Effects of the Concomitant Administration of Thyme and Tramadol on the Cerebellar Cortex of Adult Male Albino Rat

Original Article

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ABSTRACT

Introduction: Tramadol is a centrally acting opioid analgesic that used to relief pain. Although tramadol is thought to have low dependence potentials, it is used in acute or chronic pain, including postoperative, gynecologic and obstetric pain, as well as pain of various other organs. It is used in many countries including the Middle East. Its uptake can lead to bad effects on the nervous system.

Aim of the Work: To determine the harmful effects of tramadol on the cerebellar cortex of adult male rats, as well as the potential ameliorative impact of thyme when combined with tramadol.

Material and Methods: A twenty one adult male albino rats were randomized into 3 groups equally: The control group (group I) which was not received any medications, the tramadol group (group II) which was received tramadol HCL (40 mg/Kg/day) dissolved in tab water orally and the tramadol + thyme group (group III) which was received tramadol (40 mg/Kg/day) + thyme extract (500 mg/kg/day) orally. The tramadol and thyme extract were given to the rats for 8 weeks. Then the cerebella of the rats were processed to evaluate the histological, ultrastructural, immunohistochemical changes and morphometric analysis.

Results: Histologically, ultrastructurally and immunohistochemistry, the group treated with tramadol revealed remarkable degenerative and neuronal apoptotic changes of the three layers of the cerebellar cortex especially the Purkinje and the granular layers. The group treated with tramadol + thyme showed improvement in the histological, ultrastructural and immunohistochemical changes. The morphometric analysis of the present study revealed a significant difference among the three groups.

Conclusion: Tramadol intake exerted a neurotoxic effect on the structure of the cerebellar cortex of the adult male rats. Thyme extract can improve the tramadol effects but not repair it completely so this may be helpful in the management of the tramadol neuronal damage if the use of tramadol is necessary.

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Key Words: Cerebellar cortex, tramadol, thyme.

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INTRODUCTION

Tramadol (TR) is a pain reliever that acts by working on the central nervous system. It is used to treat moderate to severe pain. It is used in the treatment of rheumatoid arthritis, restless legs syndrome, motor neuron disease, labor pain, fibromyalgia, cancer, and managing low back pain, such a medicine offers a wide variety of medicinal uses[1].

TR usage is becoming more common among male and female youths who have previously struggled with drug abuse and anxiety. Many addicts are switching from heroin to TH these days[2]. Nausea, vomiting, sweating, itching, and constipation are all side effects of using this medication. Provoked seizures are most commonly thought to be caused by this drug. Long-term use of high dosages of TR is linked to severe side effects such as physical dependency and withdrawal syndrome. TR adverse effects include both traditional opiate withdrawal symptoms and unusual withdrawal symptoms, such as induced seizures[3].

TR has been shown to cross the placental barrier. Organ development, bone growth, and mortality rate are all affected by very high dosages of this medication[4].

TR should not be used during pregnancy since it might produce withdrawal symptoms in the baby. TR may be connected with a higher chance of miscarriage, but no severe defects in the infant. Prenatal TR use reduces the production of Amyloid precursor protein, which is important for neuronal survival, synapse development, and neuronal growth in the offspring's cerebellum[5].

TR produces inhibition of the antioxidant enzymes of the mitochondria of the cerebellar neurons and results in the generation of reactive oxygen species that cause damage of the cellular structures[6,7]. In the nervous

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system, the cerebellum has the greatest amounts of the neurotransmitter nitric oxide (NO), which is thought to play a role in brain ageing[8].

TR uptake causes excessive expression of the inducible nitric oxide synthytase (iONS) and though excessive production of nitric oxide which at a low concentration plays a unique role in neurotransmission and vasodilatation, whereas at higher concentrations, it is neurotoxic[9].

Thyme (TH), a member of the Lamiaceae family, is a fragrant Mediterranean native plant. TH is currently widely used as a spice, tea, and medicinal plant[10].

TH is high in vitamin C (75 percent of the daily recommended intake), vitamin A (27 percent), fiber (16 percent), riboflavin, iron (27 percent), copper (24 percent), manganese (24 percent), calcium (11 percent), phosphorus (11 percent), potassium (11 percent), and zinc (11 percent). Thymol has been shown in studies to raise the amount of good fats in cells and even the amount of DHA (docosahexaenoic acid, an omega-3 fatty acid) in kidney, heart, and brain cell membranes. This mix of oils, vitamins, and minerals, as well as the strong terpenoids rosmarinic and ursolic acids, may potentially be cancer-preventive[11].

TH is as a medical extract has antiseptic, antibacterial, antihelmintic, and antioxidant activities. It has also recently been advocated as a natural alternative to synthetic antioxidants. TH acts as a strong stimulant for the entire circulatory system, making it useful for the treatment of depression and mood disturbances[12].

TH has an important antioxidant strategy for inhibiting lipid peroxidation, which in turn is the primary cause of oxidative stress. On the neurons , combining TH extract with TR improves oxidative stress biochemical markers[13].

Cerebellar cortex is one of the highly sensitive brain regions to postnatal developmental deficit produced by drugs or chemicals. The cerebellum is among the first brain structures to begin cellular differentiation, and one of the last to fully mature[14].

MATERIAL AND METHODS

Drugs

TR was used in the form of tramadol HCl. It comes in 100 mg tablet form (Grunenthal, Italy). The dried TH leaves were purchased at a local store. The leaves of TH were ground into a fine powder. After that, 100 gm. of powder was extracted in a covered flask for 30 minutes with 200 ml of boiled distilled water (DW). After being cool, the extract was filtered. The filtrate was then dried in a vacuum. The appropriate dosages were weighed and reconstituted in 5 ml of DW[12].

Experimental Animals

In the present study a total number of 21 adult male albino rats weighing between 180-200 gm. were used. Rats were obtained from the Animal House of Assiut University. They were of similar environmental background. The animals were kept in separate cages with average temperature (22-24 C˚) and humidity in an adequately ventilated room under a regular 12h light/12h dark cycle. They were all allowed free access to food and water[9].

The Experimental Design

The rats were divided into three groups: group I (control), group II (TR-treated group), and group III (TH-plus-TR-treated group). Each animal group consisted of seven rats. For 8 weeks, the rats in group A were not given any drugs. Through an oro-gastric tube, the rats in group B were given an oral dosage of tramadol HCL (40 mg/Kg/day) suspended in tab water. The rats in group C were administered a combination of tramadol HCL (40 mg/kg/day) and TH extract (500 mg/kg/day) orally using an oro-gastric tube. This was carried out over a period of eight weeks[13,15].

Specimens'preparation

From the three groups, the adult male rats were anesthetized intraperitoneally with sodium pentobarbital (40 mg/kg) and perfused with 0.1 M sodium phosphate buffer (pH 7.4) containing 2.5 percent glutaraldehyde before being decapitated. Each rat's skull cap was opened by a circular incision from all sides after decapitation. The cerebrum was lifted and retracted using blunting procedures after the skull cap was removed. The cerebellum was prepped for the following subsequent studies in the experiment[9].

I- Histological study

i- Light microscopic study Small pieces of cerebellum were fixed in Bouin's solution for 3 hours, then dehydrated in progressively higher grades of alcohol, cleaned in xylene, and embedded in paraffin wax. For investigating the overall histological structure of the rat's cerebellar cortex, serial paraffin sagittal sections about (5 μm) were cut and prepared for gallocyanin-chrome alum staining in all groups[16].

ii- Specimens were fixed in 10% neutral buffered formalin and then processed using the labeled streptavidin–biotin immunoperioxidase. The sections were deparaffinized in xylene, rehydrated in EtOH, and washed twice in distilled water before being used. The endogenous peroxidases were suppressed by 5% hydrogen peroxide for 5 minutes. The samples were cleaned with PBS (pH 7.2). An iNOS immunoperoxidase stain was used to observe the positivity of the cytoplasm. The samples were exposed to the primary antibody at 4°C for 60 minutes prior to adding the secondary antibody. The slides were then incubated in an avidin-biotinylated peroxidase complex reagent before being exposed to the biotinylated secondary antibody for 45 minutes. The slides were stained with hematoxylin. The positive reaction appeared as a cytoplasmic brown accumulation in the cerebellar cells[17].

iii- Electron microscopic study The cerebellum was sliced into little pieces, roughly 1x1 mm in size.

The cerebella pieces were fixed in 2.5 percent buffered glutaraldehyde and subsequently processed to create semithin and ultrathin sections. The pieces were then sliced into ultrathin sections (60–80 nm) and stained with uranyl acetate and lead citrate. Finally, the slices were analysed using a JEOL-JEM-100 SX transmission electron microscope at Electron Microscopy unit, Assuit University, Egypt[18].

Morphometric study

The morphometric study included three parameters: the thickness of the molecular layer (MLT), the thickness of the Purkinje cell layer (PLT) and the thickness of the granular layer (GLT). Thicknesses of each layer were modeled as the length of the perpendicular streamlines between the two boundaries of each laminar structure of interest and calculated. From the obtained sets of semi thin sections, photographs of the same area were taken from the sampled 2 consecutive sections. It was measured at a magnification of 100/slide. The measurements were performed in 10 fields in each of five different sections taken from five different rats of each group. Leica Qwin 500 (Leica Ltd) image analyzer computer system was used to analyze all the images. The mean thicknesses of all parameters were recorded in μm[19].

Statistical analysis

The mean values of the information obtained from the image analyzer were investigated using statistical software (SPSS V23, Inc., IL, USA). The three groups' statistical data were compared using one-way analysis of variance (ANOVA). Quantitative parametric data were compared using the post-hoc Tukey test. Dunn's test preceded by the Kruskal-Wallis test were used to compare quantitative non-parametric data. Mean and standard error (SE) were used to represent quantitatively parametric data. Quantitative, non-parametric data was presented using the median and interquartile range (IQR). Statistical significance was set at $P<0.05$[20].

RESULTS

Histological results

Light microscopic results

Control group: The cerebellar cortex was covered by an intact pia mater and had three well-formed layers: the molecular layer, the Purkinje layer, and the granular layer (Plate 1-a). In the molecular layer, just a few dispersed satellite cells and basket cells could be seen. In the Purkinje layer, small rounded and big pyriform cells with central massive vesicular nuclei could be seen. Small deeply stained cells were grouped in clusters in the granular layer (Plate II-a).

TR group: The cerebellar cortex (Plates I-b,II-b) showed a wideness of the interfolial fissures with multiple spaces in molecular layer. The Purkinje cell layer was reduced in thickness. Many deformed and shrunken cells with densely stained nuclei could be observed. The granular layer showed an apparent sparing of the cells with darker staining.

TR + TH group: Examination of the cerebellar cortex (Plates I-c,II-c) showed an almost normal thickness of the three cortical layers, wideness of the interfolial fissures, an intact separated pia matter. The molecular layer showed an apparent normal distribution of its cells. The Purkinje cell layer was mostly intact with few Purkinje cells having indefinite nuclei, irregular outlines. The granular layer showed nearly normal density of the cells.

Immunohistochemistry results

Control group: A mild immunoreaction of the cytoplasm of all the cell layers was noticed in the stained sections (Plate III-a).

TR group: In the immunohistochemical-stained sections, there was a strong positive immunoreaction of the cytoplasm of several Purkinje cells. The immunological reactivity of other Purkinje cells was moderately positive (Plate III-b).

TR + TH group: In the immunohistochemical-stained slices, there was a moderate positive immunoreaction of the Purkinje cells cytoplasm (Plate III-c).

Electron microscopic results

Control group: The Purkinje cell showed a euchromatic nucleus with a folded double nuclear membrane. Its perikaryon showed many free poly ribosomes, scattered rounded and elongated mitochondria and rough endoplasmic reticulum cisternae (rER) with granular pattern. The cell membrane was visible (Plates IV-a, V-a).

TR group: Purkinje cell had a nucleus with heterochromatin and an irregular ill-defined nuclear membrane. The nuclear membrane had wide pores. Many dilated rER could be seen, damaged mitochondria, multivesicular bodies and dilated cisternae of Golgi apparatus. The cell membrane was interrupted. Extra cellular rarefaction was present (Plates IV-b,V-b).

TR + TH group: Examination of a Purkinje cell (Plates IV-c,V-c) showed a euchromatic nucleus with a folded double nuclear membrane. The cell perikaryon contained apparent normal many mitochondria and rER. Some slightly dilated rough endoplasmic reticulum and damaged mitochondria were present. The cell membrane was intact with presence of synaptic spines. The myelin sheath was preserved.

Morphometric results

For the mean MLT (μm), ANOVA analysis found a significant difference among the three groups (p *value* < 0.05). This difference indicated that TR administration produced an increase in the MLT as a sign of delay of differentiation of the cells of this layer while the combined use of TH with the TR produced a reduction effect on the thickness of this layer (Table 1,Histogram1).

In studying the mean PLT (µm), ANOVA analysis found a significant difference among the three groups (*p value* < 0.05). This difference also indicated that administration of TR decreased the PLT as a sign of degeneration and this effect is partially improved by the combined use of TH combined with TR as a sign of regeneration (Table 2,Histogram 2).

In the GLT means (µm), ANOVA analysis revealed a significant difference among the three groups (*p value* < 0.05). This significant difference denoted that administration of TR decreased the MLT, PLT and GLT of the cerebellar cortex of the adult rats as a sign of degeneration and this effect was partially by the combined use of TH with TR as a sign of regeneration (Table 3,Histogram 3).

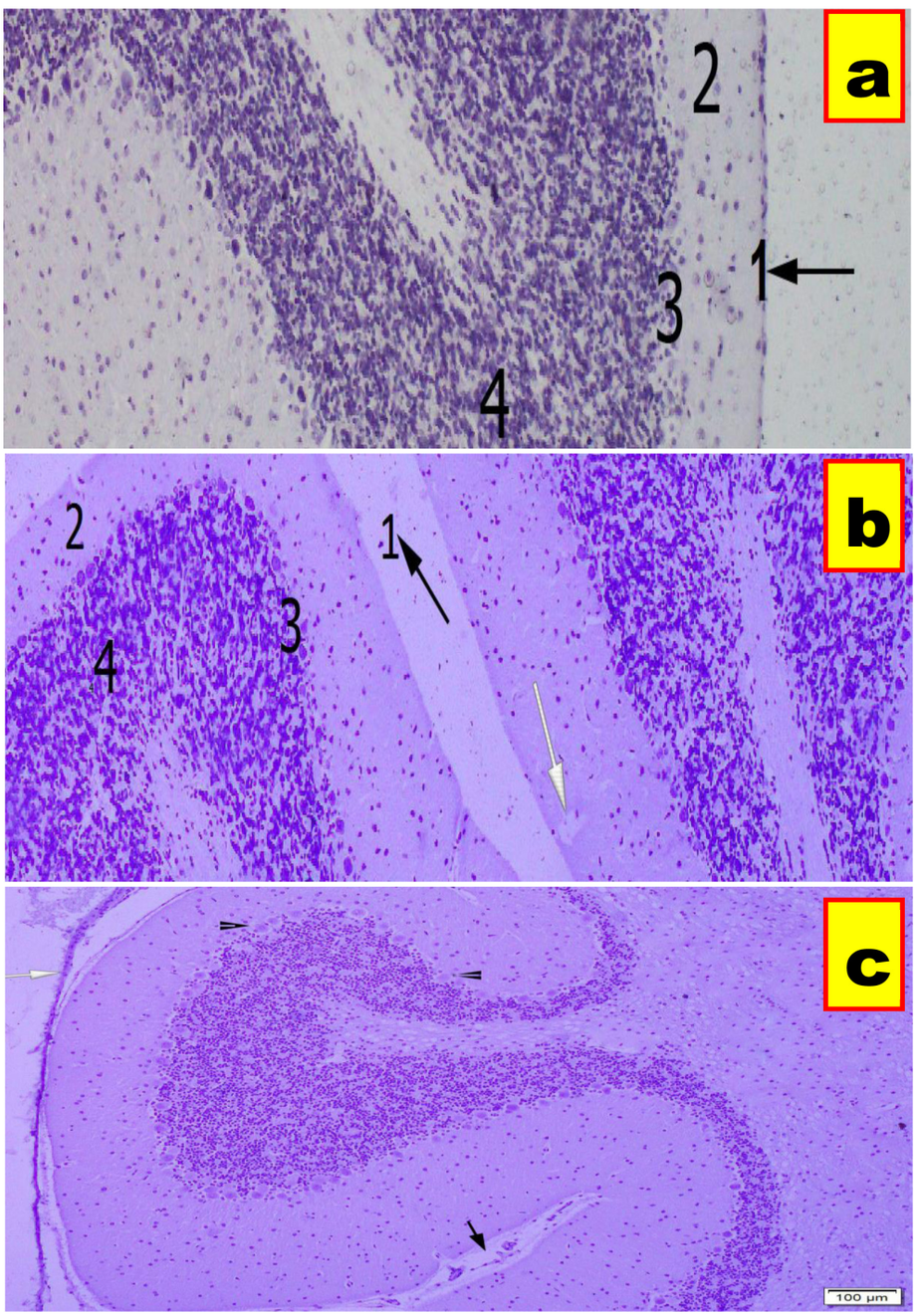


Plate I: A photomicrograph of a sagittal section of the cerebellar cortex of an adult male rat. a -Control group: The cerebellar cortex is covered by an intact pia matter (1←) and composed of well-formed three layers; The molecular layer (2), the Purkinje layer (3) and the granular layer (4). b-TR group: showing a wideness of the interfolial fissure (1←), a reduced thickness of the molecular layer (2) , Purkinje layer (3) and the granular layers (4), and vacuolations of the molecular layer (white arrow). c- TR + TH group: showing a nearly normal wideness of the interfolial fissure (black arrow). An intact separated pia matter (white arrow) and a nearly normal thickness of the three layers are observed. Few Purkinje cells are surrounded by haloes (arrow head) Gallocyanin chrome-alum stain, × 100)

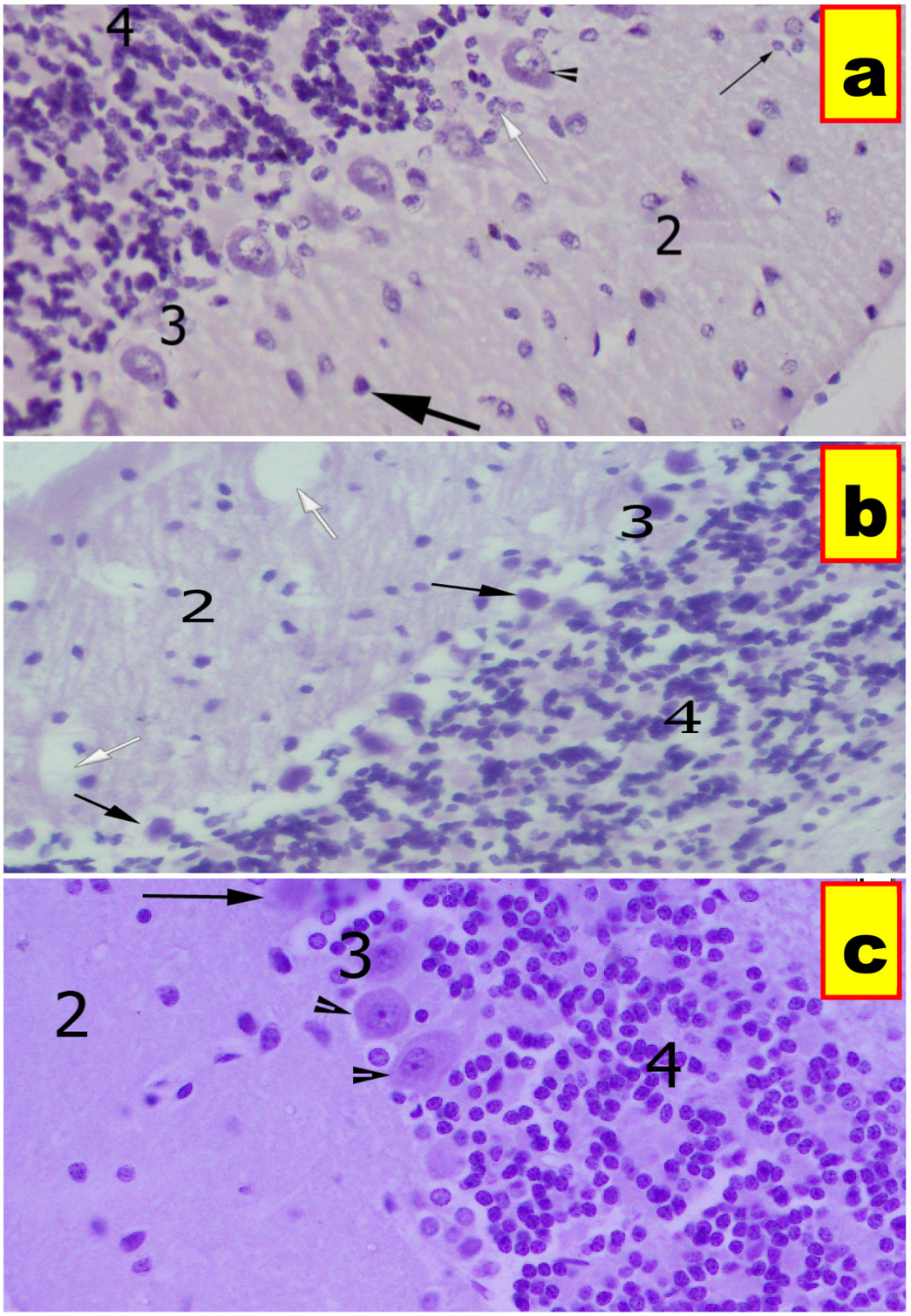


Plate II: A photomicrograph of a sagittal section in the cerebellar cortex of an adult male rat. a- Control group. The molecular layer (2) shows scattered satellite cells (thin arrow) and basket cells (thick arrow). The Purkinje layer (3) consists of large pyriform cells having central large vesicular nuclei (arrow head) and small round lightly stained glial cells (white arrow). The granular layer (4) consists of small densely stained neurons. b- TR group: The molecular layer (2) shows multiple vacuolations (white arrow).The Purkinje layer (3) contains many malformed, shrunken cells with densely stained nuclei and surrounded by haloes (black arrow). The granular layer (4) shows an apparent sparing of the cells with darker staining. c- TR + TH group: showing an apparent normal distribution of the cells of the molecular layer (2). The Purkinje layer (3) shows intact Purkinje cells with vesicular nuclei (arrow head), few Purkinje cells with indefinite nuclei and an irregular outline (arrow). The granular layer (4) shows an apparent normal density of the cells. (Gallocyanin chrome-alum stain, × 400)

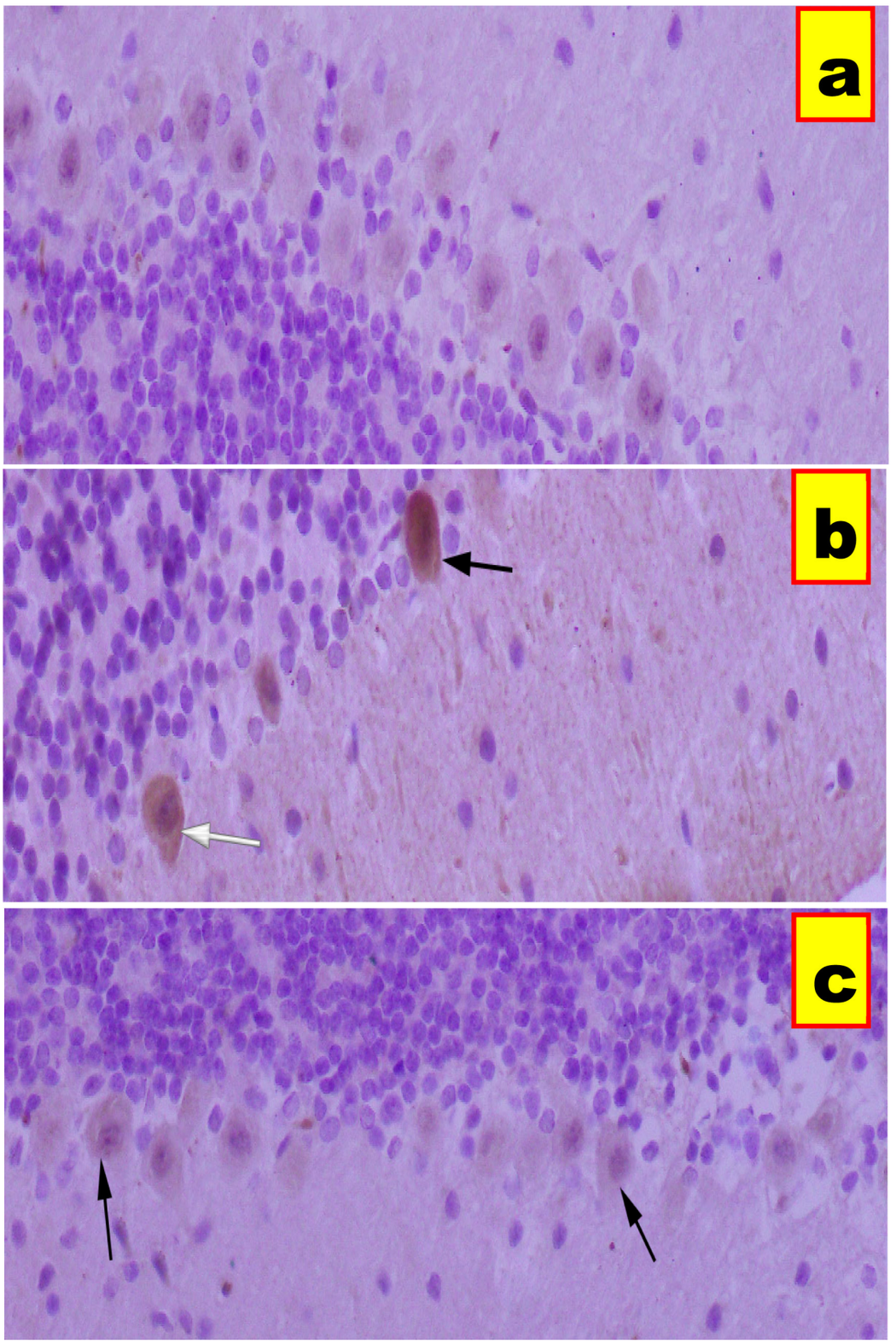
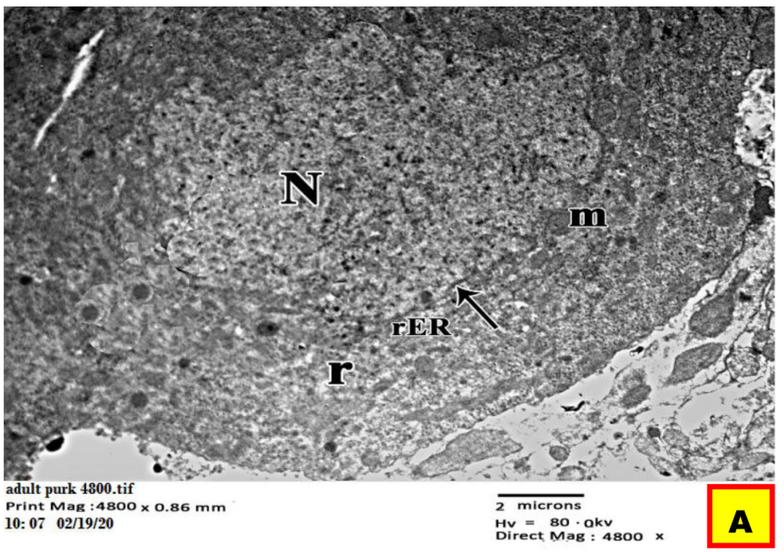
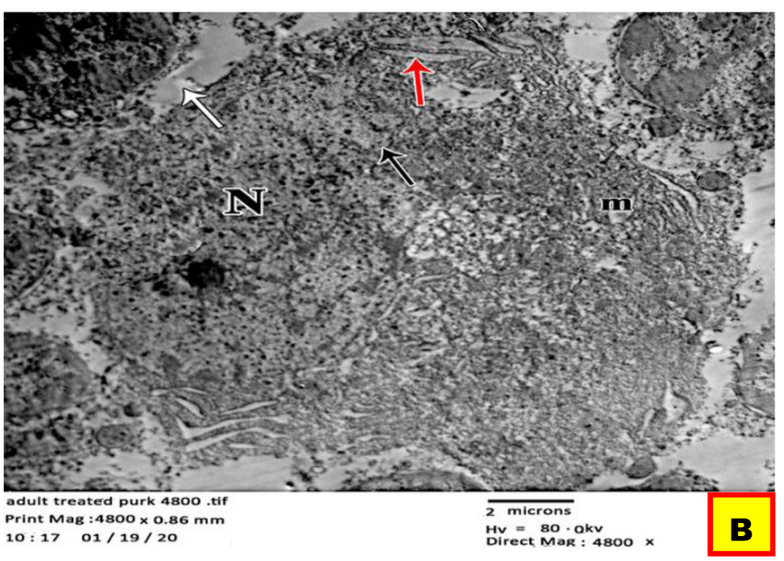


Plate III: A photomicrograph of the cerebellar cortex of an adult male rat. a- Control group: showing a mild immunoreaction of the cytoplasm of the all cell layers. b- TR group: showing a strong positive (black arrow) and a moderate positive (white arrow) immunoreaction of the cytoplasm of the Purkinje cells. c- TR + TH group: showing some Purkinje cells having a moderate positive immunoreaction in their cytoplasm (arrow). (Immunostaining for iNOS, × 400)



A-Control group: showing a Purkinje cell with a euchromatic nucleus (N) and a nuclear membrane (black arrow). The Purkinje cell perikaryon shows free ribosomes (r), scattered mitochondria (m) and rough endoplasmic reticulum cisternae (rER).

TEM.x4800)



B- TR group: showing a Purkinje cell nucleus with heterochromatin (N) and an irregular ill-defined nuclear membrane (black arrow). The cytoplasm shows many dilated rough endoplasmic reticulum cisternae (red arrow) and few scattered damaged mitochondria (m). Rarefaction of the extracellular matrix (white arrow) is noted.

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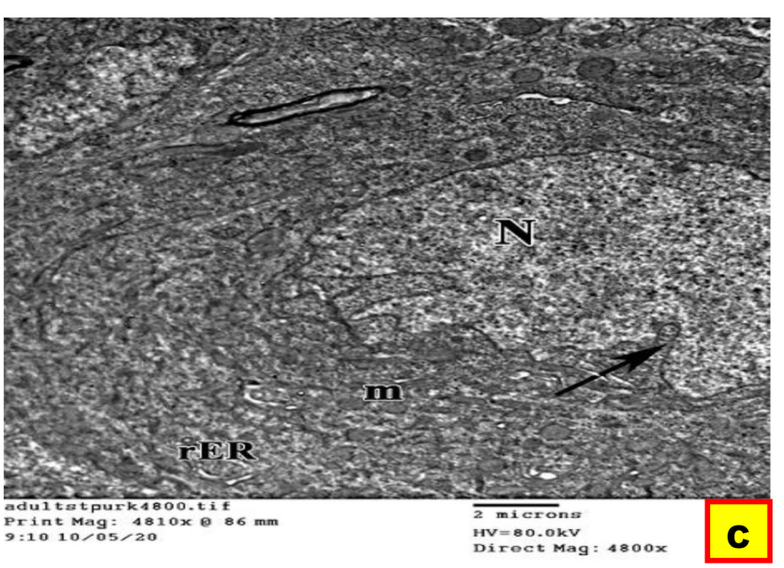

C- TR + TH group: showing Purkinje cell euchromatic nucleus (N) with a folded nuclear membrane (black arrow). The cell perikaryon contains apparent normal many mitochondria (m) and rough endoplasmic reticulum cisternae (rER).

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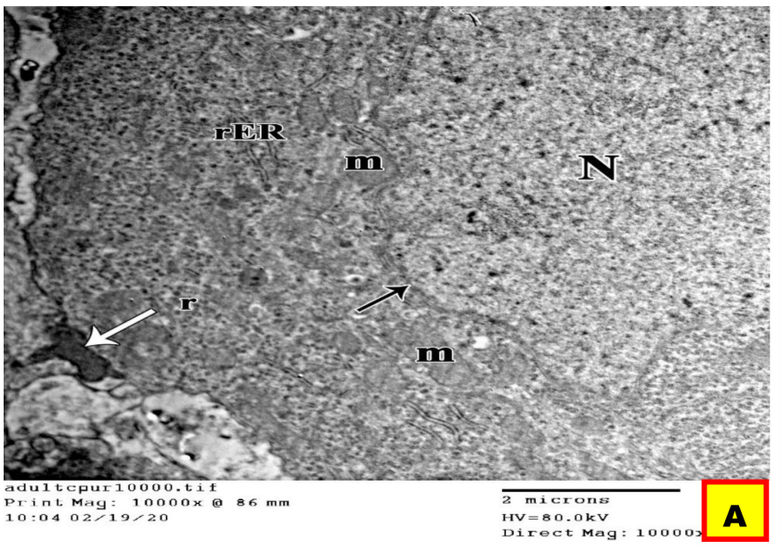
Plate IV: An electron micrograph of an ultrathin section of the cerebellar cortex of an adult male rat.



A- Control group: showing part of the nucleus (N) of the Purkinje cell having a double layer nuclear membrane (black arrow). The rounded mitochondria and sausage-shaped mitochondria (m) could be seen. The rough endoplasmic reticulum cisternae (rER) and the free ribosomes (r) appear in the cytoplasm of the neuron. The cell membrane is clearly apparent with presence of the spiny appearance (white arrow).

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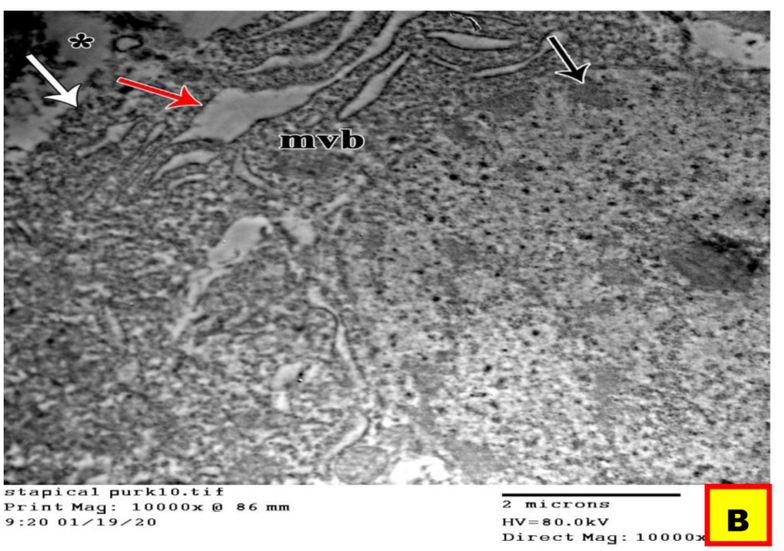

B-TR group: showing part of the Purkinje cell nucleus with heterochromatin (N). The nuclear membrane has wide pores (black arrow). The cell perikaryon contains many dilated rough endoplasmic reticulum cisternae with absence of the ribosomal granules (red arrow) and many multivesicular bodies (mvb). The cell membrane is interrupted (white arrow) with absence of spiny appearance. Extra cellular rarefaction is noted (asterisks).

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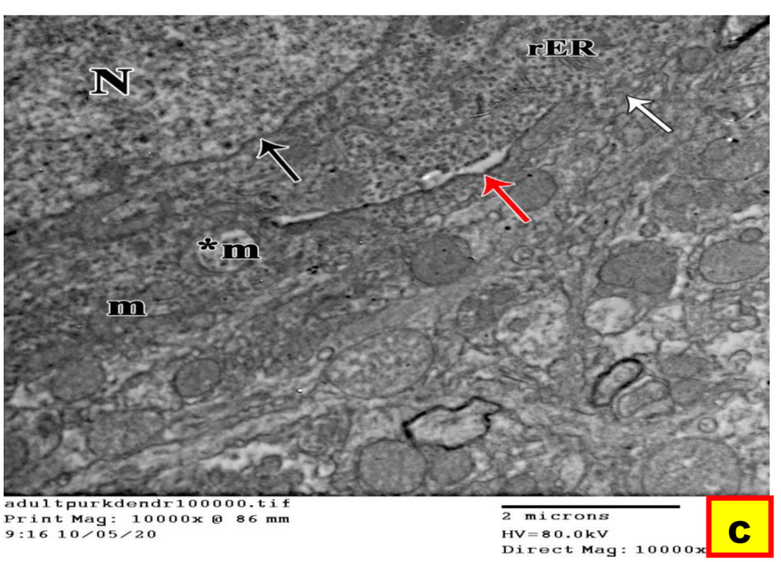


C- TR +TH group: showing part of the nucleus (N) of the Purkinje cell with a euchromatic chromatin and surrounded by regular nuclear membrane (black arrow). The cytoplasm contains intact many mitochondria (m) and rough endoplasmic reticulum (rER). Some slightly dilated rough endoplasmic reticulum (red arrow) and few damaged mitochondria (*m) are noted. The cell membrane is intact with presence of the spiny appearance (white arrow) sheath (sh) is well preserved.

(TEM.x10000)

Plate V: An electron micrograph of ultrathin sections in the different experimental groups.

Table 1: Comparison of the mean MLT (µm) in the cerebellar cortex of different experimental groups

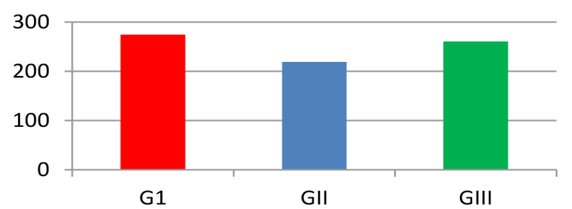
Groups	Mean ± SE
GI	99.20 ± 2.17$^{(\#\$)}$
G II	131.12 ± 3.01$^{(*\$)}$
GIII	107.57 ± 3.79$^{(\#*)}$

Table 2: Comparison of the mean PLT (µm) in the cerebellar cortex of different experimental groups

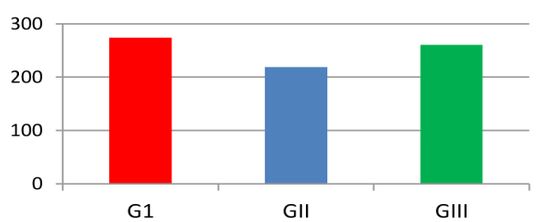
Groups	Mean ± SE
GI	16.66 ± 0.32$^{(\#\$)}$
G II	11.65 ± 0.33$^{(*\$)}$
GIII	15.03 ± 0.28$^{(\#*)}$

Table 3: Comparison of the mean GLT (µm) in the cerebellar cortex of different experimental groups

Groups	Mean ± SE
GI	274.28 ± 9.87$^{(\#)}$
G II	219.13 ± 14.91$^{(*\$)}$
GIII	260.42 ± 13.86$^{(\#)}$



Histogram 1: The relations among the mean MLT (µm) of the different groups



Histogram 2: The relations among the mean PLT (µm) of the different groups

Histogram 3: The relations among the mean GLT (µm) of the different groups

DISCUSSION

The goal of this study was to evaluate the effects of TR on the cerebellar cortex of adult male albino rats, as well as the potential ameliorative role of TH when combined with TR.

In the TR treated group, light microscopic evaluation revealed significant degenerative alterations in the current study. This was evidenced by wideness of the interfolial fissure, and the molecular layer contained multiple vacuolations. Many of the cerebellar cortex neurons were shrunken with pyknotic nuclei. These findings support the results of a study that revealed that TR use had a neurotoxic effect on the cerebellum neurons in a dose-dependent manner[9]. In this regard, other researchers noted that the histopathologic alterations in brain tissues appeared to be the result of neuronal debilitation caused by prolonged increased activity in response to TR treatment on a regular basis[21].

The noticed pericellular spaces in our study could be referred to shrinkage of cells with withdrawal of their processes secondary to cytoskeleton affection. This was explained by earlier studies that reported the long term use of opioid causes cell apoptosis[14,22].

The granular layer appeared to be reduced in thickness and showed many darkly stained cells with decrease in the cell density and absence of the cluster organization. In harmony with our results, previous studies stated that TR intake causes profound alterations in the granular layer as this layer has many intensely stained cells, karyolitic nuclei and deformed neurons[23].

The iNOS expression in the TR group showed a strongly positive cytoplasmic immunoreaction of the Purkinje cells. This outcome backs with prior research that found high

iNOS and Caspase-8 expression in the cytoplasm of cells in all zones of the adrenal gland of TR-treated rats[24]. In addition, some researchers found a significantly positive cytoplasmic reactivity for iNOS in the TR-treated rats' cerebellar cortex cells[9]. The strong positivity of immunoreaction in this study may be explained by the fact that tramadol harms vascular endothelial cells, which results in vascular congestion. Vascular congestion causes the release of the enzyme nitric oxide synthase, which raises the level of NO in the bloodstream and nitrates in the cells. In harmony with our work , earlier studies reported that the brain inflammation and neurodegeneration are responsible for the cells iNOS expression[25,26]. Furthermore, some researchers reported the presence of an immune- reaction to the involvement of iNOS in protein nitration, which might be linked to alterations in cell shape[8].

In this work the ultra-sections of the Purkinje cells of TR group showed heterochromatin of their nuclei with wide pores of the nuclear membrane. The cell perikaryon contained many dilated rER with absence of the ribosomal granules and many multivesicular bodies. The cell membrane was interrupted with absence of spiny appearance. These findings were in accordance with the outcomes of the preceding works in which the TR group showed remarkable ultra-structural alterations of the cerebellar cortex cells[9,13].

In this study, the mitochondrial alterations might be linked to oxidative stress. Mitochondria are extremely sensitive to oxidative stress. The permeability and structure of the mitochondria are altered as a result of such stress[27,28]. Increased Ca2 + release from the ER opened the mitochondrial permeability transition pores, resulting in loss of cytochrome C and activation of iNOS in stressed cells. This, in turn, created the reactive oxygen species (ROS). ROS increased Ca2 + release, building a vicious cycle that eventually resulted in cell death[29,30].

The dilated rER may be explained by lipid peroxidation brought on by TR. It could also be caused by endoplasmic reticulum stress, which could render it more vulnerable to oxidative stress as protein folding rises[31,32].

Free radical causing damage to a cell organoid may be responsible for the degenerative vacuolations and rarefaction of the cellular perikaryon[33,34].

The degeneration of synaptic spines of the cell membrane of the Purkinje cells could be attributed to the disordered neurotransmitter system after TR treatment[35,36].

By examination of the TR + TH group, we found that the concurrent use of TH extract and TR showed improvement in the immunohistochemical findings as evidence by moderate cytoplasmic immunoreaction of the cytoplasm of Purkinje cells. These findings were confirmed by the preceding work that stated that the NO plays an important role in the governance of vascular permeability and cell migration induced by proinflammatory agents and by inhibiting the expression iNOS of mRNA. TH extract dramatically inhibits the generation of NO[37].

In our study, we found that the uptake of TH extract showed improvement in the histological findings as evidenced by apparent normal wideness of the interfolial fissure. Additionally, there was an intact pia matter, the three layers appeared to be of a normal thickness, and the molecular layer's cell distribution seems to be normal. The majority of cerebellar cortex cells had normal shape, smaller pericellular gaps, and a restoration to their typical cluster organization. These results might be referred to the antioxidant activity of TH. It also indicated the thyme neuroprotective effect against the oxidative-stress producing tissue injury. In coincide with our results , some studies[36,37,38] explained that TH has a neuroprotective effect against neuronal damage caused by cerebral ischemia-reperfusion. In this regard, this might be because oxidative rER stress and apoptosis are both inhibited.

The observed enhancement in the ultra-structure findings of the cerebellar cells of the TR + TH group was evidenced by the presence of euchromatic nuclei and regular folded double nuclear membrane with normal nuclear pores width. Also, most of the cytoplasmic organelles were with normal morphology except few dilated mitochondria or few dilated rER. The myelin sheath was still present, and the cell membrane was almost preserved. We found that the uptake of thyme extract enhances the histopathological changes occurred in the adult cerebellar neurons, which is consistent with the findings of[29]. Furthermore, some studies reported that TH extract increased the number of synaptic vesicles, which in turn resulted in a rise in central neurotransmitters[37].

The noticed neuronal degenerative changes generated by TR might also be explained by the fact that high dosages of TR resulted in the production of reactive oxygen[39]. So, according to several studies, the brain is particularly vulnerable to oxidative damage. This is because of its high oxygen consumption, poor antioxidant levels, and higher polyunsaturated fatty acid levels[40]. TR use over time decreases glutathione levels in the brain, both non-enzymatic and enzymatic, as well as glutathione peroxidase activity, both of which are antioxidants[41]. Moreover, it has been recorded that oxidative alteration produces a loss of function and lowering of enzyme activity[42].

So, the improvement of the histological and imunohistochemical findings by the concomitant use of the TH extract with the TH might be due to the TH extract which has strong anti-inflammatory and antioxidant prosperities. Our results could be supported by studies that reported an improving effect of TH extract and its derivatives, due to its down-regulating effects on various inflammatory and enzymatic parameters such as C-reactive protein[43-46].

The morphometric analysis of the present study revealed that administration of TR produced an increase in the MLT as a sign of delay of differentiation of the cells of this layer while the combined use of TH with the TR produced a reduction effect on the thickness of this layer. On the other hand, administration of TR produced a decrease the PLT and GLT. This could be considered as a sign of neuro-degenerative effect of TR and this effect was partially improved by the combined use of TH as a sign of regeneration. This could be attributed to oxidative damage, apoptosis and the inhibition of neurogenesis[45]. In this regard,[47,48] reported that TH, could decrease the reactive oxygen species, then decrease protein damage .

CONCLUSION

Tramadol administration produced a neurotoxic effect on the structure of the cerebellar cortex of the adult male albino rat. Thyme extract has an improving role to the tramadol neurotoxic effects but not repairing it completely so this may be helpful in the management of the tramadol neuronal damage if the use of tramadol is necessary.

RECOMMENDATIONS

From the results of the current work, we can recommend proceeding in further studies that evaluate adding other medical flavonoids with thyme to be used as adjuvant treatment in neurological deficits affecting the cerebellar cortex.

ETHICAL APPROVAL

This experimental study was fully approved by the Local Ethical Committee and by the Institutional Review Board of Faculty of Medicine, Assiut University.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Matthiesen T, Wöhrmann T, Coogan TP, Uragg H. The experimental toxicology of tramadol: an overview. Toxicol Lett. 1998 ; 16;95(1):63–71.doi.org/10. 1016/ S0378 -4274 (98)00023-X.
2. Cicero TJ, Inciardi JA, Adams EH, Geller A, Senay EC, Woody GE, *et al*. Rates of abuse of tramadol remain unchanged with the introduction of new branded and generic products: results of an abuse monitoring system, 1994–2004. Pharmacoepidemiol Drug Saf [Internet]. 2005; 14(12):851–859. doi:org/ 10.1002 / pds.1113.
3. Kabel JS, van Puijenbroek EP. [Side effects of tramadol: 12 years of experience in the Netherlands]. Ned Tijdschr Geneeskd [Internet]. 2005;149(14):754–757.
4. Aboulhoda BE, Hassan SS. Effect of prenatal tramadol on postnatal cerebellar development: Role of oxidative stress. J Chem Neuroanat. 2018; 94:102–118. doi:org/10.1016/j.jchemneu.2018.10.002
5. Farhan TM, Kammona HR, Mubarak HJ. The evaluation of histological changes and imunohistochemical expression of amyloid precursor protein in cerebral and cerebellar cortices in newborn mice after prenatal exposure to tramadol. 2017; 6 (14): 28-43. doi: 10.20959/wjpr201714-9942
6. Lemarie A, Grimm S. Mutations in the heme b-binding residue of SDHC inhibit assembly of respiratory chain complex II in mammalian cells. Mitochondrion. 2009; 9(4):254–260. doi::org/10.1016/j.mito. 2009.03.004
7. Mohamed TM, Ghaffar HMA, El Husseiny RMR. Effects of tramadol, clonazepam, and their combination on brain mitochondrial complexes. Toxicol Ind Health. 2015;31(12):1325–1333. doi:org/ 10.1177/0748233713491814
8. Blanco S, Molina FJ, Castro L, Del Moral ML, Hernandez R, Jimenez A, *et al*. Study of the nitric oxide system in the rat cerebellum during aging. BMC Neurosci. 2010;11(1):1–14.
9. El-Bermawy MI, Salem MF. Histological changes of the albino rat cerebellar cortex under the effect of different doses of tramadol administration. Egypt J Histol. 2015;38(1):143–155. doi:org/10.1097/01. EHX. 0000461302.86011.de
10. Brown DM, Donaldson K, Borm PJ, Schins RP, Dehnhardt M, Gilmour P, *et al*. Calcium and ROS-mediated activation of transcription factors and TNF-α cytokine gene expression in macrophages exposed to ultrafine particles. Am J Physiol Cell Mol Physiol. 2004;286(2):344–353. doi:org/10.1152/ ajplung.00139.2003
11. Swayeh NH, Abu-Raghif AR, Qasim BJ, Sahib HB. The protective effects of Thymus Vulgaris aqueous extract against Methotrexate-induced hepatic toxicity in rabbits. Int J Pharm Sci Rev Res. 2014;29:187–193.
12. Kuete V. Medicinal spices and vegetables from Africa: therapeutic potential against metabolic, inflammatory, infectious and systemic diseases. Academic Press; 2017: 182-192.
13. Sarhan NR, Taalab YM. Oxidative stress/PERK/ apoptotic pathways interaction contribute to tramadol neurotoxicity in rat cerebral and cerebellar cortex and thyme enhances the antioxidant defense system: histological, immunohistochemical and ultrastructural study. Int J. 2018;4(6):124-141. doi:org/10.18203/ issn.2454-2156. IntJ Sci Rep20182083
14. Shona SI, Rizk AA, El Sadik AO, Emam HY, Ali EN. Effect of valproic acid administration during pregnancy on postnatal development of cerebellar cortex and the possible protective role of folic acid. Folia Morphol (Warsz). 2018;77(2):201–209. DOI: 10.5603/ FM. a2017.0100
15. Awadalla EA, Salah-Eldin A-E. Molecular and histological changes in cerebral cortex and lung tissues under the effect of tramadol treatment. Biomed Pharmacother. 2016;82:269–280. doi:org/ 10.1016/j. biopha.2016.04.024
16. Bancroft J, Stevens A, Turner D. Theory and practice of histological techniques 4th Ed Churchill Living Stone, New York Edinburgh. Madrid, Sanfrancisco. 1996.
17. Zhang J, Brown RP, Shaw M, Vaidya VS, Zhou Y, Espandiari P, *et al*. Immunolocalization of Kim-1, RPA-1, and RPA-2 in kidney of gentamicin-, mercury-, or chromium-treated rats: relationship to renal distributions of iNOS and nitrotyrosine. Toxicol Pathol. 2008;36(3):397–409. doi:org/10.1177/ 0192623308315832
18. Sesack SR, Miner LH, Omelchenko N. Preembedding immunoelectron microscopy: applications for studies of the nervous system. In: Neuroanatomical tract-tracing 3. Springer; 2006: 6–71.
19. Yezzi AJ, Prince JL. An Eulerian PDE approach for computing tissue thickness. IEEE Trans Med Imaging. 2003;22(10):1332–1339.
20. Altman GD. Comparing groups: three or more independent groups of observations in practical statistics for medical research. Chapman Hall. 2005; 159-162.
21. Elfeky A, Mohamed A. 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 J Forensic Med Clin Toxicol. 2017; 28(1):119–132.doi. 10.21608/ AJFM.2017. 18310

22. Ghoneim FM, Khalaf HA, Elsamanoudy AZ, Helaly AN. 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. 2014;7(11):7323- 7341.

23. Guo C, Sun L, Chen X, Zhang D. Oxidative stress, mitochondrial damage and neurodegenerative diseases. Neural Regen Res. 2013;8(21):2003-2014. doi: 10.3969/j.issn.1673-5374.2013.21.009

24. Abdelaleem SA, Hassan OA, Ahmed RF, Zenhom NM, Rifaai RA, El-Tahawy NF. Tramadol induced adrenal insufficiency: histological, immunohistochemical, ultrastructural, and biochemical genetic experimental study. J Toxicol. 2017; 1155-1169. doi: org/10.1155/2017/9815853

25. Heneka MT, Feinstein DL. Expression and function of inducible nitric oxide synthase in neurons. J Neuroimmunol. 2001;114(1–2):8–18. doi: org /10 . 1016 /S0165-5728(01)00246-6

26. Suschek C V, Schnorr O, Kolb-Bachofen V. The role of iNOS in chronic inflammatory processes in *vivo*: is it damage-promoting, protective, or active at all? Curr Mol Med. 2004;4(7):763–775. doi. org/10.2174/1566524043359908

27. Motawea SM, Amer RM, Haiba DA, Mostafa MS. Cerebral Cortical Changes in Adult Albino Rats Under the Effect of Tramadol and its Withdrawal: Histological and Morphometric Study. Egypt J Histol. 2020;43(2):412–426. doi: 10.21608/EJH. 2019.14072.1136

28. Hernández-Fonseca JP, Rincón J, Pedreañez A, Viera N, Arcaya JL, Carrizo E, *et al*. Structural and ultrastructural analysis of cerebral cortex, cerebellum, and hypothalamus from diabetic rats. Exp Diabetes Res. 2009; 329632: 1-12. doi:org/ 10.1155 /2009/ 329632.

29. Brand MD. The sites and topology of mitochondrial superoxide production. Exp Gerontol. 2010;45(7–8):466–472. doi:org/10.1016/j.exger.2010.01.003

30. Cao SS, Kaufman RJ. Endoplasmic reticulum stress and oxidative stress in cell fate decision and human disease. Antioxid Redox Signal. 2014;21(3):396–413. doi:org/10.1089/ars.2014.5851

31. Santos CXC, Tanaka LY, Wosniak Jr J, Laurindo FRM. Mechanisms and implications of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductases, mitochondrial electron transport, and NADPH oxidase. Antioxid Redox Signal. 2009;11(10):2409–2427. doi:org/10.1089/ ars.2009.2625

32. El-Newary SA, Shaffie NM, Omer EA. The protection of Thymus vulgaris leaves alcoholic extract against hepatotoxicity of alcohol in rats. Asian Pac J Trop Med. 2017;10(4):361–371. doi: org/ 10.1016 / j.apjtm.2017.03.023

33. Zarnescu O, Brehar FM, Chivu M, Ciurea AV. Immunohistochemical localization of caspase-3, caspase-9 and Bax in U87 glioblastoma xenografts. J Mol Histol. 2008;39(6):561–569.

34. Baghishani F, Mohammadipour A, Hosseinzadeh H, Hosseini M, Ebrahimzadeh-Bideskan A. The effects of tramadol administration on hippocampal cell apoptosis, learning and memory in adult rats and neuroprotective effects of crocin. Metab Brain Dis. 2018;33(3):907–916.

35. Xia W, Liu G, Shao Z, Xu E, Yuan H, Liu J, *et al*. Toxicology of tramadol following chronic exposure based on metabolomics of the cerebrum in mice. Sci Rep. 2020;10(1):1–11.

36. Setorki M, Mirzapoor S. Evaluation of Thymus vulgaris Extract on Hippocampal Injury Induced by Transient Global Cerebral Ischemia and Reperfusion in Rat. Zahedan J Res Med Sci. 2017;19(5): 291-311. doi:org/10.5812/zjrms.9216

37. Mahmoodi M, Ayoobi F, Aghaei A, Rahmani M, Taghipour Z, Hosseini A, *et al*. Beneficial effects of Thymus vulgaris extract in experimental autoimmune encephalomyelitis: Clinical, histological and cytokine alterations. Biomed Pharmacother. 2019;109:2100–2108. doi:org/10.1016/j.biopha.2018.08.078

38. Ragab IK, Mohamed HZE. Histological changes of the adult albino rats entorhinal cortex under the effect of tramadol administration: Histological and morphometric study. Alexandria J Med. 2017;53(2):123–133. doi:org/10.1016/j. ajme.2016.05.001

39. Butterfield DA, Castegna A, Lauderback CM, Drake J. Evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death. Neurobiol Aging. 2002;23(5):655–664. doi:org/ 10.1016/S0197-4580(01)00340-2

40. Abdel-Zaher AO, Abdel-Rahman MS, ELwasei FM. Protective effect of Nigella sativa oil against tramadol-induced tolerance and dependence in mice: role of nitric oxide and oxidative stress. Neurotoxicology. 2011;32(6):725–733. doi:org/10.1016/j. neuro.2011.08.001

41. Butterfield DA, Reed T, Newman SF, Sultana R. Roles of amyloid β-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment. Free Radic Biol Med. 2007;43(5):658–677. doi:org/10.1016/j. freeradbiomed.2007.05.037

42. Kanter M. Nigella sativa and derived thymoquinone prevents hippocampal neurodegeneration after chronic toluene exposure in rats. Neurochem Res. 2008;33(3):579–588.

43. Yu Y, Chao T, Chang W, Chang MJ, Lee M. Effect of thymol on oxidative stress and inflammation-related gene expression in high fat diet-induced hyperlipidemic rabbits. FASEB J. 2016;30:1174-1189. doi:org/10.1096/fasebj.30.1

44. Rahmani F, Nabi S, Idliki RB, Alimirzaei M, Barkhordar SMA, Shafaei N, *et al*. Thyme Oil Nanoemulsion Enhanced Cellular Antioxidant and Suppressed Inflammation in Mice Challenged by Cadmium-Induced Oxidative Stress. Waste and Biomass Valorization. 2022;1–8.

45. Mohammadnejad L, Soltaninejad K. Tramadol-Induced Organ Toxicity via Oxidative Stress: A Review Study. Int J Med Toxicol Forensic Med. 2022;12(1):35430,1-11. doi:org/10. 32598 / ijmtfm. v12i1.35430

46. Iranshahy M, Javadi B, Sahebkar A. Protective effects of functional foods against Parkinson's disease: A narrative review on pharmacology, phytochemistry, and molecular mechanisms. Phyther Res. 2022; 2; 36:1952–1989. Doi:org/ 10. 1002 / ptr.7425

47. Hammoudi Halat D, Krayem M, Khaled S, Younes S. A Focused Insight into Thyme: Biological, Chemical, and Therapeutic Properties of an Indigenous Mediterranean Herb. Nutrients. 2022;14(10):2104-2112. doi:org/10 .3390/ nu14102104

48. Noruzi S, Torki M, Mohammadi H. Effects of supplementing diet with Thyme (Thymuas vulgaris L.) essential oil and/or selenium yeast on production performance and blood variables of broiler chickens. Vet Med Sci. 2022; 1137-1146. doi:org / 10.1002/ vms3.736

الملخص العربى

تَأْثيراتُ التَّناوُلِ المُتَزامِنِ لِلزَّعْتِرِ وَالتَّرَامَادُولِ عَلَى القِشْرَةِ الْمُخَيَّخِيَّةِ لِذِكْرِ اَلْجُرَذِ الأَبْيَضِ البالِغِ

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الخلفية: الترامادول هو مسكن أفيوني يعمل بشكل مركزي لتسكين الألم،و يستخدم في الآلام الحادة أو المزمنة ، بما في ذلك آلام ما بعد الجراحة وأمراض النساء والتوليد ،و يمكن أن يؤدي تعاطيه إلى آثار سيئة على الجهاز العصبي.
الهدف من البحث: تحديد الآثار الضارة للترامادول على قشرة مخيخ الجرذ الذكر البالغ ، وكذلك تأثير اعطاء الزعتر المتزامن مع الترامادول.
مواد و طرق البحث: تم استخدام واحد وعشرين من ذكور الجرذان البيضاء البالغة حيث قسمت عشوائيا إلى ٣ مجموعات بالتساوي: المجموعة الضابطة (المجموعة ١) التي لم تتلق أي أدوية لمدة ٨ أسابيع ، ومجموعة الترامادول (المجموعة ٢) التي تلقت هيدرو كلوريد الترامادول عن طريق الفم (٤٠ مجم / كجم / يوم) مذابا في الماء لمدة ٨ اسابيع. أما المجموعة الثالثة فقد تم تلقيها الترامادول (٤٠ مجم / كجم / يوم) + مستخلص الزعتر (٥٠٠ مجم / كجم / يوم) عن طريق الفم لمدة ٨ أسابيع أيضا. ثم تمت معالجة مخيخ الجرذان لتقييم التغيرات النسيجية والخلوية الدقيقة وكذا المناعية النسيجية الكيميائية والتحليل القياسى لعمل نتائج إحصائية .
النتائج و الاستنتاج: من الناحية النسيجية والخلوية الدقيقة وكذا المناعية النسيجية الكيمايئية ، كشف الفحص فى المجموعة المعالجة بالترامادول عن تغيرات انتكاسية استماتية ملحوظة في الطبقات الثلاث من قشرة المخيخ ، وأظهر الفحص فى المجموعة المعالجة بالزعتر مع الترامادول تحسنا في هذه التغيرات ، أيضا كشف التحليل الاحصائى لهذه الدراسة عن وجود فرق ذى مدلول احصائى بين المجموعات الثلاث. لذا فقد خلصت الدراسة الى أن لتناول الترامادول تأثيرًا سامًا على بنية القشرة المخيخية لذكور الجرذان البالغة، و يمكن لمستخلص الزعتر أن يحسن تأثيرات الترامادول الضارة على قشرة مخيخ الجرذ ولكن لا يصلحها تمامًا ، لذا قد يكون ذلك مفيدًا تناول مستخلص الزعتر للتقليل من تلف خلايا قشرة المخيخ الناتج عن تعاطى الترامادول إذا كان استخدام الترامادول ضروريًا.