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Ameliorating Effect of 10-Dehydrogingerdione against Cerebral Cortex Damage caused by Tramadol in Adult Male Albino Rats (Histological and Immunohistochemical study)

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ABSTRACT
Background: An artificial centrally acting analgesic drug called tramadol hydrochloride is used to treat moderate to severe pain with fewer side effects than conventional opioid medicines.
Objective: This study aimed to investigate effects of tramadol on rat cerebral cortex, focusing on biochemical, histological, and immunohistochemical alterations, and possible protective effect of ginger.
Materials and methods: Fifty-five albino rats were employed. They were split into four groups: Control group (15 rats) that was further split into three equal subgroups: One received saline, one received ginger oil (200 mg/kg B.W/day), and one was kept without any medicine. Tramadol group (15 rats) received tramadol (50 mg/kg B.W/day). 3rd group received ginger oil 30 minutes before tramadol intake. In order to examine effects of stopping tramadol, 15 rats in 4th group were given tramadol for 4 weeks before being released from therapy for a further 4 weeks. Samples and specimens were prepared for biochemical and microscopic examination. **Results:** Tramadol administration revealed a histological and biochemical abnormality, where there was loss of normal arrangement of cortical layers with distorted-shape cells. In addition, there was appearance of vacuolations around them and in neuropil. In 2nd group, there was an increase in MDA level and a decrease in SOD and GPx. Ginger and tramadol-treated group showed proof of remarkable enhancement in histology of tissue and level of oxidative markers in contrast to tramadol group, while withdrawal group demonstrated partial recovery.
Conclusion: Ginger oil ameliorated tramadol- induced damage in rat's cerebral cortex.
Keywords: Tramadol, Cerebral cortex damage, 10-Dehydrogingerdione, Ginger oil, Histological and immunohistochemical.

INTRODUCTION

Opioid analgesics mitigate pain by exerting their effects primarily on the central nervous system, offering a significantly greater potency compared to non-narcotic analgesics. They are typically reserved for managing severe pain that remains unresponsive to non-narcotic treatments. Opioids are frequently and successfully used to treat acute, severe pain, offering a modest sedative effect and reducing anxiety. However, their frequent tendency to lead to abuse and addiction poses a significant issue. Additionally, high doses of potent opioids can result in fatal respiratory depression [1].

Tramadol, a synthetic opioid and 4-phenylpiperidine codeine derivative, has a low binding affinity for δ and κ opioid receptors but can bind to μ receptors. It is recommended for alleviating pain of varying intensities. With availability of many tramadol formulations, this drug is increasingly used worldwide as an alternative to high-affinity opioids, particularly in post-operative pain management. Tramadol can be administered orally, rectally, or parenterally (intramuscular, intravenous, and subcutaneous). Dosage titration should be individualized based on each patient's pain level and response [2]. Studies have demonstrated that tramadol effectively reduces moderate to severe postoperative pain in both inpatient and outpatient settings. Furthermore, tramadol administered orally or via a catheter has been shown to be as effective as other analgesics in postoperative pain management [3].

Ginger, an important medicinal herb, has been traditionally used for a wide range of conditions, including colds, headaches, toothaches, and to improve circulation in limbs. It also has a cholesterol-lowering effect. Moreover, ginger extract has been found to possess potent antioxidant activity and neuroprotective properties [4]. Tramadol's side effects vary with dosage and administration method, with intravenous and intraperitoneal routes posing higher risks. At therapeutic doses, tramadol may cause headaches, drowsiness, excessive sweating, nausea, and vomiting. Experimental studies have shown that oral or intraperitoneal administration of tramadol in rats can lead to brain changes, such as congestion and edema. Chronic use at escalating doses for over three months has also been associated with various types of neuronal degeneration [5].

Hence, this study aimed to investigate effects of tramadol on rat cerebral cortex, focusing on biochemical, histological, and immunohistochemical alterations, and possible protective effect of ginger.

MATERIALS AND METHODS

Experimental Animals: Fifty-five adult male albino rats, three to four months old, with an average weight of 150–200 grams, were utilized in this study. They were housed in Faculty of Medicine's Animal House at Benha University under carefully controlled and hygienic conditions. Rats had free access to food and water in plastic cages.

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Ethical consent: All stages of investigation were conducted in accordance with guidelines of Benha University Ethics Committee for animal experimentation and US National Institutes of Health's Guide for Care and Use of Laboratory Animals (NIH Publication No. RC 3-2-2024). Study was carried out in accordance with ethical principles for animal research, ensuring humane treatment and minimizing animal distress throughout experiment.

Chemicals

● **Tramadol hydrochloride:** Tramadol capsules were sourced from Mina Pharm, located in Heliopolis, Cairo, Egypt. The experimental solution was formulated by dissolving 50 mg of finely ground tramadol hydrochloride in 2.5 milliliters of saline, resulting in a tramadol concentration of 20 mg/ml. A dosage regimen was established wherein each rat received a daily dose of 50 mg/ kg of body weight, corresponding to an administered volume of 0.5 ml of the prepared solution [5, 6].

● **Ginger Oil:** Ginger oil was obtained from Sigma Aldrich Company. Each rat received a daily dose of 200 mg/kg body weight of ginger extract via gastric tube, one hour after tramadol injection [7].

Experimental Design: Animals were allocated into distinct cohorts through random assignment, designating them as either experimental or control groups:

- **Group I (Control group):** Comprised 15 rats, divided equally into three subgroups:
    - **Subgroup Ia:** 5 rats provided only by a regular diet and tap water.
    - **Subgroup Ib:** 5 rats received 1 ml of normal saline daily via gastric tube.
    - **Subgroup Ic**: 5 rats received 200 mg/kg body weight of ginger oil once daily via gastric tube.

All control group rats were sacrificed one month after experiment's initiation.

- **Group II (Tramadol-treated group):** Consisted of 10 rats treated with 50 mg/kg body weight of tramadol daily for 30 days.
- **Group III (Tramadol and Ginger-Treated Group):** Included 10 rats treated with same dose of tramadol as in group II, plus 200 mg/kg of ginger oil daily from start of study. These rats were sacrificed after one month.
- **Group IV (Tramadol Withdrawal Group):** Consisted of 10 rats treated with same dose of tramadol as in group II for 30 days, then left without treatment for an additional month. These rats were sacrificed two months after experiment's initiation.

All rats were sacrificed with ether at most appropriate time for each group. Following this, skull vaults were opened to expose cerebral hemispheres. One hemisphere was wrapped in aluminium foil and stored at -80 °C for biochemical studies, while other was fixed in 10% formalin for 48 hours. Samples were extracted from cerebral cortex of each hemisphere.

Biochemical analysis: The left hemisphere of each cerebrum was homogenized for biochemical analysis of oxidative stress. Homogenates were centrifuged at 10,000 g for 20 minutes at 4 °C. Supernatants were then analyzed for levels of glutathione peroxidase (Gpx), superoxide dismutase (SOD) activity, and malondialdehyde (MDA), as previously described [8].

Histopathological Study: The specimens underwent fixation in a 10% formalin solution, followed by standard processing and embedding within paraffin blocks. Thin sections, each precisely 5 µm in thickness, were subsequently prepared and subjected to histological and immunohistochemical staining for detailed examination.

- **Hematoxylin and Eosin (H & E) Stains:** Was employed as the conventional method for histological evaluation [9].
- **Periodic Acid Schiff (PAS) Stain:** Applied to evaluate metabolic disorders related to neurodegeneration, such as glycogenosis [9, 10].

Immunohistochemical Study: To investigate astrocytes, paraffin-embedded sections were subjected to immunohistochemical staining aimed at identifying GFAP (Catalog No. MA5-12023, ASTRO 6, pre-diluted IgG1, Thermo Fisher Scientific, Rockford, USA). GFAP immunoreactivity was characterized by a distinct brown staining within the astrocytic cytoplasm. The brain, striatum and cerebellum, were utilized as positive tissue controls, while negative control sections were prepared by omitting the primary antibody. Quantitative morphometric analysis was performed by enumerating GFAP-positive astrocytes across all study groups [11].

Quantitative morphometric analysis: Image software version 1.47v (National Institutes of Health, USA) was used to analyze percentage area stained with GFAP. For each rat across the three groups, the mean value was calculated from measurements obtained in three distinct, non-overlapping fields within each immunostained region.

Statistical Analysis

Data were coded, processed, and analyzed using SPSS version 26 (IBM, Armonk, New York, United States). Quantitative data normality were assessed with Kolmogorov–Smirnov test. Non-parametric data were presented as median (range), and parametric data as mean ± SD. The Kruskall-Wallis test was applied for the analysis of non-parametric datasets, whereas one-way analysis of variance (ANOVA) was utilized to assess differences among the three groups for quantitative data exhibiting a normal distribution. P-values of less than 0.05 were considered significant for all tests.

RESULTS

Light Microscopic Examination

a) Hematoxylin & Eosin (H&E) and Periodic Acid Schiff (PAS) Stains

Histopathological alterations were observed as follows:

In the control subgroup sections stained with H & E, six distinct layers of structured gray matter were discerned. Arranged from the outermost to the innermost, these strata included the molecular (plexiform) layer, external granular layer, external pyramidal cell layer, internal granular layer, internal pyramidal cell layer, and the polymorphic cell layer. The molecular layer was bordered externally by a delicate pia mater layer (Figure 1a). At higher magnification, the internal granular layer exhibited densely packed ovoid granular cells, each with a conspicuous nucleus. Inner pyramidal layer contained triangular pyramidal cells with basophilic cytoplasm, basal nuclei, and long apical dendrites. Normal eosinophilic neuropil appeared between nerve cells, filled with glial cells with small dense nuclei and blood vessels (Figure 1b).

In sections from group II (tramadol-treated group), there was a loss of normal arrangement of cerebral cortical layers, with degenerated neuropil and loss of pia mater (Figure 2a). At higher magnification, neuronal cells appeared abnormal, pyramidal cells were deformed, lacked dendrites, and had pyknotic nuclei. Both granular and glial cells were surrounded by holes and large vacuoles. Neuropil was degenerated, showing multiple vacuoles and containing a deformed neuron with acidophilic cytoplasm (red neuron) (Figure 2b).

Sections from cerebral cortex of rats in group III (treated with both tramadol and ginger oil) showed reorganization of cerebral cortex layers covered by pia mater (Figure 3a). At high magnification, some granules appeared normal with rounded cells and open-faced nuclei, while others appeared abnormal. Pyramidal cells were triangular with long dendrites, though some were distorted. Vacuolations in neuropil decreased compared to group II (Figure 3b).

In sections from group IV (tramadol withdrawal group), six layers of cerebral cortex were reorganized (Figure 4a). At high magnification, vacuolations were reduced in both neuropil and around cortical cells. Granular and pyramidal cells appeared mostly normal, although vacuolations remained around glial cells. Neurons still showed some vacuolations and degeneration (Figure 4b). PAS staining of sections from control subgroups showed a strong positive reaction (purplish-red) in pyramidal cells of cerebral cortex (Figure 5a). In contrast, sections from tramadol-treated group showed a weak PAS reaction (Figure 5b). Sections from group III displayed a moderate positive reaction (Figure 5c), while sections from group IV (tramadol withdrawal group) exhibited a weak PAS reaction in cytoplasm of pyramidal cells in cerebral cortex (Figure 5d).

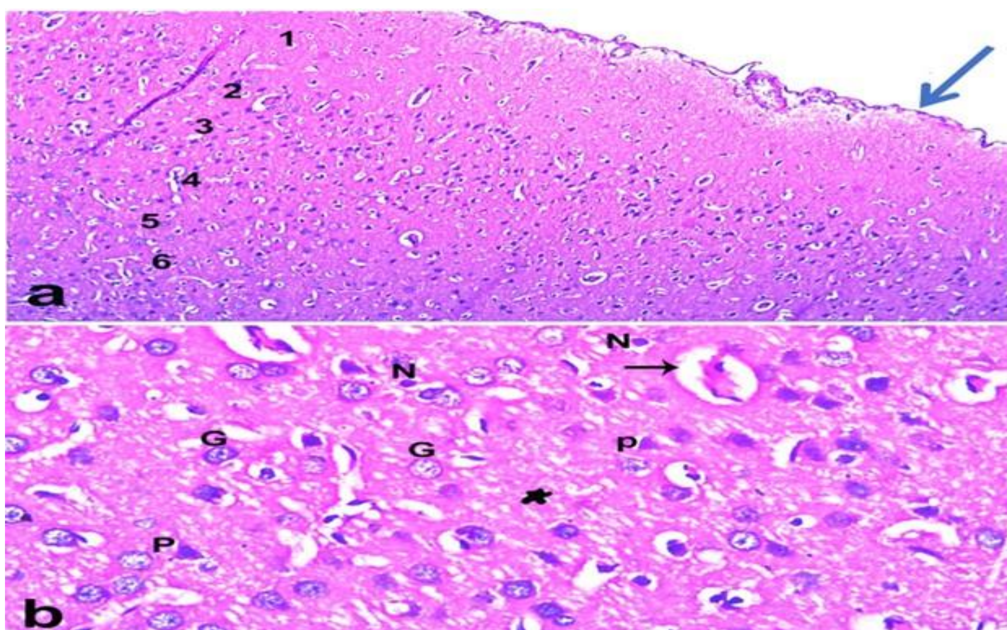


Figure (1 a & b): Photomicrographs sections in cerebral cortex of frontal lobe from control rats subgroups showed: (1a): Six layers, placed in a well-organized manner from outer to inner surface: Molecular layer (1), external granular (2), external pyramidal (3), internal granular (4), internal pyramidal (5) and polymorphic layer (6). Pia mater layer covers molecular layer (blue arrow) (H&E. x100). (1b): internal granular and internal pyramidal layers showing normal pyramidal cell (P), granular cell (G), neuroglial cell (N), and surrounding eosinophilic neuropil (star). Blood vessel is noticed (arrow) (H & E. x400).

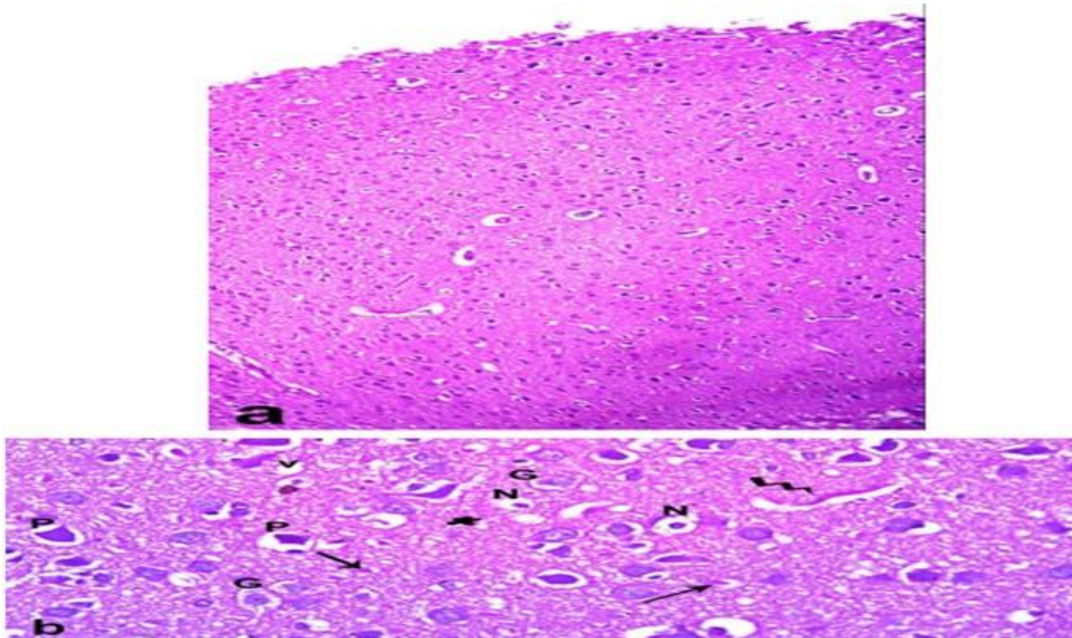


Figure (2 a & b): Photomicrographs sections in cerebral cortex of frontal lobe from rats of group II showed: (a) Loss of normal arrangement of its six layers with rarified neuropil between cells and disappearance of pia matter. (H & E. 2a x100), (b) vacuolated granular cells (G), distorted pyramidal cell with pyknotic nucleus surrounded by haloes (P), and glial cells with hole around it (N). Degenerated neuropil (star) with vacuoles (v) and abnormal neurons with acidophilic cytoplasm (red neurons) (arrow), distorted shaped blood vessel (zigzag arrow) (H & E. 2b x400).

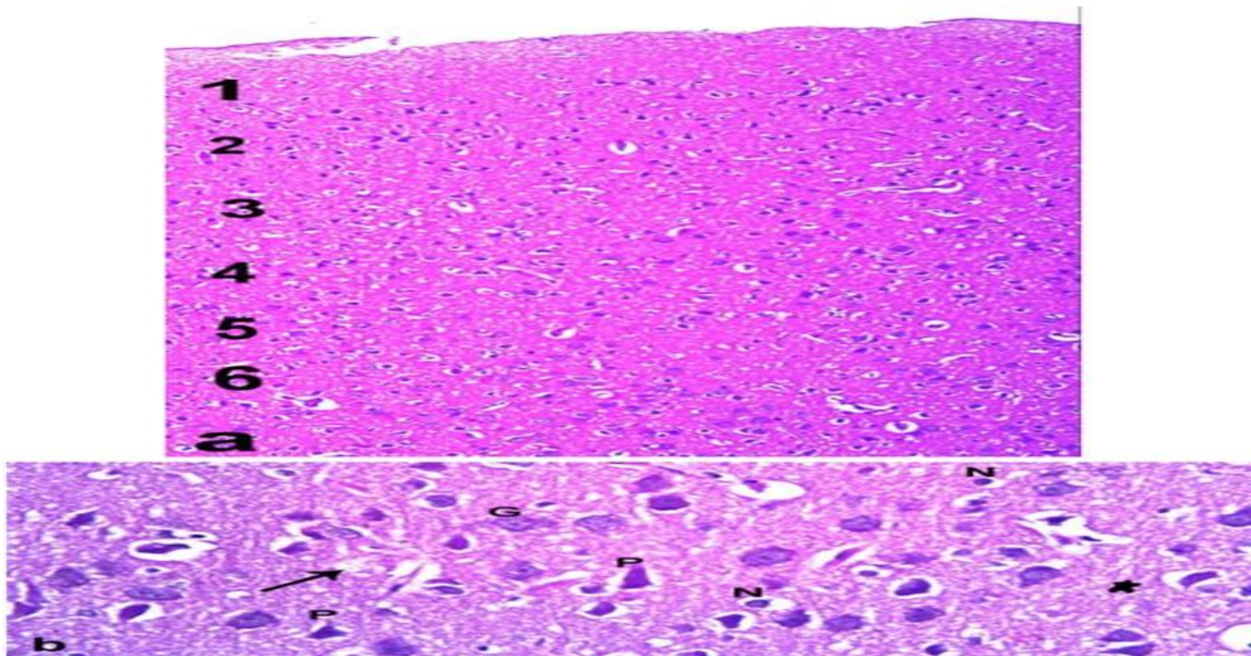


Figure (3 a & b): Photomicrographs sections in cerebral cortex of frontal lobe from rats of group III showed (a) : rearranged layers of cerebral cortex ; molecular layer (1), external granular (2), external pyramidal (3), internal glomerular (4), internal pyramidal (5), and polymorphic layer (6) covered with pia matter (zigzag). (H & E. x100). (b): Normal granular cell with prominent nuclei (G), distorted granular cell (GI), normal triangular pyramidal cell (p), some distorted pyramidal cell (pp), vacuolated glial cell (N), and eosinophilic neuropil (star). (H & E. x 400).

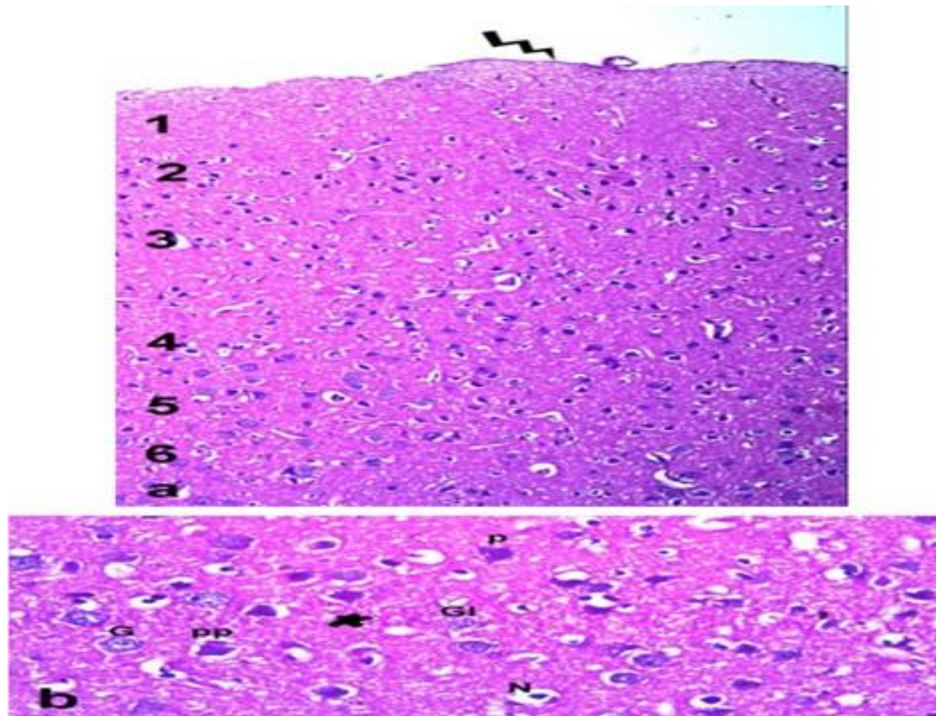


Figure (4 a & b): Photomicrographs sections in cerebral cortex of frontal lobe from rats of group IV showed: (a): Molecular (1), outer granular (2), outer pyramidal(3), inner granular (4), inner pyramidal (5), and polymorphic layer (6) of cerebral cortex (H&E. a x100). (b): normal triangular pyramidal cell with long dendrite (P), rounded granular cell with open face nucleus (G), glial cell with surround vacuolations (N). Neuropil is rarified (star) and showed vacuolations (Arrow) (H & E. x 400).

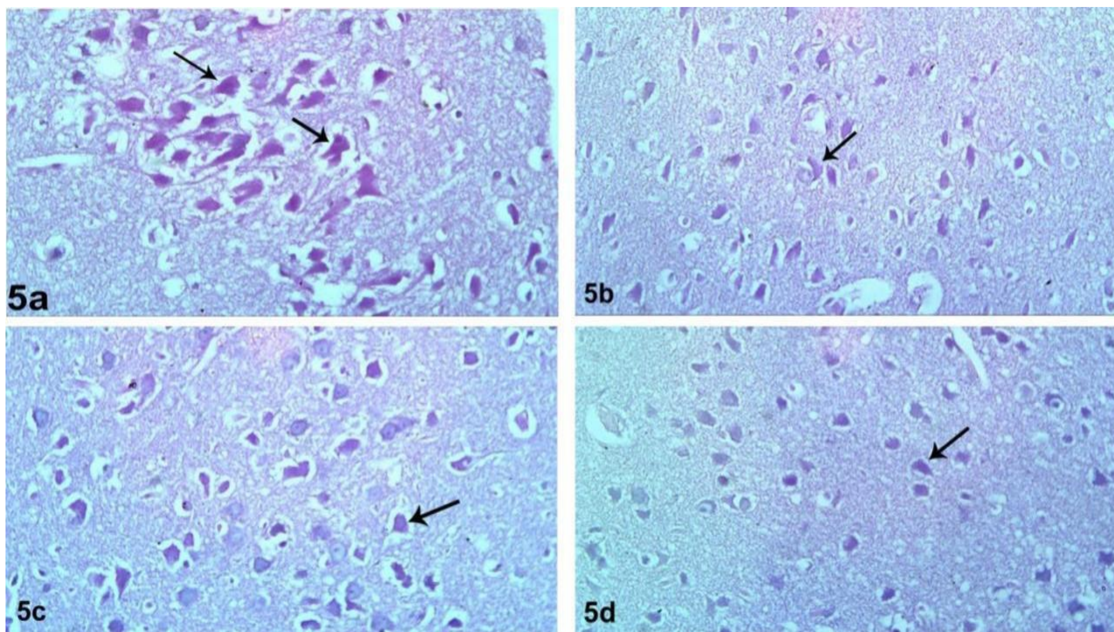


Figure (5 a-d): Photomicrographs sections in cerebral cortex of frontal lobe from rats showed: (5a): Strong positive PAS staining in pyramidal cell from rats of control groups (arrow). (5b): Negative reaction in pyramidal cells from rats of group II (arrow). (5c): Weak positive in pyramidal cells of cerebral cortex from rats of group III (arrow). (5d): Moderate reaction in pyramidal cells of cerebral cortex from rat of group IV (arrow). (PAS, x 400).

Light microscopic examination of tissue stained by glial fibrillary acidic protein (GFAP):

Sections from rats of control subgroups revealed astrocytes appeared as **very mild** GFAP-postive stained cells with a thin body and dendrites (Figure 6 a), while astrocytes in sections from rats of tramadol-treated group appeared more obvious as GFAP-positive stained cells with an increase in thickness of their body and dendrites (Figure 6 b). Astrocytes of 3rd group appeared mild GFAP-**postive and** less hypertrophied than those of 2nd group (6 c), while astrocytes in 4th group appeared with moderate positive immunoreaction (6 c & 6 d).

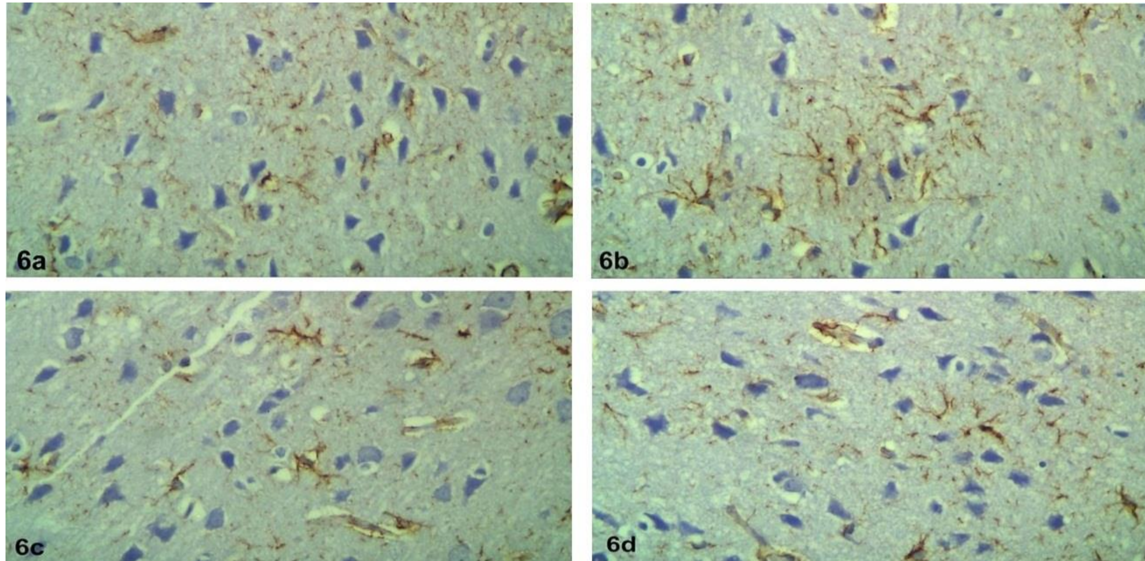


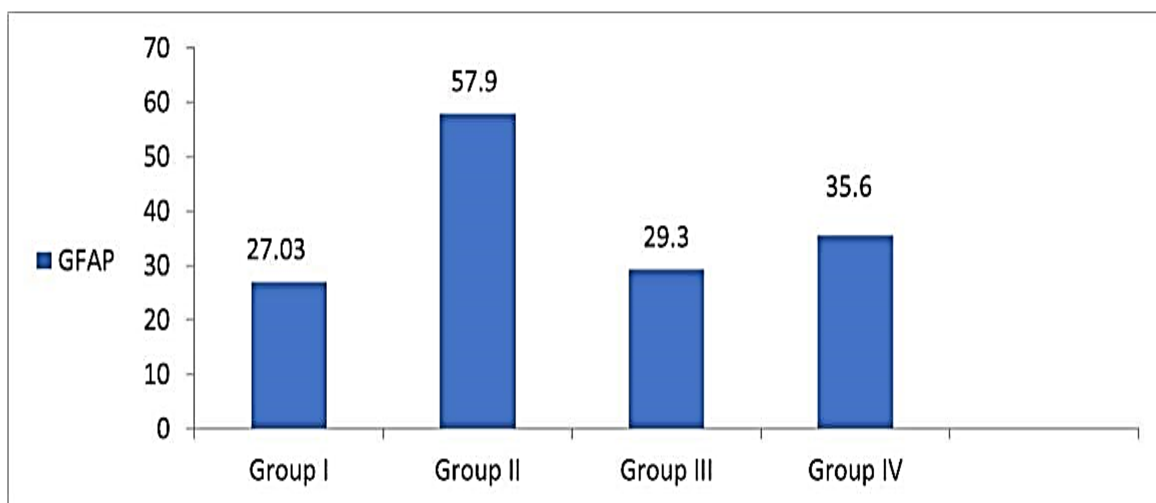
Figure (6): Photomicrographs of sections in cerebral cortex of rats showed (6 a): Group I showed mild positive GFAP immunostaining in astrocytes and their processes. (6 b) group II showed more positive GFAP immunostaining in astrocytes with increased branches of their cytoplasmic processes. (6 c) group III showed moderate positive astrocytes GFAP immunostain as compared to control group. (6 d) group IV showed astrocytes with positive GFAP immunostain (anti GFAP immunostain× 400).

Morphometric analysis of immunohistochemical stating of (GFAP)

In control group,the average quantity of astrocytes stained with anti-GFAP was 27.03 ± 0.2. Mean number of astrocytes stained with anti-GFAP in tramadol group was 57.9 ± 0.9, which was considerably higher than in control, ginger and withdrawal groups (P≤ 0.001, ≤ 0.001 and ≤ 0.01 respectively). In ginger group, percentage of anti-GFAP was 29.3 ± 0.2, which was highly significant (P≤ 0.001) in contrast to tramadol group, however there were also substantial changes in tramadol withdrawal group (35.6 ± 0.2) with (P≤ 0.04) (Table 1 and histogram 1).

Table 1: Mean number of anti-GFAP stained astrocytes in four different groups

	Control Group	Group II	Group III	Group IV	P value
GFAP +ve Astrocytes	27.03 ± 0.2[a&b]	57.9 ± 0.9 [a ,c &d]	29.3 ± 0.2 [c]	35.6 ± 0.2 [b &d]	<0.001[a] < 0.04[b] <0.001[c] <0.01 [d]



Histogram (1): Mean number of anti-GFAP stained astrocytes in different studied groups.
Data expressed as mean ± SD, *: significance ≤ 0.05; One way ANOVA method followed by post-hoc Tukey's test a: Significance Group II vs Control, b: Significance group IV vs control, c: Significance group II vs group III, d: Significance group II vs group IV.

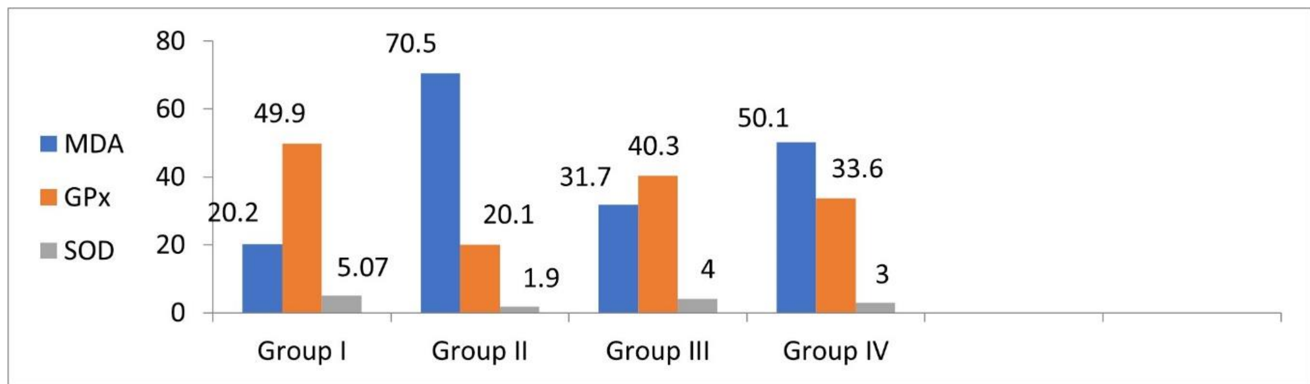
Biochemical marker of antioxidants and oxidative stress:

GPx and SOD activities were considerably diminished with tramadol group (P ≤ 0.001). However, in contrast to other groups, this group's MDA level was increased markedly (P ≤ 0.001). Co-administration of tramadol with ginger was different from tramadol group in that it leads to a noticeable rise in GPx and SOD activity as well as an obvious decrease in MDA levels (P ≤ 0.01). Moreover, ginger group's mean values for GPx and SOD activity were significantly increased while MDA values diminished significantly in contrast to tramadol group (P≤ 0.001). Moreover, in withdrawal group's mean values for GPx and SOD activity were increased while MDA values diminished in contrast to tramadol group (P≤ 0.05) (Table 2) (Histogram 2).

Table (2) showing biochemical values in different studied groups

	Group I control	Group II	Group III	Group IV	P value
Malondialdehyde (MDA) (nmol/g tissue)	20.2±0.2 a & b	70.5±0.5 a,c &d	31.7± 3.9 C	50.1±1.7 b& d	<0.001a < 0.01b <0.001c <0.05d
glutathione peroxidase (GPx) (U/g tissue)	49.9± 0.2 a & b	20.1± 0.2 a,c &d	40.3±0.7 c	33.6±0.6 b & d	<0.001a < 0.01b <0.001c <0.05d
dismutase Superoxide (SOD) (U/g tissue)	5.07 ± 0. 2 a & b	1.9± 0. 2 a,C&d	4 ± 0.1C	3±0.1 b& d	<0.001a < 0.01b <0.001c <0.05d

Data expressed as mean± SD, *: significance ≤ 0.05; One way ANOVA method followed by post-hoc Tukey's test a: Significance Group II vs Control, b: group IV Significance vs control, c: Significance group II vs group III, d: Significance group II vs group IV.



Histogram (2): Biochemical oxidative enzyme for all studied group.

DISCUSSION

The aim of current study was to examine histological and biochemical changes in rat cerebrum induced by tramadol hydrochloride, to observe effects of its withdrawal, and to explore potential healing benefits of ginger.

Our findings, after one month of tramadol administration revealed disarrangement in cerebral cortical layers. Granular, pyramidal, and glial cells in cerebral cortex displayed abnormal shapes with large vacuoles surrounding them. These results were consistent with other studies, which reported neuronal damage [12], and noted reduced nuclear condensation, cytoplasmic contraction, and a decrease in cell volume in rat brain following opioid administration [13]. Additionally, our results aligned with those of **Mohamed and Mahmoud** [14], which reported histological observations such as hypertrophy of choroid plexus, enlarged perivascular space due to hemorrhage, and loss of pyramidal cell morphology.

In this study, noticeable vacuolations within neuropil were observed following a four-week period of tramadol dosing, which were in line with findings of **Ghoneim *et al.*** [5] who reported increased apoptotic cells, substantial cortical layer disarray, hypercellularity, multinucleated large cells, and significant neuropil vacuolization. This outcome also agreed with **Zarnescu *et al.'s*** study [15], which observed vacuolations and a reduction in pyramidal cells. The vacuolations may result from destruction of cellular organelles due to free radical exposure. Furthermore, prolonged opiate administration may inhibit survival and renewal of newly formed neurons in adult brain by reducing DNA synthesis [16].

Our findings also noted presence of red neurons (neurons exhibiting hypoxic alterations) in intrinsic pyramidal and molecular layers of cerebral cortex. Some regions of neuropil displayed dilated blood vessels and inflammatory cell infiltration. These observations concurred with the resuilts of **Motawea *et al.*** [12] and **Mohamed and Mahmoud** [14], who examined the combined impacts of clonazepam and tramadol on neural tissues, observing the appearance of red neurons. Their analysis attributed this red staining to nuclear pyknosis and the depletion of Nussle bodies, which are normally stained blue in hematoxylin and eosin preparations [17].

In light of our findings, it was observed that ginger helped reverse histological and morphological abnormalities induced by tramadol. Most structural changes reverted in brain sections displaying nearly normal morphology, except for a few apoptotic pyramidal cells. Additionally, some dilated and congested blood vessels were noted. This aligned with research by **El-Akabawy and El-Kholy** [18], which demonstrated that ginger protects brain by reducing oxidative stress, apoptosis, and inflammation in rats treated with a diabetes inducer. Furthermore, study by **Hussein *et al.*** [19], which investigated ameliorative effects of propolis and ginger supplementation on brains of rats exposed to monosodium glutamate, which showed that ginger could mitigate neurochemical damage caused by monosodium glutamate in specific brain regions. Our results were also consistent with the research of **Badawy *et al.*** [10] who reported increased brain weight and improvements in hippocampus and cerebral cortex's histological structure following concurrent use of gabapentin and ginger in embryonic brains. In another study [20], ginger supplementation significantly reduced pathological changes, including neurodegeneration, perivascular edema, and gliosis, in cerebrum and cerebellum following combined fluoride and dimethoate toxicity. Furthermore, ginger's positive effects are also mediated by enhancing neurogenesis, reducing acetylcholinesterase (AChE) expression, and modifying astrocytic response to damage. Study of **Shanmugam *et al.*** [21] reported ginger's potential anti-inflammatory and antioxidant properties. These effects are likely due to phenolic-ketone derivatives, like gingerols and shogaols, which have been shown to mitigate reactive oxygen species (ROS)-induced CNS damage. Additionally, studies indicate that ginger supplementation reduces lipid peroxidation in brain tissue [19] .

Nevertheless, in this experiment cellular damage was significantly reduced in groups that stopped tramadol treatment for one month compared to group receiving tramadol alone and showed slightly altered cerebral cortex architecture, although a small number of pyramidal cells were reduced and encircled by halos. These findings were in agreement with those of **Motawea *et al.*** [10] who observed subtle architectural alterations within specific regions of the cerebral cortex post-tramadol withdrawal. While a subset of granular and pyramidal cells exhibited persistent deformities, alongside with the continued presence of red neurons, the pyramidal cells in both the external and internal cortical layers predominantly retained a normal morphology. Similarly, other studies [22] reported a significant reduction in cellular damage in withdrawal group compared to tramadol-treated groups, noting that apoptotic changes caused by tramadol regressed, and apoptotic index returned to near-normal levels, although it still differed significantly from control. Additionally, **Iversen** [23] documented a gradual amelioration of opioid-induced toxic encephalopathy over a four-week exposure period, with near-total recovery observed by six months. This finding lends additional support to the notion of partial regenerative capacity in brain tissue following the cessation of tramadol. This is due to nervous system's limited regenerative capacity, which distinguishes it from other body organs [22]. While, full recovery from neurotoxicity may take several months, it is often at least partially reversible.

Analysis of PAS-stained sections revealed an exceedingly weak PAS reaction within the neuronal cells of the tramadol group, with this diminished staining particularly pronounced in degenerated cells. This result aligned with study by **Elsukary *et al.*** [24], which compared neurotoxicity between tramadol and pregabalin in rats. Similar findings were reported in **Badawy *et al.'s*** study [10], indicating that long-term opioid exposure can directly impact progenitor neuron populations in adult brain, reducing proliferation and survival of new neurons. This effect is associated with opioid interaction with μ-opioid receptors, leading to decreased DNA synthesis and cell proliferation. Concurrent administration of ginger induced a strong PAS response within granular and pyramidal cells, while the withdrawal group demonstrated a moderate PAS reaction specifically in pyramidal cells.

Our results also showed that tramadol significantly increased number of GFAP-positive neurons, which is consistent with findings by **Liu *et al.'s*** study [13]. underlying theory is that microglial cells mediate neuroinflammatory process in CNS [21], releasing inflammatory cytokines that contribute to neurodegeneration [25]. This migration and activation lead to an inflammatory response in prefrontal cortex [26]. This reactive gliosis, likely due to oxidative stress, occurs following long-term tramadol administration for a month [12]. Our study demonstrated that ginger treatment mitigated gliosis caused by tramadol, which aligned with earlier research on diabetic rats, where antioxidant properties were believed to reduce reactive gliosis through free radical scavenging [27].

Biochemical findings from this study revealed that albino rats treated with tramadol for one month had significantly elevated MDA levels and significantly reduced SOD and GPx levels compared to control group. These results are consistent with **Mohamed and Mahmoud's** study [14], which showed that tramadol administration in albino rats altered several antioxidant enzymes, including glutathione-S-transferase, quinone reductase, catalase, superoxide dismutase, peroxidase, and glutathione reductase, resulting in decreased cerebral lipid peroxidation and GSH levels. This is also in agreement with **El Baky and Hafez's** study [28], which found that tramadol administration raised plasma MDA levels while significantly reducing plasma GPx and SOD levels. Similarly, **Alfred *et al.*** [29] reported alterations in glutathione reductase and GPx activity, and an increase in ROS production caused by tramadol administration was shown to decrease SOD and CAT activity in albino rats.

The marked decline in brain SOD and GPx activity, coupled with high MDA levels, may be ascribed to increased lipid peroxidation, which leads to tissue damage and breakdown of antioxidant defences. Supporting this hypothesis are the results of **Abdel-Zaher *et al.'s*** study [30], which demonstrated that tramadol administration in mice led to GPx depletion, enhancing lipid peroxidation in kidney cortical slices, causing mitochondrial damage and inhibiting protein synthesis. MDA serves as a highly sensitive biomarker for lipid peroxidation and a reliable indicator of oxidative stress magnitude. It is well-documented that GPx enzymes rely on a transition metal as a cofactor. Tramadol may interfere with these metal cofactors, consequently diminishing the enzymatic activity [31].

Conversely, our findings showed that co-administration of ginger with tramadol improved oxidative stress parameters, restoring antioxidant enzymes by increasing GPx and SOD levels and reducing MDA levels. These results align with other studies [20, 32], which reported that ginger enhanced antioxidant marker enzyme levels in brains of diabetic rats. Withdrawal group exhibited a non-significant increase in SOD concentration and brain GPx activity, along with a reduction in MDA, compared to tramadol group. These results align with findings from **Elwy and Tabl** [33], which demonstrated that stopping tramadol for 30 days significantly increased GSH levels, CAT activity, and SOD in liver tissue. According to **Hussein *et al.*** [34] the brain tissue of tramadol withdrawal groups exhibited GPx activity and

GSH levels that were marginally elevated, though not significantly, in comparison with those observed in the therapeutic and toxicity tramadol groups.

Limitations: This study was limited by its use of a single animal model, which may not fully represent complexity of tramadol-induced neurotoxicity and protective effects of ginger in humans. Additionally, short duration of treatment and withdrawal periods may not capture long-term effects of tramadol use and cessation. Further studies with extended observation periods and diverse models are needed to better understand chronic impacts and potential therapeutic role of ginger in neuroprotection.

CONCLUSIONS

Tramadol administration adversely affected cerebral cortex at both histological and biochemical levels. However, ginger administration exerted a protective effect on brain, and tramadol withdrawal led to partial recovery from many of its degenerative effects.

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Conflict of Interest: Nil.

REFERENCES

1. **Camilleri M, Lembo A, Katzka D (2017)**: Opioids in Gastroenterology: Treating Adverse Effects and Creating Therapeutic Benefits. Clin Gastroenterol Hepatol., 15: 1338-49.
2. **Elghait A, Mostafa T, Gameaa F *et al.* (2022)**: Comparative Histological Study on Effect of Tramadol Abuse on Testis of Juvenile and Adult Male Albino Mice. Anat Cell Biol., 55: 341-55.
3. **Nossaman V, Ramadhyani U, Kadowitz P *et al.* (2010)**: Advances in perioperative pain management: use of medications with dual analgesic mechanisms, tramadol & tapentadol. Anesthesiol Clin., 28: 647-66.
4. **Zahedi A, Fathiazad F, Khaki A *et al.* (2012)**: Protective effect of ginger on gentamicin-induced apoptosis in testis of rats. Adv Pharm Bull., 2: 197-200.
5. **Ghoneim F, Khalaf H, Elsamanoudy A *et al.* (2014)**: Effect of chronic usage of tramadol on motor cerebral cortex and testicular tissues of adult male albino rats and effect of its withdrawal: histological, immunohistochemical and biochemical study. Int J Clin Exp Pathol., 7: 7323-41.
6. **Matthiesen T, Wöhrmann T, Coogan T *et al.* (1998)**: experimental toxicology of tramadol: an overview. Toxicol Lett., 95: 63-71.
7. **El-Borm H, Gobara M, Badawy G (2021)**: Ginger extract attenuates labetalol induced apoptosis, DNA damage, histological and ultrastructural changes in heart of rat fetuses. Saudi J Biol Sci., 28: 440-7.
8. **Shi Z, Cao J, Chen J *et al.* (2009)**: Butenolide induced cytotoxicity by disturbing prooxidant-antioxidant balance, and antioxidants partly quench in human chondrocytes. Toxicol In Vitro, 23: 99-104.
9. **Suvarna K, Layton C, Bancroft J (2018):** Bancroft's theory and practice of histological techniques: Elsevier health sciences, 9: 2018.
10. **Badawy S, Hammad S, Amin S *et al.* (2014)**: Effects of Dependence of Tramadol, Diazepam and Their Combination on Brain of Albino Rats: Biochemical, Histological and Immunohistochemical Study. Ain Shams Journal of Forensic Medicine and Clinical Toxicology, 23: 139-47.
11. **Afsari Z, Renno W, Abd-El-Basset E (2008)**: Alteration of glial fibrillary acidic proteins immunoreactivity in astrocytes of spinal cord diabetic rats. Anat Rec (Hoboken), 291: 390-9.
12. **Motawea S, Amer R, Haiba D *et al.* (2020)**: Cerebral Cortical Changes in Adult Albino Rats under Effect of Tramadol and its Withdrawal: Histological and Morphometric Study. Egyptian Journal of Histology, 43: 412-26.
13. **Liu L, Lu J, Wang X *et al.* (2013)**: Neuronal apoptosis in morphine addiction and its molecular mechanism. Int J Clin Exp Med., 6: 540-5.
14. **Mohamed H, Mahmoud A (2019)**: Chronic exposure to opioid tramadol induces oxidative damage, inflammation and apoptosis, and alters cerebral monoamine neurotransmitters in rats. Biomed Pharmacother., 110: 239-47.
15. **Zarnescu O, Brehar F, Chivu M *et al.* (2008)**: Immunohistochemical localization of caspase-3, caspase-9 and Bax in U87 glioblastoma xenografts. J Mol Histol., 39: 561-9.
16. **Eisch A, Barrot M, Schad C *et al.* (2000)**: Opiates inhibit neurogenesis in adult rat hippocampus. Proc Natl Acad Sci U S A., 97: 7579-84.
17. **Garman R (2011)**: Histology of central nervous system. Toxicol Pathol., 39: 22-35.
18. **El-Akabawy G, El-Kholy W (2014)**: Neuroprotective effect of ginger in brain of streptozotocin-induced diabetic rats. Ann Anat., 196: 119-28.
19. **Hussein U, Hassan N, Elhalwagy M *et al.* (2017)**: Ginger and Propolis Exert Neuroprotective Effects against Monosodium Glutamate-Induced Neurotoxicity in Rats. Molecules, 22: 1928.
20. **Sharma P, Verma P, Sood S *et al.* (2023)**: Protective Effect of Quercetin and Ginger (Zingiber officinale) extract against Dimethoate Potentiated Fluoride-Induced Nephrotoxicity in Rats. Foods, 12: 1899.
21. **Shanmugam K, Ramakrishna C, Mallikarjuna K *et al.* (2010)**: Protective effect of ginger against alcohol-induced renal damage and antioxidant

enzymes in male albino rats. Indian J Exp Biol., 48: 143-9.
22. **Khodeary M, El-Din A, Kholy S (2010)**: a Histopathological and Immunohistochemical Study of Adult rats' Brain after Long-term Exposure to Amadol (Tramadol Hydrochloride). Mansoura J Forensic Med Clin Toxicol., 18: 1-24.
23. **Iversen L (2003)**: Cannabis and brain. Brain, 126: 1252-70.
24. **Elsukary A, Helaly A, El Bakary A *et al.* (2022)**: Comparative Study of Neurotoxic Effects of Pregabalin Versus Tramadol in Rats. Neurotox Res., 40: 1427-39.
25. **Hoogland I, Houbolt C, van Westerloo D *et al.* (2015)**: Systemic inflammation and microglial activation: systematic review of animal experiments. J Neuroinflammation, 12: 114.
26. **Dheen S, Kaur C, Ling E (2007)**: Microglial activation and its implications in brain diseases. Curr Med Chem., 14: 1189-97.
27. **Baydas G, Nedzvetskii V, Tuzcu M *et al.* (2003)**: Increase of glial fibrillary acidic protein and S-100B in hippocampus and cortex of diabetic rats: effects of vitamin E. Eur J Pharmacol., 462: 67-71.
28. **El Baky A, Hafez M (2017)**: NOS Expression in Oxidative Stress, Neurodegeneration and Male Infertility Induced by Abuse of Tramadol. Biochemistry & Pharmacology: Open Access, 6: 1-6.
29. **Alfred O, Ibiam U, Emmanuel I *et al.* (2022)**: Effects of tramadol administration on some biochemical parameters in Wister albino rats under non-induction of pain. Multidisciplinary Journal for Applied Research in Engineering and Technology, 2: 137-67.
30. **Abdel-Zaher A, Abdel-Rahman M, Elwasei F (2011)**: Protective effect of Nigella sativa oil against tramadol-induced tolerance and dependence in mice: role of nitric oxide and oxidative stress. Neurotoxicology, 32: 725-33.
31. **Ismail M, Al-Naqeep G, Chan K (2010)**: Nigella sativa thymoquinone-rich fraction greatly improves plasma antioxidant capacity and expression of antioxidant genes in hypercholesterolemic rats. Free Radic Biol Med., 48: 664-72.
32. **Wang Y, Qin Z (2010)**: Molecular and cellular mechanisms of excitotoxic neuronal death. Apoptosis, 15: 1382-402.
33. **Elwy A, Tabl G (2014)**: Effects of chronic usage of tramadol, acetaminophen and tramacet on some biochemical and immunological changes in male rats. J Drug Res Egypt, 35: 63-71.
34. **Hussein S, Ismail H, Abdel Aal S (2017)**: Effect of tramadol drug on some biochemical and immunological parameters in albino male rats; evaluation of possible reversal following its withdrawal. Benha Veterinary Medical Journal, 33: 418-29.