Original Article

Role of Melatonin in Ameliorating the Harmful Effects of Tramadol on the Frontal Cortex of Adult Male Albino Rat (Histological and Immunohistochemical Study)

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ABSTRACT

Introduction: Tramadol is the foremost utilized opioid in moderate to severe pain. Previous studies reported changes in the rat's brain after either oral or intraperitoneal tramadol administration. Melatonin 'neurohormone' has antioxidant and anti-apoptotic effects on the brain.

Aim of the Work: This work aimed to assess the role of melatonin in ameliorating the harmful impacts of tramadol on the frontal cortex of adult male albino rat.

Materials and Methods: forty adult male albino rats were used, their ages ranged from 6-8 months and their weight ranged from 180-200gm. Rats were equally distributed for four groups: Group I (control group): Rats were divided into two subgroups: Ia: rats were kept without any treatment for thirty days and group Ib: rats received intraperitoneal injection of 0.1 mL of 0.9% saline and 2% ethanol daily for thirty days. Group II (Melatonin group): each rat was intraperitoneally injected of 10mg/kg/day of melatonin for thirty days. Group III (Tramadol group): each rat received 50mg/kg/day tramadol orally by orogastric tube for thirty days. Group IV (Tramadol+ Melatonin group): each rat was injected with melatonin and received oral tramadol as in the groups II and III respectively for thirty days.

Results: Tramadol administration resulted in disarrangement of the grey matter layers of rats' frontal cortices, depletion of glycogen and synaptophysin of neural cells with increased apoptosis and astrocytosis. However, concomitant administration of melatonin with tramadol showed regular structure of rats' frontal cortices with attenuated neural apoptosis, proper contents of glycogen, synaptophysin and astrocytes.

Conclusion: Melatonin has a good protective effect against the harmful impacts of tramadol on the frontal cortex of adult male albino rat.

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Key Words: Cortex, melatonin, tramadol.

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INTRODUCTION

Pain is a disturbing sensation, but it is crucial for survival. It occurs with tissue injury or irritation. Analgesics (pain killers) are of two types, opioids and non-opioids. For moderate to severe pain not reacting to non-opioid pain killers, opioids are utilized, they are much stronger and act on the central nervous system directly to relief pain[1-2]. However, Opioids frequently lead to addiction and when used in large dosages, may result in severe respiratory side effects or even death[3-4].

Tramadol is a synthetic analogue of codeine with central impact and is the foremost utilized opioid in moderate to severe pain. Tramadol has minimal respiratory and cardiovascular impacts in comparison with other opioids (e.g., morphine, pethidine, and oxycodone). It is purchasable in many forms such as tablets, suppositories and solutions for injection[5-7]. Its adverse effects depend on the way of administration and dose. Intravenous and intraperitoneal routes are considered more hazardous than the other routes. Though, previous studies reported changes in the rat's brain after tramadol administration either by oral or by intraperitoneal routes[8,9]. Egyptian Health Authorities moved tramadol on the list of highly addictive substances in 2012 from schedule three to one[10].

Melatonin is a neurohormone naturally formed from the essential amino acid tryptophan and secreted mainly from the pineal gland (other organs secrete it gut, retina, testis and ovary). It has many functions such as biological clock regulation, modulation of gene transcription and protection of cells from oxidative stress[11–21]. Melatonin effects as antioxidant and anti-apoptotic on the brain have been proved[22]. Its part as a free radical scavenger for oxidative injury of the brain was reported in several past studies[23–26].

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Therefore, this work was planned to assess the role of melatonin in ameliorating the harmful impacts of tramadol on the frontal cortex of adult male albino rat.

MATERIALS AND METHODS

Chemicals

Tramadol was obtained from Hikma Pharmaceutical Company Giza, Egypt. The 200mg tablet was suspended in 10 ml of distilled water.

Melatonin was obtained from Sigma, Chemical, USA (1g powder in a glass bottle). Each 10mg of the powder was dissolved in 0.5 mL (1:1 2% ethanol and 0.9% saline)[27].

Animals

Forty male adult albino rats were used, their ages extended from 6-8 months[28] and their weight extended from 180-200gm. Rats were hold at the medical research center (Faculty of Medicine, Ain-Shams University). The experiment was endorsed by the Research Ethics Committee of Faculty of Medicine, Ain-Shams University (FMASU R44/2023). All rats have been subjected to the same circumstances all through the experiment. Rats have been kept at room temperature with suitable ventilation in metal cages, standard light-dark cycles. They were free get to water and food and equally distributed in four groups (10 rats each):

Group I (control group): Rats were divided into two subgroups Ia (five rats): they remained without any treatment for thirty days and Ib (five rats): they received intraperitoneal injection of 0.1 mL (ethanol in saline) daily for thirty days.

Group II (Melatonin group): each rat was intraperitoneally injected of 10mg/kg/day of melatonin[27] for thirty days.

Group III (Tramadol group): each rat received oral dose of 50mg/kg/day tramadol by orogastric tube for thirty days[29].

Group IV (Tramadol+ Melatonin group): each rat was injected with melatonin and received oral tramadol as in the groups II and III respectively for thirty days.

Processing of samples

At the conclusion of the thirty days, rats were sacrificed (under anesthesia by inhalation of diethyl ether 1.9%). The skulls were carefully opened, and the cerebral hemispheres were then delicately retrieved and washed with saline. Each hemisphere was settled in buffered formalin (10%) then processed and inserted in paraffin blocks.

Paraffin pieces were cut (5µm thickness) and stained for histological, and immunohistochemical examination. Hematoxylin and Eosin stain (Hx. &E.) for histological examination[30], Periodic Acid–Schiff stain (PAS) for detection of glycogen[31] and for immunohistochemical staining, deparaffinized sections were mounted on positively charged slides for:

Caspase-3 "for apoptosis" sections were incubated with rabbit cleaved caspase 3 polyclonal antibody at a dilution of 1:200 (In *vitro* gen, Sweden AB Stockholm Sweden). The secondary antibody was anti mouse antibody diluted of 1:500 (In *vitro* gen, Molecular Probes, Eugene, Oregon, USA).

Glial fibrillary acidic protein (GFAP) "for glial cells" The primary antibody was rabbit anti GFAP diluted in phosphate-buffered saline (PBS) 1:200 (Sigma company), the secondary antibody was biotin anti-rabbit diluted in PBS 1:200 (BSA; Sigma Chemical Co.) and synaptophysin for detection of synapses, anti-synaptophysin mouse monoclonal antibody IgG (1:200) and rabbit monoclonal IgG (1:200) were used (Abcam, Cambridge, UK). Positive immune reaction appeared brown coloration of the cytoplasm with caspase 3, brown coloration of astrocytes with GFAP and brown coloration of cell bodies plasma membranes and dendrites/axon with synaptophysin. The used counterstain was Mayer's hematoxylin. In negative controls, the primary antibody was supplanted by PBS. Tonsils for caspase-3, cerebellum for GFAP and colon for synaptophysin were used as positive controls[32-34].

Light microscope (Olympus) with a digital automatic camera (BX3M series, Olympus, Tokyo, Japan) at Anatomy department, Faculty of Medicine, Ain Shams University was used in examination and photographing of the stained sections.

Morphometric analysis

Morphometric analysis was done using image-J software version 1.48v (National Institute of Health, Bethesda, Maryland, USA), ten randomly chosen non-overlapped fields of ten different sections of the same group were used for measuring the mean area % of PAS reaction, caspase 3, GFAP and synaptophysin positive immune reactions. The magnification used for PAS, caspase 3 and GFAP was x400 with an objective lens of x40 and a magnification of x100 with an objective lens of x10 for synaptophysin. Pixels were calibrated for the certain measurements using a stage micrometer.

Statistical analysis

Data analysis was accomplished by SPSS free software version 20 (IBM Corp., Armonk, NY, USA). By One-way ANOVA and Bonferroni Post Hoc Test, the differences between every two groups of the groups I, III, IV were compared. Data provided as the mean value ± standard deviation. Outcomes were considered nonsignificant with a $P\ value > 0.05$, high significant with a $P\ value \leq 0.001$ and significant with a $P\ value \leq 0.05$.

RESULTS

Hx. & E. stain

Both group I and II appeared having regular grey matter layers of the frontal cortex: molecular layer, outer granular layer, outer pyramidal layer, inner granular layer, inner pyramidal layer, and polymorph layer. The

granule cells showed up with rounded cell bodies and large vesicular nuclei whereas the pyramidal cells showed up with triangular cell bodies, basophilic cytoplasm, and vesicular nuclei. The homogenous eosinophilic ground substance was filled with neuropil and small blood vessels (Figures 1,2).

Group III showed disorganized grey matter layers occupied mainly by deeply stained cells. Most of the granule cells and pyramidal cells showed up distorted with dark stained nuclei. Perineural vacuolations and dilated congested blood vessels were also noticed (Figures 3,4).

Group IV showed regular layers of the grey matter. The granule cells were having rounded cell bodies and vesicular nuclei. The pyramidal cells showed up with triangular cell bodies and vesicular nuclei (Figures 5,6).

PAS stain

Group I and group II showed strong positive cytoplasmic reaction of pyramidal and granule cells (Figure 7). Group III showed weak reaction of most pyramidal and granule cells (Figure 8). Group IV showed strong reaction of most pyramidal and granule cells (Figure 9).

Caspase-3 immunohistochemical stain

Group I and group II showed negative cytoplasmic immune reaction of almost all granule and pyramidal cells (Figure 10). Group III showed strong positive immune reaction of almost all granule and pyramidal cells (Figure 11). Group IV showed negative immune reaction of most granule and pyramidal cells (Figure 12).

GFAP immunohistochemical stain

Group I and group II showed star-shaped astrocytes having small bodies and thin processes (Figure 13). Group III showed large astrocytes with thick processes (Figure 14). Group IV showed small astrocytes with thin processes (Figure 15).

Synaptophysin immunohistochemical stain

Group I and group II showed strong positive reaction of the cell bodies plasma membranes and dendrites/axon between the cells (Figure 16). Group III showed weak positive reaction of the cell bodies plasma membranes and of dendrites/axon between the cell bodies (Figure 17). Group IV showed moderate to strong positive reaction of the cell bodies plasma membranes and of dendrites/axon between the cell bodies (Figure 18).

Morphometric results and statistical analysis

Morphometric measures for the mean area % PAS reaction, caspase 3, GFAP and synaptophysin immune reactions were illustrated in (Tables (1-4), Histograms (1-4)). Statistical analysis revealed highly significant statistical difference between group I and group III and between group III and group IV and non-significant statistical differences between group I and group IV for all measures.

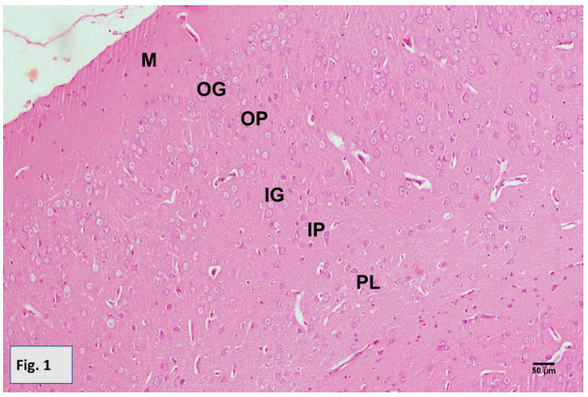


Fig. 1: A photomicrograph of a section of group I frontal cortex showing regular layers of grey matter, molecular (M), outer granular (OG), outer pyramidal (OP), inner granular (IG), inner pyramidal (IP) and polymorph (PL). (Hx. &E., x40, 50µm scale bar)

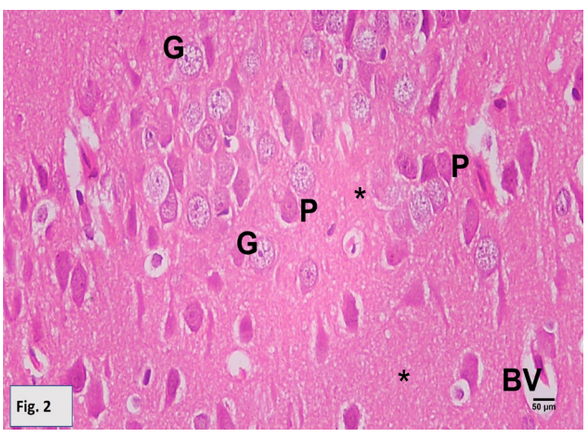


Fig. 2: A photomicrograph of a section of group I frontal cortex showing granule cells (G) having rounded cell bodies and large vesicular nuclei and pyramidal cells (P) with triangular cell bodies, basophilic cytoplasm and vesicular nuclei. Notice, the homogenous eosinophilic neuropil (*) with small blood vessels (BV) between the cells. (Hx. &E., x400, 50µm scale bar)

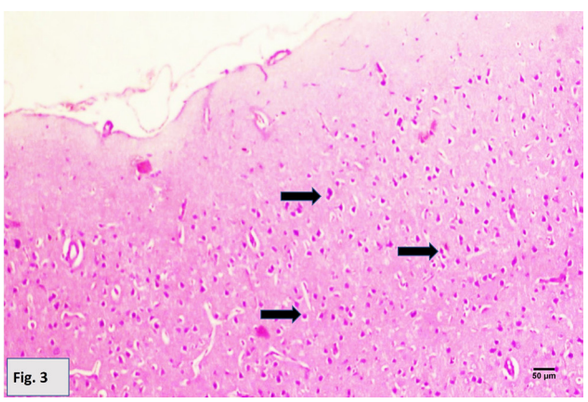


Fig. 3: A photomicrograph of a section of group III frontal cortex showing disorganized grey matter layers occupied mainly by deeply stained cells (black arrows). (Hx. &E., x40, 50µm scale bar)

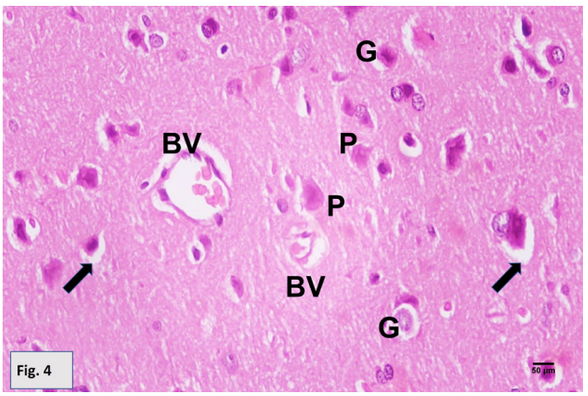


Fig. 4: A photomicrograph of a section of group III frontal cortex showing hardly identified granule cells (G) and pyramidal cells (P) having dark stained nuclei with perineural vacuolations (black arrow). Notice dilated congested blood vessels (BV). (Hx. &E., x400, 50µm scale bar)

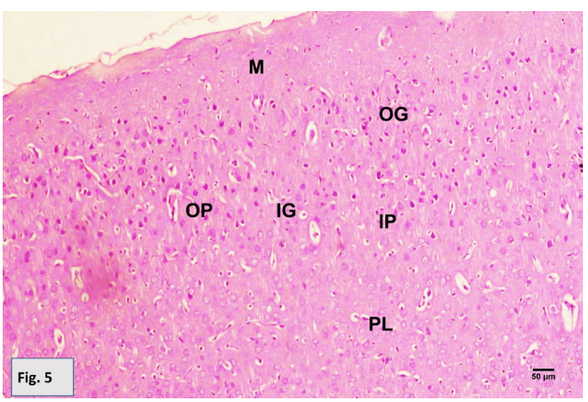


Fig. 5: A photomicrograph of a section of group IV frontal cortex showing regular layers of grey matter, molecular (M), outer granular (OG), outer pyramidal (OP), inner granular (IG), inner pyramidal (IP) and polymorph (PL). (Hx. &E., x40, 50µm scale bar)

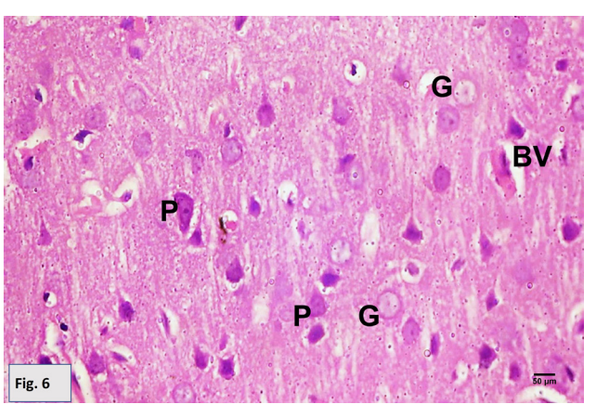


Fig. 6: A photomicrograph of a section of group IV frontal cortex showing granule cells (G) having rounded cell bodies and large vesicular nuclei and pyramidal cells (P) with triangular cell bodies and vesicular nuclei. (Hx. &E., x400, 50µm scale bar)

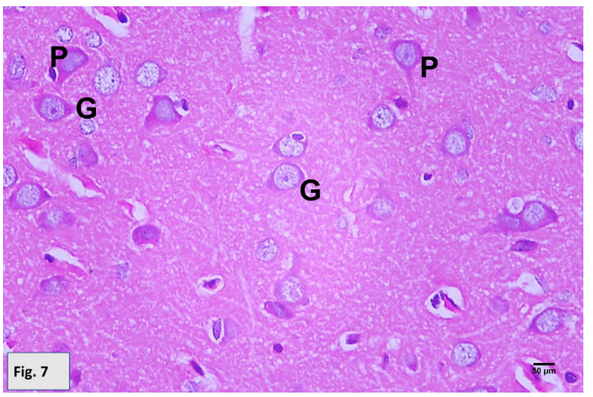


Fig. 7: A photomicrograph of a section of group I frontal cortex showing strong positive cytoplasmic reaction for PAS of pyramidal cells (P) and granule cells (G). (PAS x400, 50µm scale bar)

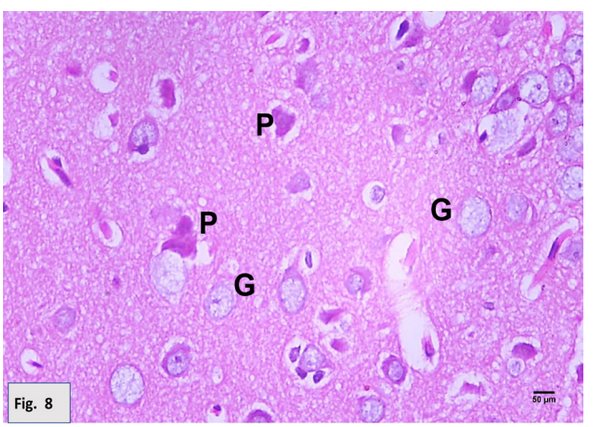


Fig. 8: A photomicrograph of a section of group III frontal cortex showing weak cytoplasmic reaction for PAS of most pyramidal cells (P) and granule cells (G). (PAS x400, 50µm scale bar)

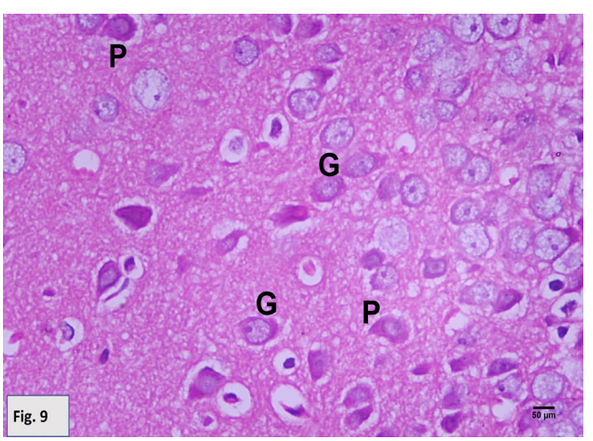


Fig. 9: A photomicrograph of a section of group IV frontal cortex showing strong positive cytoplasmic reaction for PAS of most pyramidal cells (P) and granule cells (G). (PAS x400, 50µm scale bar)

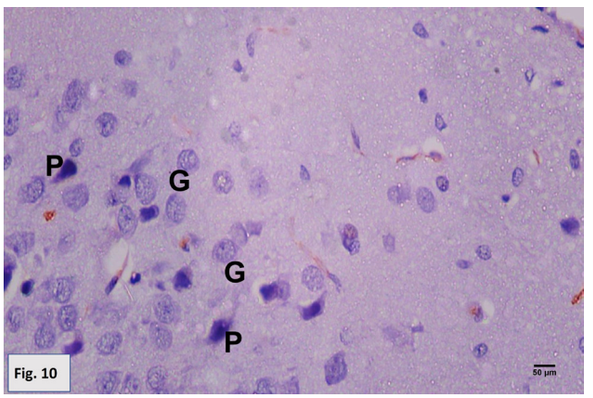


Fig. 10: A photomicrograph of a section of group I frontal cortex showing negative cytoplasmic immune reaction for caspase-3 of granule (G) and pyramidal cells (P). (Caspase-3 x400, 50µm scale bar)

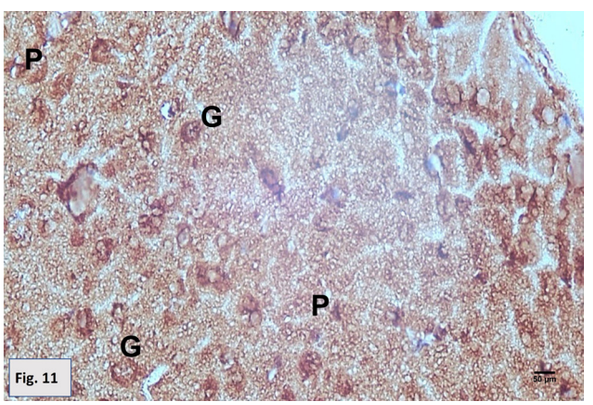


Fig. 11: A photomicrograph of a section of group III frontal cortex showing positive cytoplasmic immune reaction for caspase-3 of most granule (G) and pyramidal cells (P). (Caspase-3 x400, 50µm scale bar)

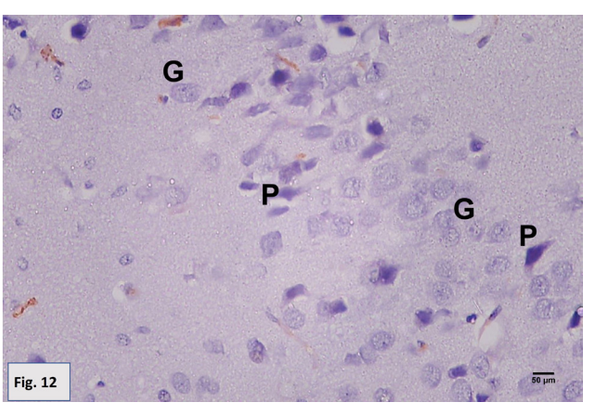


Fig. 12: A photomicrograph of a section of group IV frontal cortex showing negative cytoplasmic immune reaction for caspase-3 of granule (G) and pyramidal cells (P). (Caspase-3 x400, 50µm scale bar)

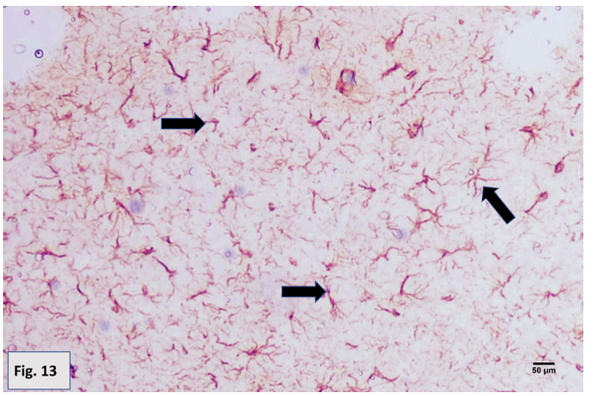


Fig. 13: A photomicrograph of a section of group I frontal cortex showing positive immune reaction for GFAP of star-shaped astrocytes (black arrows) having small bodies and thin processes. (GFAP x400, 50µm scale bar)

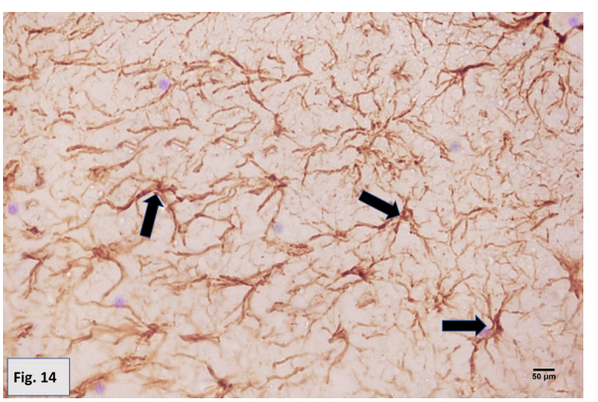


Fig. 14: A photomicrograph of a section of group III frontal cortex showing positive immune reaction for GFAP of large astrocytes (black arrows) with thick processes. (GFAP x400, 50µm scale bar)

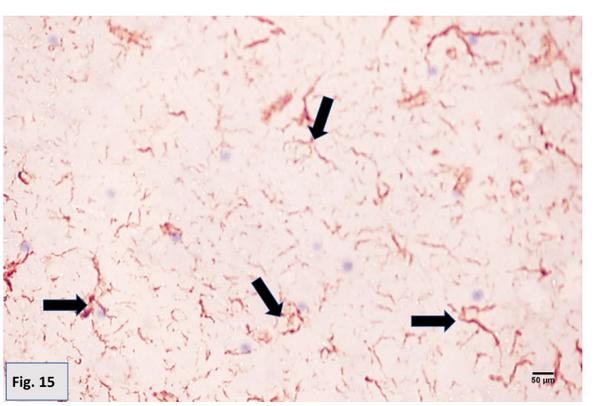


Fig. 15: A photomicrograph of a section of group IV frontal cortex showing positive immune reaction for GFAP of star-shaped astrocytes (black arrows) having small bodies and thin processes. (GFAP x400, 50µm scale bar)

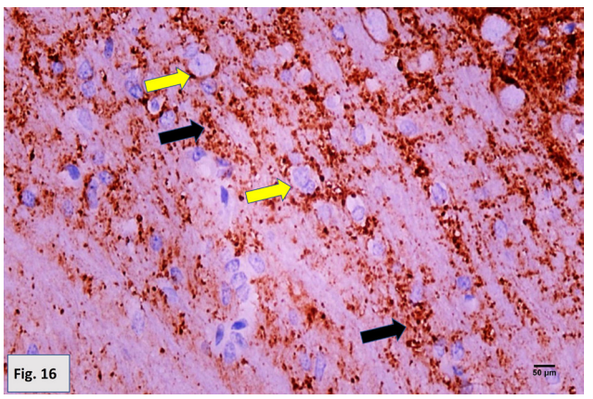


Fig. 16: A photomicrograph of a section of group I frontal cortex showing strong positive immune reaction for synaptophysin of cell bodies plasma membranes (yellow arrows) and of dendrites/axon between the cells (black arrows). (Synaptophysin x400, 50µm scale bar)

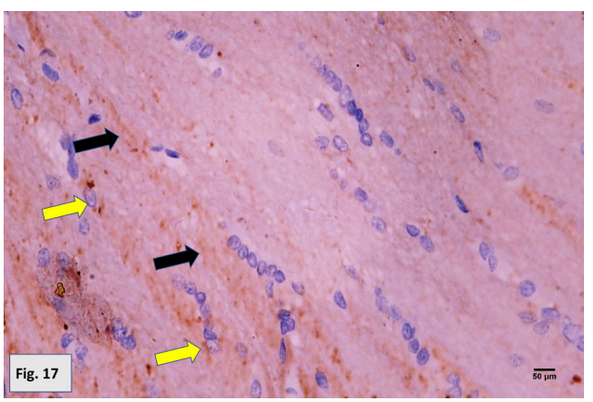


Fig. 17: A photomicrograph of a section of group III frontal cortex showing weak positive immune reaction for synaptophysin of cell bodies plasma membranes (yellow arrows) and of dendrites/axon between the cells (black arrows). (Synaptophysin x400, 50µm scale bar)

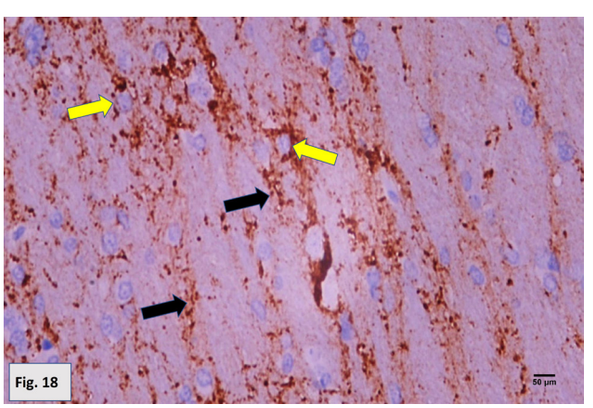


Fig. 18: A photomicrograph of a section of group IV frontal cortex showing moderate to strong positive immune reaction for synaptophysin of cell bodies plasma membranes (yellow arrows) and of dendrites/axon between the cells (black arrows) (Synaptophysin x400, 50µm scale bar)

Table 1: illustrating the comparison of the mean area% (between the groups I, III and IV) of PAS. P value (*) non-significant and (**) highly significant *P-value*)

		(Group I) (Control)	(Group III) (Tramadol)	(Group IV) (Tramadol+ Melatonin)
Area% of PAS (mean ± standard deviation)		43.65 ± 1.5	29.28 ± 8.8	43.07 ± 0.8
P-value Between the groups	I&III		0.00001**	
	I&IV		0.2*	
	III&IV		0.00001**	

Table 2: illustrating the comparison of the mean area% (between the groups I, III and IV) of caspase 3. *P value* (*) non-significant and (**) highly significant *P-value*).

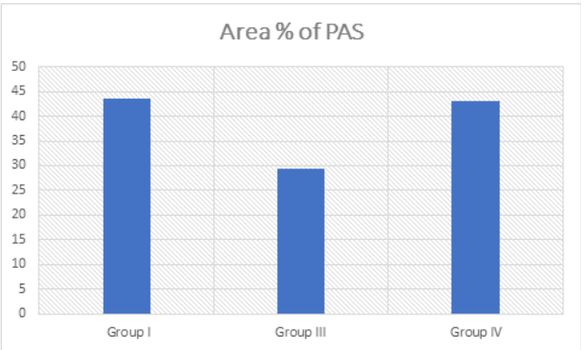
		(Group I) (Control)	(Group III) (Tramadol)	(Group IV) (Tramadol+ Melatonin)
Area% of caspase 3 (mean ± standard deviation)		2.36 ± 0.6	26.68 ± 1.7	2.86 ± 0.4
P-value Between the groups	I&III		0.00001**	
	I&IV		0.6*	
	III&IV		0.00001**	

Table 3: illustrating the comparison of the mean area% (between the groups I, III and IV) of GFAP. *P-value* (*) non-significant and (**) highly significant

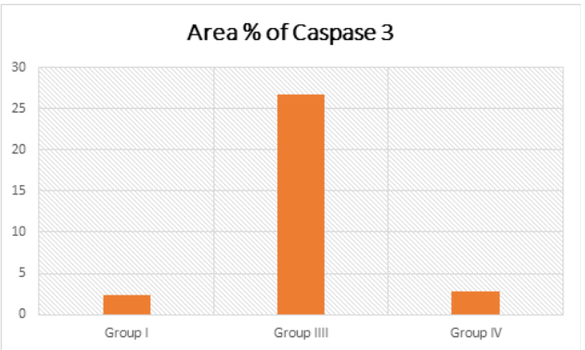
		(Group I) (Control)	(Group III) (Tramadol)	(Group IV) (Tramadol+ Melatonin)
Area% of GFAP (mean ± standard deviation)		10.43 ± 0.8	21.38 ± 0.5	10.54 ± 0.4
P-value Between the groups	I&III		0.00001**	
	I&IV		0.5*	
	III&IV		0.00001**	

Table 4: illustrating the comparison of the mean area% (between the groups I, III and IV) of synaptophysin. *P-value* (*) non-significant and (**) highly significant

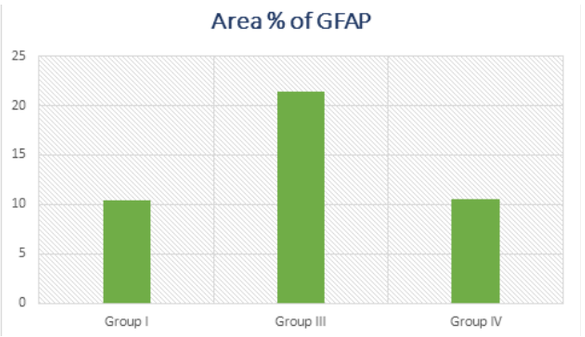
		(Group I) (Control)	(Group III) (Tramadol)	(Group IV) (Tramadol+ Melatonin)
Area% of synaptophysin (mean ± standard deviation)		58.87 ± 4.8	23.24 ± 1.6	57.16 ± 4.1
P-value Between the groups	I&III		0.00001**	
	I&IV		0.09*	
	III&IV		0.00001**	



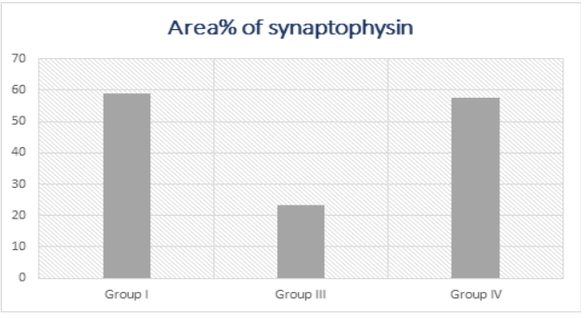
Histogram 1: illustrating the morphometric comparison of the mean area% (between the groups I, III and IV) of PAS



Histogram 2: illustrating the morphometric comparison of the mean area% (between the groups I, III and IV) of caspase 3



Histogram 3: illustrating the morphometric comparison of the mean area% (between the groups I, III and IV) of GFAP



Histogram 4: illustrating the morphometric comparison of the mean area% (between the groups I, III and IV) of synaptophysin

DISCUSSION

The role of melatonin in ameliorating the harmful impacts of tramadol on the frontal cortex of adult male albino rat was assessed in the present study. Tramadol is a central pain relieving with μ-opioid receptor low affinity, it also prohibits norepinephrine and serotonin reuptake which constitute the main analgesic mechanism of action[35]. Despite being one of the opioid analgesics, it has a superior tolerability and fewer adverse effects compared to oral nonsteroidal anti-inflammatory drugs and the other opioids[36].

Administration of tramadol for thirty days in the present study revealed marked histological changes of rats' frontal cortices, which were confirmed by the morphometric study and the statistical analysis. The histological changes were in the form of disarrangement of the grey matter layers, neural degeneration, perineural vacuolations, dilated congested blood vessels and increased cellular apoptosis. Abou El Fatoh *et al*.[37] reported neural degeneration with congested blood vessels following tramadol administration in rats. In addition, Liu *et al*.[38] detailed that opioids administration resulted in neuronal degeneration by increasing apoptotic cells in rats' brain. The presence of perineural vacuolations and degeneration of rat's brain cells were attributed in a previous study to the decrease in protein synthesis by the cells and their exposure to free radicals which leads to oxidative damage which influence the capacity of cells to transfer impulses and eventually cause cell death[39].

It has been demonstrated that opioid administration in rats is related to prominent changes within the principal proteins that participate in apoptosis initiation process[40] leading to pro-apoptotic Fas-receptor up regulation, anti-apoptotic oncoprotein Bcl-2 down regulation and increasing intracellular pro-apoptotic components such as caspase-3[41].

Additionally, in the current study tramadol induced cytoplasmic glycogen depletion of most granule and pyramidal cells. This glycogen depletion goes with the detected apparent neural cells degeneration in the present study. Badawy *et al*.[42] attributed the decrease in the glycogen content of the cerebral cortex with tramadol administration in rats to the destruction of the neurons.

Chronic opiate exposure has been proved to decrease the survival and the proliferation of new neurons in the human brain as they act directly on neurocytes progenitor through μ-opioid receptor decreasing their proliferation and DNA synthesis[43].

Administration of tramadol in the present study also revealed astrocytosis and decreased synaptophysin content. Glial cells maintain the central nervous system integrity, keep the neurons from stress-induced injury and improve neuronal survival during different pathological conditions[44]. The presence of unusual rise within the number of hypertrophic astrocytes in rat's brain is associated with neural degeneration as the swollen astrocytes encircle

the degenerated neural cells[45]. Astrocytes are the initial responders to brain tissue injury as they are the foremost copious glial cells in the brain. After brain tissue damage they stimulate other cellular responses and react rapidly through increasing the production of GFAP which leads to proper glial scar formation[46,47].

Regarding synaptophysin, it is an integral membrane glycoprotein in neurons with imperative role in synaptic transmission[48]. Synaptic loss is considered an indicator for axonal damage or axonal transport disturbances in various neurological pathologies of the brain[49]. Mitochondrial dysfunction, oxidative stress, hindrance of synaptogenesis and enhanced apoptosis were described as the causes of opioids neurotoxicity[50,51].

In previous studies, complete reversibility of tramadol neurotoxic impacts wasn't accomplished after its withdrawal. Apoptotic neurons and cytoplasmic vacuolations were still detected months after its withdrawal[52-54].

In contrary, concomitant administration of melatonin with tramadol for thirty days in the present study showed regular structure of the frontal cortices' grey matters, preserved granule and pyramidal cells with proper glycogen content. Lahiri *et al*.[55] declared that the cerebral cortex contains high level of melatonin than other tissues and exogenous melatonin administration in mice resulted in rise of its level both in the blood and in tissues (e.g., cerebral cortex, heart and kidneys). Baptista *et al*.[56] reported that melatonin administration associated with or without insulin protected the frontal cortices of the diabetic rats from axonal degeneration, decreased glycogen content and high apoptotic index.

Although melatonin is naturally secreted in the body, synthetic melatonin is considered a safe dietary supplement needed to improve outcomes and treatment efficacy of some neurological disorders such as in insomnia, neurodegenerative diseases, pain and mental disorders, also it is recommended as an adjuvant in treatment of some types of cancer and metabolic disorders[57]. Melatonin roles as free radical scavenger, antiapoptotic and anti-inflammatory on the central nervous system have been proved[58-60]. It is a powerful antioxidant with a great capability to neutralize free radicals than other antioxidants[61]. Melatonin has prominent hydrophilic and lipophilic properties, so it crosses effortlessly all biological membranes and enters the cells to reach the subcellular compartments, this capability permits it to diminish the harm of the oxidative radicals in both aqueous and lipid environments of the cells[62].

In addition, administration of melatonin with tramadol in the current study attenuated apoptosis and astrocytes reactivity and preserved the synaptophysin content. A prior study on co-administration effects of melatonin and tramadol on rats' testes elucidated that melatonin has attenuated the mitochondrial injury and cellular apoptosis that occur with tramadol. Melatonin cellular protective effects were attributed to its ability to increase the anti-apoptotic gene expressions and to diminish pro-apoptotic gene expressions. Also, to its antioxidant effects that maintain the mitochondrial membrane integrity[63]. Additionally, melatonin reduced neuronal cell apoptosis and astrocyte reactivity in hippocampus and dentate gyrus after brain injury in mice[64]. In a previous study, administration of melatonin concomitant with nonsteroidal anti-inflammatory drugs proved to be effective in restoring neurogenesis and neuroplasticity after neural damages by oxidative inflammation in patients with neurodegenerative diseases[65]. Finally, Kaewsuk *et al*[66] also reported that melatonin preserved synaptophysin content in the neonatal rat brain from methamphetamine-induced reduction.

CONCLUSION

Melatonin has a good protective effect against the harmful impacts of tramadol on the frontal cortex of adult male albino rat, through attenuating neurons degeneration, apoptosis and astrocyte reactivity, in addition to preservation of glycogen and synaptophysin contents.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دور الميلاتونين في تخفيف الآثار الضارة للترامادول على القشرة الأمامية لذكر الفـأر الأبيض البالغ (دراسة نسيجية وكيميائية مناعية)

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المقدمة: ترامادول هو أكثر المواد الأفيونية استخدامًا في حالات الآلام المتوسطة والشديدة. أبلغت الدراسات السابقة عن تغييرات في دماغ الفئران بعد تناول الترامادول عن طريق الفم أو داخل الصفاق. الميلاتونين "الهرمون العصبي" له تأثيرات مضادة للأكسدة ومضادة لموت الخلايا المبرمج فى المخ.

الهدف: يهدف هذا العمل إلى تقييم دور الميلاتونين في تخفيف الآثار الضارة للترامادول على قشرة المخ الأمامية لذكور الفئران البيضاء.

المواد والطرق: تم استخدام ٤٠ من ذكور الفئران البيضاء البالغة والذين تتراوح أعمارهم بين ٦-٨ شهور واوزانها تتراوح بين ١٨٠-٢٠٠ جم. تم توزيع الفئران بالتساوي على أربع مجموعات: المجموعة الأولى (المجموعة الضابطة): تم تقسيم الفئران إلى مجموعتين فرعيتين: Ia تم الاحتفاظ بالفئران دون أي علاج لمدة ثلاثين يومًا والمجموعة Ib: تلقت الفئران حقنة داخل الصفاق بمقدار ٠,١ مل من ٠,٩ ٪ ملحي و ٢ ٪ إيثانول يوميًا لمدة ثلاثين يومًا. المجموعة الثانية (مجموعة الميلاتونين): تم حقن كل فأر داخل الصفاق بمقدار ١٠ ملجم / كجم / يوم من الميلاتونين لمدة ثلاثين يومًا. المجموعة الثالثة (مجموعة ترامادول): تلقى كل فأر ٥٠ مجم / كجم / يوم ترامادول عن طريق الفم عن طريق أنبوب المعدة لمدة ثلاثين يومًا. المجموعة الرابعة (مجموعة الترامادول + الميلاتونين): تم حقن كل فأر بالميلاتونين وتلقى ترامادول بالفم كما في المجموعتين الثانية والثالثة على التوالي لمدة ثلاثين يومًا.

النتائج: أدى تناول الترامادول إلى اختلال طبقات الجزء الرمادى فى قشرة المخ، ونضوب الجليكوجين ، والسينابتوفيسين فى الخلايا وزيادة موت الخلايا المبرمج وزيادة الخلايا النجمية في قشرة المخ الأمامية للفئران. ومع ذلك فقد أظهر تناول الميلاتونين المتزامن مع ترامادول بنية منتظمة لقشرة المخ الأمامية للفئران ، وتنكس لموت الخلايا المبرمج ، واظهرت محتوى مناسب من الجليكوجين والسينابتوفيسين والخلايا النجمية.

الخلاصة: الميلاتونين له تأثير وقائي جيد ضد الآثار الضارة للترامادول على قشرة المخ الأمامية لذكر الفـأر الأبيض البالغ.