Original Article

Histological and Immunohistochemical Study on the Effect of Tramadol Abuse on Cerebral Cortex and Hippocampus in Male Albino Rabbits

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ABSTRACT

Background: Tramadol is an analgesic used in moderate and severe pain however, tramadol abuse among adolescence leads to various alterations in brain histological structure that leads to psychiatric and physical abnormalities.

Aim of the Work: This study aimed to evaluate the effects of tramadol abuse in male albino rabbits on the cerebral cortex and the hippocampus and the effect of its withdrawal.

Material and Methods: Thirty male albino rabbits were divided into three groups; the control group, the tramadol treated group in which rabbits were received tramadol 42 mg/ kg/ day for ten days then the dose was increased to 84 mg/kg/day for another ten days then 168 mg/kg/day for another ten days. The tramadol recovery group in which rabbits were received the same doses as the previous group then the tramadol intake was stopped and the rabbits were sacrificed after 4 weeks. In the present study, the histological and immunohistochemical studies of the cerebral cortex and hippocampus were evaluated.

Results: tramadol abuse among the tramadol treated group caused neuronal cell disorganization in cerebral cortex and hippocampus in the form of cell apoptosis, wide intercellular space, degenerative vacuolation and diffuse chromatolyses and strong positive Caspase–3 antibody reaction. After stoppage of tramadol there were many normal neurons with decease the percentile of cell apoptosis, wide intercellular space, degenerative vacuolation and diffuse chromatolyses and mild positive Caspase–3 antibody reaction.

Conclusion: Tramadol abuse induced neurotoxicity and histological changes in brain tissue although, after stoppage of tramadol intake there was incomplete regression of brain histopathological alterations.

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Key Words: Caspase–3 antibody, cerebral cortex, hippocampus, tramadol.

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INTRODUCTION

Tramadol is a centrally acting synthetic analgesic with opioid activity, widely used in moderate to severe pain. Compared to the classical opioid analgesic morphine, the tramadol is considered to be a relatively safe analgesic. It is used as an analgesic in cancer, in chronic neuropathic pain and postoperatively[1].

Tramadol can also bring on feelings of intense euphoria, which may be what prompts some individuals to start abusing it. Tramadol abuse among adolescents becomes a widely spread all over the world[2].

Tramadol abuse among adolescence has many health and social consequences Adolescence is a critical period for neurodevelopment. Exposure to addictive substances during this period leads to various alterations in brain histological structure that can be translated into functional consequences throughout life[3].

Although tramadol is a weak opioid, its long-term use may result in wide psychiatric and physical symptoms. The chronic administration of tramadol is associated with oxidative stress, inhibition of neurogenesis, apoptosis and mitochondrial dysfunction[4].

The frontal lobe control emotions, behavior, attention, judgment and plays a key role in future planning including self management and decision-making Tramadol abuse lead to behavior and emotional disturbances, loss of confidence, neglect healthy social interactions and education regression[5].

The hippocampus belongs to the limbic system and plays important roles in learning and consolidation of information from short-term memory to long-term memory and in spatial memory. The hippocampus plays an important role in formation of new memories about experienced events[6].

Some of the new studies have also demonstrated that tramadol administration impairs memory function in rodent models by activation of μ-opioid receptors[7].

The present study was designed to study the effects of tramadol abuse in male albino rabbits on the cerebral cortex and the hippocampus and the effect of its withdrawal.

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MATERIAL AND METHODS

Animals

Thirty male albino rabbits aged 4 to 12 weeks equal to the human adolescence age[8]. The rabbits weighing 1-1.5 kg were included in the study obtained from the animal house, Moshtohor Faculty of Veterinary Medicine, Benha University. These rabbits were kept in spacious cages, two rabbits per cage, with free access to food and water. All animals received human care with the Animal Care Guidelines of the National Institutes of Health and the Ethical Committee for scientific research approved the design of the experiments.

Drugs

Commercial available tramadol hydrochloride tablet each contained 200 mg were purchased from October Pharma S.A.E., Egypt, under license of German Grunenthal Company. The tablet 200 mg was dissolved in 20 ml of distilled water. Thus every 1 ml of the solution contained 10 mg of the drug. The animals were weighted and received the calculated dose of the drug according to their weight.

Experimental design

The rabbits were divided into three groups as follows:

Control group: it included ten male albino rabbits; each rabbit was given regular diet

Tramadol treated group: it included ten male albino rabbits, rabbits were received tramadol 42 mg/ kg/ day for ten days then the dose was increased to 84 mg/kg/day for another ten days then 168 mg/kg/day for another ten days orally by orogastric feeding tube[9].

Tramadol recovery group: it included ten male albino rabbits, that were received the same doses as the previous group then the tramadol intake were stopped and the rabbits were sacrificed after 4 weeks.

The animals were sacrificed by ether inhalation then; craniotomy and laminectomy were performed within 30 min after death. Then the brains were collected carefully and washed with normal saline. The frontal and temporal lobe of the brain from each group were fixed with 10% formalin for five days. After fixation, the tissue specimens were dehydrated, cleared with xylene and embedded in paraffin. The frontal and temporal lobe blocks were cut into 5 µm coronal serial sections (5 sections in each animal at 100 µm intervals) and stained for histopathological study.

Histopathology study

Hematoxylin and eosin staining

The brain was fixed in 10% formalin saline. After fixation, the right cerebral hemisphere was embedded in paraffin blocks and processed for the preparation of serial coronal sections, 5 u. thicknesses, and then sections were examined. Five microscopic fields were chosen randomly per slide and five slides per animal were evaluated for any histopathological changes. The collected data for all groups were estimated as a percentage[10].

Cresyl violet staining

Sections were stained in 0.1% cresyl violet solution (dissolved in 0.01% glacial acetic acid) at 37°C for 10 minutes, rinsed quickly in distilled water, differentiated in 95% ethyl alcohol for 30 seconds. Sections were dehydrated in 100% alcohol 2 × 5 minutes, dewatered through graded ethanol, and permeabilized with xylol until the cover slips were mounted using neutral resin for permanent preservation, used to stain the Nissl substance (rough endoplasmic reticulum) appears dark blue due to the staining of ribosomal RNA in neurons[11].

Immunohistochemical staining for Caspase–3

Apoptosis was immunohistochemically localized using Caspase–3 antibodies. Paraffin sections (4 µm thick) were incubated with a rabbit monoclonal caspase–3 antibody using the avidin biotin peroxidase method[12].

Statistical Analysis

The collected data were summarized in term of frequency and percentage using the computerized Statistical Package for Social Science (SPSS; Version 20.0 for Windows, SPSS Inc., Chicago, IL).

RESULTS

Rabbit behavioral changes

All animals were observed daily for any abnormal behavior.

Control group

The rabbits were feel comfortable, interest in their food, play with each other and not aggressive when handled.

Tramadol treated group

During the first ten days they behaved normally except for sedation and lack of interest in their food before the designed dose. During the second ten days, the rabbits became restless, irritable being aggressive when handled and some had tremors one to two hours before the designed dose, after the dose the rabbits became calmness, very inactive and anorexia had developed. As the dose increased the rabbits became more irritable with bulging eyes, some of them became aggressive to other rabbits, anorexia and loss of body weight and some symptoms like tremors and salivation were also noted.

Tramadol recovery group

During the first ten days of withdrawal the tremors, irritability, salivation and anorexia were exaggerated. But these behaviors were gradually improved by time till the end of the fourth week.

Histopathological examination

Hematoxylin and eosin stained sections of the frontal

lobe from rabbits in control group showed the cerebral cortex from the frontal lobe with its sex layers and normal blood vessel (Figure 1). Layer I the molecular layer consisted of large amount of fibers and poorly cellular, layer II the outer granular layer consisted mainly of cell islands with large rounded vesicular nuclei were detected (Figure 2), layer III the pyramidal layer consisted of large pyramidal cells with basophilic cytoplasm, large vesicular nucleus and long apical dendrite. The glial cells with small dense nuclei were also seen (Figure 3), layer IV the inner granular layer is characterized by presence of many small granule cells, layer V the layer of large pyramidal cells and layer VI contained cells with various size and shape (Figure 4).

The hippocampus from the temporal lobe sections showed the following three layers molecular consisted mainly of fibers and some non pyramidal cell, pyramidal layer consisted mainly of pyramidal cell and some granular flask shaped cell and polymorphic layer consisted of few cells called interneurons and large amount of fibers (Figures 5,6).

Hematoxylin and eosin stained sections of cerebral cortex of the frontal lobe from rabbits in treated group showed neuronal cell disorganization and dilated blood vessels and capillary (Figure 7), 100% of cerebral cortex sections contained apoptotic cells characterized by neuronal shrinkage and chromatin condensation, 65% degenerative vacuolization, 45% wide intercellular space, 30% diffuse chromatolysis of nuclear chromatin with absence of nucleoli and 20% multinuclear cells were present (Figure 8) (Table 1).

The hippocampus from the temporal lobe sections showed marked neuronal degeneration as 100% of sections contained apoptotic cells characterized by neuronal shrinkage and chromatin condensation and 55% diffuse chromatolysis of nuclear chromatin with absence of nucleoli detected in pyramidal layer, 35% wide intercellular space. Also 20% of brain sections contained red neurons due to hypoxia and it was subsequent to apoptosis, the red coloration was due to degeneration of nucleus and loss of nissl bodies which are normally stained blue in H&E (Figures 9,10) (Table 2).

Hematoxylin and eosin stained sections of the frontal lobe from rabbits in tramadol recovery group showed return of cerebral cortex towards normal morphology as shown by remarkable regression of the total degenerative changes that induced by tramadol in all brain sections (Figure 11). Most of neuronal cells appeared normal while 30% of brain sections contained apoptotic cells, 15% degenerative vacuolization and 10% showed wide intracellular space (Figure 12) (Table 1). Nearly normal appearance of the hippocampus with its three layers molecular, pyramidal and polymorphic (Figure 13). While 25% of hippocampus sections showed apoptotic neurons, 10% wide intracellular space, 5% showed degenerative vacuolization and 3% red neurons (Figure 14) (Table 2).

Cresyl violet staining

The Nissl substance appeared dark blue (basophilic) due to the staining of ribosomal RNA in neurons. The control group showed basophilic condensations of Nissl granules around the nucleus inside the nerve cells in cerebral cortex and hippocampus sections (Figures 15 and 18). In tramadol treated group there were neurons with absent nucleoli, neurons with dark shrinkage cytoplasm and swollen neurons (Figures 16 and 19). In tramadol recovery group some neurons showed Nissl granules around the nucleus and other neurons showed absence of nucleoli, few neurons with dark shrinkage cytoplasm and swollen neurons in hippocampus sections (Figure 17), some neurons showed absence of nucleoli in cerebral cortex sections (Figure 20).

Caspase -3 immunostaining

Positive immunohisto-chemical staining of caspase-3 demonstrated as brown cytoplasmic staining index for the degree of nuclear apoptosis. The control group showed normal negative reaction of brain tissue to the Caspase–3 antibody (Figures 21 and 24). In tramadol treated group there were many neurons with strong positive Caspase–3 reactions in their cytoplasm (Figures 22 and 25). While the caspase-3 immunostaining of brain sections from tramadol recovery group showed few neurons with mild positive reactions to the Caspase–3 antibody in their cytoplasm (Figures 23 and 26).

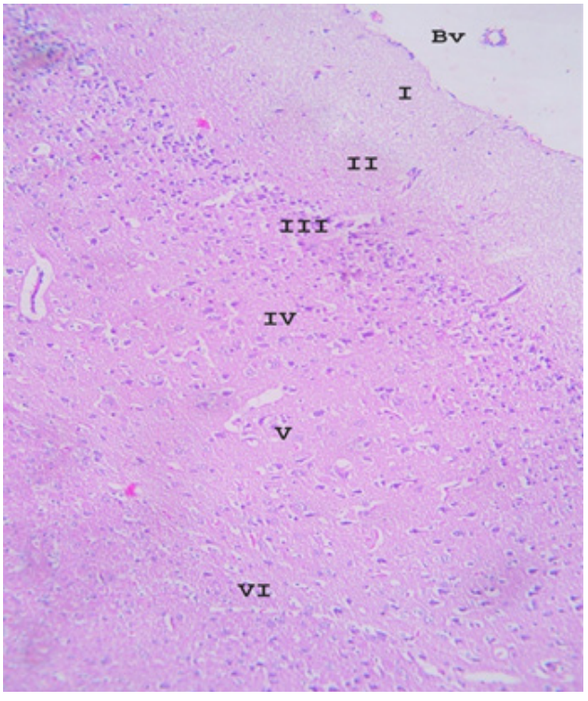

Fig. 1: A photomicrograph of a coronal section in cerebral cortex of frontal lobe from rabbits of the control group showing neuronal cells in layers I, II, III, IV, V, VI and normal blood vessel (Bv). (H& E X 100)

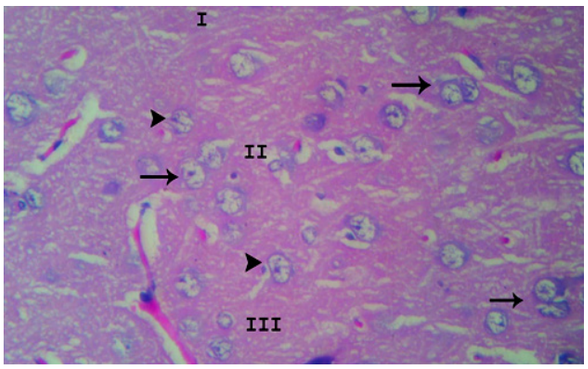

Fig. 2: A photomicrograph of a coronal section in cerebral cortex of frontal lobe from rabbits of the control group showing first layer (I) poorly cellular, second layer (II) contained cell islands of granular cell with large rounded vesicular nuclei (arrow) and single granular cell (arrow head) and (III) the third layer. (H& E X 400)

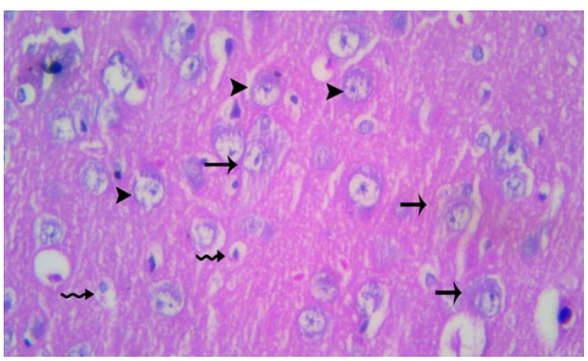

Fig. 3: A photomicrograph of a coronal section in cerebral cortex of frontal lobe from rabbits of the control group showing layer III contained pyramidal cell with basophilic cytoplasm, large vesicular nucleus and long apical dendrite (arrow), granular cell (arrow head) and glial cell with small dense nuclei were also seen (wavy arrow). (H& E X 400)

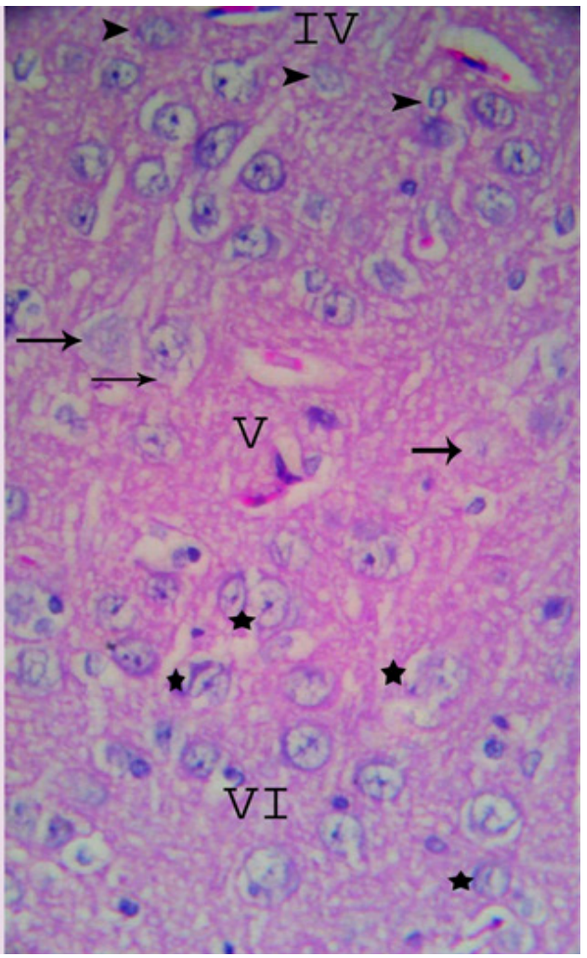

Fig. 4: A photomicrograph of a coronal section in cerebral cortex of frontal lobe from rabbits of the control group showing layer IV is characterized by the presence of many small granule cells (arrow head), layer V the layer of large pyramidal cells (arrow) and layer VI contained cells with various size and shape (star) (H& E X 400)

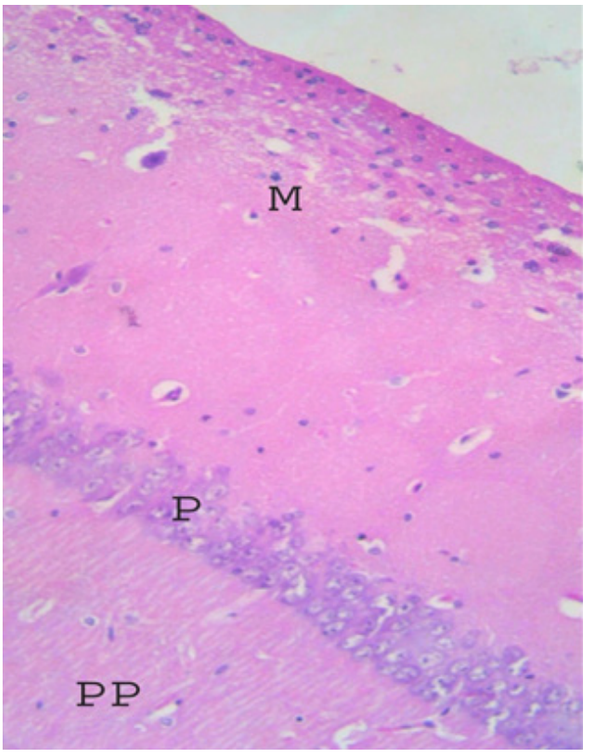

Fig. 5: A photomicrograph of a coronal temporal lobe section in the hippocampus from the control group showing molecular layer consisted mainly of fibers and some non pyramidal cell (M), pyramidal (P) and polymorphic (PP) layers. (H& E X 100)

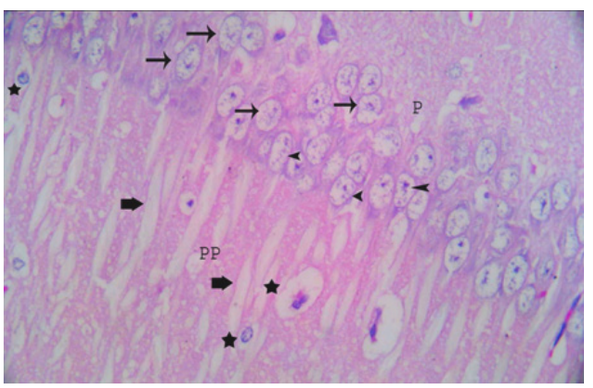

Fig. 6: A photomicrograph of a coronal temporal lobe section in hippocampus of control group showing pyramidal layer (P) contained pyramidal cell (arrow), granular flask shaped cell (arrow head), polymorphic layer (PP) contained interneuron (star) and fibers (thick arrow). (H& E X 400)

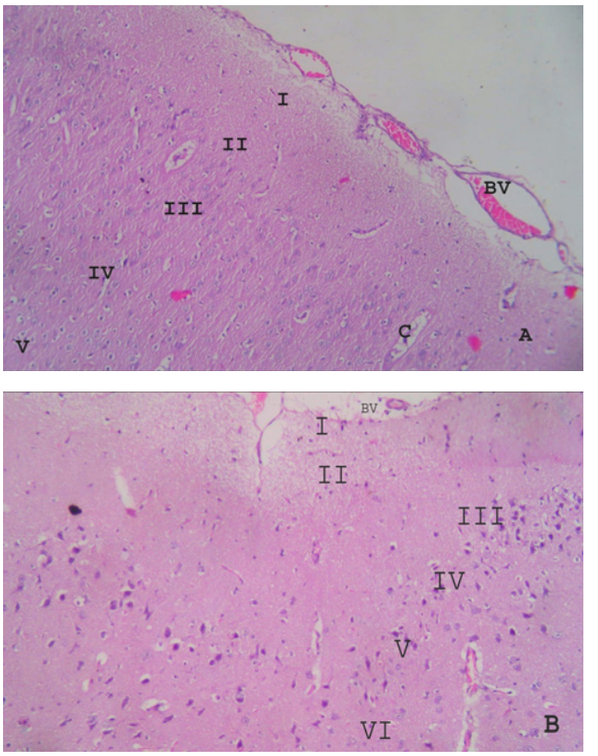

Fig. 7: A and B photomicrographs of a coronal section of cerebral cortex in frontal lobe from tramadol treated group showing layers I, II, III, IV, V and VI with neuronal disorganization and dilated blood vessel (Bv) and cabillary (C). (H& E X 100)

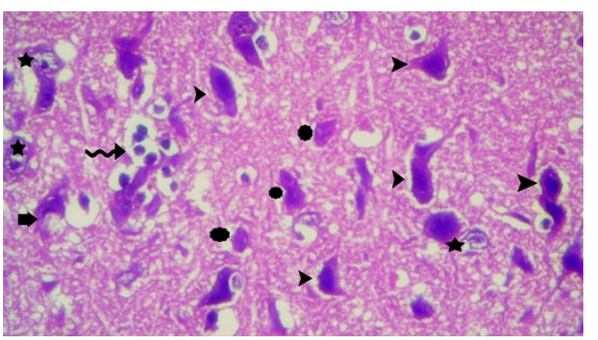

Fig. 8: A photomicrograph of a coronal section of cerebral cortex in frontal lope from tramadol treated group showing apoptotic cells characterized by neuronal shrinkage and chromatin condensation (arrow head), degenerative vacuolization (thick arrow), diffuse chromatolysis with absence of nucleoli (flower), wide intercellular space (star) and multinucleated cell (wavy arrow). (H& E X 400)

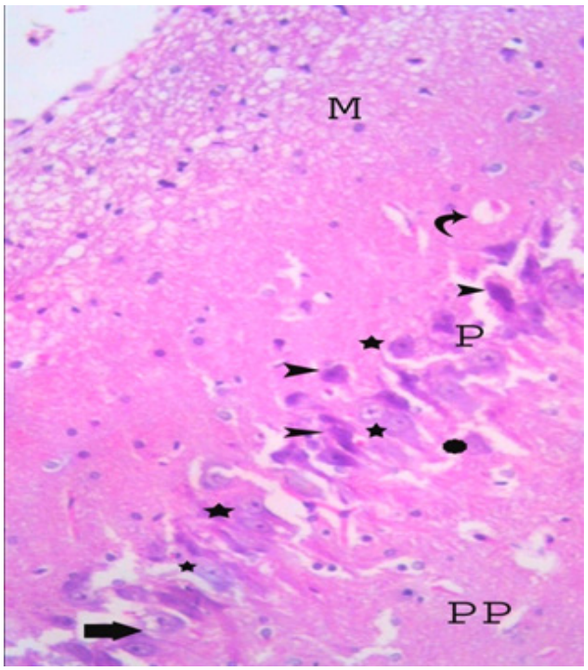

Fig. 9: A photomicrograph of a coronal temporal lobe section in the hippocampus from tramadol treated group showing molecular layer (M), pyramidal layer (P) contained apoptotic cells characterized by neuronal shrinkage and chromatin condensation (arrow head), wide intercellular space (star), and diffuse chromatolysis with absence of nucleoli (flower), red neuron (curved arrow) and polymorphic layer (PP). (H& E X 100)

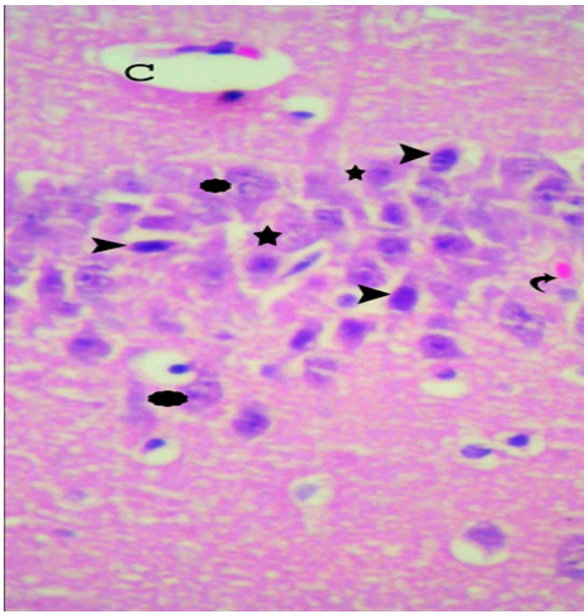

Fig. 10: A photomicrograph of a coronal temporal lobe section in the hippocampus from tramadol treated group showing apoptotic cells characterized by neuronal shrinkage and chromatin condensation (arrow head), wide intercellular space (star), diffuse chromatolysis with absence of nucleoli (flower), red neuron (curved arrow) and dilated capillary (C)(H& E X 400)

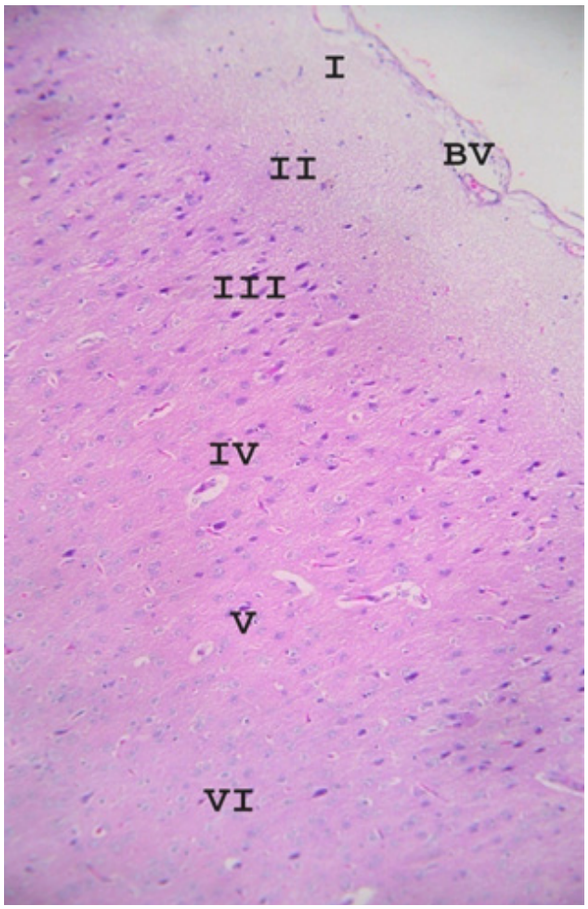

Fig. 11: A photomicrograph of a coronal section in cerebral cortex of frontal lobe from the recovery group showing nearly normal cerebral cortex layers I, II, III, IV, V, VI and normal blood vessel (Bv). (H& E X 100)

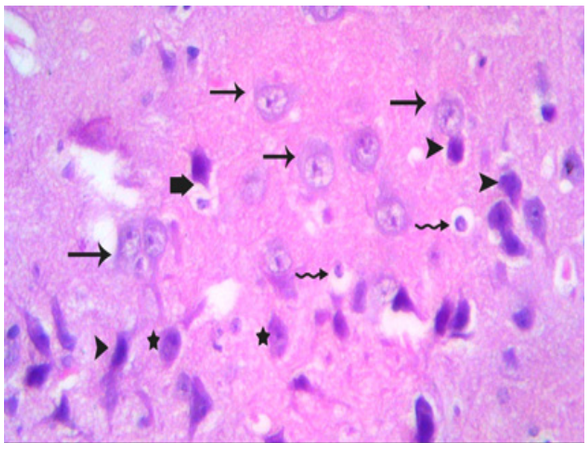

Fig. 12: A photomicrograph of a coronal section in cerebral cortex of frontal lobe from tramadol recovery group showing normal cells (arrow), some apoptotic cells (arrow head), degenerative vacuolization (thick arrow), wide intercellular space (star) and glial cell (wavy arrow). (H& E X 400)

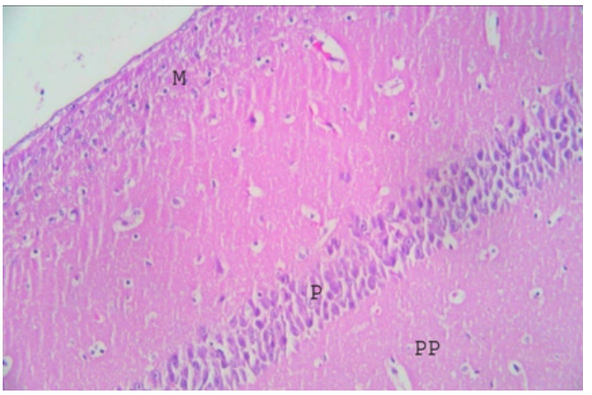

Fig. 13: A photomicrograph of a coronal temporal lobe section in hippocampus from tramadol recovery group showing nearly normal molecular (M), pyramidal (P) and polymorphic (PP) layers. (H& E X 100)

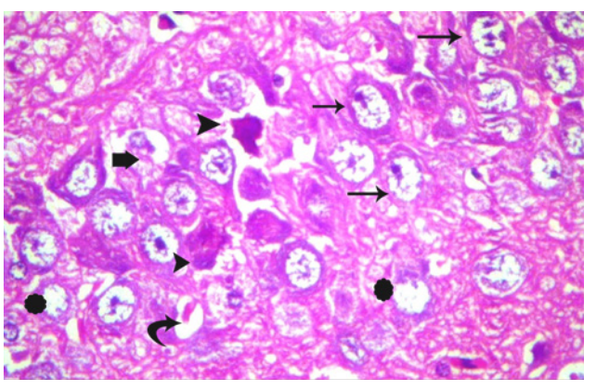

Fig. 14: A photomicrograph of a coronal temporal lobe section in hippocampus from tramadol recovery group showing normal cells (arrow), some apoptotic cells (arrow head), diffuse chromatolysis with absence of nucleus (flower), degenerative vacuolization (thick arrow) and red neuron (curved arrow). (H& E X 400)

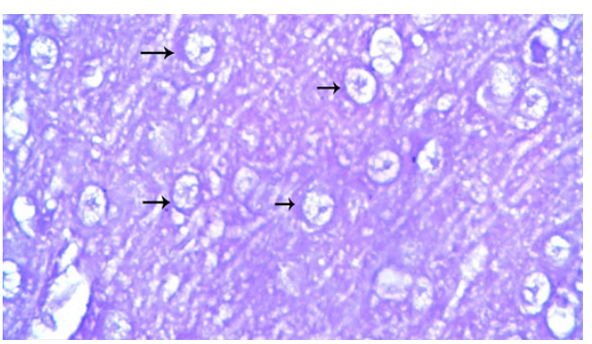

Fig. 15: A photomicrograph of a coronal section in cerebral cortex of frontal lobe from the control group showing the Nissl granules around the nucleus inside the nerve cell (arrow). (Cresyl violet × 400)

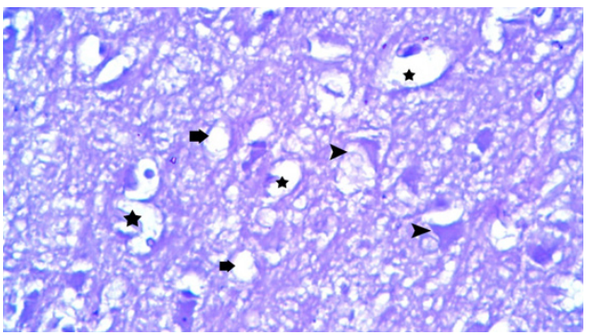

Fig. 16: A photomicrograph of a coronal section in cerebral cortex of frontal lobe from the tramadol treated group showing neurons with absent of nucleoli (thick arrow) neurons with dark shrinkage cytoplasm(arrow head) and swollen neurons (star). (Cresyl violet × 400)

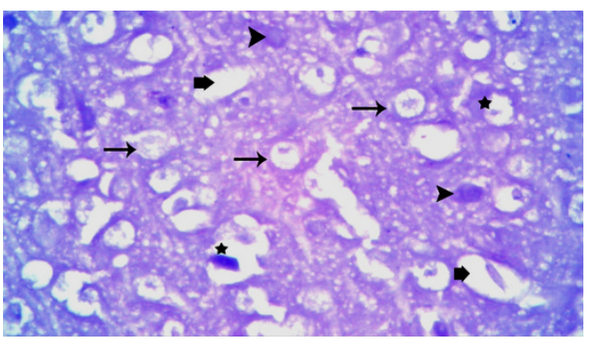

Fig. 17: A photomicrograph of a coronal section in cerebral cortex of frontal lobe from the tramadol recovery group showing some neurons have Nissl granules around the nucleus (arrow) and others neurons with absent nucleus (thick arrow), swollen neurons (star) and dark shrinkage cytoplasm (arrow head). (Cresyl violet × 400)

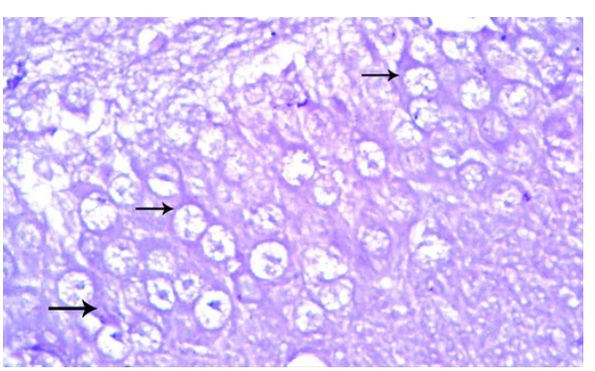

Fig. 18: A photomicrograph of a coronal section in hippocampus of temporal lobe from the control group showing the Nissl granules around the nucleus inside the nerve cell (arrow) (Cresyl violet × 400)

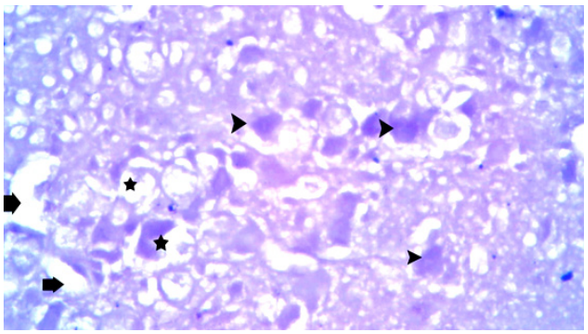

Fig. 19: A photomicrograph of a coronal section in hippocampus of temporal lobe from the tramadol treated group showing neurons with absent of nucleoli (thick arrow) neurons with dark shrinkage cytoplasm(arrow head) and swollen neurons (star) (Cresyl violet × 400)

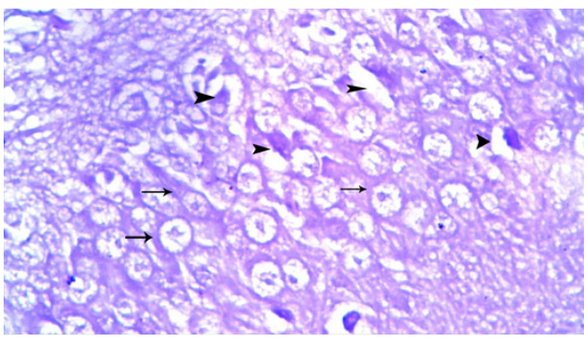

Fig. 20: A photomicrograph of a coronal section in hippocampus of temporal lobe from the tramadol recovery group showing some neurons have Nissl granules around the nucleus (arrow) and others neurons with absent nucleus (arrow head). (Cresyl violet × 400)

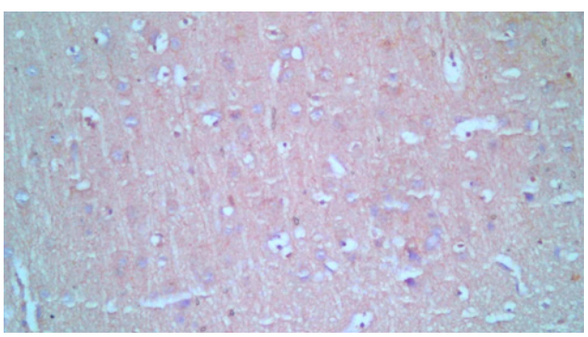

Fig. 21: A photomicrograph of a coronal section in cerebral cortex of frontal lobe from the control group showing the normal negative reaction of brain tissue to the Caspase–3 antibody (Caspase immunostaining × 400)

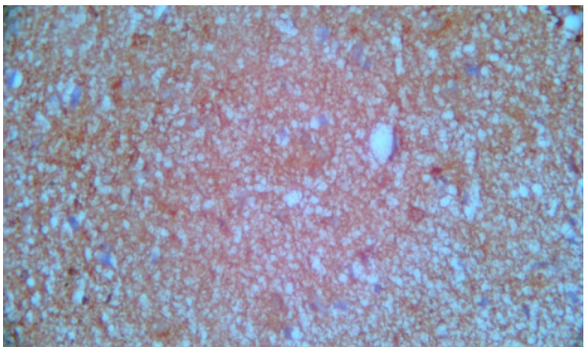

Fig. 22: A photomicrograph of a coronal section in cerebral cortex of frontal lobe from the tramadol treated group showing many neurons with strong positive reaction of brain tissue to the Caspase–3 antibody (Caspase immunostaining × 400)

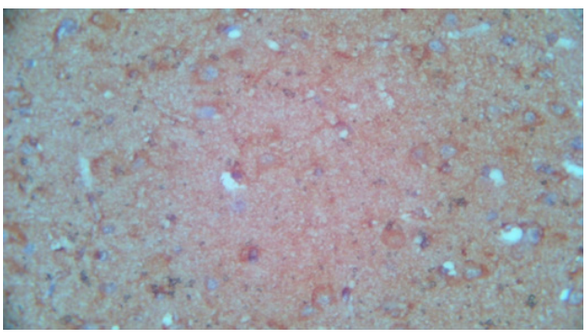

Fig. 23: A photomicrograph of a coronal section in cerebral cortex of frontal lobe from the tramadol recovery group showing some neurons with positive reaction of brain tissue to the Caspase–3 antibody (Caspase immunostaining × 400)

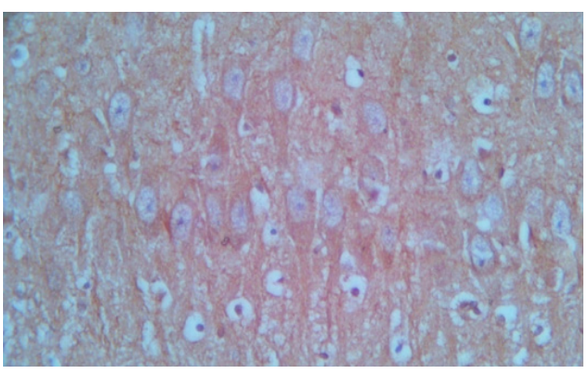

Fig. 24: A photomicrograph of a coronal temporal lobe section in hippocampus from the control group showing the normal negative reaction of brain tissue to the Caspase–3 antibody (Caspase immunostaining × 400)

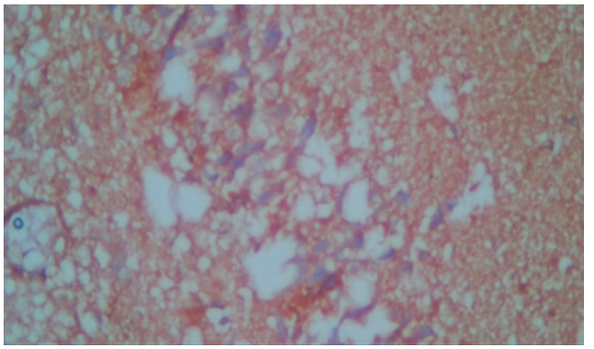

Fig. 25: A photomicrograph of a coronal temporal lobe section in hippocampus from the tramadol treated group showing many neurons with strong positive reaction of brain tissue to the Caspase–3 antibody (Caspase immunostaining × 400)

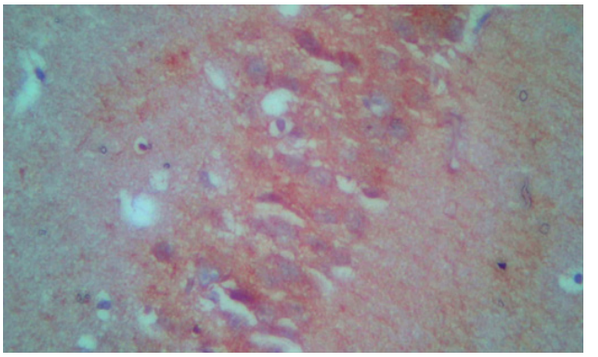

Fig. 26: A photomicrograph of a coronal temporal section in hippocampus from the tramadol recovery group showing some neurons with positive reaction of brain tissue to the Caspase–3 antibody (Caspase immunostaining × 400)

Table 1: Frequency distribution of the histopathological changes in cerebral cortex sections of the rabbits in all studied groups

	apoptotic cells	Degenerative vaculation	Wide Intercellular space	Diffuse chromatolysis	Multinuclear cells
Control group %	0%	1%	0%	1%	0%
Tramadol treated group %	100%	65%	45%	30%	20%
Tramadol recovery group %	30%	15%	10%	5%	2%

Table 2: Frequency distribution of the histopathological changes in hippocampus sections of the rabbits in all studied groups

	apoptotic cells	Intercellular odema	Diffuse chromatolysis	Red neurons
Control group %	0%	1%	1%	0%
Tramadol treated group %	100%	55%	35%	20%
Tramadol recovery group %	25%	10%	1%	3%

DISCUSSION

Tramadol abuse has increased in the Middle East region, tramadol use was common among adolescents and over one third of tramadol users had drug-related problems[3].

Chronic use of tramadol leads to impairment in learning and memory because of their effect on the frontal cortex and the hippocampus[13].

The present study was designed to study the effects of tramadol abuse in male albino rabbits on the cerebral cortex and the hippocampus and the effect of its withdrawal.

In this study the rabbit's behaviors changed after tramadol administration as the following, during the first ten days they behaved normally except for sedation and lack of interest in their food before the designed dose. During the second ten days, the rabbits became restless, irritable being aggressive when handled and some had tremors one to two hours before the designed dose, after the dose became calmness, very inactive and anorexia had developed. As the dose increased the rabbits became more irritable with bulging eyes, some of them became aggressive to other rabbits, anorexia and loss of body weight. Some symptoms like tremors and salivation were also noted. This result was in the same current with the result of[9] they found that tramadol abuse during adolescence caused behavior alteration in the form of antidepressant–like effect and development of depression in rats was associated with reduction the intake of a sucrose solution. In the same way[14] reported that tramadol administration in adult rats resulted in behavioral changes as restlessness, irritability defensive and aggressive reactions.

In this study after stoppage of tramadol in take the rabbit's behaviors during the first ten days of withdrawal were exaggerated there were tremors, irritability, salivation and anorexia. But these behaviors were gradually improved by the time till the end of the fourth week. This was in agreement with[14] they reported that after withdrawal of tramadol there were gradual improvement in rats aggressive behaviors after two months and these behaviors were milder and improved rapidly after lofexidine administration during the recovery period.

In the present study hematoxylin and eosin stained sections of cerebral cortex from rabbits in tramadol treated group showed neuronal cell disorganization, 100% of cerebral cortex sections contained apoptotic cells characterized by neuronal shrinkage and chromatin condensation, 65% intercellular edema as the cells appeared with visible intact nuclei with increased

cytoplasm/ nucleus ratio, 45% degenerative vacuolization, 30% diffuse chromatolysis of nuclear chromatin with absence of nucleoli detected in layers I, II, III, IV, V and VI. 20% multinuclear cells were present in layers III. Dilated congested blood vessels and capillary and by cresyl violet staining there were neurons with absent nucleoli, neurons with dark shrinkage cytoplasm and swollen neurons. On the contrary to the present study[9] studied the neurotoxic effect of tramadol and cannabis on adolescence male albino rats, they found that abuse of tramadol or cannabis, alone and in combination, caused antidepressant effect, impaired spatial memory and raised serotonin levels in the cerebral cortex and hippocampus that induced oxidative stress.

Also[15] studied the effect of tramadol on motor cortex and added that the pyramidal and granular cells appeared shrunken and apoptotic[16]. Reported that administration of tramadol increased density of α1–adrenoceptors in the rat brain cortex. Brain tissues showed congestion of submeningeal blood vessels and neural degeneration.

In the present study hematoxylin and eosin stained sections of the hippocampus from rabbits in tramadol treated group showed marked neuronal degeneration as 100% of hippocumpus sections contained apoptotic cells characterized by neuronal shrinkage and chromatin condensation and 55% intercellular edema, 35% diffuse chromatolysis of nuclear chromatin with absence of nucleoli detected in pyramidal layer, 40% extensive degenerative vacuolization, 20% of hippocumpus sections contained red neurons and by cresyl violet staining there were neurons with absent nucleoli, neurons with dark shrinkage cytoplasm and swollen neurons. A previous study[17] concluded that Tramadol had degenerative effects on both lateral and medial entorhinal areas.

In corroboration[18] studied the effect of acute and chronic administration of tramadol on spatial memory in rats and they revealed that tramadol impaired memory when administered acutely or chronically. Single dose administration of tramadol showed more destructive effect than multiple doses of tramadol on the memory. Also[19] founded that tramadol and cannabis exerts different effects on Acetylcholinesterase, butyrylcholinesterase and paraoxonase1activities which could contribute to memory problems and the decline in cognitive function in chronic users.

In the present study hematoxylin and eosin stained sections of cerebral cortex from rabbits in tramadol recovery group showed return of cerebral cortex towards normal morphology as shown by remarkable regression of the total degenerative changes that induced by tramadol in all brain sections. Most of neuronal cells appeared normal while 30% of brain sections contained apoptotic cells, 15% intracellular edema and 10% showed degenerative vacuolization and by cresyl violet staining there were some neurons showed Nissl granules around the nucleus and other neurons showed absence of nucleoli. This was in agreement with[14] they found slight improvement in the histo-morphological changes in cerebral cortex, however with lefoxidine administration the cerebral cortex sections showed nearly normal appearance of the neurons and neuropil, return of brain tissues towards normal morphology.

In this study hematoxylin and eosin stained sections of hippocampus from rabbits in tramadol recovery group showed nearly normal appearance of the hippocampus with its three layers molecular, pyramidal and polymorphic. While 25% of hippocampus sections showed apoptotic neurons, 10% intracellular edema 5% showed degenerative vacuolization and 3% red neurons and by cresyl violet staining there were some neurons showed Nissl granules around the nucleus and other neurons showed absence of nucleoli, few neurons with dark shrinkage cytoplasm and swollen neurons. In the same way[9] they found that after withdrawal, the antidepressant effect was reversed, improvement in antioxidants and apoptotic markers and incomplete regression of brain histopathological alteration but[20] found that 2 weeks after stoppage of tramadol treatment as a recovery period there were decrease in serum level of urea, creatinine, sialic acid, interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α) and brain tissue nuclear factor kappa B (NF-kB), myeloperoxidase (MPO) concentration in withdrawal rats when compared with these levels in tramadol treated groups.

In the present study after caspase-3 immunohisto-chemical staining of brain tissue that demonstrated the apoptotic neurons as brown cytoplasmic staining, caspase 3 immunostaning sections in tramadol treated group showed many neurons with strong positive reactions to the Caspase–3 antibody in their cytoplasm. While caspase 3 immunostaning sections of the brain from tramadol withdrawal group showed few neurons with mild positive reactions to the Caspase–3 antibody in their cytoplasm. This was in agreement with[21] they reported that on molecular level, the expression of the pro-apoptotic Bax and Caspase-3 showed a significant increase because the anti-apoptotic Bcl-2 decreased markedly indicating that tramadol is harmful at cellular level and induce apoptotic changes in brain tissues[22]. They concluded that administration of tramadol disturbed learning and memory and it has neurotoxicity effects on inducing dark neurons formation and apoptosis in rat hippocampus.

Concluded[14] that repeated Tramadol administration causes degenerative changes on the rat brain which increased with increasing the period of administration. Some improvement was detected after stoppage and more improvement with Lofexidine treatment in withdrawal period[23]. Concluded that administration of tramadol have histological abnormalities on both cerebral cortex and testicular tissues associated with oxidative stress in these organs. Also, apoptosis were increased in both organs which regress after withdrawal.

CONCLUSION

Based on the results of the present study, it can be

concluded that tramadol abuse induced neurotoxicity and histological changes in brain tissue. After stoppage of tramadol intake there was incomplete regression of brain histopathological alterations.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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الملخص العربى

دراسه هستولوجيه وهستوكميائيه مناعيه على تاثيرتعاطي الترامادول على القشرة المخية والحصين في الأرانب البيضاء الذكور

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المقدمه: الترامادول هو مسكن يستخدم في الألام البسيطه والشديده ، لكن تعاطي الترامادول بين المراهقين يؤدي إلى تغيرات مختلفة في التركيب النسيجي للمخ مما يؤدي إلى مشاكل نفسية وجسدية.

الهدف من هذا الدراسه: تهدف هذه الدراسة إلى تقييم آثار تعاطي الترامادول في الأرانب البيضاء الذكور على القشرة المخيه والحصين وتأثير انسحابه.

طرق البحث: اشتملت الدراسه على ثلاثين من الأرانب الذكور البيضاء وقسمت إلى ثلاث مجموعات. المجموعة الضابطة، المجموعة التي عولجت بالترامادول وتم اعطاء الأرانب ترامادول ٤٢ ميلي غرام لكل كيلوغرام في اليوم لمدة عشرة أيام ثم زادت الجرعة إلى ٨٤ ميلي غرام لكل كيلوغرام في اليوم لعشرة أيام أخرى ثم ١٦٨ ميلي غرام لكل كيلوغرام في اليوم لعشرة أيام أخرى. مجموعة التعافى من الترامادول و تم فيها اعطاء الأرانب نفس جرعات الترامادول كما في المجموعة السابقة ثم توقف اعطاء الترامادول للارانب لمدة ٤ أسابيع، ثم تقييم الدراسات النسيجية والنسيجيه المناعية للقشرة المخيه والحصين.

النتائج: تسبب تعاطي الترامادول في عدم تنظيم الخلايا العصبية في القشرة المخية والحصين حيث ادى الى موت الخلايا المبرمج وانحلال الكروماتين، وايجابية تفاعل الأجسام المضادة لـكاسبيز ٣. وبعد توقف تعاطى الترامادول، وجد العديد من الخلايا العصبية السليمه وكذالك قلت نسبة موت الخلايا المبرمج مع موت وانحلال الكروماتين و القليل من تفاعل الأجسام المضادة لـكاسبيز ٣.

الخلاصة: تعاطي الترامادول يؤدى الى السميه العصبيه وتغيرات نسيجية في المخ، بعد توقف تعاطى الترامادول لم يتم تعافى انسجة المخ بالكامل.