Histological study on the effect of different doses of tramadol administration on adult male albino rat cerebellum

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Abstract: Background: Tramadol hydrochloride is a centrally acting synthetic analgesic drug used to treat moderate to severe pain. Long-term use of high doses of tramadol may be associated with physical dependence and a withdrawal syndrome. **Objective:** This study was designed to demonstrate the histological changes and immunohistochemical findings in the cerebellum of the adult male albino rats induced by different doses of tramadol administration. **Materials and methods:** 50 adult male albino rats were divided into two main groups: control group (Group I), received a balanced diet for 2 weeks and experimental group (Group II), divided into two subgroups; IIA, divided into IIA_1, injected with 0.7 mg as a single daily dose of tramadol solution for 2 weeks and IIA_2, injected with 0.7 mg as a single daily dose of tramadol solution for 2 weeks, then left for another 2 weeks without injection for recovery and IIB, divided into two subgroups IIB_1, injected with 1.8 mg as a single daily dose of tramadol solution for 2 weeks and IIB_2, injected with 1.8 mg as a single daily dose of tramadol solution for 2 weeks, then left for another 2 weeks without injection for recovery. At the end of the experiment, the rats were sacrificed and decapitated. The cerebellum was removed and prepared for histological and immunohistochemical studies. **Results:** Group II A_1showed mild congested blood capillary. It also showed degenerated Purkinje cells characterized by deformity in the nuclear membrane appeared as finger projections. Group II A_2showed mild restoration of the normal histomorphological structures. It also showed degenerated Purkinje cell with projections of its nuclear membrane and remnants of electron dense bodies. Group II B_1showed congested blood vessels with empty spaces in the Purkinje layer with disarrangement of Purkinje cells. It also showed an irregular-shaped, necrotic Purkinje cells. Group II B_2showed mild congested blood vessels with still presence of some apoptotic Purkinje cells and mild degenerated nerve axons with some disarrangement of myelin sheath. **Conclusion:** The results of this study provided evidence that tramadol intake exerts a neurotoxic effect on the cerebellar structure in an ascending manner according to the dose administered.

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1. Introduction:

Tramadol hydrochloride is a centrally acting synthetic analgesic drug used to treat moderate to severe pain. This drug has a wide range of applications such as in the treatment of rheumatoid arthritis, restless legs syndrome, motor neuron disease, fibromyalgia, and to control labor pain (1). The most commonly reported adverse reactions of this drug are nausea, vomiting, sweating, itching, and constipation. It is the most frequently suspected cause of provoked seizures. Long-term use of high doses of tramadol may be associated with physical dependence and a withdrawal syndrome. Tramadol causes typicalopiate-like withdrawal symptoms as well as atypical withdrawal symptoms including seizures (2). Repeated tramadol administration in patients might lead to accumulation of toxic metabolites in the body, increase the risk for pharmacokinetic interactions, and/or decrease the clearance of tramadol, thus increasing its potential for toxicity (3). In addition, other studies have been revealed that chronic tramadol administration in rats is associated with significant changes in the principle proteins involved in the apoptosis signaling which collectively leads to induction of apoptosis (4).

2. Materials and methods:

50 adult male albino rats were used in this study weighing about 200 g / each. They divided into two main groups:

Control group (Group I):

It includes 10 rats that were received a balanced diet and free water supply for 2 weeks.

Experimental group (Group II):

It includes 40 rats that were divided equally into two subgroups IIA and IIB:

Subgroup IIA: It includes 20 rats that were divided equally into: **IIA_1**, injected intraperitoneally with 0.7 mg as a single daily dose of tramadol solution for 2 weeks (this dose is equivalent to the human dose of 40 mg as a single daily dose) and **IIA_2**, injected intraperitoneally with 0.7 mg as a single daily dose of tramadol solution for 2 weeks, then left for another 2 weeks without injection with tramadol for recovery (5).

Subgroup IIB: It includes 20 rats that were divided equally into: **IIB_1**, injected intraperitoneally with 1.8 mg as a single daily dose of tramadol solution for 2 weeks (this dose is equivalent to the human dose of 100 mg as a single daily dose) and **IIB_2**, injected intraperitoneally with 1.8 mg as a single daily dose of tramadol solution for 2 weeks, then left for another 2 weeks without injection with tramadol for recovery (6).

The drug was purchased from the October Pharma, Egypt in the form of an ampoule (100 mg/2 ml). The ampoule was diluted in saline to 100 ml volume; thus, the concentration became 1 mg/ml. Subgroup IIa and subgroup IIb were injected with 0.7 and 1.8 ml of diluted tramadol, respectively (5).

At the end of the experiment, the rats of each group will be sacrificed and decapitated. The cerebellum specimens were fixed in Bouin's solution and processed for paraffin blocks. Sections were cut into 5µm thickness and stained with Hx & E for routine histological study, silver stain for dendrites, caspase-3 immune-stain for detection of apoptosis (7). Other specimens were fixed in 3% buffered glutaraldehyde, semithin sections were cut into 1 µm thickness, stained with toluidine blue stain for detection of structure of the Purkinje cells (5).

Ultrathin sections (70 nm) were obtained and stained with uranyl acetate and lead acetate and were examined using JEOL JEM - 2100(TEM) in the Electron Microscopic Unit, Mansoura University (8).

Statistical analysis:

Data was statistically analyzed using SPSS (Statistical package for social science). Data was expressed as mean ± SD and analyzed by using student's t- test for comparison between two groups. Differences were regarded as non-significant $P > 0.05$, significant $P < 0.05$, highly significant $P < 0.01$ and very highly significant when $P < 0.001$ (9).

3. Results:

A- Histological results:

Group I (control):

Sections that stained with Hx. & E. showed the normal histomorphological structures of the cerebellum including white mater, Purkinje layer that formed of cells arranged in one row, the granular layer with a large number of small deeply stained cells and cerebellar islands in between, and the molecular layer that formed mainly of few cells (figs.1-2). Other sections that stained with silver showed Purkinje cells of normal sizes with its chromatin and normal prominent dendrites which appeared gold brown between the molecular layer and the granular layer (figs.3-4). Also, sections that stained with toluidine blue showed the normal structure of the molecular layer with few small cells, the Purkinje cells which appear pyriform in shape with large rounded vesicular nuclei and prominent nucleoli, and the granular layer that have small cells with deeply stained nuclei (fig.5). Other sections that stained with caspase 3 showed negative stainable Purkinje cells (fig.6).

Sections that examined by TEM showed the normal structure of granular cells with large nuclei, condensed chromatin and a thin rim of cytoplasm beside a few myelinated nerve fibers and numerous dendrites of Purkinje cells (fig.7). It also showed the normal ultra-structures of Purkinje cells with a large nucleus having normal chromatin, normal cytoplasmic organelles including Golgi apparatus and many mitochondria (fig.8).

Group II A_1:

Sections that stained with Hx. & E. showed mild congested blood capillary in white mater, loss of some Purkinje cells, chromatolysis of nuclei and eosinophilic cytoplasm of others with nearly normal others with empty spaces in between (figs.9-11). Also showed numerous pyknotic cells with mild edema within the molecular layer (figs.12-13). Other sections that stained with silver showed small sized Purkinje cells with dendritic destruction (fig.14). Also, sections that stained with toluidine blue showed loss of the pyriform shape of Purkinje cells with irregular large pyknoticnuclei. Anumerous dark-stained granular cells also appeared (fig.15). Other sections that stained with caspase 3 showed moderately stainable Purkinje cells surrounded by vacuoles and positive numerous small cells (figs.16-18).

Sections that examined by TEM showed the structure of normal granular cell with a large nucleus, condensed chromatin and a thin rim of cytoplasm. Another granular cells showed irregularity in its cell membrane, an indented nucleus with dispersed chromatin, gaps in its nuclear membrane with some vacuoles, besides mildly degenerated vacuolated myelin sheath of nerve fibers (figs.19-20). It also showed degenerated Purkinje cells characterized by deformity in the nuclear membrane appeared as finger projections, dispersed chromatin, disappearance of normal cytoplasmic organelles with small atrophied mitochondria besides numerous large dense bodies,

dilated Golgi apparatus and destroyed cisternae of rough endoplasmic reticulum (figs.21-22).

Group II A_2:

Sections that stained with Hx. & E. showed mild restoration of the normal histomorphological structures including Purkinje layer between molecular layer and granular layer (fig.23). Also some normal Purkinje cells with abundant cytoplasm and prominent chromatin could be seen among many shrunken Purkinje cells with pyknotic nuclei and eosinophilic cytoplasm. The normal Purkinje cells lost the normal arrangement in one line but instead they were seen among cells of the granular layer (figs.24-26). Other sections that stained with silver showed some restoration of Purkinje cells' dendrites, but the cells were still surrounded by empty vacuolation (figs.27-28). Also, sections that stained with toluidine blue also showed nearly normal Purkinje cells, while other cells appeared shrunken with pyknotic nuclei and darkly stained cytoplasm (fig.29). Other sections that stained with caspase 3 showed some Purkinje cells with mild +ve stain beside some other small cells with marked immunopositivity (figs.30-31).

Sections that examined by TEM showed restoring of granular cells with rearranged chromatins, and axonal myelin sheaths besides numerous interstitial electron dense bodies (figs.32-33). It also showedone degenerated Purkinje cell with projections of its nuclear membrane and remnants of electron dense bodies (fig.34). It also showed part of cytoplasm of Purkinje cell with normal mitochondria and axonal myelin sheath (fig.35).

Group II B_1:

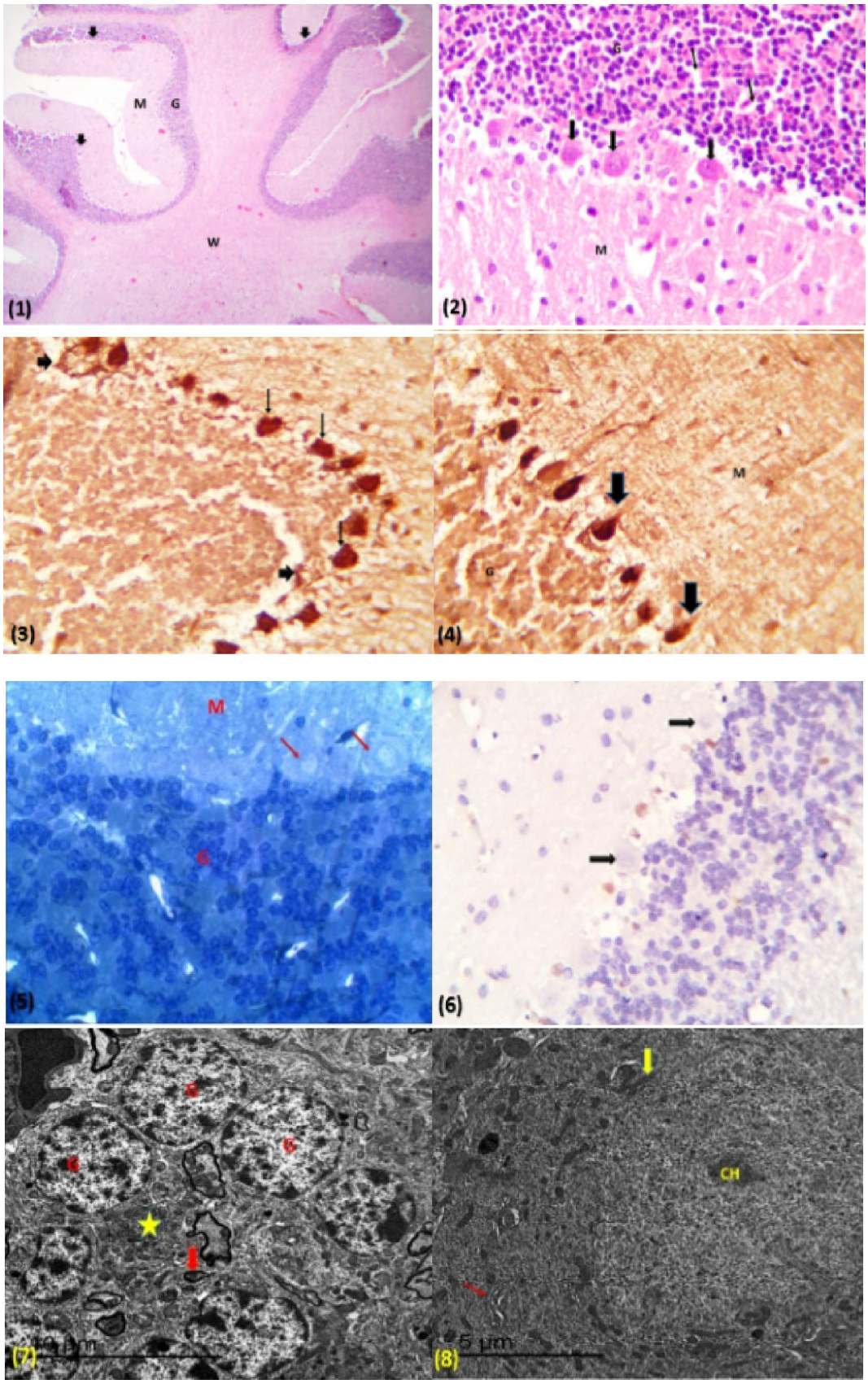
Sections that stained with Hx. & E. showed congested blood vessels with empty spaces in the Purkinje layer (fig.36). Accumulation with disarrangement of Purkinje cells were observed in some sections (fig.37). The cells appeared with deeply stained acidophilic cytoplasm and pyknotic nuclei disarranged among the granular cell layer which showed hypocellularity (fig.38). Empty spaces and appearance of remnants of Purkinje cells (cell ghosts) were also seen in Purkinje cell layer (fig.39). Mild demyelination in the molecular layer, some congestion and wide cerebellar island were observed in other sections (fig.40). Numerous empty spaces and wide separation of white matter fibers was noticed (fig.41). Focal gliosis was seen in some sections represented by focal accumulation of neuroglia cells among white matter (fig.42). Other sections that stained with silver showed loss of Purkinje cells in some area leaving remnants of cells (cell ghosts), while other Purkinje cells appeared with condense chromatin and loss of their dendrites beside empty areas (fig.43). Also, sections that stained with toluidine blue showed an irregular shaped Purkinje cells with an irregular nucleus, and another cells with karyolytic nuclei besides the granular layer that contains small cells with no nuclei (fig.44). Other sections that stained with caspase 3 showed marked +ve staining of shrunken apoptotic Purkinje cells as well as numerous small cells. Also showed marked immunopositive staining of shrunken pyramidal neurons beside vacuolation (fig.45).

Sections that examined by TEM showed apoptotic granular cells in the granular layer with faint chromatin and a thin cytoplasm (fig.46). It also showed an irregular-shaped, necrotic Purkinje cell with an irregular shrunken nucleus and an ill-defined nuclear membrane with disappearance of numerous cytoplasmic organelles and degenerated axons with disarrangement of myelin sheath (figs.47-48).

Group II B_2:

Sections that stained with Hx. & E. showed nearly normal cerebellar layers including normal Purkinje cells with mild congested blood vessels (figs.49-50), with still presence of some apoptotic Purkinje cells with chromatolysis surrounded by wide spaces. Narrowing and hypocellularity of the granular layer was also observed (fig.51). It also showed moderate demyelination and chromatolysis of pyramidal neurons in the white matter (fig.52). Other sections that stained with silver showed mild restoration of Purkinje cells dendrites with condense chromatin beside mild edema (figs.53-54). Also, sections that stained with toluidine blue also showed restoration of some Purkinje cells characterized by prominent dendrites with still presence of chromatolysis within a few small sized other Purkinje cells (fig.55). Other sections that stained with caspase 3 showed negative immunopositive staining of some Purkinje cells, with presence of mild to moderate immunopositive staining of other Purkinje cells surrounded by mild vacuolation beside marked positive small cells (figs.56-57).

Sections that examined by TEM showed nearly normal granular cells and still presence of mild degenerated nerve axons with some disarrangement of myelin sheath (figs.58-59). It also showed regenerative attempts of Purkinje cell with irregular nucleus with active small-sized dilated Golgi apparatus, rough endoplasmic reticulum, small-sized mitochondria, some electron dense bodies and short dendrites (fig.60).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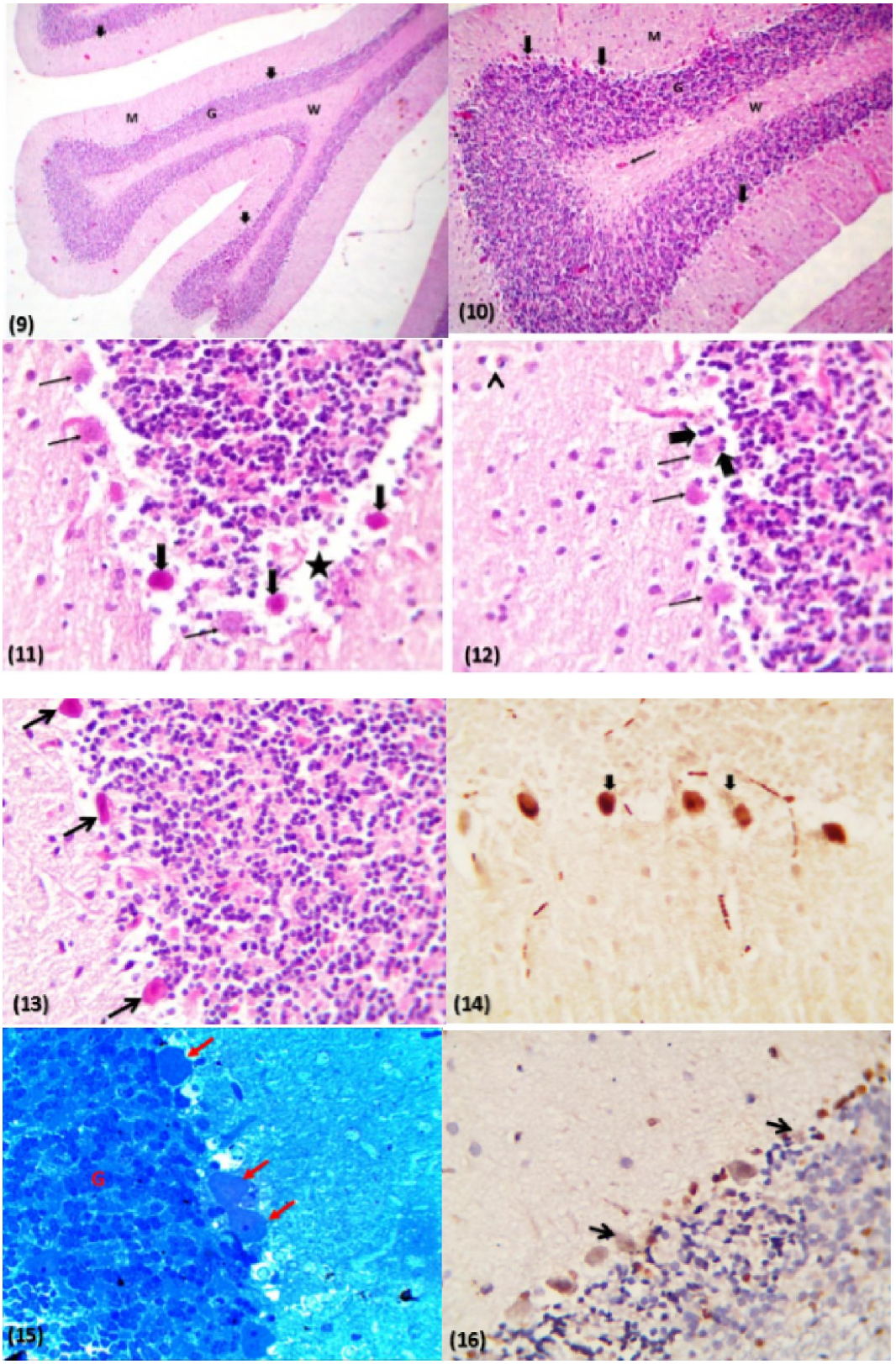
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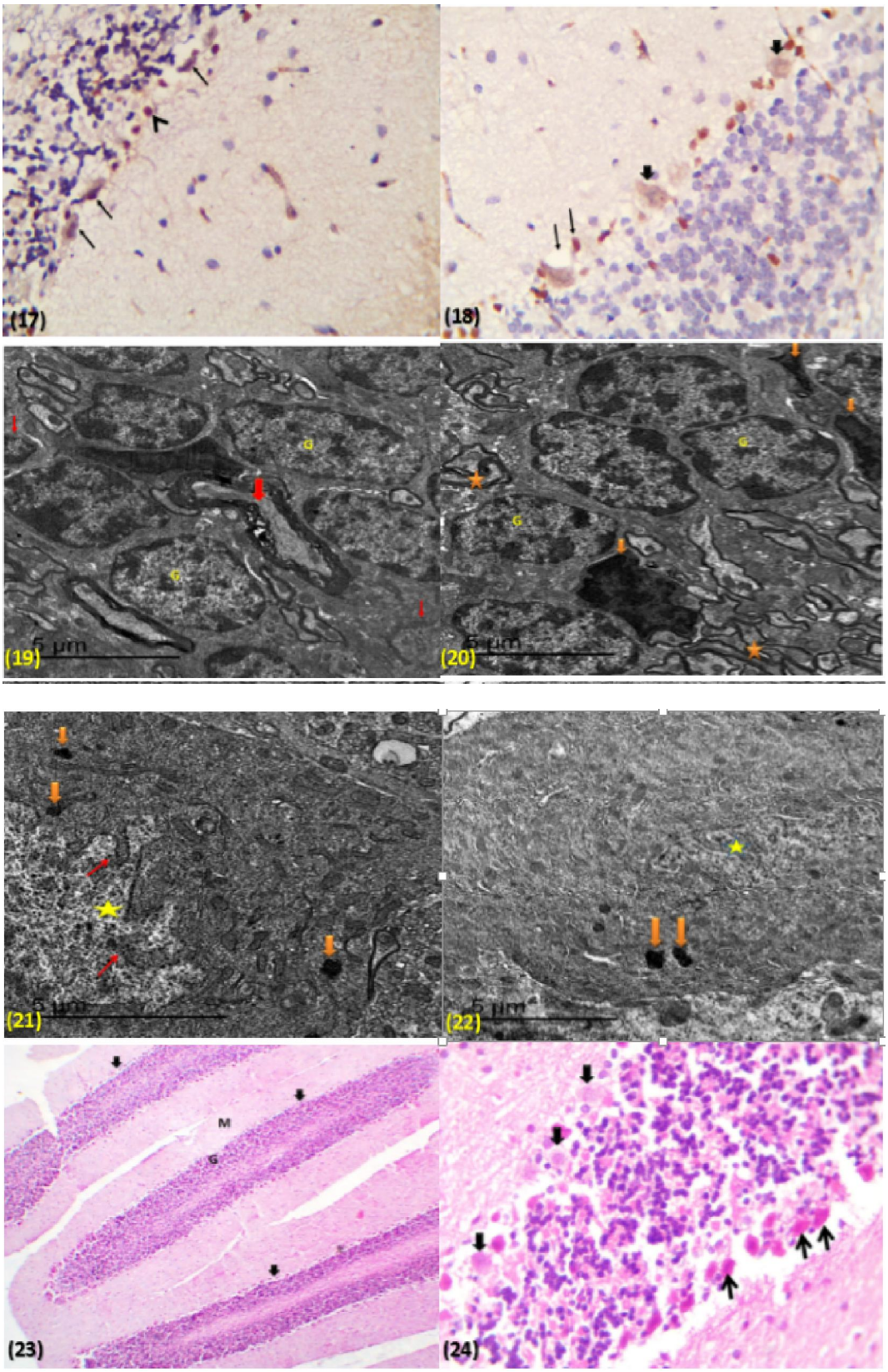
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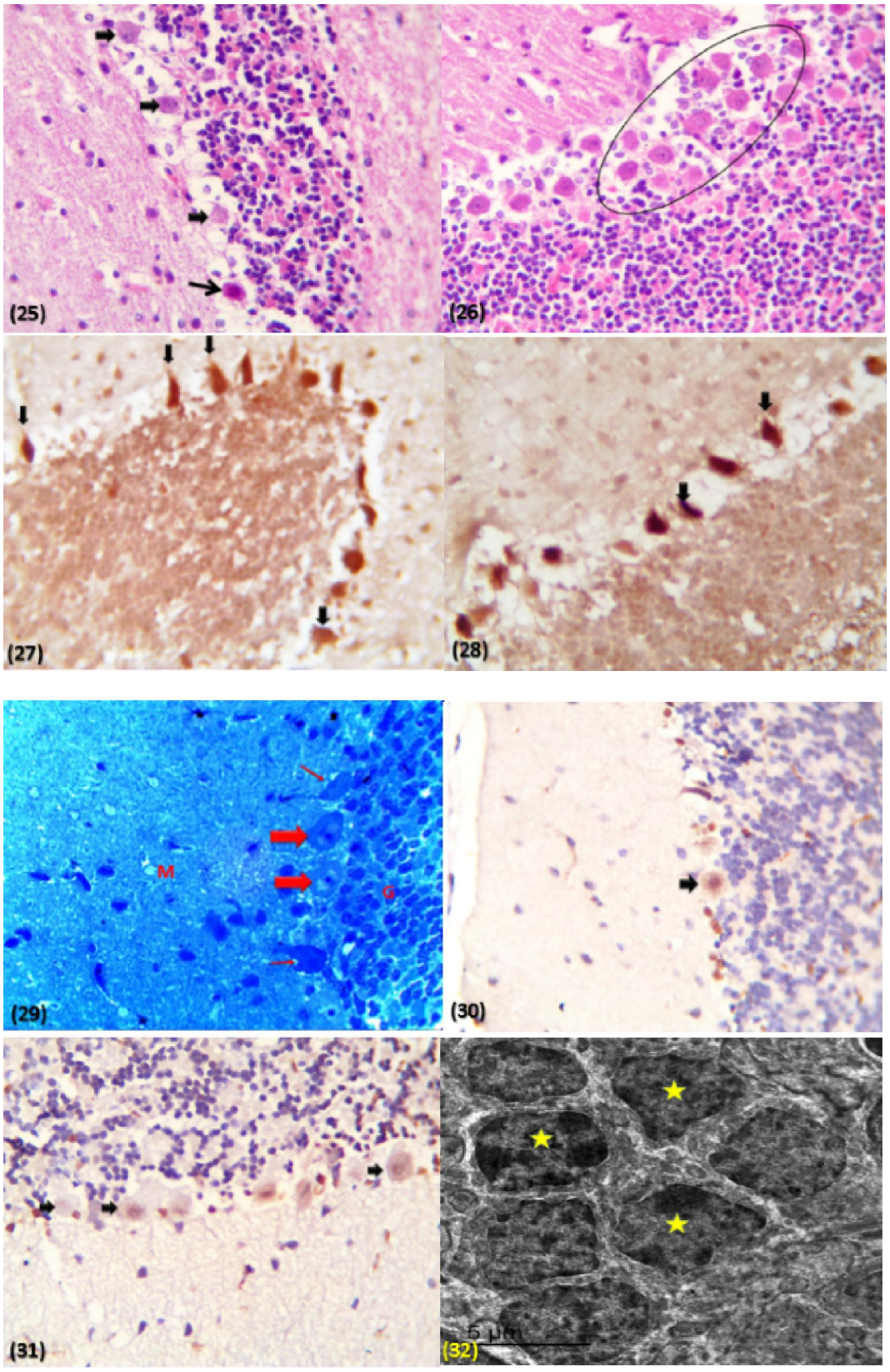
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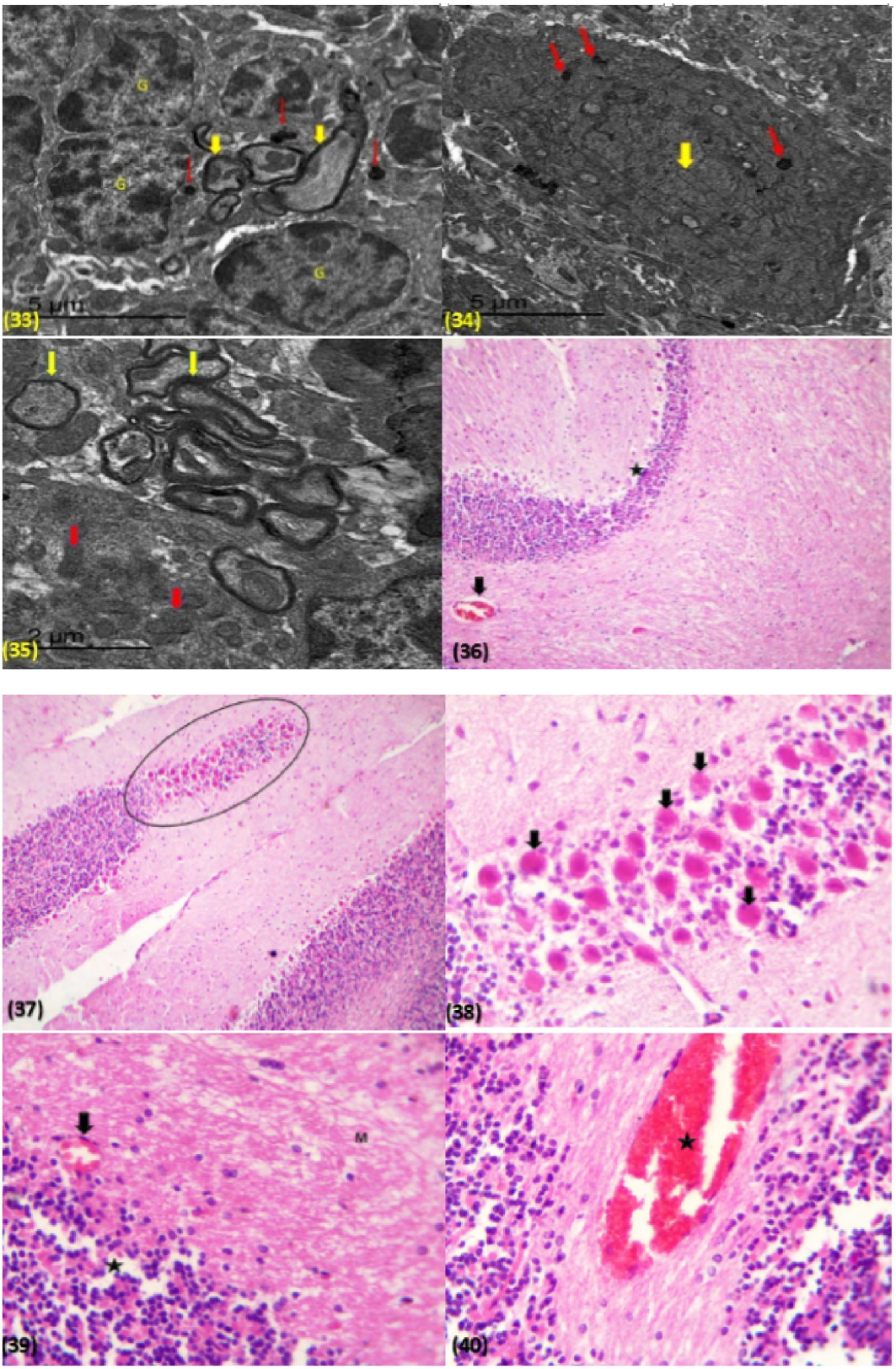
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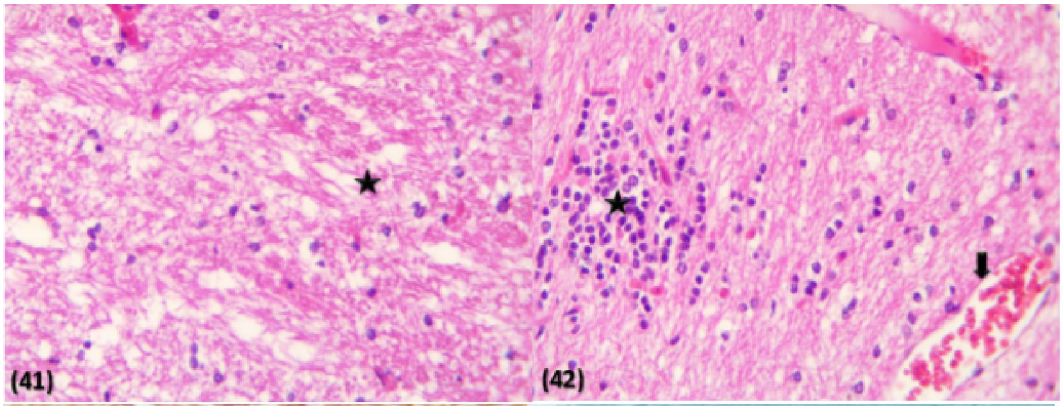
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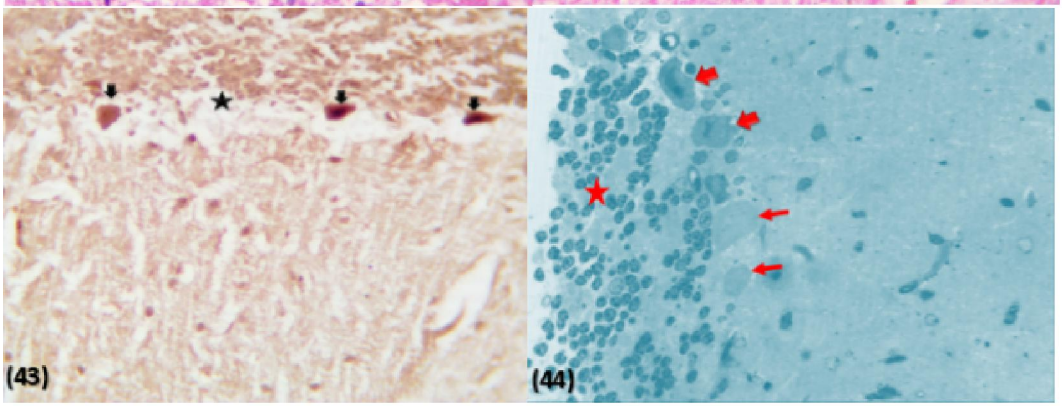
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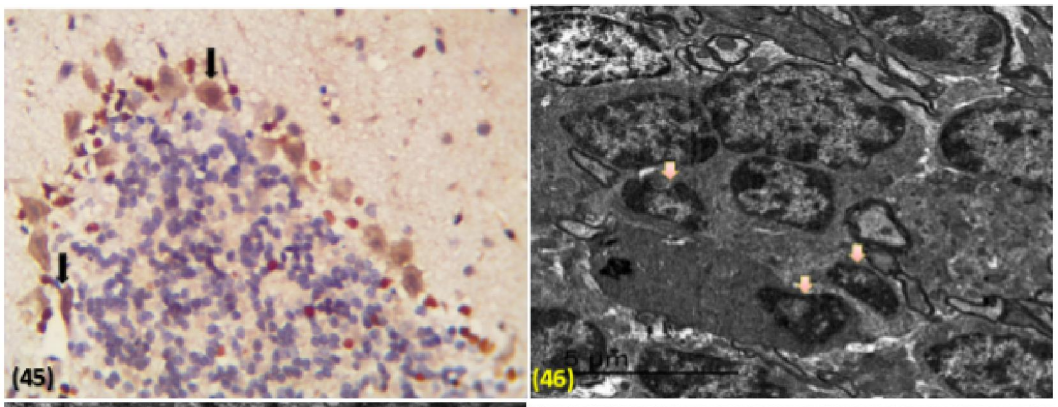
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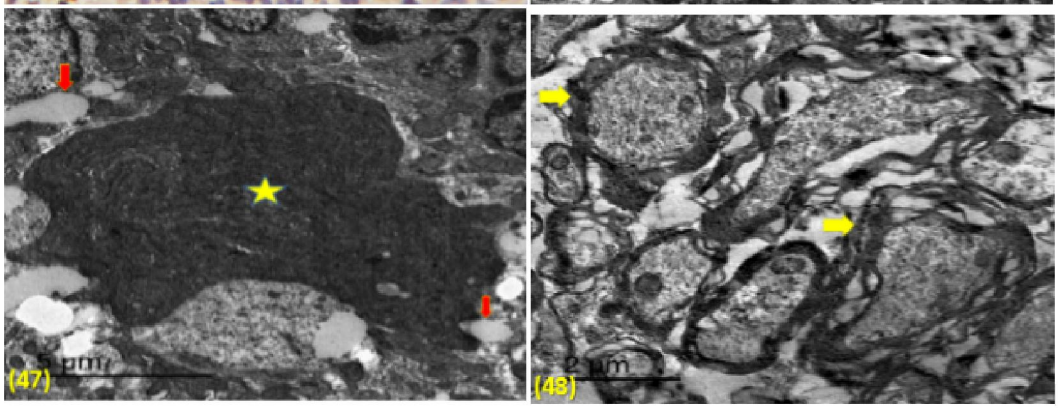
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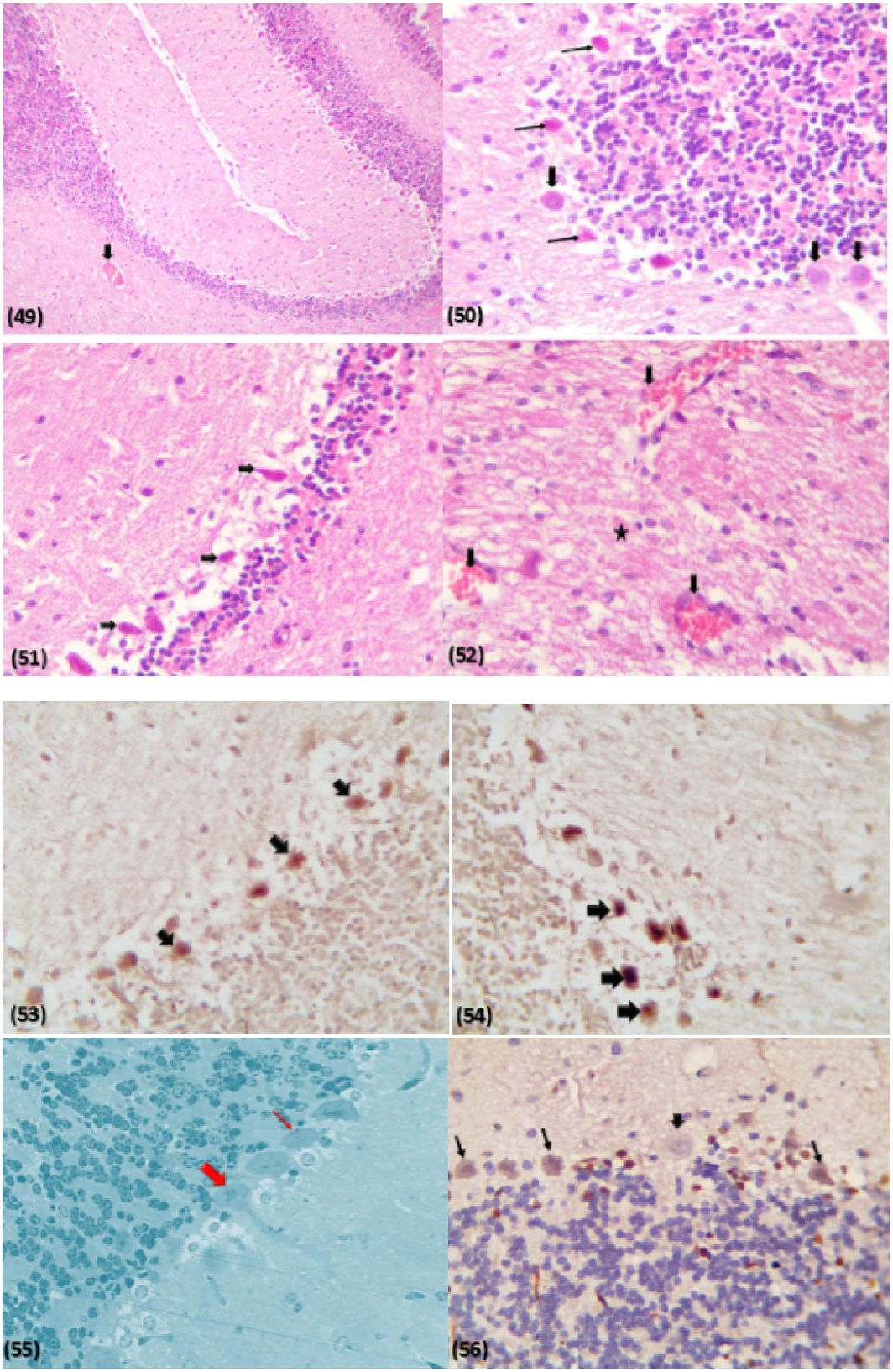
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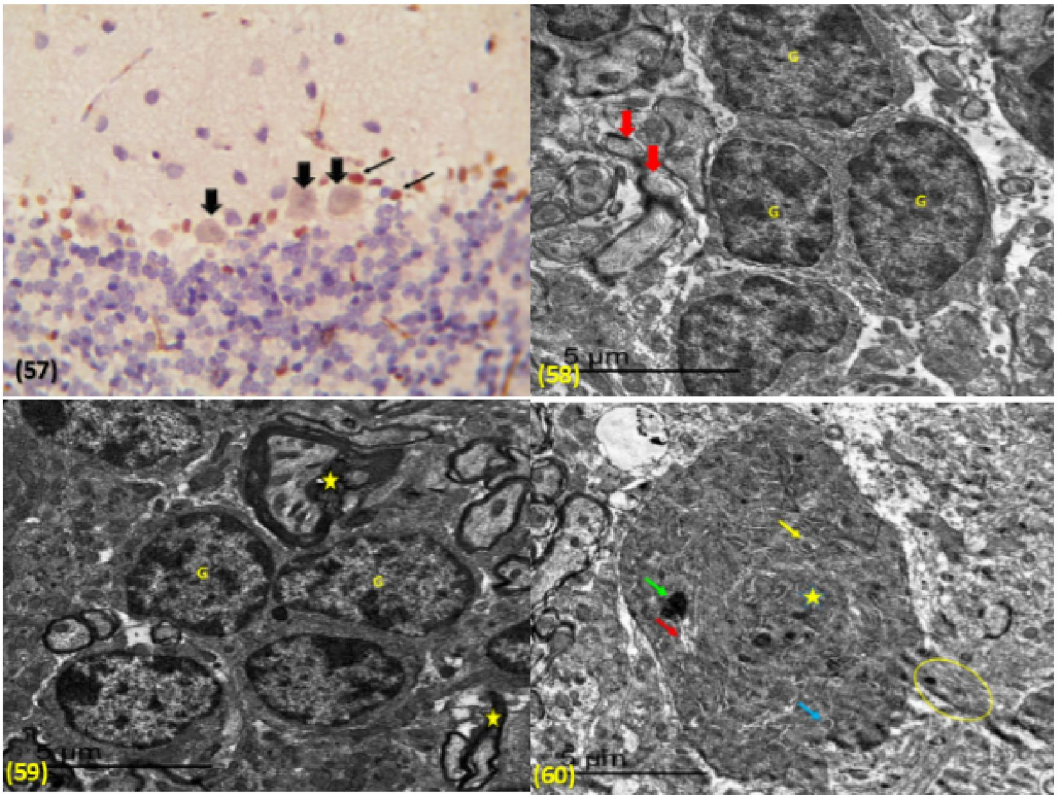

B- Quantitative study:

Table (1)

Groups	Caspase 3 Density Mean ± S. D	P values
Group I (control)	124.95 ± 16.78	P1 = 0.0017
Group II A_1	154.35 ± 7.85	P2 = 0.7553
Group II A_2	132.70 ± 8.10	P3 = <0.0001
Group II B_1	190.53 ± 7.14	P4 = 0.1152
Group II B_2	141.62 ± 8.19	P5 = 0.0244
		P6 = 0.0002
		P7 = 0.3201
		P8 = <0.0001
		P9 = 0.6502
		P10 = <0.0001

P1: compare between Group I and Group II A_1, **P2:** compare between Group I and Group II A_2, **P3:** compare between Group I and Group II B_1, **P4:** compare between Group I and Group II B_2, **P5:** compare between Group II A_1 and Group II A_2, **P6:** compare between Group II $A_1$and Group II B_1, **P7:** compare between Group II A_1 and Group II B_2, **P8:** compare between Group II A_2 and Group II B_1, **P9:** compare between Group II A_2 and Group II B_2, **P10:** compare between Group II B_1 and Group II B_2.

Table (2)

Lesions	Group I (control)	Group II A_1	Group II A_2	Group II B_1	Group II B_2
Purkinje cells shrinkage	-	++	+	+++++	+++
Neuronal necrosis	-	++	+	+++++	+++
Neuronal degeneration	-	++	+	+++++	+++
Hemorrhages	-	++	+	+++++	+++
Demyelination	-	++	+	+++++	+++
Caspase 3 immunopositivity	-	++	+	+++++	+++

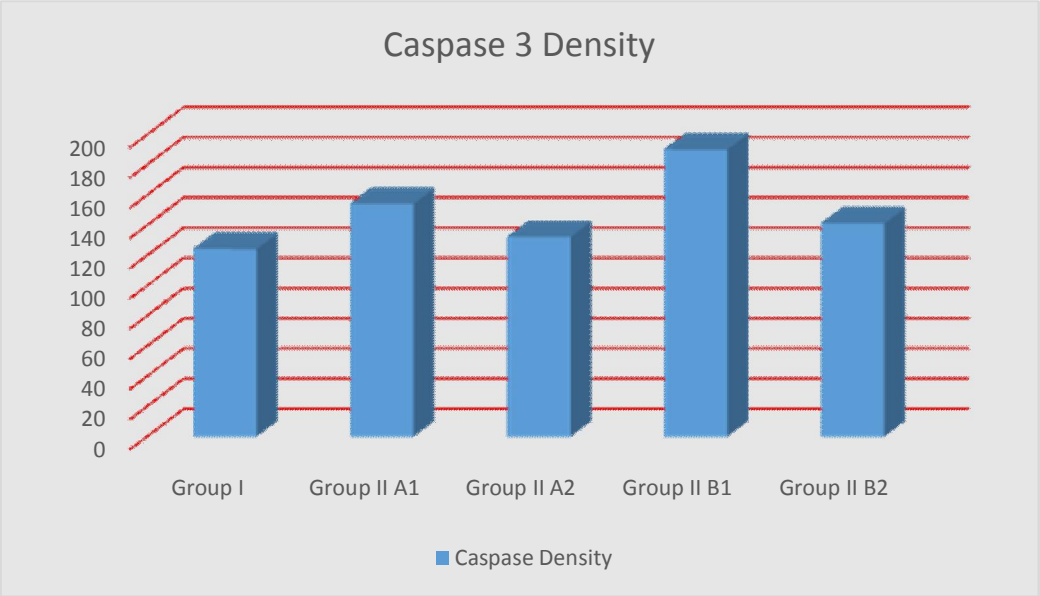

Diagram (1)

4. Discussion:

TrHC is available in a variety of pharmaceutical formulations for various routes e.g. Subcutaneous, intra-muscular, intra-venous, sub-lingual, and oral drug delivery. TrHC is rapidly and almost completely absorbed after oral administration. The mean peak plasma concentration occurs after 2 hours and its bioavailability is approximately 70% as a result of the first-pass metabolism in the liver. About 20% of the drug is bound to plasma proteins and the mean half-life is 6 hours (10). This study was designed to demonstrate the histological changes and immunohistochemical findings in the cerebellum of the adult male albino rats induced by different doses of tramadol administration because of the increase of opiate's users worldwide.

In this study, light microscopic results of the Group I (control) showed the normal histomorphological structures of the cerebellum which was formed of an outer cortex of white matter and grey matter and inner medulla of white matter. The grey matter was formed of three layers from inside to outside, the molecular layer that formed mainly of few cells and many fibers, the Purkinje layer that formed of single cell layer arranged in one row and the granular layer which was formed of large number of small deeply stained cells and cerebellar islands of synaptic connection in between the cells. These results were in harmony with (11) & (12) and (13). The transmission electron microscope results of this group were coincided with what observed by L/M. It showed the normal structure of the Purkinje cells that appeared with a large nucleus having normal chromatin, normal cytoplasmic organelles including Golgi apparatus and many mitochondria. The granular cells also appeared with large nuclei, condensed chromatin and a thin rim of cytoplasm beside a few myelinated nerve fibers and numerous dendrites of Purkinje cells. These results were coincided with what reported by (5) and (14).

Concerning light microscopic results of Group II A_1- that injected intraperitoneally with 0.7 mg as a single daily dose of tramadol solution for 2 weeks-mild congested blood capillaries were appeared in white mater, loss of some Purkinje cells, chromatolysis and eosinophilic cytoplasm of others with nearly normal others with empty spaces in between. Also showed numerous pyknotic cells with mild separation within the molecular layer. These results are in agreement with the finding of (15) & (16) and (17) who reported that the wide spaces which were found in between the cells could be due to loss of some cells or due to accumulation of edema fluid and disruption of blood supply by the effect of the drug. They also reported that intraperitoneal administration of 12.5 mg tramadol/kg body weight (low dose) did not lead to notableside effects. However, intraperitoneal administration of 25–50 mg/kg (high dose) was associated with variable degrees of side effects. Also, (18) reported that cell death in neurodegeneration occurs through the intrinsic mitochondrial apoptotic pathway. They added that all neurodegenerative features were caused by the overproduction of mitochondrial reactive oxygen species with oxidative stress on the neurons. The electron microscopic study of this group revealed degenerated Purkinje cells, each one characterized by large nucleus with deformity in its nuclear membrane appeared as finger projections, dispersed chromatin,

disappearance of normal cytoplasmic organelles with small atrophied mitochondria besides numerous large dense bodies, dilated Golgi apparatus and destroyed cisternae of rough endoplasmic reticulum. These results were similar to that reported by (14) who added that administration of morphine sulfate in adult rats significantly reduced the size and numbers of Purkinje cells, with loss of their specific shape, which could have been because of apoptosis and/or necrosis.

However, light microscopic results of the Group II A_2- that injected intraperitoneally with 0.7 mg as a single daily dose of tramadol solution for 2 weeks, then were left for another 2 weeks without injection - showed some restoration of the normal histomorphological structures including Purkinje layer between molecular layer and granular layer. The Purkinje cells appeared with abundant cytoplasm and prominent chromatin, but still present many shrunken Purkinje cells with pyknotic nuclei and eosinophilic cytoplasm. These results were in agreement with (4) who reported that there was some return of brain tissues towards normal morphology after stoppage of drug intake. This means some improvement of the oxidative stress on the nervous tissue. Also (12) reported that restoration of the normality of Purkinje cells dendrites and chromatins appeared, but still surrounded by empty vacuolation. Moreover, (19) reported that numerous nearly normal Purkinje cells appeared with still present many shrunken others with pyknotic nuclei and eosinophilic cytoplasm in between the granular layer and the molecular layer. The transmission electron microscope results of this group were coincided with what observed by L/M. It showed restoration of granular cells with rearranged chromatins, and axonal myelin sheaths besides numerous interstitial electron dense bodies. These results were in harmony with what was reported by (20). It showed also restoration of the Purkinje cytoplasmic organelles with normal mitochondria, and still presence of projection of its nuclear membrane besides remnants of electron dense bodies and also showed rearranged axonal myelin sheath similar to that reported by (4).

However, light microscopic results of the Group II B_1- that injected intraperitoneally with 1.8 mg as a single daily dose of tramadol solution for 2 weeks - showed severe changes in the form of many congested blood vessels with marked empty spaces in the Purkinje layer with prominent focal apoptosis with multilayer deposition of Purkinje cells in between deeply stained granular cells. It also showed few cells with pyknotic nuclei beside mild demyelination in the molecular layer and wide cerebellar islands, congested blood vessels in the granular layer. Also mild to moderate vacuolation with severe demyelination of nerve axons and focal aggregation of the neuroglia cells were appeared in the white matter. These findings were in agreement with (21) who clarified that chronic use of morphine and/or tramadol in increasing doses causes neuron degeneration in the rat brain, which could contribute toward cerebral dysfunction and reported that congestion may have been because the vascular endothelial cells were affected, leading to the release of NO, which is an endothelial relaxing factor and reported that the role of NO is concentration dependent; at a low concentration, NO has been shown to play a unique role in neurotransmission and vasodilatation, whereas at higher concentrations, it is neurotoxic. Also (22) reported that Purkinje cells after tramadol injection appeared suffering from condense chromatin with loss of their dendrites beside empty areas and loss of other Purkinje cells. They reported that the cause of reduction in the thickness of the Purkinje layer may be decreased Purkinje cell size with a defective function. However, (14) reported that morphine induced a significant decrease in the molecular and granular layers' thickness of the rat cerebellum, considering them as signs of chromatolysis and gliosis. Moreover, (23) reported that irregular shaped Purkinje cells appeared with an irregular nucleus, and another cells appeared with karyolytic nuclei besides the granular layer that contains small cells with no nuclei. They explained that prolonged exposure to neuronal insult could lead to an adaptive response in the form of crowding of Purkinje cells. That is in a trial to re-establish the synaptic contact with other neurons to perform their function. Also, the observed darkly stained nuclei of the granular layer that clumped were considered to be secondary to the changes that occurred in the Purkinje cells. Sections of this group that examined in this study by TEM showed the structure of granular cells with apoptotic nuclei, faint chromatin and a thin cytoplasm. These results were in agreement with those of (24) who explained the cerebellar-damaging effects of opioids including tramadol to be because of reduced Purkinje cell proliferation, cell differentiation, and increased Purkinje cell death. They added that the neurotoxic effects of opioids that caused cell death were induced by mitochondrial damage. Other authors reported that another possible mechanism of opioids that causes alteration of the Purkinje cells might be blockage of neuronal activity, causing the neurons to receive internal signals to self-destruct (25). This group also showed an irregular-shaped, necrotic Purkinje cells, each one appeared with an irregular shrunken nucleus and an ill-defined nuclear membrane with disappearance of numerous cytoplasmic organelles, and degenerated axons with disarrangement of myelin sheath similar to that reported by (14) who reported ultrastructural damaging effects of long-term use of

morphine on the cerebellar cortex in the form of fragmentation of the cisternae of both types of endoplasmic reticulum, obvious destruction of the mitochondrial inner membrane, and cristae-mediated cell death. In addition, abnormal nuclei with a deformed perforated nuclear membrane with degeneration of the synapses could interpreted as a sign of necrosis.

However, light microscopic results of the Group II B_2- that injected intraperitoneally with 1.8 mg as a single daily dose of tramadol solution for 2 weeks, then were left for another 2 weeks without injection – showed mild to moderate improvement in the cerebellar layers including some normal Purkinje cells with mild congested blood vessels, with still presence of some apoptotic Purkinje cells with chromatolysis surrounded by edema and narrowing of the granular layer. Also showed moderate demyelination and chromatolysis of pyramidal neurons in the white matter. These were results coincided with that revealed by (12) & (26) and (27). The transmission electron microscope results of this group were coincided with what observed by L/M. It showed nearly normal granular cells and still presence of mild degenerated nerve axons with some disarrangement of myelin sheath. These results were coincided with what reported by (20) who stated that rats examined after the withdrawal recovery period showed some reduction in cellular damage when compared to tramadol treated groups. It showed also regenerative attempts of some Purkinje cells with still present irregular nucleus having finger projections, vacuolated cytoplasm with dilated Golgi apparatus, dilated rough endoplasmic reticulum, atrophied mitochondria, electron dense bodies, with short dendrites similar to that reported by (4).

The current study showed that Caspase 3-immune-histochemically positive cells which isindicated as apoptotic index was increased in group II $A_1$and with more increase in group II B_1, while it was decreased in group II $B_2$and more decrease in group II $A_2$when compared to group I (control) with its negative results. This indicates that there is an increase in apoptosis in cerebellum in rats receiving tramadol which was subsides when the drug become withdrawn. These findings coincide with that reported by (12) & (28) and (29).

The results of this study provided evidence that tramadol intake exerts a neurotoxic effect on the cerebellar structure in an ascending manner according to the dose administered.

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References:

1. Gana TJ., Pascual ML., Fleming RR., Schein JR., Janagap CC., Xiang J. and Vorsanger GJ. (2006): Extended-release tramadol in the treatment of osteoarthritis: a multicenter, randomized, double-blind, placebo-controlled clinical trial. Curr Med Res Opin; pp: 1391–1401.
2. Kabel JS. and Van Puijenbroek EP. (2005): Side effects of tramadol: 12 years of experience in the Netherlands. Ned Tijdsch Geneeskd; pp: 754–757.
3. De Decker K., Cordonnier J., Jacobs W., Coucke V., Schepens P. and Jorens PG. (2008): Fatal intoxication due to tramadol alone: case report and review of the literature. Forensic Sci Int; pp: 79–82.
4. Fatma M. Ghoneim., Hanaa A. Khalaf., Ayman Z. Elsamanoudy. and Ahmed N. Helaly. (2014): Effect of chronic usage of tramadol on motor cerebral cortex and testicular tissues of adult male albino rats and the effect of its withdrawal: histological, immunohistochemical and biochemical study. Int J Clin Exp Pathol; pp: 7323-7341.
5. Manal I. El-Bermawy. and Maysa F. Salem. (2015): Histological changes of the albino rat cerebellar cortex under the effect of different doses of tramadol administration. The Egyptian Journal of Histology: Volume 38; pp: 143–155.
6. Shadnia S., Soltaninejad K., Heydari K., Sasanian G. and Abdollahi M. (2008): Tramadol intoxication: a review of 114 cases. Hum Exp Toxicol; pp: 201–205.
7. Bancroft JD. and Stevens A. (1996): Theory and practice of histological techniques. 4th ed. New York, London, Tokyo: Churchill Livingstone.
8. Hayat MA. (1989): Principles and techniques of electron microscopy. 3rd ed. London: MacMillan Press.
9. Rosner B. (1990): Fundamentals of Biostatistics, 3rd edition BWS Kent publishing Co. Boston; pp: 111- 115.
10. Eassa BI. and El-Shazly MA. (2013): Safety and efficacy of tramadol hydrochloride on treatment of premature ejaculation. Asian JAndrol; pp: 138–142.
11. Hauser K. F., Houdi A. A., Turbek C. S., Elde R. P. and Maxson, W. (2000): Opioids intrinsically inhibit the genesis of mouse cerebellar granule neuron precursors in vitro: Differential impact of μ and δ receptor activation on proliferation and neurite elongation. European Journal of Neuroscience; pp: 1281–1293.

12. Ahmed K. Elden Elfeky. and Abdelnaby S. Mohamed. (2017): Effects of Tramadol Addiction on Brain of Adult Male Albino Rats and Role of lofexidine during Withdrawal Period: A Biochemical, Histopathological and Immunohistochemical Study. Ain Shams Journal of Forensic Medicine and Clinical Toxicology; pp: 119-132.
13. Ilhami Celik., Muzaffer Seker., and Ahmet Salbacak. (2018): Histological and histomorphometric studies on the cerebellar cortex and silver stained nucleolus organizer regions of Purkinje neurons in chronic morphine-treated rats. Veterinarski Arhiv; pp: 75-88.
14. Bekheet SH., Saker SA., Abdel-Kader AM. and Younis AE. (2010): Histopathological and biochemical changes of morphine sulphate administration on the cerebellum of albino rats. Tissue Cell; pp: 165–175.
15. McKay. B. E. and Turner R. W. (2005): Physiological and morphological development of the rat cerebellar Purkinje cell. The Journal of physiology; pp: 829-850.
16. Cannon CZ., Kissling GE., Hoenerhoff MJ., King-Herbert AP. and Blankenship-Paris T. (2010): Evaluation of dosages and routes of administration of tramadol analgesia in rats using hot-plate and tail-flick tests. Lab Anim (NY); pp: 342–351.
17. Samaka R. M., Girgis N. F. and Shams T. M. (2012): Acute toxicity and dependence of tramadol in albino rats: relationship of nestin and notch 1 as stem cell markers. J. of American Science; pp: 313-327.
18. DiMauro S. and Schon EA. (2008): Mitochondrial disorders in the nervous system. Annu Rev Neurosci; pp: 91–123.
19. Omar N. M. (2016): Nigella sativa oil alleviates ultrastructural alterations induced by tramadol in rat motor cerebral cortex. Journal of Microscopy and Ultrastructure; pp: 76-84.
20. Khodeary MF., Sharaf El-Din AI. and El Kholy A. (2010): Histopathological and immunohistochemical study of adult rats' brain after long term exposure to amadol (tramadol hydrochloride). Mansoura J Forensic Med Clin. Toxicol; pp: 1-24.
21. Atici S., Cinel L., Cinel I., Doruk N., Aktekin M., Akca A., Camdeviren H. and Oral U. (2004): Opioid neurotoxicity: comparison of morphine and tramadol in an experimental rat model. International journal of neuroscience; pp: 1001-1011.
22. Liu L. W., Lu J. and Wang XH. (2013): Neuronal apoptosis in morphine addiction and its molecular mechanism. Int J Clin Exp Med; pp: 540-5.
23. Abou El Fatoh MF., Farag M. and Sayed AE. (2014): Some Biochemical, Neurochemical, Pharmacotoxicological and Histopathological alterations induced by long-term administration of tramadol in male rats. Int J Pharm Sci; pp: 565-571.
24. Mao J., Sung B., Ji RR. and Lim G. (2002): Neuronal apoptosis associated with morphine tolerance: evidence for an opioid-induced neurotoxic mechanism. J Neurosci; pp: 7650–7661.
25. Farber NB. and Olney JW. (2003): Drugs of abuse that cause developing neurons to commit suicide. Brain Res Dev; pp: 37–45.
26. Demeri O., Celik I., Seker M., Sur E., Ozkan Y., Aydin MF. and Salbacak A. (2002): Histological and morphometric studies of the cerebellar cortex and nucleolus organiser region (Ag-NORs) in Purkinje neurons of chronic morphine treated rats. Proceedings of the XVII International Symposium on Morphological Science; Timisoara, Romania.
27. Barsotti CE., Mycyk MB. and Reyes J. (2003): Withdrawal syndrome from tramadol hydrochloride. Am J Emerg Med; pp: 87–88.
28. Zarnescu O., Brehar FM. and Chivu M. (2008): Immunohistochemical localization of caspase-3, caspase-9 and Bax in U87 glioblastomaxenografts. J Mol. Histol; pp: 561-569.
29. Sharifipour M., Izadpanah E., Nikkhoo B., Zare S., Abdolmaleki A., Hassanzadeh K., Moradi F. and Hassanzadeh K. (2014): A new pharmacological role for donepezil: attenuation of morphine-induced tolerance and apoptosis in rat central nervous system. J Biomed Sci; pp: 21–6.

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