Effect of Tramadol on the Ovaries of Female Mice

Original Article

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ABSTRACT

Backgroud: Tramadol hydrochloride (TrHCl) is considered a synthetic analgesic drug, showing opioid and non- opioid characteristics, the central nervous system is the main system affected by it. It is used to relieve pain, but it can be used to treat other neurotic and psychotic diseases as well.

These characteristics comes from that TrHCl acts on opioid receptors, having a weak agonistic effect, and it affects monoamine receptors by blockage of noradrenaline (NE) and serotonin (5-HT) reuptake, this will lead to the suppression of pain transmission in the nervous system.

Frequent studies concentrated on the effects of TrHCl on the functions of the testis with carelessness to its effects on the functions of the ovaries.

Aim of the Work: The aim of this study was to investigate the effects of tramadol hydrochloride (TrHCl) on female reproductive function, ovarian toxicity and examine the morphology of the ovaries of mice exposed to tramadol hydrochloride (TrHCl).

Materials and Methods: Forty female mice were divided randomly into two groups of 20: one control group and one (TrHCl) treated group. The TrHCl treated group received 100mg per kilogram body weight of TrHCl for 2months.

The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen, and progesterone (P) were measured. Evaluation of the ovaries was conducted after staining with hematoxylin-eosin, and immunohistochemical studies.

Results: The concentration of reproductive hormones was significantly lower in the (TrHCl)-treated group. In addition, the total number of follicles of all types was significantly lower in the (TrHCl) -treated group.

Conclusion: These results suggest that female reproductive function is inhibited by (TrHCl). Thus TrHCl may thus significantly reduce the fertility of female mice.

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Key Words: Mice, ovarian toxicity, tramadol.

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INTRODUCTION

Tramadol hydrochloride (TrHC) is considered a synthetic analgesic drug[1-3].

TrHC is related to morphine, and codeine, but it is less potent than both of them[4-6].

TrHC is more preferable than other opioid drugs because of its unique pharmacological actions, as it has a lower possibility of adverse effects and of occurrence of abuse[7].

Several investigators have shown that exposure of adult male to intake of opioids inhibits the production of hypothalamic, pituitary, adrenal, and gonadal hormones.

These changes have occurred in men, who developed lower blood levels of gonadotrophins, cortisol in addition to testosterone few hours following acute intake[8].

This syndrome caused by opioids and leading to hypo gonadotrophic.

Hypo gonadism in males is termed opioid-induced androgen deficiency (OPIAD)[9-11].

Also studies showed lower levels of adrenal, and gonadal hormones with chronic use of opioids[8,10].

Opioid endocrinopathy in women who consumed opioids was infrequently reported although hypo menorrhea, amenorrhea and sexual dysfunction are dominant in women who addicted heroin[8,12,13].

AIM OF THE WORK

The aim of this study was to investigate the effects of tramadol hydrochloride (TrHCl) on female reproductive function, ovarian toxicity and examine the morphology of the ovaries of mice exposed to tramadol hydrochloride (TrHCl).

MATERIALS AND METHODS

The present study was carried out on 40 normal adult female mice. All mice were kept in good ventilation and aerated room. Excess diet was given during the experimental period. They were allowed to free access of water.

Animals were divided into two groups; control (n=20) and tramadol-treated group (n=20).

Applied tramadol HCL doses (100 mg /Kg body weight) were suspended in saline solution and daily orally administered for 60 days.

At the end of the experiment, the mice were weighed,

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then blood samples were taken for measurement of LH, FSH, estrogen and progesterone levels.

Histological study

Ovaries of control group and tramadol treated group were incised, 10% phosphate buffered formalin with pH 7.4 was used for its fixation, then ascending grades of ethyl alcohol were used for its dehydration. Serial 5 µm sections were cut and then were stained with (H&E). These sections were examined under light microscopy, and Photographed[14].

Immunohistochemical study

Paraffin sections were immersed in xylene and then monoclonal antibody anti-caspase 3 was applied to the sections using the avidin-biotin peroxidase complex for the detection of apoptosis[15,16].

Image Analysis Study

Quantitation of caspase-3 immunostaining was performed using digital image analysis system in combination with analysis software program. This software package utilizes the sum of the optical density of cytoplasm and nucleus to calculate the amount of cytoplasmic and nuclear immunostain . Also area percent and intensity of caspase 3 were measured[17]. This was performed in Histology Department, Faculty of Medicine.

Quantitation of immunoperoxidase staining

Quantitation of caspase-3 immunostaining was performed using Cell A pro image analysis system in combination with the quantitative proliferative index Cell A pro software program (cell analysis system). This software package utilizes the sum of the optical density of cytoplasm and nucleus to calculate the amount of cytoplasmic and nuclear immunostain present. Also area percent and intensity of caspase 3 were measured.

Sequential fields were selected randomly and the mean of positively immunostained cells were taken[17].

Statistical analysis

The Data was collected and entered into the computer. Statistical analysis was done using Statistical Package for Social Sciences (SPSS/version 21) software.

The statistical test used as follow

Quantitative data were described using mean, standard deviation, For normally distributed data, comparison between two independent population were done using independent t-test. Significance of the obtained results was judged at the 5% level, where values ≤ 0.05 are significant.

RESULTS

In the control group the body weight was within normal range while in tramadol-treated group, there was a decrease in the body weight.

In the control group the ovary was pearl-shaped,covering its surface was a single layer of ovarian surface epithelium, directly beneath it there was a dense fibrous tissue capsule the tunica albuginea.

The ovary had a cortex and a vascular medulla

The cortex showed numerous follicles under different stages of development, including primordial follicles, primary follicles, secondary follicles, antral follicles, and graffian follicles.

In tramadol-treated group, there was a detected damage of the growing follicles. Many of the mature follicles appeared atretic in the form of cystic-like structure. There was a decrease in the number of follicles.

On the other side it increased ovulation, so the number of corpus luteum increased, and the corpus luteum produced was degenerated. The ovary was congested (Figures 1,2).

ovaries in the luteal phase labeled immunohistochemically with caspase 3 antibody and DAB chromogen (brown reaction) demonstrating moderated caspase 3 positive reaction in the corpora lutea at different stages of maturation in control ovaries and intense positive caspase 3 reaction in tramadol treated ovaries (Figures 3,4)

There was diminished all hormonal levels LH, FSH, estrogen and progesterone in Tramadol treated group.

The body weight in control group was ranged from 28.3-29.7 gm, with a mean value 28.97±0.41 gm, while in experimental group the weight ranged from 20.0-24.1 gm, with a mean of 21.82±1.52 gm, on comparing the two studied groups regarding weight, it was found that there was a significant decrease in weight in experimental group less than control group (p <0.05) (Table 1).

The follicles number in control group was ranged from 21-25 with a mean of 23.4±1.45, while in experimental group the number of follicles ranged from 12-22 with a mean of 16.6±4.16, there was a highly significant decrease in number of follicles in experimental group less than the control group (p <0.01) (Table 2).

The hormonal assay of the two studied groups showed that the level of FSH in control group was 0.0737±0.02 while in experimental group was 0.059±0.03, there was a significant decrease in FSH in experimental group less than the control group (p <0.05). The LH in control group was 0.289±0.04 while in experimental group was 0.191±0.04, the control group show a significant increase in LH more than the experimental group (p < 0.01). The oestrogen in control was 12.1±0.58, while in experimental group was 9.96±1.36, there was a highly significant decrease in oestrogen in experimental group less than the control group (p <0.01). Finally the progesterone in control group was 5.21±0.72, while in the experimental group was 3.962±0.64, there was a significant increase of progesterone in control more than the experimental group (p <0.01) (Table 3).

The following graphs show comparison between control and tramadol treated groups in the immunohistochemical reaction as regards the mean area percent of caspase 3, the mean optical density of caspase 3, and the mean intensity of caspase 3 (Graphs 1,2,3).

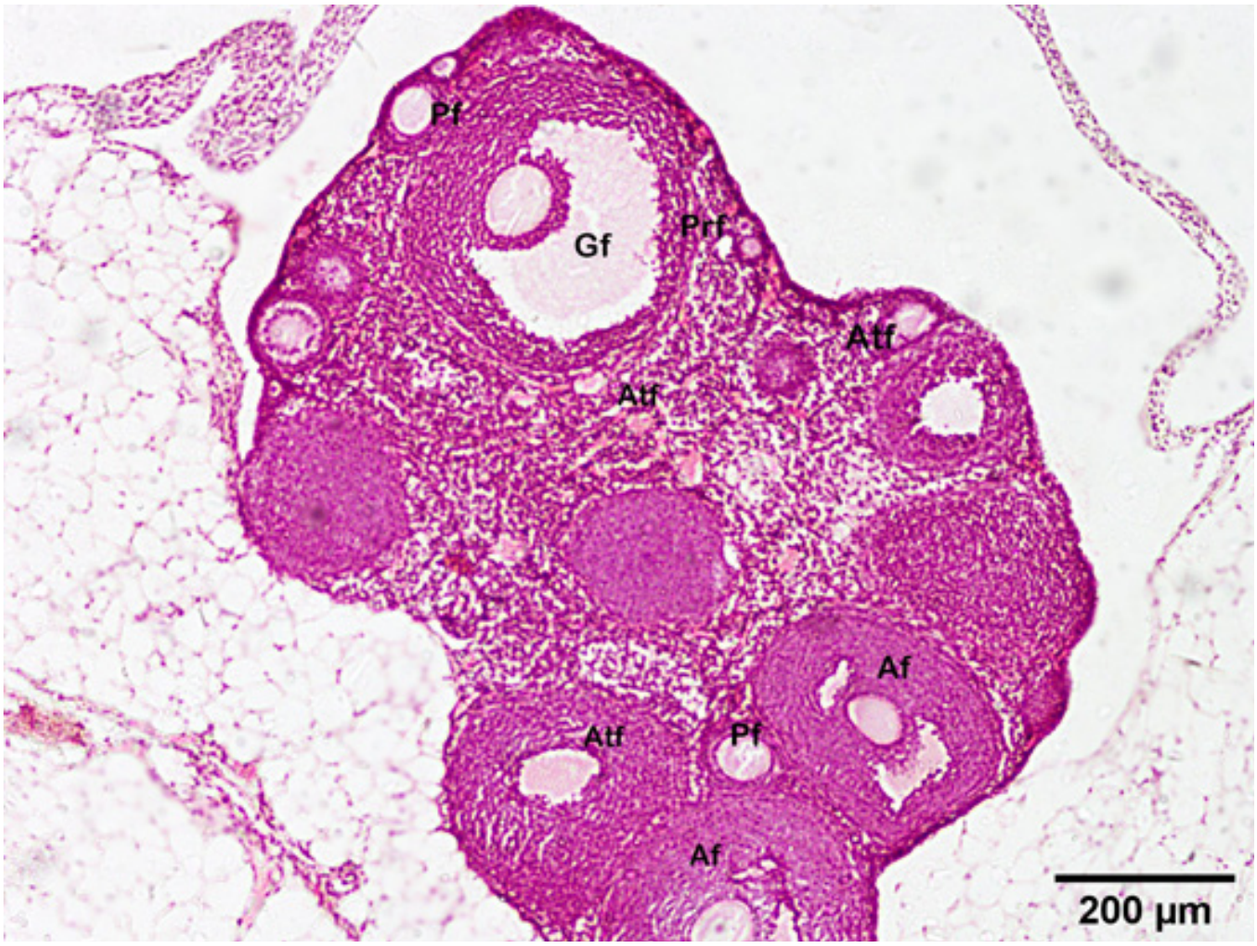


Fig. 1: Photomicrograph of the ovary of the control group, showing normal structure of the ovary. Notice the presence of adequate number of primordial follicles (Prf), primary follicles (Pf), antral follicles (Af) and graffian follicles (Gf). Atretic follicles (Atf) appear in a normal range. (100X, H&E stain)

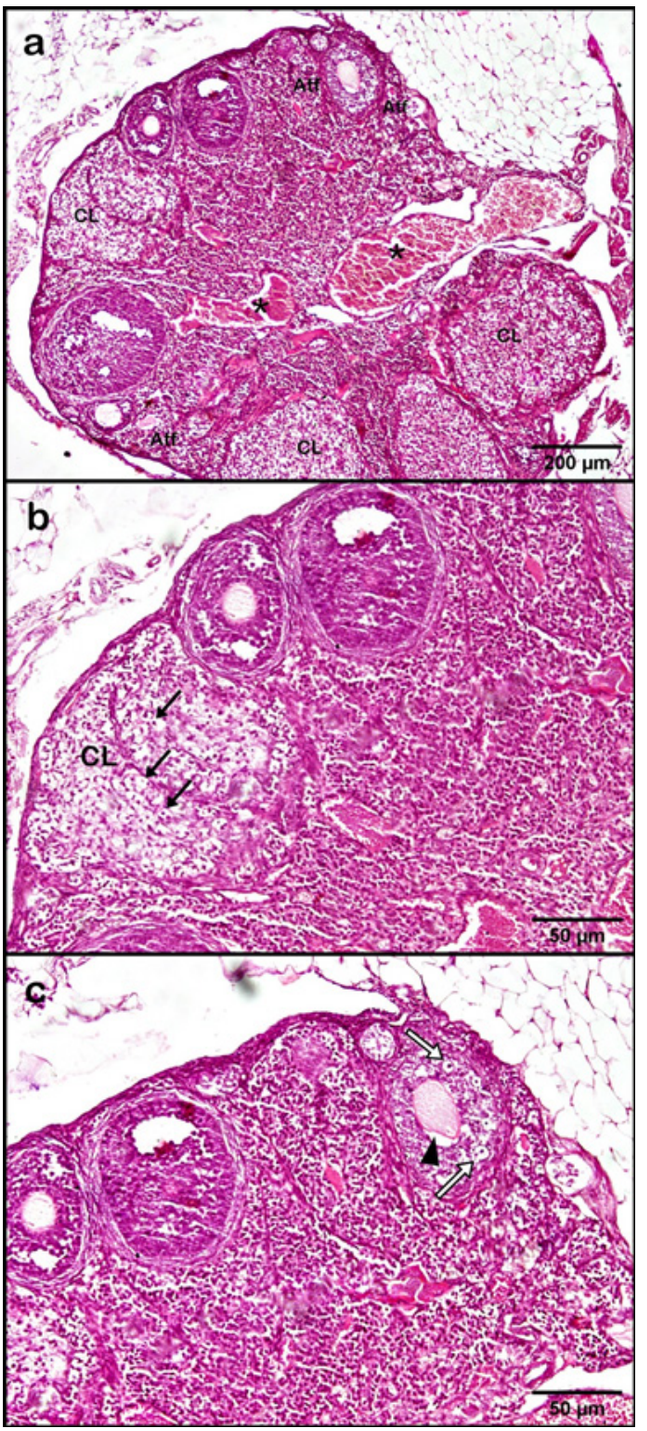


Fig. 2: Photomicrographs of the ovary of the (tramadol) group, (a) showing disturbed ovarian structure with congested blood vessels (*). Notice degenerative changes in the ovarian follicles. Notice the increased number of the atretic follicles (Atf) and the corpus luteum (CL). (100X, H&E stain) (b) Corpus luteum shows, degenerating corpus luteal cells; with pyknotic nuclei and abundant clear foamy cytoplasm (black arrow). (400X, H&E stain) (c) Antral follicle (Af) shows, degenerative changes in the ovum; shrinkage of the ooplasm (black arrow head) and some granulosa cells show pyknosis of the nucleus and dissolution of the cytoplasm (white arrow). (400X, H&E stain).

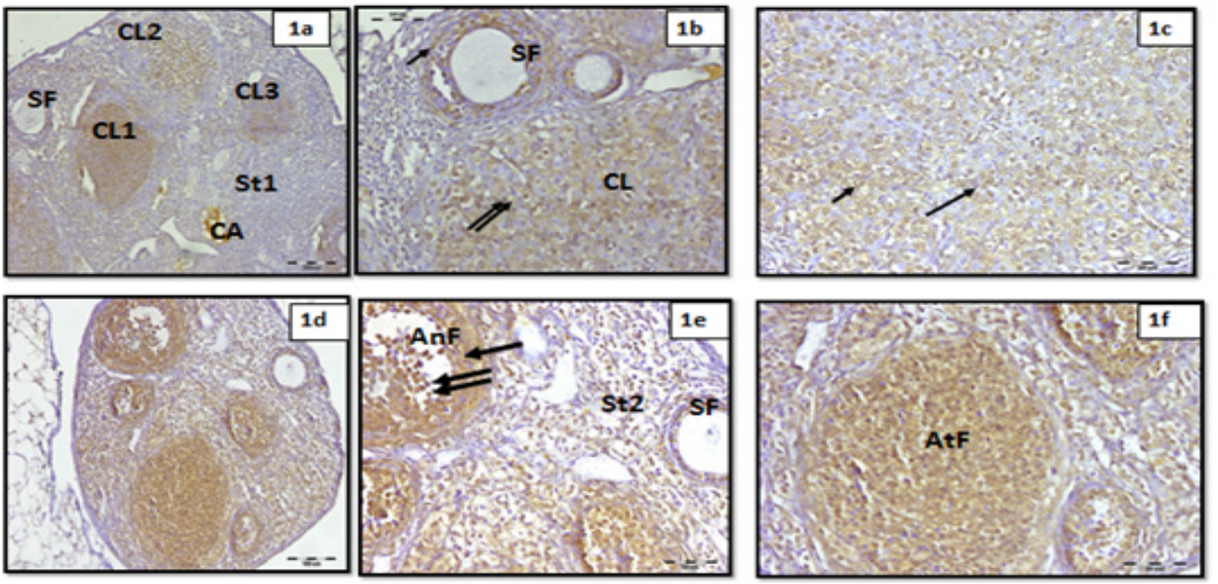


Fig. 3: Control Group: Light photomicrograph of control mice ovaries in the luteal phase labeled immunohistochemically with caspase 3 antibody and DAB chromogen (brown reaction) demonstrating: 3a: low power view showing distribution of moderated caspase 3 positive reaction in the corpora lutea at different stages of maturation (CL1, CL2, CL3) and in cell remnants within the corpus albicans (CA). Note the negative reaction in the stromal cells (St1) in this section. 3b: positive caspase 3 reaction in individual follicular cells (arrow) of the secondary growing follicle (SF) and clusters of granulosa lutein cells (double arrows) of the corpus luteum (CL). 3c: High power magnification depicting moderate reaction in the cytoplasm and nuclei of granulosa cells forming the corpus luteum in 3b. 3d: low power view of another control ovary in advanced luteal phase showing moderate caspase3 reaction in antral follicle (AnF), secondary growing follicle (SF) and atretic follicle (AtF). 3e: Higher magnification of 3d depicting the distribution of caspase 3 reaction in all layers formed by follicular cells (double arrow) and cells shed in the antral space (arrow) of the antral follicle (AnF). Note the dispersed fine positive reaction in the nuclei of luteinized vaculated cells (St2) in the stroma. 3f: High power view of the atretic follicle (AtF) in 3d revealing diffuse moderate caspase 3 reaction in the nuclei of the bulk of forming apoptosing cells. (Mic. Mag. 3a, 3d x 100; 3b, 3e, 3f x 200; 3c x 400)

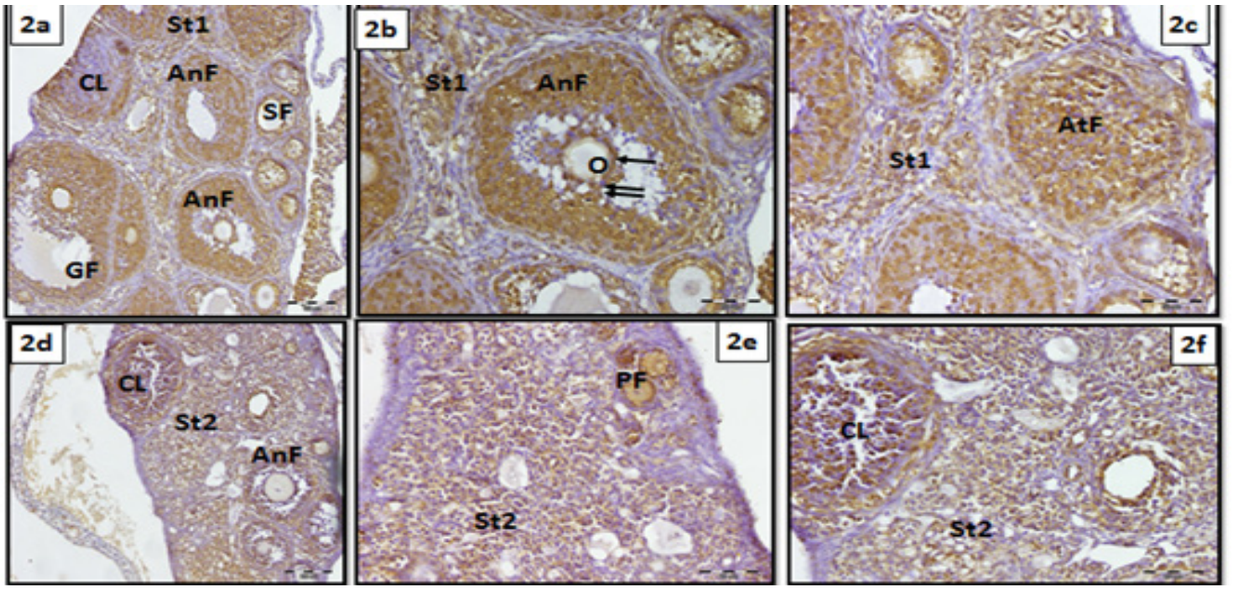


Fig. 4: Treated Group: Light photomicrograph of mice ovaries treated with Tramadol and labeled immunohistochemically with caspase 3 antibody and DAB chromogen (brown reaction) demonstrating: 4a: low power view showing intense positive caspase 3 reaction in the follicular cells lining the Graafian follicle (GF) , antral follicles (AnF), secondary follicle (SF), corpus luteum (CL) and clusters of stromal cells (St1). 4b: Higher magnification of the antral follicle (AnF) in 4a showing intense caspase reactivity in the corona radiate cells (arrow) and cumulus oophorus (double arrow) surrounding the ovum (O). 4c: The atretic follicle (AtF) of the same ovary in 4a reveals dense positive reaction for caspase 3 in the forming mass of apoptosing cells.
4d: low power view of another tramadol treated ovary in an advanced luteal phase showing intensified caspase 3 reaction in the stromal cells (St2), corpus luteum (CL) and antral follicle (AnF). 4e: A strong reaction is depicted in the follicular cells surrounding the ovum of primordial follicles (PF) in 4e ovary. 4f: The whole mass of granulosa cells in the corpus luteum (CL) in 4d exhibit intensive positive reaction for caspase 3 as well as the luteinized cells in the stroma (St2). (Mic. Mag. 4a , 4d x 100; 4b,4c,4e, 4f x 200)

Table 1: Comparison between the two studied groups regarding the body weight

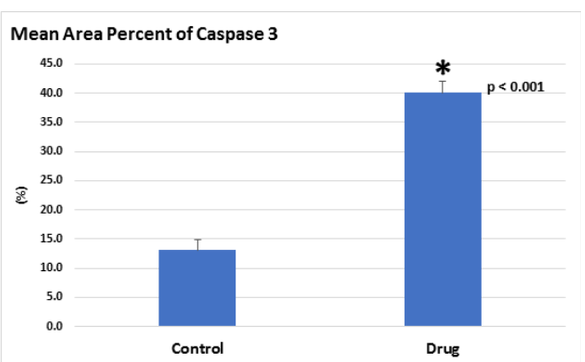
Body weight	Control	Experimental
Range	28.3-29.7	20.0-24.1
Mean ± S.D.	28.97 ± 0.41	21.82 ± 1.52
t-test	4.65	
P value	0.0021*	

Table 2: Comparison between the two studied groups regarding the No. of follicles

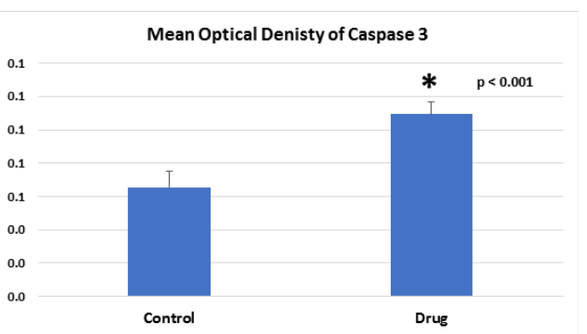
No. of follicles	Control	Experimental
Range	21-25	12-22
Mean ± S.D.	23.4 ± 1.45	16.6 ± 4.16
t-test	5.02	
P value	0.0016*	

Table 3: Comparison between the two studied groups regarding the hormonal assay

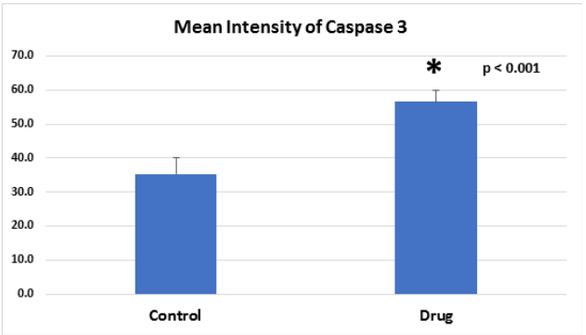
Hormonal	Control	Experimental	t-test	*P value*
FSH				
Range	0.04-0.1	0.02-0.09	1.96	0.042*
Mean ± S.D.	0.0737 ± 0.02	0.059 ± 0.03		
LH				
Range	0.22-0.32	0.11-0.26	4.02	0.0021*
Mean ± S.D.	0.289 ± 0.04	0.191 ± 0.04		
Oestrogen				
Range	11.3-12.9	6.98-11.5	4.82	0.00012*
Mean ± S.D.	12.1 ± 0.58	9.96 ± 1.36		
Progesterone				
Range	4.3-6.1	3.1-5.2	3.96	0.0033*
Mean ± S.D.	5.21 ± 0.72	3.962 ± 0.64		



Graph 1: Bar chart showing comparison between control and tramadol treated groups in the immune histochemical reaction as regards the mean area percent of caspase 3.



Graph 2: Bar chart showing comparison between control and tramadol treated groups in the immune histochemical reaction as regards the mean optical density of caspase 3.



Graph 3: Bar chart showing comparison between control and tramadol treated groups in the immune histochemical reaction as regards the mean intensity of caspase 3.

DISCUSSION

The worldwide usage of narcotic drugs among young people especially tramadol directed me to carry out this work to show its effects on the female ovaries.

In this study tramadol caused decreased body weight in mice treated with 100mg/kg for two months. This was similar to other opioids such as morphine. This is as mentioned Siddiqui *et al*[18]. This disagreed with Ahmed & Kurkar who found that tramadol did not cause decrease in body weight of rats[19]. This difference between the two studies might be due to the difference in animals used which were mice in this study and rats in theirs.

The present study showed that tramadol administration in mice trigger ovulation blockade marked by low levels of LH and FSH. These results agree with the findings of Melad G. Paulis, Mohamed F. Abbas[20].

It also agreed with studies done by Ahmed and Kurkar[19]. They found that rats which administered subcutaneous tramadol injections (40mg/kg body weight) three time per week for two months showed reduced plasma levels of LH, and FSH.

Also these results agreed with studies carried on other opioids as mentioned by Morley, P faus& Gorzalka[21,22]. It was due to that opioids decreased GnRH release causing decreased LH and FSH release from the pituitary as explained by Colameco & Coren, and Daniell[23,24]. The present study showed that tramadol decreased estrogen and progesterone hormones. This was in accordance with the study of Pang *et al*[25]. which emphasized on the inhibitory effects of opoids on ovulation and consequently on ovarian hormones in rats, and due to the fall of pituitary hormones LH and FSH as emphasized by Ching *et al*[26].

The present study showed that tramadol caused decrease in the number of different ovarian follicles, so this would affect the fertility .There was increase in the ovulation, so the number of corpora lutea increasd. But the corpora lutea produced were degenerated, so it would not carry out its function. The ovaries were congested. This was in agreement with Heba Atef who showed severe decrease in the number ovarian follicles and increased number of atretic follicles, cystic follicles, and corpora lutea. The stroma of the ovary was increased, congested and hypercellular. This decrease in the number ovarian follicles was associated with ovarian dysfunction[14].

Application of other similar drugs as naloxone the antagonist of opioids to female rats caused failure of their reproductive functions. The two drugs Clonidine and yohimbine caused estrogen decrease, and increase in the number of cysts and corpora lutea[14].

A previous study conducted by Melad G. Paulis, Mohamed F. Abbas stated that Tramadol causes damage through oxidative stress. Oxidative stress triggers the pathway of apoptosis which involves the enhanced expression of caspase 3 enzyme. This agreed with the findings of the present study that showed intensified apoptosis in rats treated with tramadol in comparison with the control group that showed mild apoptosis in the atretic follicles which is normally found.

This was also similar to findings of Ahmed & Kurkar and El- Gaafarawi[27], who studied tramadol effect on male rats. This occurred with other opioids on animal Payabvash *et al*.[28], Zhou *et al*.[29], and human studies Safarinejad *et al*.[30].

CONCLUSION

This study suggests that female reproductive function and hormones are inhibited by Tramadol. Thus Tramadol may significantly reduce the fertility of female mice.

CONFLICT OF INTERETS

There are no Conflicts if Interest.

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الملخص العربى

تأثير الترامادول على مبايض اناث الفئران

نسرين مصطفى الحمصانى

قسم التشريح وعلم الاجنة ـ كلية الطب ـ جامعة الإسكندرية

يعتبر هيدروكلوريد الترامادول عقارًا مسكنًا صناعيًا ، يظهر خصائص أفيونية المفعول وغير أفيونية المفعول ، والجهاز العصبي المركزي هو الجهاز الرئيسي الذي يتأثر به. يتم استخدامه لتسكين الآلام ، ولكن يمكن استخدامه أيضًا لعلاج الأمراض العصبية والذهانية الأخرى.

تأتي هذه الخصائص من أن الترامادول يعمل على مستقبلات المواد الأفيونية ،حيث له تأثير ناهض ضعيف ، ويؤثر على مستقبلات مونوامين عن طريق وقف امتصاص النورأدرينالين والسيروتونين ، مما يؤدي إلى تقليل انتقال الألم في الجهاز العصبي.

ركزت الدراسات المتكررة على تأثيرات الترامادول على وظائف الخصية مع الإهمال لتأثيرها على وظائف المبايض. لذلك كان الهدف من هذه الدراسة هو التحقيق في آثار هيدروكلوريد الترامادول على الوظيفة الإنجابية للإناث ، وسمية المبيض وفحص مورفولوجيا مبيض الفئران المعرضة لهيدروكلوريد الترامادول.

تم تقسيم أربعين أنثى فئران بشكل عشوائي إلى مجموعتين من ٢٠: مجموعة تحكم ضابطة ومجموعة معالجة ب هيدروكلوريد الترامادول. تلقت المجموعة المعالجة ١٠٠ مجم لكل كيلوغرام من وزن الجسم من هيدروكلوريد الترامادول لمدة شهرين .

تم قياس مستويات الهرمون المنبه للبصيلات والهرمون المصفر والإستروجين والبروجسترون . تم إجراء تقييم للمبايض بعد تلطيخها بالهيما توكسيلين و الايوزين ودراسات كيميائية مناعية. كان تركيز الهرمونات التناسلية أقل بشكل ملحوظ في المجموعة المعالجة بـهيدروكلوريد الترامادول. بالإضافة إلى ذلك ، كان العدد الإجمالي للبصيلات من جميع الأنواع أقل بشكل ملحوظ في المجموعة المعالجة بـهيدروكلوريد الترامادول . تشير هذه النتائج إلى أن وظيفة التكاثر الأنثوية معطلة بواسطة هيدروكلوريد الترامادول وبالتالي قد يقلل هيدروكلوريد الترامادول بشكل كبير من خصوبة إناث الفئران.