

Neurodegeneration and Oxidative Stress in Brain Tissues Induced by Tramadol with the Protective Effects of Royal Jelly in Albino Rats

Atef M. Khalil^{1*}, Amany Sayed Mowas¹, Mohammed H. Hassan², Hassan Ahmed³, Obeid Shanab⁴

¹Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, South Valley University, 83523 Qena, Egypt, ²Department of Medical Biochemistry, Faculty of Medicine, South Valley University, 83523 Qena, Egypt, ³Department of Physiology, Faculty of Veterinary Medicine, South Valley University, 83523 Qena, Egypt, ⁴Department of Biochemistry, Faculty of Veterinary Medicine, South Valley University, Qena 83523, Egypt

Abstract

Tramadol hydrochloride (TH) is an opioid centrally acting analgesic used to treat moderate to severe acute and chronic pains. Therefore, it became the most prescribed opioid worldwide. In this study, we investigated the neurodegenerative disorders of tramadol in brain tissues and the protective role of royal jelly. Twenty male albino rats allocated into four groups: Group 1, served as a control group, and Group 2, administered with tramadol at a dose of 20 mg/kg/b. W for 60 days. Group 3: rats administered with tramadol at a dose of 20 mg/kg/b. W for 60 days and treated with royal jelly (RJ) in a 100 mg/kg dose. b.w. Group 4: Rats inoculated with royal jelly (RJ) at a dose of 100 mg/kg. b.w. Blood samples were collected for hematological and biochemical analysis. Brain tissues were harvested for neurodegeneration biomarkers detection and histopathological examinations. Administration of tramadol revealed a significant decrease in Hb concentration, RBCs count, PCV %, Lymphocytes %, and platelets number, while WBCS count, Neutrophils, and monocytes % increased. Also, Tramadol induced a decrease in glucose-6-phosphate dehydrogenase (G6PD) while creatine kinase -BB (CK-BB) and neuron-specific enolase enzymes (NSE) were decreased. Tramadol increased the lipid peroxidation MDA, while total antioxidants capacity (TAC) and glutathione reductase (GSH) concentrations were decreased. Histopathologically, tramadol-induced neurodegenerative changes in brain neurons manifested by acute necrosed neurons with gliosis and vascular congestions. The administration of royal jelly improved the previous deleterious effects by decreasing brain tissue oxidative stress. Tramadol misuse caused neurodegenerative effects and was relieved by RJ administration.

Keywords: Tramadol, Royal jelly, Oxidative Stress, Brain, Neurodegeneration, Misuse

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*Corresponding Author: Atef M. Khalil

E-mail: atefh4668@gmail.com

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Introduction

Drugs are chemicals which can be obtained naturally or synthesized to be used for many of medical uses. However, the frequent and not needed use of some of these drugs resulting in short-lived or chronic addiction (Murthy et al., 2010). Nowadays drug addiction has become a irritable social problem in the modern world as well as it involves lifetime exposure of about 46% of the general population (Pantelias and Grapsa, 2011). Addiction of both synthetic (tramadol, oxycodone, buprenorphine and heroin) and natural (Codeine and morphine) opioids is well known and can be diagnosed in many peoples (Moratti et al., 2010; Meyer et al., 2014). Tramadol was synthesized and medically used as an analgesic drug in Germany 35 years ago. Tramadol possess weak opioid effect, and it acts centrally, as well as inhibiting and preventing catecholamine and serotonin (5-HT) reuptake to make analgesia (Liu and Liu, 2013). The analgesic effect of tramadol achieved through two complementary and synergistic mechanisms; the first mechanism is by stimulation the μ -opioid receptors (Ide et al., 2006), and the second one is done by decreasing the neuronal usage of noradrenaline and serotonin neurotransmitters (Raffa et al., 1992). The addiction of tramadol is resulting from long term use for treatment and relive pain-related disorders and when it used as an alternative drug in addicted patients treatment (Drugs and Therapy Bulletin, 2002). The long-term use and addiction of tramadol has pathological and chemical effects on the cellular level are not clearly understood (Atici et al., 2005). Both renal and hepatic disorders are developed during treatment and addiction with tramadol because its metabolism and extraction are

done in the liver and kidney (Wu et al., 2001; Atici et al., 2005).

Oxidative stress is an oxidative condition in the tissues due to increase the reactive oxygen species(ROS), resulting in various histopathological disorders. Many pathological damage in different body organs are related to activation of the oxidative stress, such as cardiovascular disease, diabetes mellitus, Tumors, rheumatoid arthritis, and finally end by neurodegenerative disorders in the brain and spinal cord (Valko et al., 2009). A lot of side effects related to tramadol administration according to the dose administered for example nausea, vomiting, sweating, itching, constipation, headache, and central nervous system stimulation. Neurotoxicity is commonly seen in peoples addicted with tramadol because of the ability of the drug to pass the blood-brain barrier, which reported in many human studies (Bekjarovski et al., 2012). From the recorded neurotoxicity signs are the generalized tonic-clonic seizures. Furthermore, long term administration of tramadol caused neuronal degeneration in the rat's brain, which attributed to cerebral dysfunction (Atici et al., 2005), in addition to changing the brain's neurotransmitter concentrations (Bloms-Funke et al., 2011).

Royal Jelly (RJ) is one of the honeybee products obtained from the worker honeybees' hypopharyngeal and mandibular glands as it used as the primary food for queens and in the first three days of life (Han et al., 2011). RJ has many recognized pharmacological activities, such as anti-hypercholesterolemia, antioxidant and hypoglycemic effects (Gou et al., 2007, Munstedt et al., 2009). Moreover, RJ has suitable proteins with

high peptides level and essential amino acids. It also possess high antioxidant influences and scavenging ability against the toxic free radicals (Guo et al., 2008). The protein content of RJ has Polyphenols and phenols which are responsible for antioxidant activity (Viuda-Martos et al., 2008). Many experimental studies revealed that RJ has high protective powers in different tissues, where it acts as anti-inflammatory and lower the blood glucose concentration (Elnagar, 2010, Hidaka et al., 2006), Therefore, this study was designed to investigate the possible neurodegenerative disorders in the brain due to tramadol administration with recording the ameliorative effects of Royal Jelly in adult male rats through evaluation of neurodegenerative, lipid peroxidation and oxidative stress biomarkers as well as the histopathological findings.

Materials and Methods

Ethics Statement

The research bioethics committee(RBC) of Faculty of Veterinary Medicine, South Valley University has approved the experimental work under approval number "VM/SVU/23(2)-34" . All the experimental protocols and procedures, including the rules for the animal welfare and uses for experiments. All the experimental animals were accommodated following the complete guidelines of the animal care and away from suffering stressors.

Animals and Husbandry

Twenty adult male albino rats 6 to 8 weeks of age weighing 90-125 g. the experimental animals were purchased from the animal house Cairo, Egypt. Rats were housed in clean, well-ventilated metal cages with a metallic grid. They were housed in the experimental room, the temperature was managed at 22-24 °C and

kept under a normal light/dark cycle. The balanced feed pellets were given to rats with water daily ad libitum. The animals were observed for two weeks before starting the experiment for acclimatization.

Drug

Tramadol HCL, commercially known as Tamol- x 225, was purchased from Royal Company, Cairo, Egypt. Each tablet contained 225 mg of the drug. Tramadol HCl tablets were dissolved in saline solution to obtain a concentration equal to 40 mg/kg body weight/day and were given orally for 60 days.

Royal Jelly (RJ)

Pure RJ was purchased from a local Healthy Food Store, Qena city, Egypt. According to the manufacturer's reports, RJ is the fresh secreted mixture of nutritional glands of the head of a bit of worker bee. RJ was produced pure and free from applied chemicals or antibiotic sprays.

Royal Jelly Preparation

For preparing the RJ solution. 10 g of RJ was dissolved in 10 ml normal saline to extract RJ stoke with the concentration of 100 mg/kg/day. The prepared extract is freshly used daily by gently pipetting into the stomach tube. The remaining RJ was then placed at 4 °C in the refrigerator.

Experimental design

Twenty male albino rats were used. Animals were randomly divided into four groups (N= 5) and administrated tramadol alone or combined with RJ for 60 days as follows: Group 1: served as a control group and was given NaCl 0.9% orally. Group 2: Tramadol (Tramadol Group) administrated orally at a dose of 20 mg /kg body weight. Group 3: (Tramadol +RJ group), tramadol at a dose of 20 mg /kg body weight and Royal jelly at a dose of 100 mg/kg body weight orally. Group 4: Royal jelly (RJ

group) administrated orally at a dose of 100 mg/kg body weight.

Sample collection

At the end of the experiment, rats were sacrificed. Blood samples were collected directly from the heart and divided into two portions. Portion one was added on a tube with anticoagulant (EDTA) for whole blood samples for complete blood count (CBC). The second portion was added to the plain tube for serum collection and kept frozen at -20 °c until used for biochemical analysis of creatine kinase (CK-BB). The abdominal The brain was extracted for histological examination.

Neurodegenerative markers in serum

Creatine Kinase (CK-BB) kit (CAT NO: 238 001) was purchased from Diagnostic spectrum company, Dokii, Egypt. For detection of creatine kinase in serum of rat. Using automated analyzer Mindray (Model, Manufacturer, Country).

Tissue homogenate preparation

Immediately after euthanasia and brain extraction, the brain tissue homogenate was prepared, according to Behairy et al., (2020). In the cold phosphate-buffered saline (PBS, 0.01 mol/L, pH 7), the brain tissue was homogenized using the glass homogenizer (1:9 W/V). The resulting homogenates will be centrifuged for 5 min at 5000× g; the supernatants will be filtered with a Millipore filter (0.45 µm) to eliminate tissue debris. Neurodegenerative biomarkers (G6PD and NSE) and antioxidant parameters (MDA, TAC and GSH) were evaluated by the spectrophotometric method.

Neurodegenerative biomarkers analyses in brain tissues

Following brain dissecting, small fresh specimens from the brain were collected and divided into two parts. The first part was rapidly washed and added in plastic

tubes, then was kept at -80 °c for Glucose 6 phosphate dehydrogenase enzyme (G6PD) detected using Glucose 6 Phosphate Dehydrogenase Assay Kit 5 x 96T purchased from Abcam Company (CAT NO: ab102532) and rat Neuron-Specific Enolase, detected by NSE ELISA Kit 5 x 96T purchased from Wuhan Humaei Biotech Co., Ltd (CUSABIO), China (CAT NO: CSB-E14065p).They were measured colorimetrically by Eliza done on brain tissue extract.

Anti-oxidant capacity determination

From collected, frozen brain tissue extract, Lipid peroxidation Malondialdehyde (MDA) (CAT NO: MD 25 29) and oxidative system detection, Total Antioxidant Capacity (TAC) (CAT NO: TA 25 13), and Glutathione Reductase (GSH) (CAT NO: GR 25 23) kits were purchased from (Bio diagnostic Company, Giza, Egypt) and analyzed by colorimetric method using mindary chemical analyzer (Mindray Ba-88A, Mindray, China).

Histopathological examination

Following complete necropsy of the experimental male rats, the second part of the brain tissues was rapidly fixed in 10% formalin solution for at least 24 h. After that, these specimens were processed through the conventional paraffin embedding techniques (dehydration in ascending grades of ethyl alcohol, clearing in different changes of xylene and embedding in various modifications of melted paraffin wax at 60 °c). Paraffin blocks were cut by microtome into 5 microns thick sections, which were stained by Hematoxylin and Eosin (H&E), according to the method described by Levick, (1989).

Statistical analysis

All data in mentioned groups were analyzed using the one-way ANOVA

followed by the Tukey-Kramer post-hoc multiple comparisons test. The significance level was considered as $p < 0.05$ (Which software is used).

Results

Hematological analyses

Administration of tramadol revealed a significant ($p < 0.05$) decrease in Hemoglobin (Hb) contents, RBCs count as well as Packed cell volume. In contrast, a consequential ($p < 0.05$) increase was disclosed in the number of circulatory platelets compared to the control group. However, Co-administration of tramadol with Royal jelly showed improvement of the former parameters, whose value recovered around the control ones. as shown in Table(1). Moreover, administration of tramadol denoted a significant ($p < 0.05$) increase in the total white blood cell (WBCs) count when compared with the control group. On the contrary, the WBCs count ameliorated

when tramadol is associated with the Royal Jelly administration. For Differential leukocyte count, tramadol inoculation induced a significant ($p < 0.05$) decrease in the percentage of circulatory lymphocytes while neutrophils and monocytes significantly ($p < 0.05$) increased in comparison to the control group. The group administered tramadol, and Royal Jelly showed improvement in all the previous WBCs associated parameters (Table2). Surprisingly, the administration of Royal Jelly not only ameliorated the deleterious effect on the percentage of lymphocytes induced by tramadol but also increased their values significantly ($p < 0.05$) compared with the tramadol-treated group. However, Royal Jelly alone could not recover the percentage of neutrophils and monocytes, which appeared significantly ($p < 0.05$) lower than that in the tramadol-treated group.

Table 1: Effects of daily oral administrations of tramadol (20 mg/kg b.wt.) for 60 days with ameliorative effect of RJ on hematological parameters in male albino rats. Data presented as Mean \pm SEM. N=5. The different lowercase letters indicate significant as $p < 0.005$.

Groups	Control	Tramadol	Tramadol + RJ	RJ
Parameters				
HB g/dl	14.55 \pm 1.17 ^a	11.05 \pm 0.29 ^b	13.15 \pm 0.53	13.05 \pm 0.98
RBCs $\times 10^6$ mm ³	6.16 \pm 0.19 ^a	4.28 \pm 0.47 ^b	5.28 \pm 0.36	5.98 \pm 0.55
PC %	34.6 \pm 2.57 ^a	26.58 \pm 0.96 ^b	31.88 \pm 2.51	31.15 \pm 3.75
Platelets $\times 10^9$ /L	345 \pm 52.18 ^a	489 \pm 21.19 ^b	385 \pm 34.00	366 \pm 19.72

Table 2: Effects of daily oral administrations of tramadol (20 mg/kg b.w) for 60 days with ameliorative effect of RJ on WBCS count and differential leucocytes count in male albino rats. Data presented as Mean \pm SEM. N=5. The different lowercase letters indicate significant as $p < 0.005$.

Groups	Control	Tramadol	Tramadol + RJ	RJ
WBCs $\times 10^3$ mm ³	9.55 \pm 1.59 ^a	15.62 \pm 1.8 ^b	11.62 \pm 0.62	10.1 \pm 1.29
Lymphocytes %	78.7 \pm 2.91 ^a	67.7 \pm 1.12 ^b	74.57 \pm 1.27	85.45 \pm 1.29 ^a
Neutrophiles %	4.37 \pm 1.33 ^a	11.35 \pm 0.96 ^b	6.55 \pm 1.01	3.80 \pm 0.46 ^a
Monocytes %	15.92 \pm 1.49 ^a	20.05 \pm 1.44 ^b	14.22 \pm 0.29 ^a	8.9 \pm 0.91 ^a

Neurodegenerative Biomarkers

Fig 1: shows the effects of tramadol administration in a dose of (20 mg/k.g. b.w) in albino rats on some of the biochemical parameters, including Glucose 6 phosphate dehydrogenase (G6PD) enzyme, Creatine Kinase -BB (CK-BB) enzyme and Neuron-specific enolase Enzyme (NSE) for 60 days. Oral inoculation of rats with tramadol drug caused a significant decrease in the concentration of G6PD ($p < 0.005$) in comparison to the control group. The G6PD level improved significantly after Royal Jelly was given in co-administration with tramadol compared to the tramadol group. Royal Jelly administration for the rats induced a significant ($p < 0.05$) increase in the level of G6PD enzymes compared to the control, tramadol and tramadol + RJ groups. In addition, the results exhibited that administration of tramadol induced a

significant increase in the level of CK-BB ($p < 0.005$) compared with control rats. Co-administration of tramadol with Royal Jelly improved the concentration of the enzyme serum level when compared with the tramadol group. When the royal jelly was given alone to the rats, a significant decrease was noticed in the CK-BB enzyme concentration ($p < 0.01$) compared with tramadol and tramadol + RJ groups. Also, administration of tramadol to the experimental rats induced a significant ($p < 0.05$) increase in the neuron-specific enolase enzyme compared to the control group. However, the level of NSE was corrected when Royal Jelly was given with Tramadol compared with the tramadol-treated group. Additionally, the treatment with Royal Jelly denoted a significant ($p < 0.05$) decrease in the level of the NSE enzyme in comparison to Tramadol and tramadol+ RJ groups.

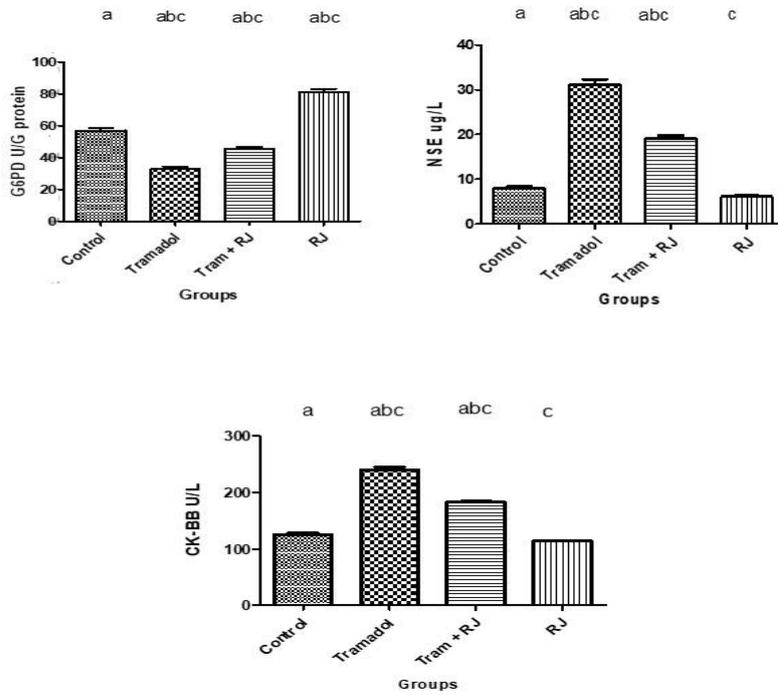


Figure 1: effects of tramadol administration on the neurodegenerative markers in brain tissues with ameliorative effects of Royal jelly. Administration of tramadol induced significant decrease to G6PD while CK-BB and NSE were decreased. Giving royal Jelly improved the changes in parameters concentrations. Bar Values (means ±SE) when n=5 and P<0. 005. Same letters indicates significant difference

Lipid peroxidation

Administration of rats with tramadol drug orally for successive 60 days induced a significant increase in the concentration of MDA (p<0.005) in comparison to the control group. The concentration of MDA improved significantly after administration

of royal jelly in co-administration with tramadol compared to the tramadol-treated group. Royal Jelly administration to the rats for 60 days induced a significant decrease in the level of MDA enzymes compared to control, tramadol and tramadol + RJ groups, as cleared in Fig. (2).

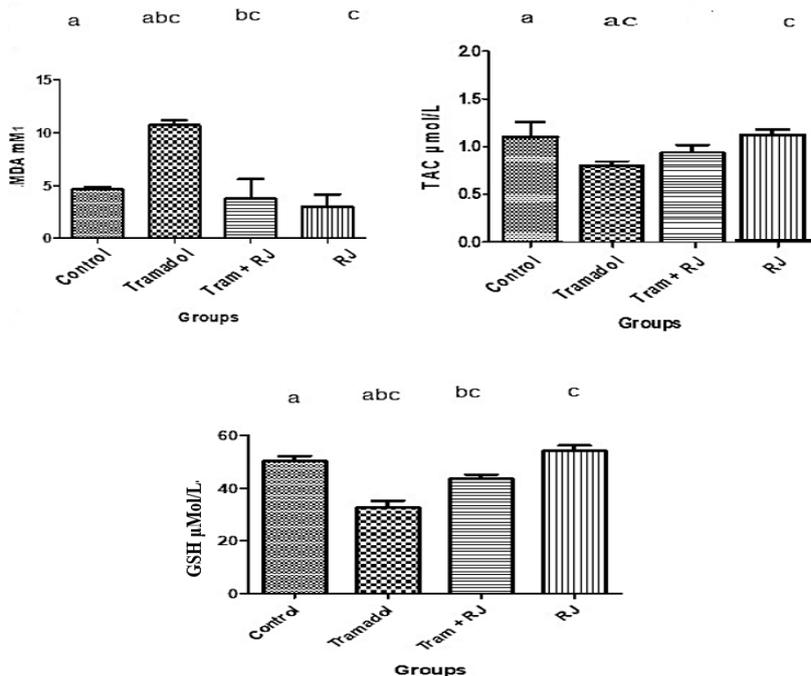


Figure 2: effects of tramadol administration on the neurodegenerative markers in brain tissues with ameliorative effects of Royal jelly. Administration of tramadol induced significant increase in MDA while TAC and GSH were decreased. Giving royal Jelly improved the changes in parameters concentrations. Bar Values (means ±SE) when n=5 and P<0. 005. Same letters indicates significant difference.

Antioxidant findings

The effects of tramadol administration in a dose of (20 mg/k.g.b.w) in albino rats for frequent 60 days were seen in Fig (2). The administration of tramadol caused a significant decrease in the level of TAC ($p < 0.05$) when compared with control animals. Co-administration of tramadol with Royal Jelly improved the concentration of TAC in comparison to the Tramadol treated group. When the Royal Jelly was given alone, the animals exhibited a significant increase in the level of TAC when compared with Tramadol and Tramadol+ Royal Jelly. At the same time, when the tramadol drug was given to the rats induced a significant decrease in the level of GSH compared to the control group. This adverse effect improved after the Royal Jelly administration. Also, Royal Jelly improves the oxidative status by increasing the level of GSH compared to Tramadol and Tramadol+ Royal Jelly groups.

Histopathology

Histopathological examination of control and Royal jelly-treated rats

revealed a delicate pia matter layer followed by the normal histological structure of brain sections consisting of an outer molecular layer, external granular and pyramidal layers followed by inner fine and pyramidal layers, and ends with a polymorphic layer. Also, normal blood capillaries are admixed with normal nerve cells distributed in the well-arranged six layers and typically appear as eosinophilic ground substance (neuropil) Fig (3). Tramadol-treated groups revealed signs of neuronal toxicity, such as disorganized cortical layers and acute red degenerative neurons, which have hypereosinophilic cytoplasm and pycnotic nucleus; some other degenerated features including prominent vacuolation of neuropil. Microglioses appeared in some regions while others have dilated and congested vasculature Fig (4). Rats treated with tramadol and Royal jelly revealed no marked histological changes. Still, they showed evidence of normal morphology of brain tissue, mild blood congestion, normal neurons with no degenerative changes, and normal cortical layer distribution Fig (5).

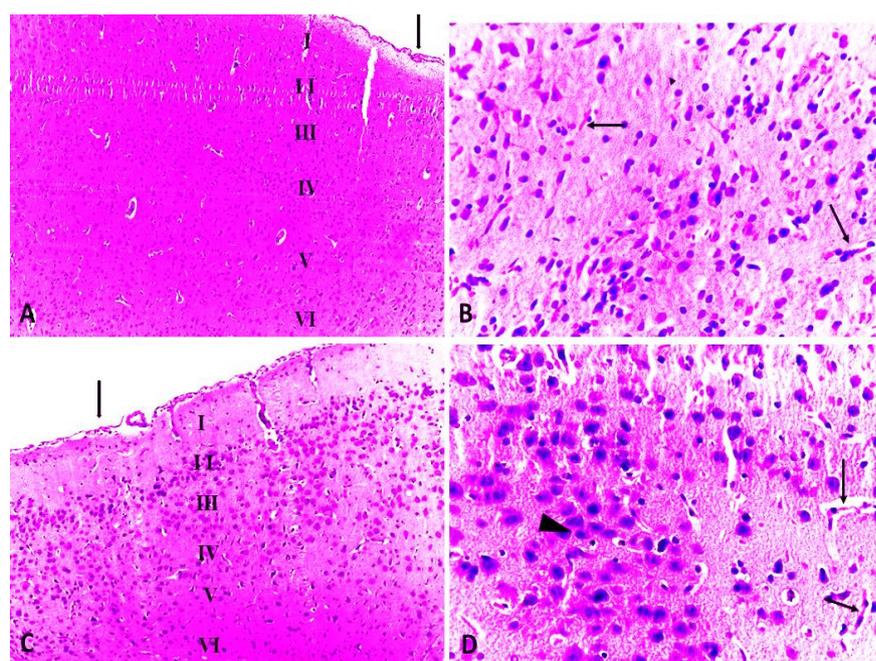


Figure 3:

Histopathological section of the cerebral cortex of the control rats (A & B), and Royal jelly treated groups (C & D). Normal histological structures of the cerebral cortex are arranged in 6 layers (I, II, III, IV, V, VI) (A & C), thin pia matter layer (Thick arrow) (A & C), normal neurons (Arrowhead) (D), normal blood capillaries (Thick arrows) (B&D). (H&E: A&C, X 100; B, X 200; D, X 400).

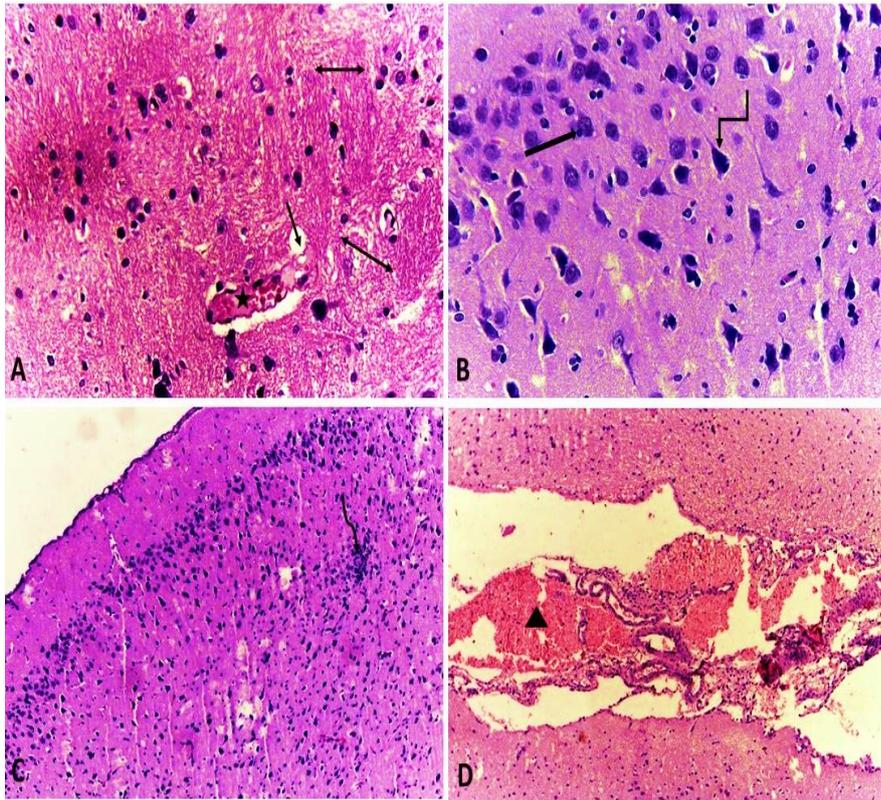


Figure 4:

Histopathological section of the cerebral cortex of tramadol-treated groups revealed neuropil vacuolation (Double line arrow & Thin arrow) (A), congestion (Asterisk) (A), gliosis (Connector curved arrow) (C), red neurons (Connector elbow arrow) (B), Degenerated neurons (Thick arrow) (B), meningeal congestion (Arrowhead) (D). (H&E: C&D, X 100; A, X 200; B, X 400).

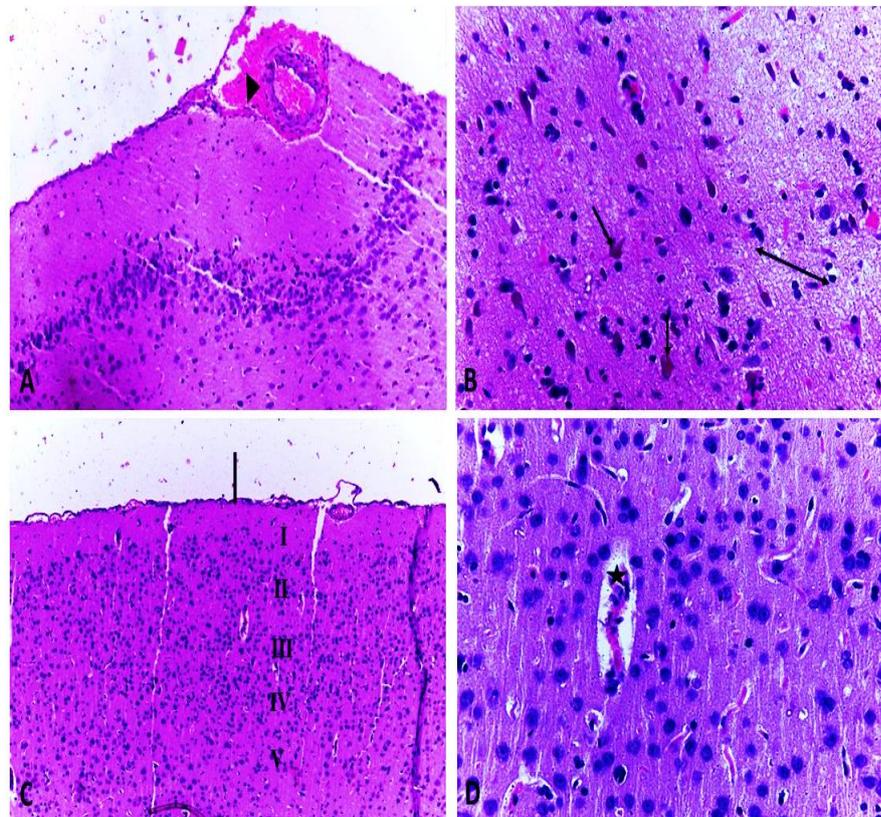


Figure 5:

Histopathological section of the cerebral cortex of rats of the tramadol and Royal jelly treated groups revealed restoration of brain normal tissue. Mild congestion is observed (arrowhead) (A), (Asterisk) (D), Thin pia matter layer (Thin arrow) (C), normal arranged cortical layers (I, II, III, IV, V, VI) (C), no evidence of neuropil vacuolation (Double line arrow) (B) and few numbers of necrotic neurons (Thin arrow) (B). (H&E: A&C, X 100; B, X 200; D, X 400).

Discussion

Tramadol hydrochloride (TH), is used to treat acute and chronic pain and widely prescribed all over the world (Grond and Sablotzki, 2004, Ebrahimzadeh et al., 2013). It interferes with the neuronal release and reuptake of serotonin and norepinephrine, that influences its analgesic effect (El-Sayed et al., 2013). In this study, we investigated the neurodegenerative effects of tramadol on the brain tissues of rats with the ameliorative effects of Royal jelly. The frequent use of tramadol without medical description causes many health hazards on the body, which include brain damage (Ibrahim and Hala, 2017), liver and kidney injuries (Heba and Azza, 2016), Hormonal (Essam et al., 2015) and behavioral signs (Yamasaki et al., 2015). In our study, tramadol administration in albino rats caused hematological, biochemical and histopathological alterations related to the brain. A significant decrease in Hb concentration, RBCs count, and PCV percentage was reported, the same results were reported by Aziza et al., (2019). The decline in the Hb level, as well as RBCs count, may attribute to many mechanism the first one through the direct inhibitory effect of tramadol on erythrocytes formation in the bone marrow as reported by Aldiwan et al., (2015), second mechanism through deficiency of G6PD enzyme which decrease in Hemoglobin concentration and RBCs (Cappellini and Fiorelli, 2008). In the same, tramadol administration induced a significant increase in WBCs count that agree with the report of Aziza et al., (2019). The rise in WBCs count was associated with a substantial decrease in the peripheral circulatory lymphocyte percentage and increased Neutrophils and monocytes % in

the circulation. Our results for lymphocytes % were opposite to results obtained by Aldalou et al. (2014), who reported that tramadol administration caused increased WBCs count and Lymphocytes percentage. The increase in the WBCs count was due to the stimulation of the immune system by inflammations induced by tramadol, as mentioned by Aldiwan et al., (2015). The decrease in circulatory lymphocytes %, was due to the migration and passing of the circulatory lymphocytes via the blood-brain barrier (BBB) and cerebrospinal fluid barrier (CSFB) to share in the process of inflammation and specific cytokines production as (Prass et al., 2003; Liesz et al., 2009a) and in the neuronal degeneration (Sommer et al., 2017). In accordance, the increase in neutrophils and monocytes attributed to their participation in the acute inflammatory changes was induced by tramadol in other organs such as the liver and kidney as well as testicular tissues. Additionally, tramadol administration caused an increase in the platelets count (thrombocytosis). This agrees with results recorded by Aziza et al., (2019). The increase in thrombocytes was due to the inflammatory changes induced by tramadol, such as cellular and vascular degeneration, as described by Yan et al., (2013).

For better understanding of the impacts of tramadol when given for long days, some biomarkers related to the neuron and brain damage such as G6PD, CK-BB and NSE enzymes were detected. In this study, tramadol inoculation to the experimental rats induced deficiency in the level of G6PD enzyme compared to the control animals. Our results confirmed by the study of Winnie et al., (2013), who reported that the Reactive Oxygen Species

(ROS) induced neurodegenerative changes in G6PD deficient aged mice. Deficiency of G6PD initiate ROS production which induce brain damage resulting in synaptic inhibition in the hippocampus with lowering the number of Purkinje cells (Margret et al., 2020) . NSE is physiologically situated in the healthy neuron and cannot released extracellular fluids. However, when neuronal axons are injured, NSE is fastly upregulated to maintain homeostasis (Wu et al., 2004). In our study, administration of tramadol induced a significant increase in the concentration of CK-BB, which was associated with pathological damage in the rat's brain tissues, where increase the circulating CK-BB level is associated with acute cerebrovascular diseases (Young, 2001).

Further, it was described in many previous reports that tramadol has a significant oxidative effect on the brain and other body tissues leading to tissues damages (Barbosa et al., 2021). The oxidative stress induced by tramadol in the brain was reported by Lemarie & Grimm, (2009); Mohamed et al., (2019), they explained that tramadol inhibits mitochondria's mechanical pathways that generate reactive oxygen species.

In our study, continuous administration of tramadol for 60 days induced an increase in the lipid peroxidation status, representing an increased MDA level and decrease in the concentration of TAC and GSH enzymes . This is in agreement with El-Gaafarawi, (2006) and Abdel-Zaher et al., (2011). The increase in MDA concentration was attributed to ROS resulting from tramadol toxicity (Rabei, 2011).

In this study, the histological section from the brain tissues was harvested to

investigate the cerebral dysfunction and cortical layer disorganization, and neuronal degeneration caused by tramadol administration to rats for successive 60 days. Brain tissues exhibited signs of neuronal toxicity, appearing as acute red degenerative changes appeared as shrunken neurons with a pyknotic nucleus and highly eosinophilic cytoplasm which are the histopathological marker of apoptosis due to hypoxic changes, the same results reported by Mohamed, et al., (2019); Zhuo et al., (2012). Vacuolation of neuropil and focal Microglioses. neuropil degeneration results from cell organoid damage resulting from free radicals' exposure (Zarnescu et al., 2008; Brown et al., 2004). Some areas of brain tissue revealed dilated congested vasculature admixed with red neurons which contributed to cerebral dysfunction (Mohamed et al., 2019). In our histological findings, meningeal congestion and gliosis were prominent features in some cases of tramadol-treated groups. Loss of pyramidal cells, increase perivascular spaces in addition to sub meningeal blood vessels congestion and neuronal degeneration (Abou El Fatoh et al., 2014). Our results come in accordance results reported by Fatma et al., (2014). Histopathological damages result from oxidative stress on the brain tissues (Barbosa et al., 2021).

Royal Jelly has important pharmacological activities: strong antioxidant, neurotrophic, lowering blood glucose and lipids, protect the liver and kidney, lower the blood pressure, antitumor, antibiotic, anti-inflammatory, immunomodulatory and anti-allergic, general tonic, antiaging etc (Mărghitaş, 2008). RJ contains large quantities of acetylcholine, which act as neurotransmitter in both the peripheral and

central nervous systems as well as it used as neuromodulator in the motor division of the somatic nervous system (Mateescu, 2005). In our study, co-administration of Royal jelly with tramadol induced improvement to the hematological, biochemical and histopathological changes induced by tramadol administration for 60 days. The progress of the harmful effects resulted from tramadol administration was attributed to the antioxidant activity of royal jelly which in turn decreases the cellular damage as well as its role in enhancing the immune system and inhibiting the inflammatory events in the site of injury (Mateescu, 2005). In recent research, it was proved that RJ enhanced and increases the differentiation of all types of brain cells from neural stem cells and neurogenesis promotion in the association with the unsaturated fatty acid present in the chemical content of RJ, 10-hydroxy-trans-2- decanoic acid (HDEA) (Hattori et al., 2007). The same action of Royal jelly against the possible damages induced by tramadol was confirmed via the histopathological examination of the brain tissue sections.

Conclusion

From the previous results, chronic tramadol administration is an important factor in the histopathological alterations in different areas of brain tissue. RJ treatment-induced improvement in the chemical responses and inflammatory processes resulting in restoration of the normal histological features of brain tissue and normal neurons function.

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