamolan, Tramal, Ultradol, Ultram, Ultramex, and Contramal.

Previously Tramadol was used for treating the problem of premature ejaculation (**Safarinejad et al., 2013**), and as a medications or aphrodisiacs for an expansive base of individuals which led to the ban on trading. Tramadol has pernicious impact on the fertility procedure of both male and female; it increases apoptic spermatogenic and ovarian cells. Therefore through causing a monstrous decay of endometrial organ with the uterine tube, and complication of the seminiferous tubules with relatively missing of sperm and similarly diminished spermatogenic cells **(Heba, 2015)**. long-term organization of tramadol effects on sperm quality and testicular tissues and these impacts are dose dependent **(Omid et al., 2014),** It significantly reduced serum LH, FSH, testosterone and cortisol hormones, but elevated prolactin (PRL) and estradiol (E2) in male rats **(Inass, 2006)**.

Lethal cases have been accounted for because of tramadol overdose. In those examples, demise has been ascribed to cardiopulmonary capture and hepatic disappointment **(Daubin et al., 2007)** as well as hypoglycemia (**Mugunthan and Davoren, 2012)**. There are a few studies concerning with the effect of tramadol on gonads **(Heba, 2015)**. So the present work will focus on the toxicity of tramadol on ovaries and testis.

MATERIALS AND METHODS

Drug: Tramadol: Tamol-X (Tramadol HCl), 225 mg per capsules. It is an odorless, white to off-white crystalline powder is promptly dissolvable in both water and ethanol (1). Its chemical name is (+) cis-2-[(dimethylamino) methyl]-1-(3-m ethoxyph-enyl) cyclohexanol hydrochloride. It was obtained from October Pharma Com. Giza, Egypt

Experimental Design

60 adult albino rats(male and female) were divided into 6 groups as following: Group (1): It contain (10 male albino rats) of The rats were orally received NaCl 0.9% without any chemical substances given by oral gavage daily for successively 60 days.. It used as a control male rats, Group (2): 10 male rats were orally received a high dose of tramadol as 90 mg/kg b.wt dissolved in 10 ml saline, given by oral gavage, daily for successively 60 days according to **Matthiesen et al., (1998),** Group (3): 10 male rats were orally received a low dose of tramadol as 45 mg/kg b.wt dissolved in 5 ml saline. given by oral gavage, daily for successively 60 days**.** Group (4): 10 female rats were orally received NaCl 0.9% without any chemical substances given by oral gavage, daily for successively 60 days, it used as a control female rats**.** Group (5): 10 female rats were orally received a high dose of tramadol at 90 mg/kg b.wt dissolved in 10 ml saline. given by oral gavage, daily for successively 60 days**.**Group (6): 10 female rats were orally received a low dose of tramadol at 45 mg/kg b.wt dissolved in 5 ml saline. given by oral gavage, daily for successively 60 days. All animals of Groups (1,2,3,4,5,6) were sacrificed after blood samples were collected by "Retro orbital sinus " under anesthesia, for biochemical and hematological analysis. Tissues specimens from the internal organs including testes and ovaries were removed and fixed in a suitable fixative (formalin 10%) for 24 h. then kept in 70% ethyl alcohol for the histopathological examination. Epiddydimals (cauda regions) of Testes were removed for sperms count.

Investigations required

Fertility tests including Testosterone, Progesterone, Follicle stimulating hormone (FSH), and Luteinizing hormone (LH) hormones were determined using Intact Immunoassay is a two-site ELISA [Enzyme-Linked Immuno Sorbent Assay] kits according to manufacture structure.

Histopathological Examinations

The sacrificed animals were immediately examined for any gross lesions. Then, tissues specimens from the genital organs including the testes and ovaries were collected, fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections about 4-5 µm thicknesses were prepared and stained with Harries hematoxylin and eosin for microscopical examinations according to **(Culling et al., 1985)**.

Sperm count

Samples of mature sperm were collected from the caudal region of epididymis by mincing it finely. Sperm concentration was analyzed using the haemocytometer method. Sperm suspensions from the caudal epididymis were diluted 1:200 with fixative solution (sodium acid carbonate-formaldehyde solution) and counted according to the procedure indicated in the WHO laboratory manual (**Omid et al., 2014).**

Statistical Analysis

The variability degree of results was expressed as Mean ± Standard Deviation (Mean ± S.D). The data were statistically analyzed by One-Way ANOVA analysis of variance (Prism Computer Program) and the least significant difference (L.S.D) was used to test the difference between treatments. Results were consider statistically significant when $P < (0.05)$.

RESULTS

Table (1), (Graphs 1,2) included significant decrease in sperm count of high dose of tramadol (G.2) and low dose of tramadol (G.3), when compared with control(G1) at $P<0.05$. Both FSH and LH hormones level exhibited significant decrease in high dose, and low dose of tramadol when compared with control group at $P<0.05$. A significant increase appeared in FSH level in low dose of tramadol (G.3) when compared with high dose of tamadol (G.2). LH level showed a significant increase in low dose of tramadol (G.3) when compared with high dose of tramadol (G.2) at $P<0.05$.

Testosterone hormone recorded significant decrease in high and low doses of tramadol in comparison with control.

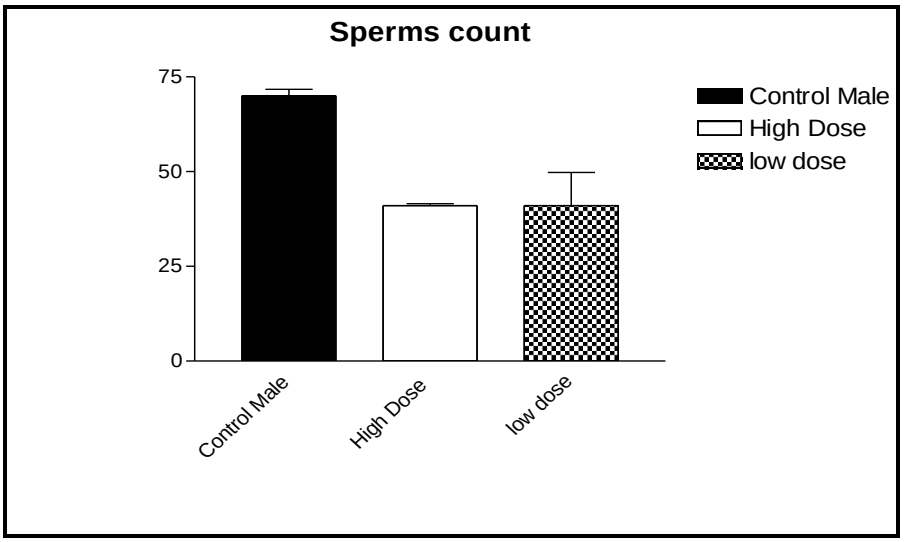
The obtained data in **Table (2), (Graph 3)** revealed significant decrease in level of FSH and LH hormones of the female groups in (high & low doses of tramadol), when compared with control (G. 4) at $P<0.05$. Also a significant decrease in FSH level in low dose of tramadol (G.6) when compared with high dose of tramadol (G.5), while a significant increase in LH level in low dose of tramadol(G.6) when compared with high dose of tramadol (G.5). Progesterone hormone was not statistically changed in high dose of tramadol when compared with control female rats but the Progesterone hormone in low dose of tramadol (G.6) was significant increase when compared with control female rats & the high dose of tramadol (G.5) at $P<0.05$.

ISSN: 0975-8585

Table (1): Effect of oral administration of high and low dose (90 mg/kg b.wt and 45 mg/kg b.wt respectively) of tramadol on fertility tests including (sperm count, FSH (IU/l), LH (IU/l), and Testosterone H. (IU/L)) of male albino rats .

Parameters / Groups	Fertility Tests			
	Sperm Count M.± S.D.	**FSH (IU/L)** M.± S.D.	**LH (IU/L)** M.± S.D.	**Testosterone H. (IU/L)** M.± S.D.
Group (1) (Control)	70.0±3.8	1.9±0.13	2.1±0.15	4.6±0.36
Group (2)(90 mg/kg tramadol)	42.4±2.1^{-a}	0.036±0.01^{-a}	0.64±0.11^{-a}	0.16±0.01^{-a}
Group (3)(45 mg/kg tramadol)	41.8±6.1^{-a}	0.19±0.01^{-a+b}	0.79±0.09^{-a+b}	0.20±0.02^{-a}

The results presented Mean ± S.D of 10 rats
a → significantly different from control group(G.1).
b → significantly different from high dose group (G.2).
\+ Significant increase at ($p<0.05$).
– Significant increase at ($p<0.05$).



Graph (1): Effect of oral administration of high and low dose (90 mg/kg b.wt and 45 mg/kg b.wt respectively) of tramadol on sperm count of male albino rats.

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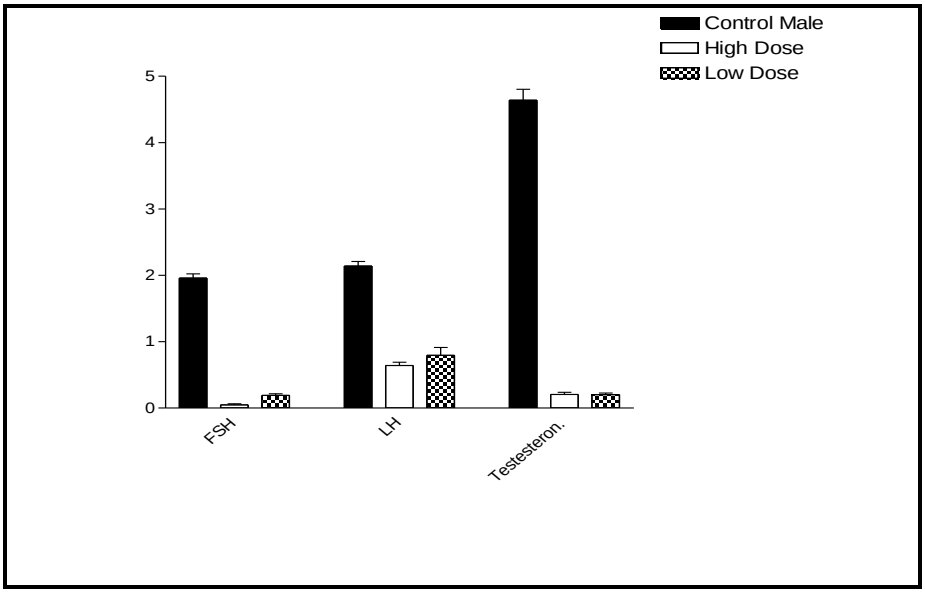

Graph (2): Effect of oral administration of high and low dose (90 mg/kg b.wt and 45 mg/kg b.wt respectively) of tramadol on fertility tests including (FSH (IU/l), LH (IU/l), and Testosterone H. (IU/L)) of male albino rats.

Table (2): Effect of oral administration of high and low dose (90 mg/kg b.wt and 45 mg/kg b.wt respectively) of tramadol on fertility tests including (FSH (IU/l), LH (IU/l), and Progesterone H (IU/L)) of female albino rats.

Parameters / Groups	Fertility Tests		
	FSH (IU/L) M.± S.D.	**LH (IU/L)** M.± S.D.	**Progesterone H.(IU/L)** M.± S.D.
Group (4) (Control)	3.049±0.2	2.9±0.27	4.7±0.27
Group (5) (90 mg/kg tramadol)	1.6±0.46 $^{-a}$	1.6±0.11 $^{-a}$	4.4 ±0.24
Group (6) (45 mg/kg tramadol)	0.84±0.07 $^{-a-b}$	2.0±0.12 $^{-a+b}$	7.2±0.53 $^{+a+b}$

The results presented Mean ± S.D of 10 rats

a → significantly different from control group(G.4).

b → significantly different from high dose group (G.5).

\+ Significant increase at ($p<0.05$).

– Significant increase at ($p<0.05$).

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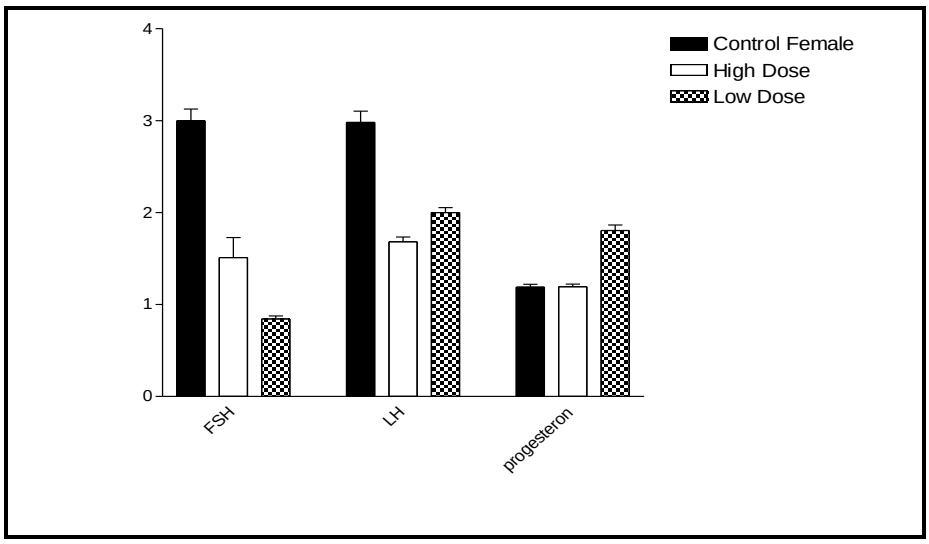

Graph (3): Effect of oral administration of high and low dose (90 mg/kg b.wt and 45 mg/kg b.wt respectively) of tramadol on fertility tests including (FSH (IU/l), LH (IU/l), and Progesterone H (IU/L)) of female albino rats.

HistoPathological results

Testes

Control testes**(G.1)** showed seminiferous tubules lined with germ cells, which progress to spermatocytes and ended to become sperm, in addition to sertoli cells which support the lining epithelium of tubules **(Fig. 1)**. High dose of tramadol **(G.2)** revealed atrophy in seminiferous tubules with edema leading to detachment of germ cells from basement membrane **(Fig. 2)**. There was degeneration detected in all germ cells in seminiferous tubules **(Fig. 3)**. Low dose of tramadol **(G.3)** showed slightly degenerative change in some seminiferous tubules **(Fig. 4)**.

Ovary

Ovary of the control group **(G.4)** showed many primordial follicles, which are found around the edges of the cortex. There are fewer follicles in different stages of development **(Fig. 5)**. While, high dose of tramadol **(G5)** showed follicular cyst represented by large dilated antrum with pale eosinophilic follicular fluid and degeneration in granulosa cells, besides multiple layers in granulosa cells lining portion of cyst and other was detected **(Fig. 6)**. Moreover, ovaries in high dose of tramadol **(G.5)** exhibited dysgerminoma which characterized by large, round, polyhedral to ovoid with vesicular cytoplasmic with round to ovoid granular chromatin resemble seminoma **(Fig.7)**. Degenerative change in the granulose cells of secondary follicles was observed **(Fig. 8)**. Ovaries of low dose of tramadol **(G.6)** displayed comprise in graffian follicles leading to degeneration in granulose cells and pressure ova **(Fig.9)**. Hyperplasia in granulose cells in graffian follicles with degeneration in other was also detected in low dose of tramadol **(G.6)**, besides loss atrial fluid and ova **(Fig. 10**).

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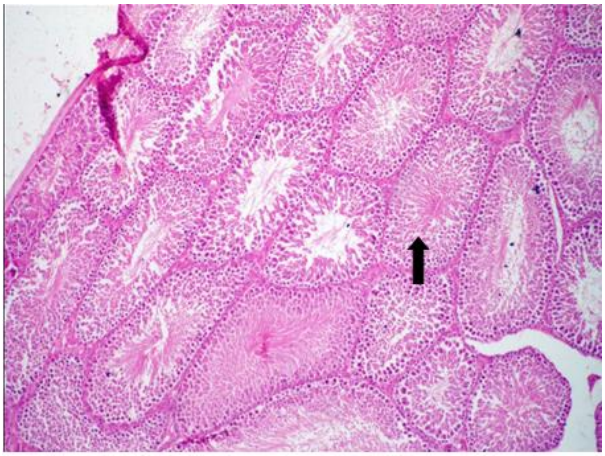

Figure 1: Testis in (G.1) control showed seminiferous tubules lined with germ cells, progress to spermatocytes and ended to become sperm, in addition to Sertoli cells which support the lining epithelium of tubules. (H&E., X 80)

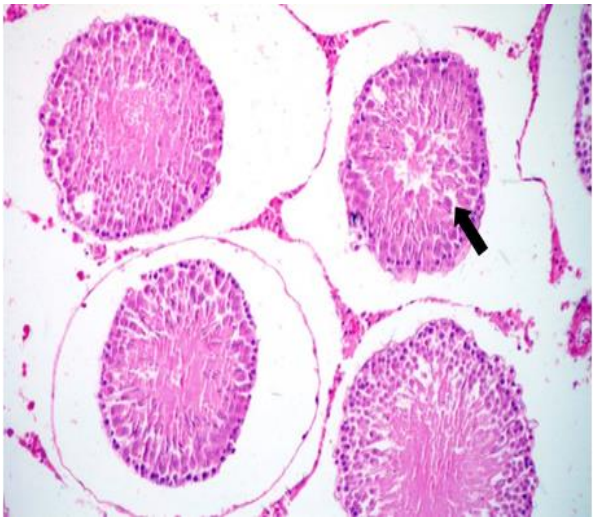

Figure 2: Testis in high dose (H D) of tramadol (G.2) showed atrophy in seminiferous tubules with edema leading to detachment of germ cells from basement membrane.
(H&E., X 150)

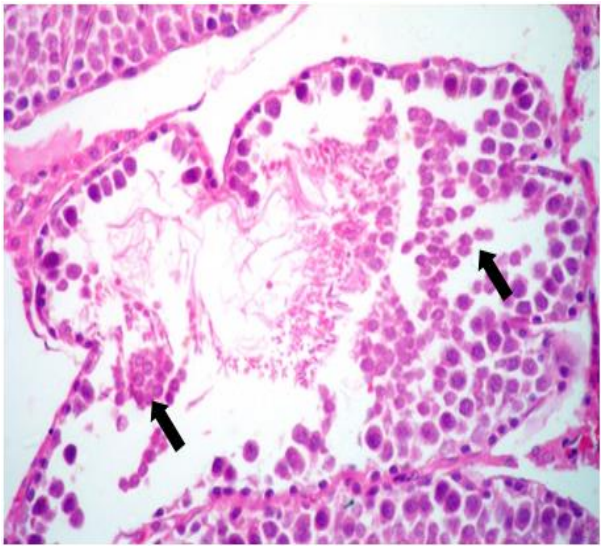

Figure 3: High power of (figure 2), Testis in high dose of tramadol (G.2) showed degeneration in all germ cells in seminiferous tubules.(H&E., X 300)

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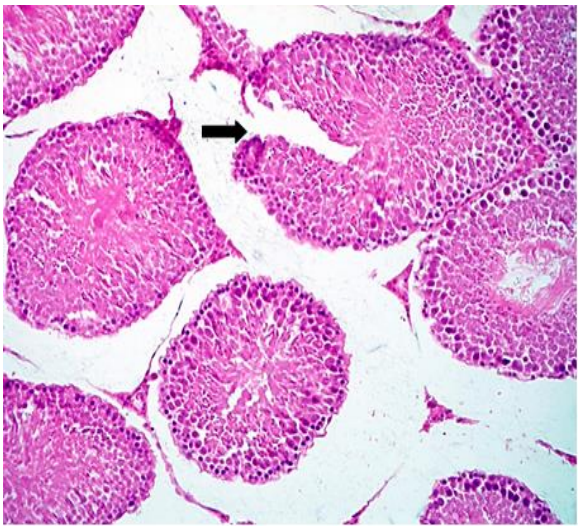

Figure 4: Testis in Low dose (G.3) of tramadol showed slightly necrosis and degenerative change in the germinal cells in some seminiferous tubules. (H&E., X 150)

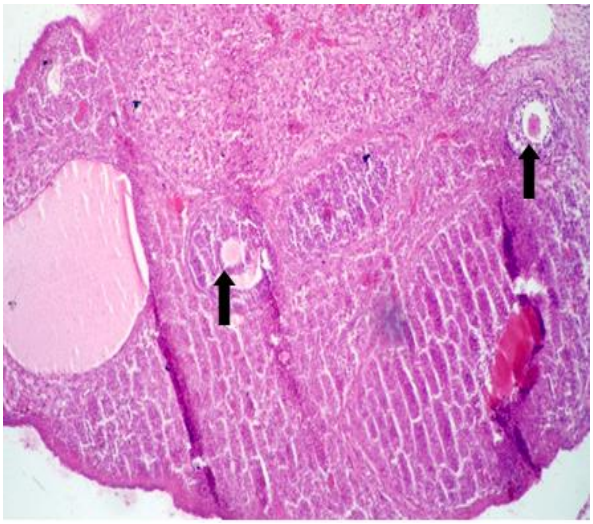

Figure 5: Ovary in (G.4) control showed ovary contains many primordial follicles, which are found around the edges of the cortex. There are fewer follicles in different stages of development (arrows). (H&E., X 150)

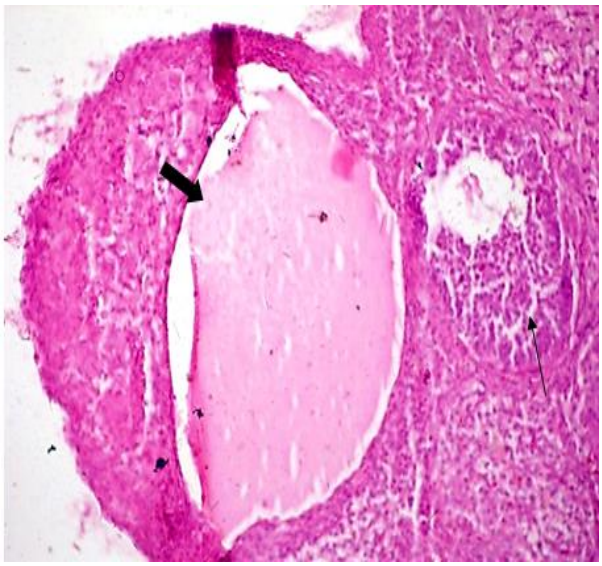

Figure 6: Ovary in high dose of tramadol (G.5) showed follicular cyst represented by large dilated antrum with pale eosinophilic follicular fluid and degeneration in granulosa cells (thick arrow) besides, multiple layers in granulosa cells lining portiof cyst and other (thin arrow)(H&E., X 300)

ISSN: 0975-8585

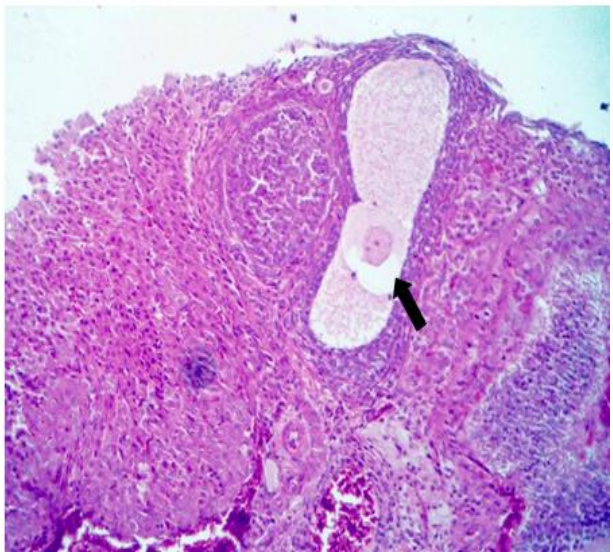

Figure 7: Ovary in high dose of tramadol (G.5) showed dysgerminoma which characterized by large, round, polyhedral to ovoid with vesicular cytoplasmic with round to ovoid granular chromatin resemble seminoma (arrows). (H&E., X 150)

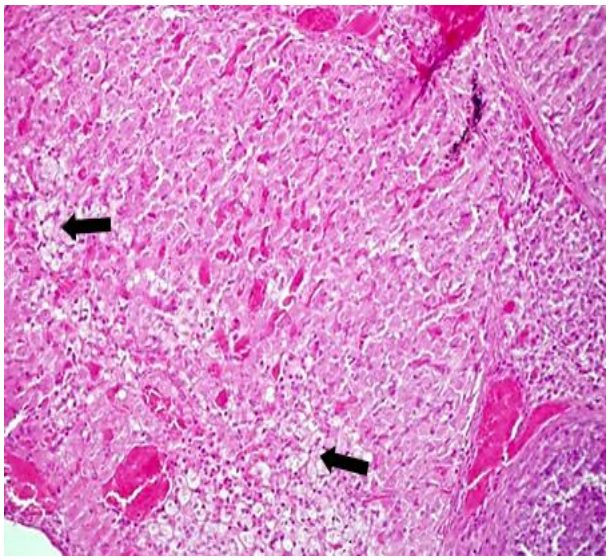

Figure 8: Ovary in high dose of tramadol (G.5) showed degenerative change in the granulose cells of secondary follicles. (H&E., X 150)

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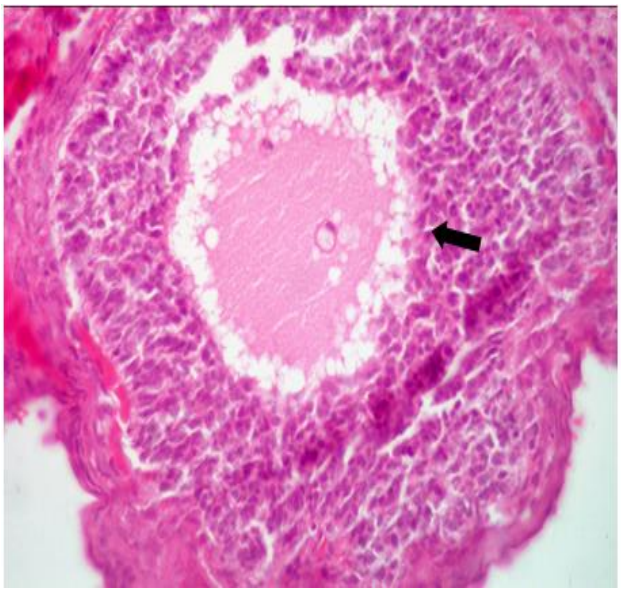

Figure 9: Ovary in low dose of tramadol (G.6) showed comprise in graffian follicles leading to degeneration in granulose cells and pressure ova.
(H&E., X 80)

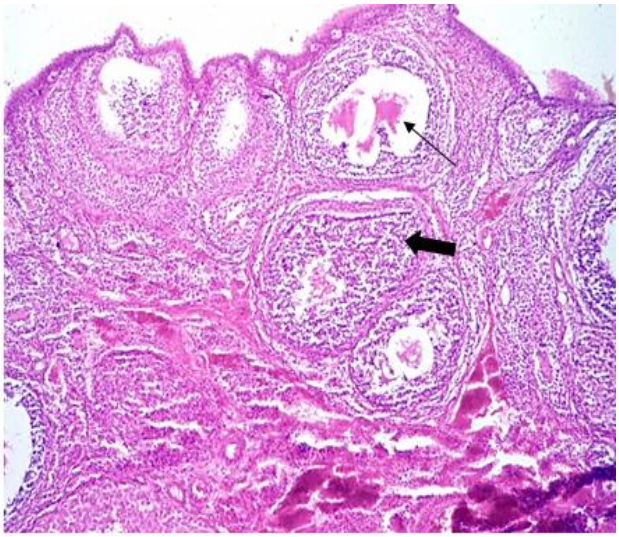

Figure 10: High power of (figure 9), Ovary in low dose of tramadol (G.6) showed hyperplasia in the granulose cells in graffian follicles with degeneration in other (thick arrow) besides, loss atrial fluid and in ova (thin arrow). (H&E., X 150)

DISCUSSION

Tramadol is a manufactured midway acting opioid pain relieving which is fundamentally utilized for treatment of direct to serious agony **(Nossaman et al., 2010).**

Recently, abuse and dependence of tramadol as well as toxicity and tramadol-related deaths have been increasingly reported **(Tjäderborn et al., 2007)**. So the present study deal with hazardous effect of tramadol on fertility in both female and male during treatment. The current study showed that tramadol at high and low doses reduced the sperm count compared with the control group. Histopathological observations also supported the biochemical data of this study which indicated severe lesions in the tissues of testis after Tramadol administration, also the histopathological studies in the present work represented by

atrophy in seminiferous tubules with edema leading to detachment of germ cells from basement membrane (figure 2), In addition, the numbers of primary spermatocytes and rounded spermatids in the seminiferous tubules of treated group were significantly low as compared with that observed in the control group. These findings are probably were due to destruction of the spermatogonia as a result of low testosterone level, which is necessary for normal sperm development. **Agarwal and Parbakaran, (2005)** reported that spermatozoa are exceptionally vulnerable to harm by exorbitant convergences of receptive oxygen species because of the high substance of polyunsaturated unsaturated fats inside their plasma membrane. Lipid peroxidation demolishes the structure of the lipid matrix in the membranes of spermatozoa, and it is related with loss of motility and hindrance of spermatogenesis. The decreased FSH and LH levels associated with tramadol administration through affecting of opioid on gonadotropin-releasing hormone (GnRH) **(Bliesener et al., 2005)**. **El-Gaafarawi, (2006)** observed the reduction of serum levels of LH, FSH and testosterone after paroxetine treatment. Furthermore, opioid-binding sites have been found in Sertoli cells, suggesting an additional peripheral site of action capable of blocking hormone synthesis and/or increasing hormone degradation. Also, **Ceccarelli et al., (2006)** reported that decreasing testosterone generation, morphine, tramadol and buprenorphine credited to build aromatization of testosterone to estradiol. Opiates cause loss of drive and erectile and ejaculatory dysfunctions among men. Sedative manhandle may bring about hypogonadism, basically by diminish in arrival of gonadotropin-discharging hormone (GnRH), testosterone deficiency and infertility **(Lamont and Mathews, 2007and Vuong et al., 2010)**. Also **Caju et al., (2012)** who observed reduction of Sertoli and Leydig cells in adult albino rats presented to serious and interminable measurements of morphine, They clarified this testicular change by disorders in the endocrine and paracrine capacities that can indirectly impact the last size of sertoli cell populace through cluttered LH, estradiol, somatotropin, somatostatin, prolactin and GnRH followed up on either on hypothalamus or directly on pituitary organs. Also **Heidari et al., (2012)** mentioned that opiate induced reduction of serum testosterone level though induction of abnormal structural and functional abnormalities in the secondary sex organs. Many studies focused on testicular function tramadol with negligence on ovarian function **(Cicero et al., 2002, Ahmed & Kurkar (201**4). So in This investigation showed that tramadol organization in rats diminished both pituitary sex stimulating hormones; LH and FSH. It entrenched that opioids diminish the release of GnRH or interfere with its typical pulsatility at the level of the hypothalamus, bringing about a diminished arrival of LH and FSH from the pituitary and an optional fall in gonadal steroid generation **(Daniell, 2008 , Colameco & Coren, 2009 and Katz & Mazer, 2009).** But progesterone hormone insignificantly decreased in rats treated with 90mg/kg. These discoveries are reliable with a few reports of the inhibitory impacts of morphine on ovulation in rats (**Packman and Rothchild, 1976; Pang et al., 1977**) and with opioid mediated inhibition of gonadotropin release and decreases in circulating reproductive hormones (**Ching, 1983; Howlett and Rees, 1986**). This impact is credited to the beforehand specified fall on LH and FSH. Likewise, it was discovered that opioid receptors have additionally been restricted in ovarian tissue societies and opioids have been appeared to specifically smother ovarian steroid creation in vitro (**Kaminski, 2006**). **As regard** histological examination of the ovaries, the results went in the same line as hormonal, estrous cycle. higher doses of tramadol administration induced ovarian folliclar cysts represented by large dilated antrim with pale eosinophilic follicular fluid and degeneration in the granulosa cells, bedsides dysgerminoma characterized by vesicular cytoplasmic with round to ovoid granular chromatin resemble seminoma. Moreover, ovaries at lower doses of tramadol showed degeneration in granulose cells and pressure ova with degeneration and loss atrial fluid and ova. The present results agree with the study carried out by **Marwa and Adel Kurkar, (2014)** who reported that rats received subcutaneous injections of tramadol (40 mg⁄ 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 were found cause reproductive failure **(Du Toit et al., 2006)**. It was found to decrease estradiol and increase the bulk of cysts and corpus lutea **(Zangeneh et al., 2011)**. Thienorphine was a partial opioid agonist with long-lasting antinociceptive was found to induce a concentration-dependent decrease in frequency and amplitude of the contraction on the isolated oestrus and pregnant uterine strips **(Zhou et al., 2013)**. Tramadol caused concentration-dependent inhibition of potassium chloride-induced myometrial contractility (**Shah et al., 2013)**. 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 **(Környei et al., 1997)**.

ISSN: 0975-8585

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