Dose-Response:
An International Journal
October-December 2024:1–13

DOI: 10.1177/15593258241290458
journals.sagepub.com/home/dos
Sage

Cucurbita Pepo L. Seed Oil Modulates Dyslipidemia and Neuronal Dysfunction in Tramadol-Induced Toxicity in Wistar Albino Rats

Ezebuilo Ugbala Ekpono[1], Patrick Maduabuchi Aja[2,3], Udu Ama Ibiam[2], Peter Chinedu Agu[2], Ejike Daniel Eze[4], Adam Moyosore Afodun[5], Osita Gabriel Okoye[1], Josiah Eseoghene Ifie[3], and Ayomide Victor Atoki[3]

Abstract

Objective: The modulating effects of *Cucurbita pepo* seed oil (CPSO) on dyslipidemia and neuronal dysfunction in tramadol toxicity were studied.

Methods: Fifty-six albino rats were divided into seven groups of eight rats each after a 2-week acclimatization period. All animals had unrestricted access to water and feed, and treatments were administered orally once daily for 42 days. Glutamate dehydrogenase and glutaminase activities were assessed using brain homogenate, while lipid profiles were analyzed in serum samples.

Results: Tramadol toxicity was evidenced by significant ($P < 0.05$) increases in brain glutamate dehydrogenase along with significant ($P < 0.05$) decreases in the activities of glutaminase in the group administered only tramadol. Also, serum levels of total cholesterol, LDL-C and triglycerides also increased significantly ($P < 0.05$) following administration of tramadol with decreased level of HDL-C ($P < 0.05$). However, treatment with CPSO significantly restored the activities and levels of the altered biochemical parameters in a dose-dependent manner. The results of the biochemical investigation using the lipid profile and the enzymes of glutamate metabolism were corroborated by the results obtained from the histopathological examination of the brain.

Conclusion: The results of this study therefore suggest that tramadol-induced dyslipidemia and neuronal dysfunction be managed and prevented by the administration of *Cucurbita pepo* seed oil.

Keywords

Cucurbita pepo, dyslipidemia, neuronal dysfunction, glutamate dehydrogenase, glutaminase, and tramadol

Introduction

Tramadol is a synthetic analog of codeine which is a widely used opioid, especially as an analgesic for moderate to severe pain relief.[1] The analgesic effect of tramadol is attributable to its agonistic action on opioid receptors and its inhibitory effect on neuronal reuptake of serotonin and norepinephrine; which leads to the blockage of the transmission of nociceptive stimuli in the

[1] Department of Science Laboratory Technology, Federal Polytechnic, Oko, Nigeria
[2] Department of Biochemistry, Faculty of Science, Ebonyi State University, Abakaliki, Nigeria
[3] Department of Biochemistry, Faculty of Biomedical Sciences, Kampala International University, Kampala, Uganda
[4] Department of Physiology, School of Medicine, Kabale University, Kabale, Uganda
[5] Department of Anatomy and Cell Biology, Faculty of Health Sciences, Busitema University, Uganda

Received 16 May 2024; accepted 4 September 2024

Corresponding Author:
Ayomide Victor Atoki, Department of Biochemistry, Kampala International University, Western Campus, Ishaka-Bushenyi, Uganda.
Email: atokiav@kiu.ac.ug

central nervous system.[1–3] At a therapeutic dose, tramadol does not cause major side effects in comparison to other opioid analgesics and is useful for the management of neurological problems like anxiety and depression.[3] Unfortunately, multiple adverse effects were developed in patients with prolonged tramadol therapy, including neurological disorders like seizures, serotonin syndrome, Alzheimer's disease, and Parkinson's disease.[4] These adverse effects are corroborated with experimental animal studies showing associated prolonged tramadol treatment with several organ systems dysfunction, including damage to the cerebral cortex, lung, testis, and liver.[5–7]

With an expected increase in the number of prescriptions of tramadol to patients experiencing chronic painful conditions, there is the likelihood of patient addiction according to several clinical studies.[8–10] The addictive effects may be connected to the ability of tramadol to inhibit the gamma-aminobutyric acid (GABA) receptors, interfere with the dopamine synthesis and release as well as induce oxidative stress.[4,11,12] Several studies have implicated oxidative stress in cardiovascular diseases and its associated conditions such as heart failure, endothelial dysfunction, and myocardial ischemia-reperfusion injury.[13,14] Additionally, increased Reactive Oxygen Species (ROS) production compromises the function of endothelial nitric oxide synthase enzyme (eNOS) and nitric oxide (NO) production as observed in endothelial dysfunction in several vascular pathological conditions.[15–17]

Over the last decade, there has been an overwhelming increase in the use of various herbal supplements and extracts in most developing and developed countries about the management of several adverse effects of drugs.[18,19] The pumpkin, *Cucurbita pepo* L. (*C. pepo*), is a green vegetable belonging to the Cucurbitaceae family and it is one of the natural products used in supplementations; it is leafy and its fruits contain various seeds varying in size, color, shape and weight.[20,21] It is rich in many antioxidants and beneficial nutritional supplements such as essential fatty acids, amino acids (tyrosine and L-phenylalanine), phytosterols (betasitosterol), â-carotenes, lutein and selenium.[22,23] Notwithstanding the usefulness of the various parts of this plant, pumpkin seed oil (PSO) supplementation has been used in folk medicine for the prevention of changes in plasma lipids, treatments of hypertension and atherosclerosis.[24,25]

Though it seems several studies have been designed around pumpkin plant and its supplementation potentials, there is still limited literature specifically describing the effects of tramadol on glutaminase, glutamate dehydrogenase, heart and blood vessels. Therefore, it is imperative to investigate the modulating effects of *Cucurbita pepo* L. seed oil on dyslipidemia and neuronal dysfunction associated with tramadol-induced toxicity in Wistar albino rats.

Materials and Methods

Plant Materials and Extraction Procedure

Fresh seeds of *Cucurbita pepo* L. were collected from Agharaoza village in the Izzi Local Government Area of Ebonyi State and were identified by Prof. S. C. Onyekwelu; a Taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. The extraction of the pumpkin's seed oils (CPSO) was by the method described by Mathangi.[26] The seeds were washed to remove dirt, sundried, and manually blended to obtain a powder. Exactly, 250 mL n-hexane was used to extract 30 g of the powdered seeds in a Soxhlet extractor at 70°C for 6 hr. The crude oil extract (CPSO) was obtained by removing the solvent from the oil after extraction using a rotary evaporator. The crude CPSO obtained was clarified using a method described by Nwozo.[27]

Animal Source and Handling

A total of 56 adult male Wistar albino rats (200 – 250 g) were purchased from the Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu, Nigeria. The animals were kept in standard cages for 2 weeks for acclimatization in the Animal House of the Department of Biochemistry, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. During this time, the animals were fed with normal laboratory feeds and clean water ad libitum. All animal procedures were performed following our institutional research and ethics committee approval number (EBSU/BCH/ET/21/007) and in line with the ethical guidelines for the care and use of animals for scientific purposes developed by the Animal Use and Care Committee of Nigeria, 2004.

Experimental Design

After acclimatization, the rats were randomly divided into seven experimental groups (n = 8). We chose the CPSO doses based on the reports of Agu et al.[28] and Aja et al.[29] Group 1 (normal control; NC): rats were fed on pellets and allowed free access to water; Group 2 (saline group; NSC): the animals received 5 mL/Kg body weight of normal saline. Group 3-6 received 100 mg/Kg body weight (b.wt.) of tramadol; TM.[30,31] Group 3 (100 mg/Kg b.wt. TM): the rats were left untreated; Group 4, 5 and 6 (TM-CPSO treated groups): the animals received 5 mL/Kg, 2.5 mL/Kg and 1.5 mL/Kg body weight of CPSO respectively; Group 7 (CPSO group): the animals received 5 mL/Kg body weight of CPSO only.[32,33] All the administrations were done by oral intubation once daily for six weeks.

Sample Collection

After the drug and extracts administrations, the animals were mildly anaesthetized using halothane and sacrificed by cervical dislocation to obtain the blood and brains for both biochemical and histological analyses. Blood samples were collected via the femoral vein, and centrifuged to obtain the serum which was stored for lipid profile analysis. Five of the harvested brains were randomly selected and rinsed with normal saline and 0.1 mol/L hydrochloric acid buffer (pH 7.4) was added for

homogenization. The homogenate was centrifuged at 3000 r/min for 15 min at 4°C and the supernatant was obtained for the determination of glutathione dehydrogenase and glutaminase activities. The remaining brain tissues per group were also further routinely processed for the histopathological examination of the brain for cellular integrity.

Determination of Neuronal Dysfunction

Assays of glutamate dehydrogenase (GDH) and glutaminase activities were used to determine neuronal dysfunction. GDH activity was assayed according to the method of Lee and Lardy[33] while glutaminase activity was assayed by the method of Chetan.[34]

Determination of Lipid Profile

Total cholesterol (Chol.) and triglyceride (TAG) concentrations were determined by enzymatic saponification methods according to Kendall[35] and Sardesai and Manning,[36] respectively. High-density lipoprotein cholesterol (HDL-C) concentration was determined by the centrifugation method of Albers.[37] The equation method of Friedewald[38] was used in the determination of LDL-cholesterol concentrations of the samples.

Histopathological Examination

Each rat's brain tissues were removed gently and fixed with formalin. The fixed brain tissues were stored in 70% ethyl alcohol. After 24 hours, the fixed tissues were processed to paraffin embedding and sectioned according to the method of Bancroft and Gamble[39] for histopathological examination. We longitudinally sectioned the brain tissue with a microtome and stained it with haematoxylin and eosin (H and E) to examine microscopic histopathological alterations. We examined the stained slides under a light microscope. We used it as a scale bar: 50 µm, magnification: ×400, Measured length: 3 scale bar units; actual size: 3 × 50 µm = 150 µm.

Statistical Analysis

Data were analyzed with one-way ANOVA using GraphPad Prism (Version 8.0.2). The data are presented as the mean ± standard error of means (SEM). The data means were compared using a post-hoc one-way ANOVA at $P < 0.05$. Groups' mean values were compared between groups using Turkey's multiple comparisons test, revealing significant differences at $P < 0.05$.

Results

Effects of Cucurbita Pepo *Seed Oil (CPSO) on Neuronal Function*

Glutamate dehydrogenase activity was significantly ($P < 0.05$) elevated (Figure 1) while glutaminase was significantly ($P < 0.05$) lowered (Figure 2) in tramadol-only treated rats (Group 3; 100 mg/Kg b.wt. TM) when compared to controls. CPSO treatment only (Group 7; 5 mL/Kg b.wt. CPSO) showed no significant difference in the glutamate dehydrogenase activity (Figure 1) while the glutaminase activity was significantly increased (Figure 2) when compared to the controls. CPSO treatments significantly decreased the activity of glutamine dehydrogenase and significantly increased the activity of glutaminase when compared to the group that received tramadol only (Group 3; 100 mg/Kg b.wt. TM) without treatment (Figures 1 and 2, respectively).

Effects of Cucurbita Pepo *Seed Oil (CPSO) on the Lipid Profile*

The TChol., LDL-C, and TAG levels in the tramadol-only administered group (Group 3; 100 mg/Kg b.wt. TM) were significantly increased when compared to the controls while HDL-C levels were significantly decreased (Figures 3–6 respectively). Administration of only CPSO (Group 7; 5 mL/Kg b.wt. CPSO) showed a significant decrease in LDL-C and HDL-C levels with no significance in cholesterol and TAG levels when compared to the controls. When compared to group 3 (100 mg/Kg b.wt. TM only), significant increase in HDL-C levels and a decrease ($P < 0.05$) in TChol., LDL-C and TAG were observed in the treatment groups except group 6 administered 1.5 mL/Kg b.wt. CPSO showed a significant increase in LDL-C. The result equally revealed that there was no significant ($P < 0.05$) difference in the lipid profiles of the group administered 5 mL/Kg b.wt. CPSO when compared with the normal controls while the groups administered 2.5 mL/Kg b.wt. CPSO and 1.5 mL/Kg b.wt. CPSO showed a differential response.

Results of the Histopathological Examination of the Brain

Histological analysis of the brains of albino rats co-administered 100 mg/Kg body weight of tramadol and CPSO at doses of 5 mL/Kg, 2.5 mL/Kg, and 1.5 mL/Kg are presented in Figures 7–13. The photomicrograph of the brains of group 1 rats (normal control) is presented in Figure 7(A)–(B). The result of a section of the brain showed a normal brain with a molecular layer (ML), granular layer (GL), and well-outlined Purkinje cells within the Purkinje layer. The photomicrograph of the brain of group 2 albino rats administered only normal saline is presented in Figure 8(A)–(B). The result of a section of the brain in this group showed a normal brain with mild clumping of the Purkinje cells (CPC). The result of the section of the brain of group 3 animals administered with tramadol showed severe degeneration with severe fatty change (FC) (steatosis), severe vacuolation (V), and necrosis (N) within the molecular layer and granular layer in 9A and moderate vacuolation in 9B. Administration of 100 mg/Kg b. wt tramadol and 5 mL/Kg b. wt of CPSO in treatment group 4

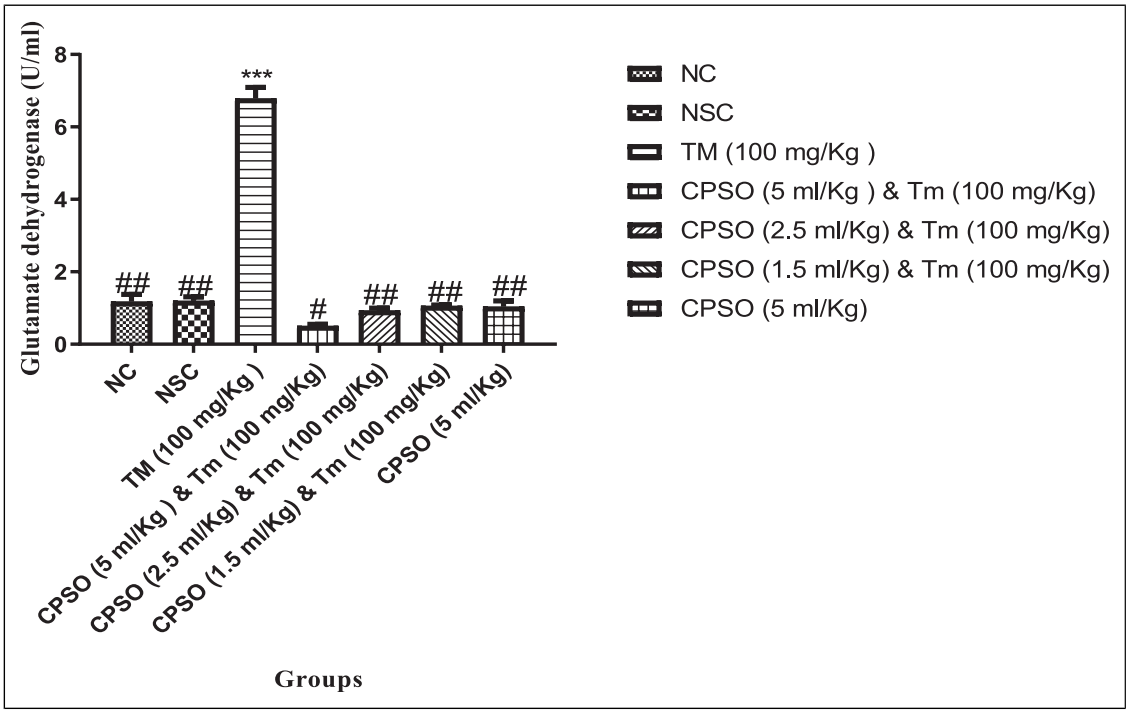

Figure 1. Glutamate Dehydrogenase Activity of Tramadol-Induced Toxicity in Albino Rats Treated with CPSO. Data are shown as mean ± SEM (n = 5). The mean values of groups were compared using Tukey's multiple comparisons test, revealing significant differences at $P < 0.05$. CPSO: *Cucurbita pepo* seed oil, NC: Normal control, NSC: Normal saline control, TM: Tramadol control.

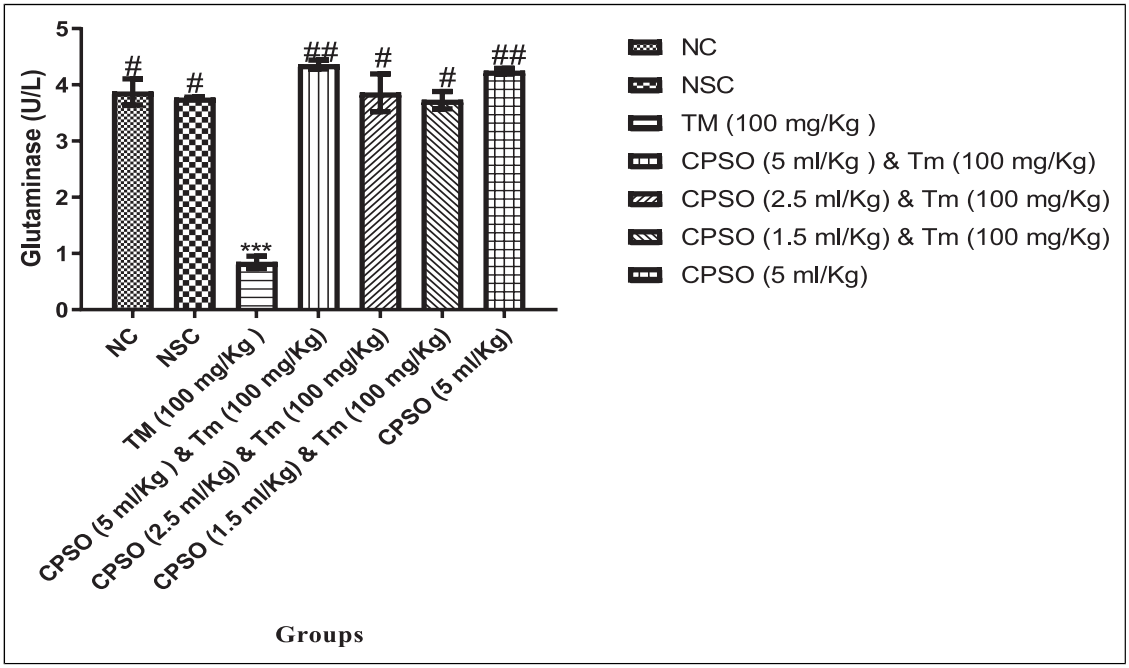

Figure 2. Glutaminase Activity of Tramadol-Induced Toxicity in Albino Rats Treated with CPSO. Data are shown as mean ± SEM (n = 5). The mean values of groups were compared using Tukey's multiple comparisons test, revealing significant differences at $P < 0.05$. CPSO: *Cucurbita pepo* seed oil, NC: Normal control, NSC: Normal saline control, TM: Tramadol control.

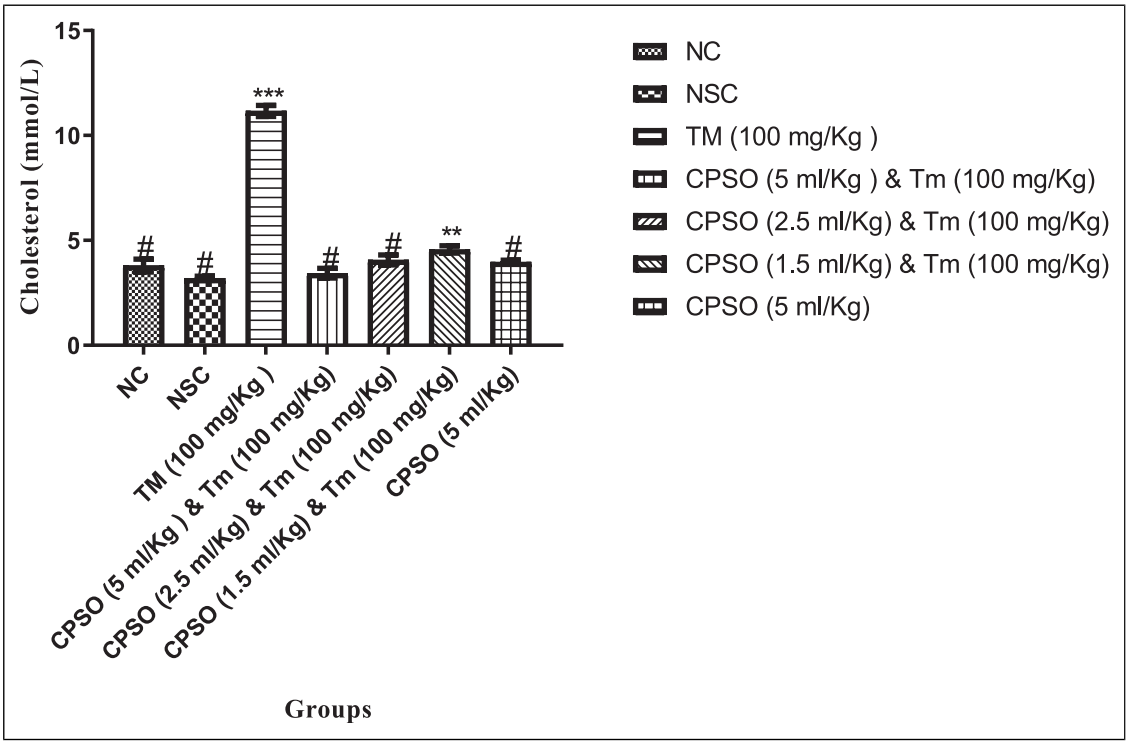

Figure 3. Cholesterol Level of Tramadol-Induced Toxicity in Albino Rats Treated with CPSO. Data are shown as mean ± SEM (n = 8). The mean values of groups were compared using Tukey's multiple comparisons test, revealing significant differences at $P < 0.05$. CPSO: *Cucurbita pepo* seed oil, NC: Normal control, NSC: Normal saline control, TM: Tramadol control.

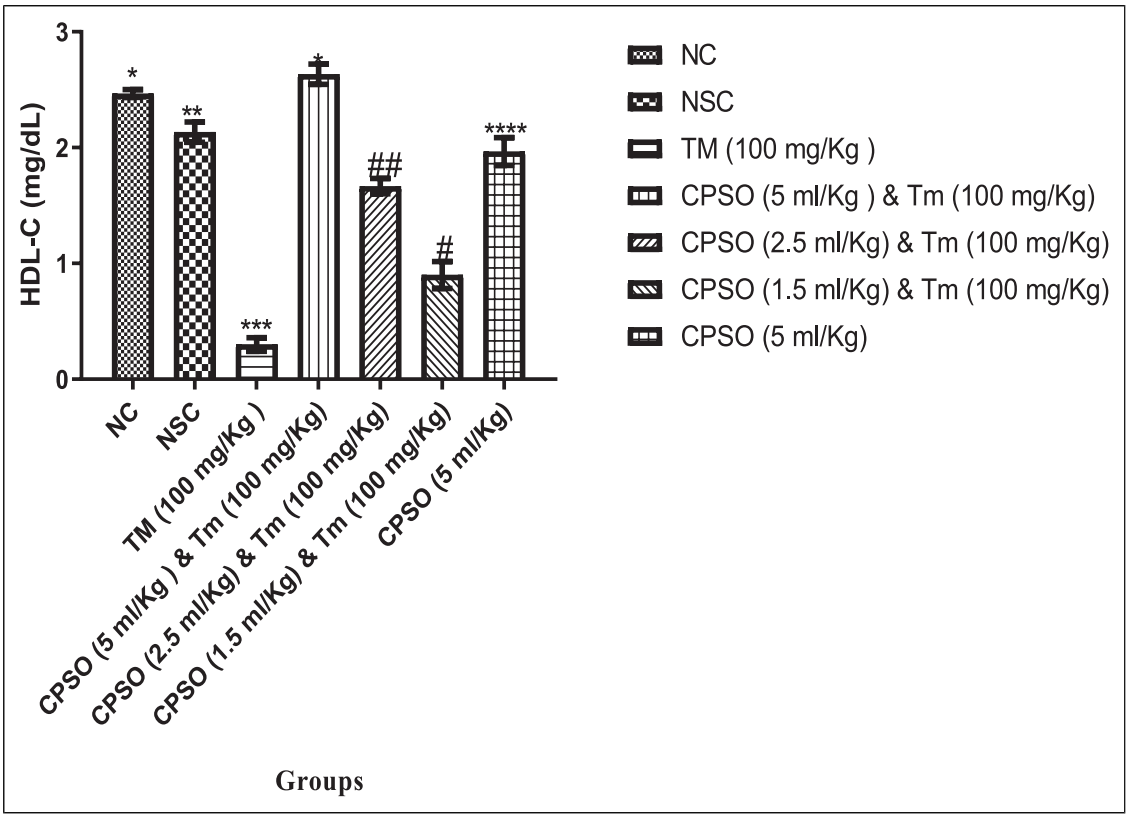

Figure 4. HDL–Cholesterol Level of Tramadol-Induced Toxicity in Albino Rats Treated with CPSO. Data are shown as mean ± SEM (n = 8). The mean values of groups were compared using Tukey's multiple comparisons test, revealing significant differences at $P < 0.05$. CPSO: *Cucurbita pepo* seed oil, NC: Normal control, NSC: Normal saline control, TM: Tramadol control.

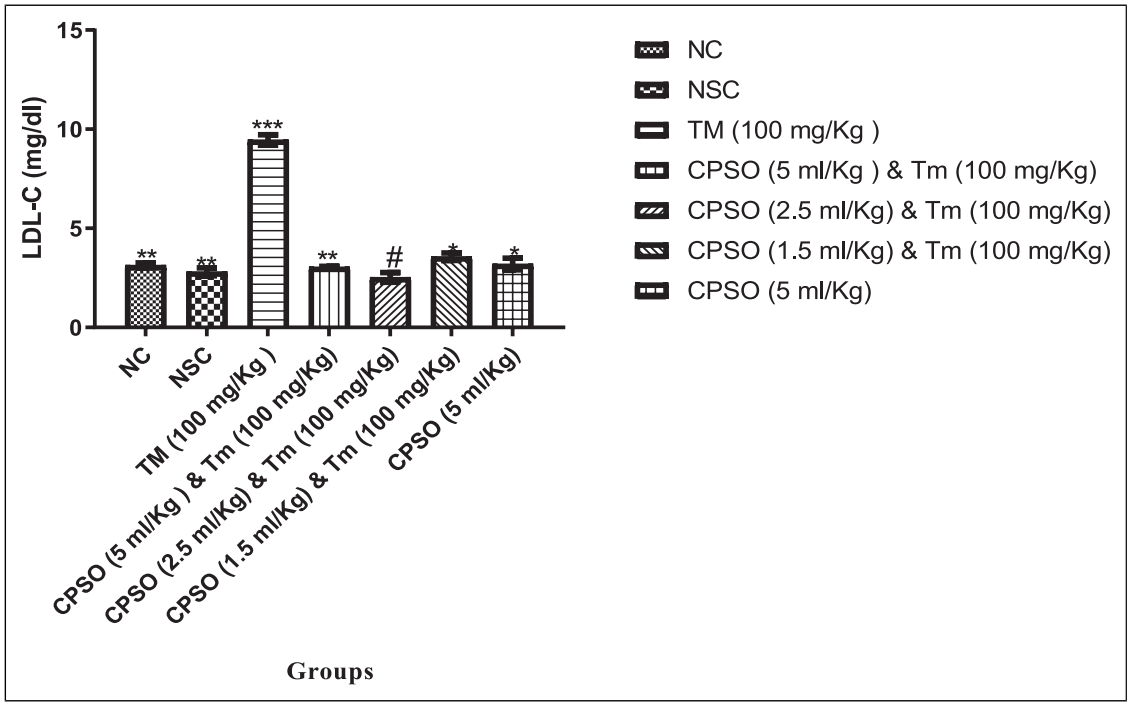

Figure 5. LDL-cholesterol Level of Tramadol-Induced Toxicity in Albino Rats Treated with CPSO. Data are shown as mean ± SEM (n = 8). The mean values of groups were compared using Tukey's multiple comparisons test, revealing significant differences at $P < 0.05$. CPSO: *Cucurbita pepo* seed oil, NC: Normal control, NSC: Normal saline control, TM: Tramadol control.

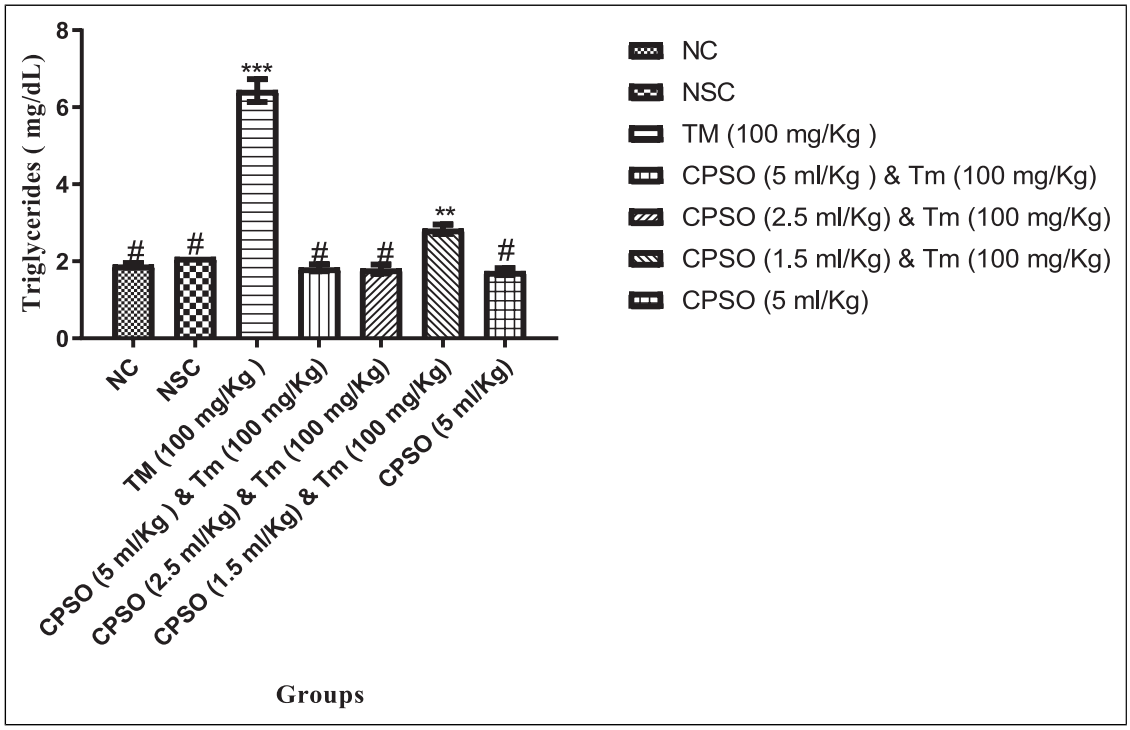

Figure 6. Triglycerides Level of Tramadol-Induced Toxicity in Albino Rats Treated with CPSO. Data are shown as mean ± SEM (n = 8). The mean values of groups were compared using Tukey's multiple comparisons test, revealing significant differences at $P < 0.05$. CPSO: *Cucurbita pepo* seed oil, NC: Normal control, NSC: Normal saline control, TM: Tramadol control.

showed moderate healing with mild fatty change (FC) (steatosis), mild vacuolation within the molecular layer and granular layer in 10B, and mild vacuolation in 10A (Figure 10(A)–(B)). Administration of 100 mg/Kg b. wt tramadol and 2.5 mL/Kg b. wt of CPSO in treatment group 5 showed mild to moderate healing with mild focal loss of tissue (LT) within the molecular layer in 11A and moderate aggregate of inflammatory cell (AIC) and mild fatty change (FC) within the molecular layer in 11B as presented in Figure 11(A)–(B). Administration of 100 mg/Kg b. wt Tm and 1.5 mL/Kg b. wt of CPSO in treatment group 6 showed mild healing with moderate necrotic(N) appearance of the Purkinje layer with non-distinct Purkinje cell outline in 12A and moderate fatty change (FC) within the molecular layer in 12B as presented in Figure 12(A)–(B). Administration of only 5 mL/Kg b. wt of CPSO in treatment group 7 (Figure 13(A)–(B)) showed a normal brain. However, a mild vacuolation (V) and mild fatty change (FC) were observed in 13B.

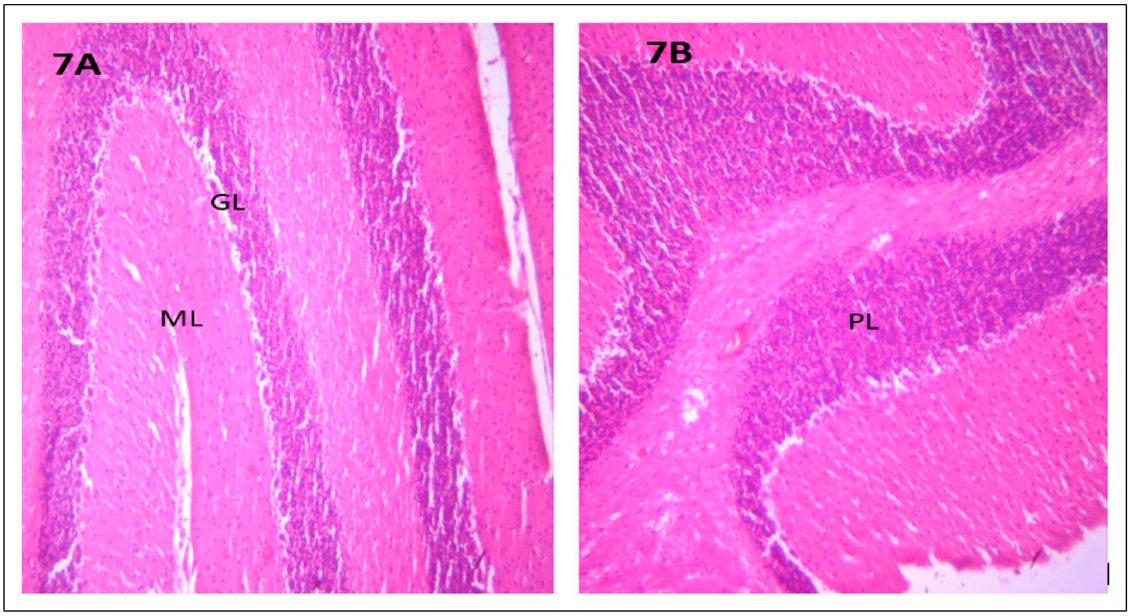

Figure 7. Photomicrograph of the brain of the normal control group (x100/400) (H/E). Normal brain with molecular layer (ML), granular layer (GL) and well-outlined Purkinje cells within the Purkinje layer.

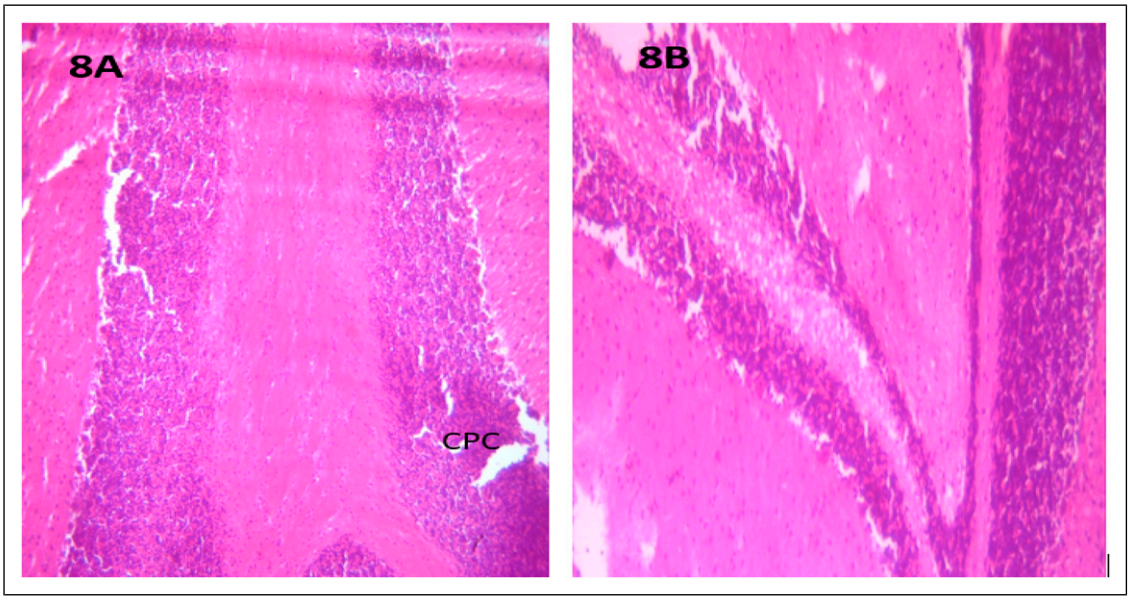

Figure 8. Photomicrograph of the brain of group 2 administered normal saline (x100/400) (H/E). Normal brain with mild clumping of the Purkinje cells (CPC) in 8A observed.

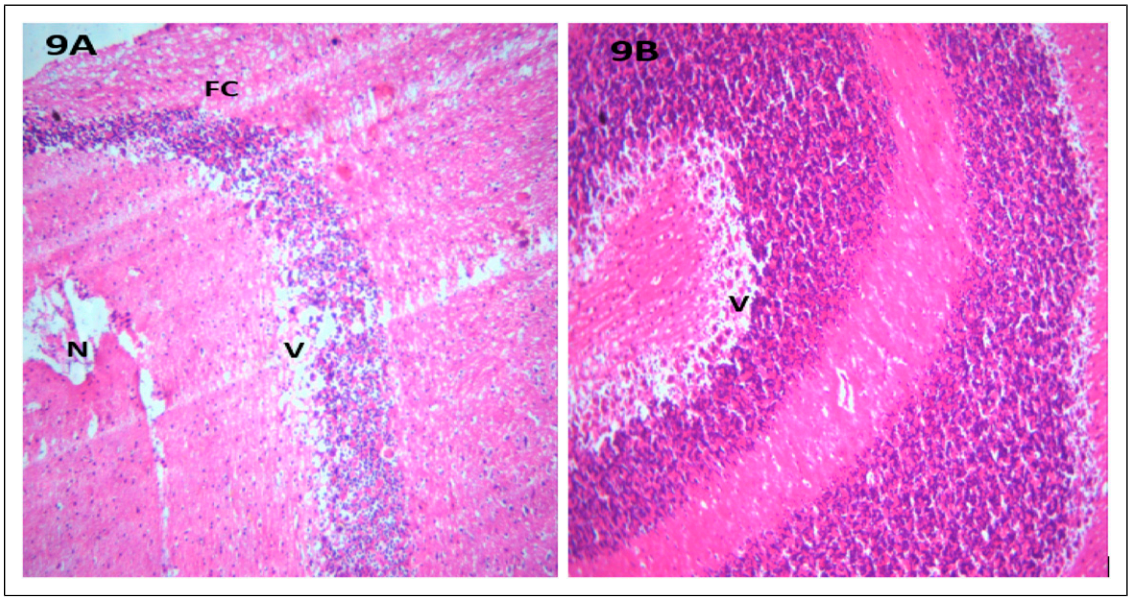

Figure 9. Photomicrograph of the brain of the group administered only tramadol (x100/400) (H/E). Severe degeneration with sever fatty change (FC) (steatosis), severe vacuolation (V) and necrosis (N) within the molecular layer and granular layer in 9A and 9B.

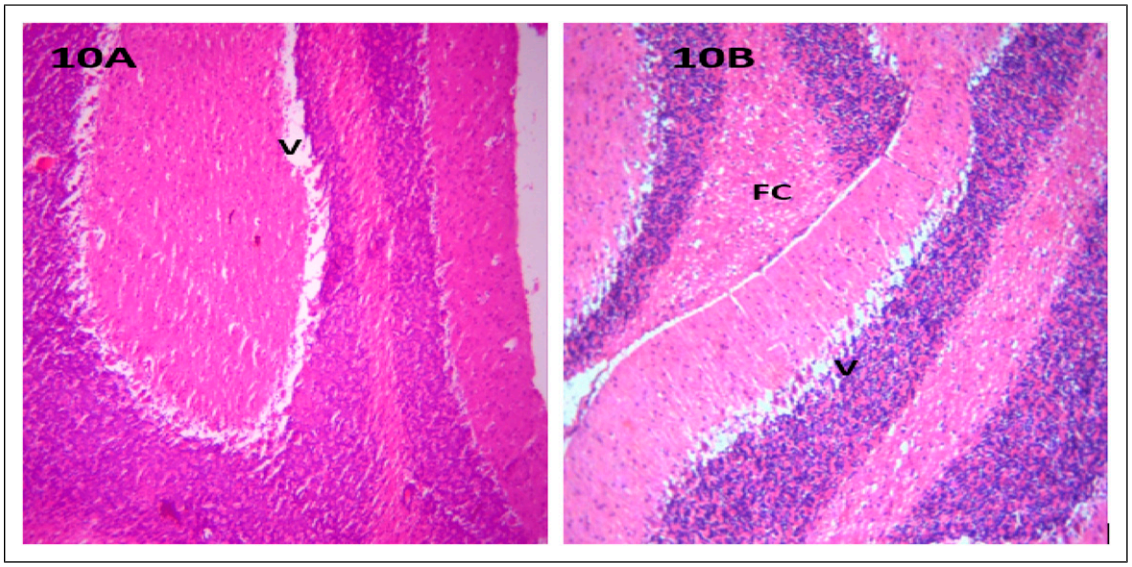

Figure 10. Photomicrograph of the brain of the group administered tramadol + 5 mL/Kg b.wt. CPSO (x100/400) (H/E). Moderate healing with mild fatty change (FC) (steatosis), mild vacuolation within the molecular layer and granular layer in 10B and mild vacuolation in 10A.

Discussion

Despite its effectiveness in managing moderate to severe pain, tramadol has been associated with damage of several tissues.[7] Additionally, chronic use of tramadol was linked to increased dyslipidemia and neuronal disorders.[14] The potential of CPSO to modulate the associated toxicities of chronic tramadol treatment on rats' brains and lipid profiles was evaluated in this study.

The result revealed a significant increase in GDH activity and a significant decrease in glutaminase activity in the brain following the administration of the 100 mg/Kg b.wt. Tramadol is about normal control as shown in Figures 1 and 2. A similar result was reported by Chetan[34] who revealed a significant increase in the activities of glutamate dehydrogenase (GDH), aspartate aminotransferase (AAT), and glutamine synthetase (GS) and a significant decrease in glutaminase activity in the brain following tramadol administration in albino rats when compared with the control rats. The result is expected as glutamate is the predominant excitatory amino acid neurotransmitter in the mammalian central nervous system acting on

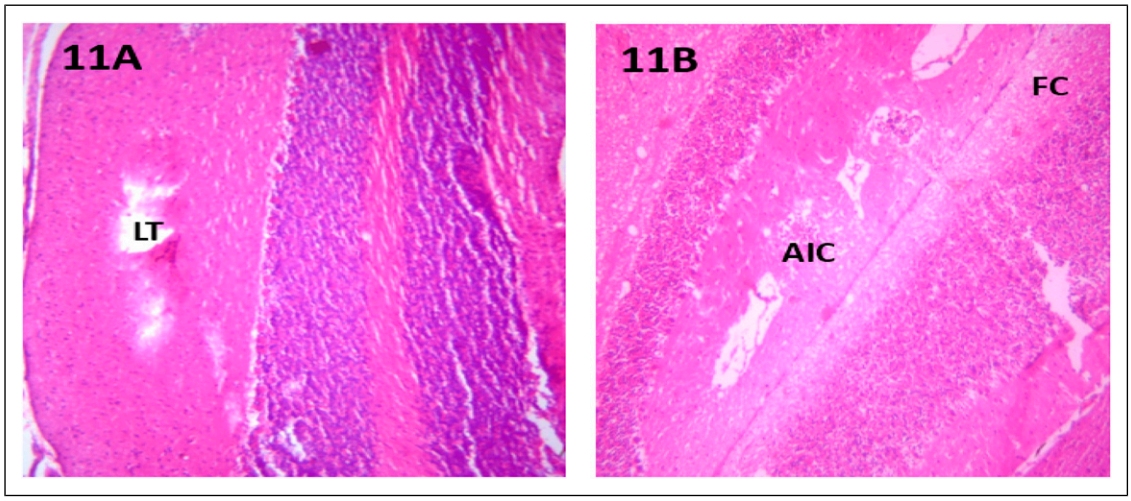

Figure 11. Photomicrograph of the brain of the group administered tramadol + 2.5 mL/Kg b.wt. CPSO (x100/400) (H/E). Mild to moderate healing with mild focal loss of tissue (LT) within the molecular layer in 11A and moderate aggregate of inflammatory cells (AIC) and mild fatty change (FC) within the molecular layer in 11B.

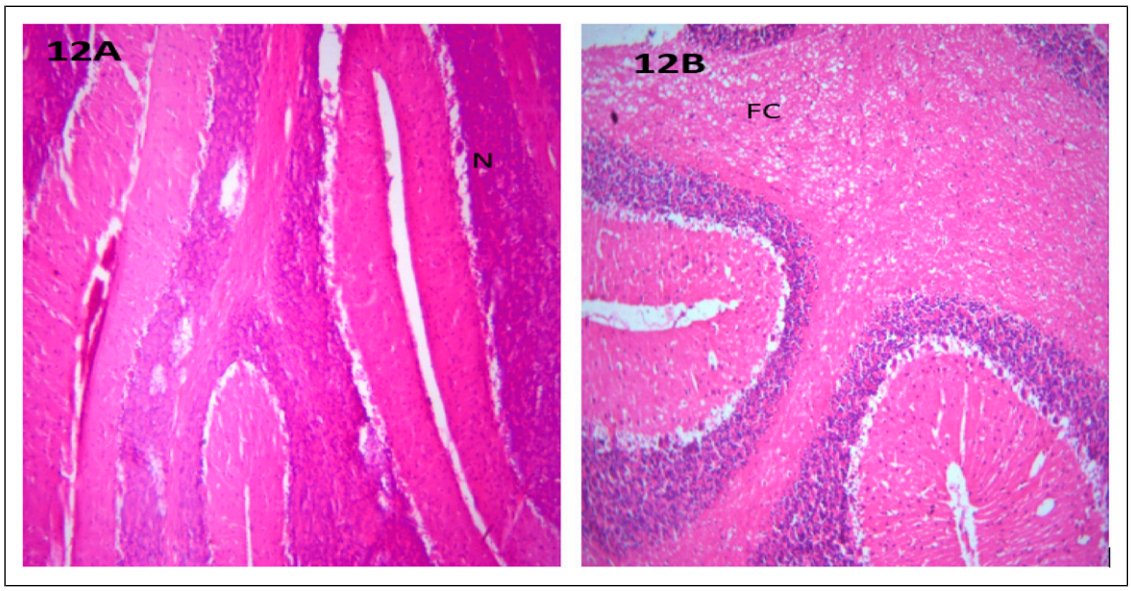

Figure 12. Photomicrograph of the brain of the group administered tramadol + 1.5 mL/Kg b. wt. CPSO) (x100/400) (H/E). Mild healing with moderate necrotic (N) appearance of the Purkinje layer with non-distinct Purkinje cells outline in 12A and moderate fatty change (FC) within the molecular layer in 12B.

N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and metabotropic receptors.[40] Several studies have demonstrated that the excitatory amino acid (EAA) receptor system is involved in the process of opioid dependence and chronic administration of opioid receptor agonists decreases the normal activation of NMDA receptors.[41–43] Glutamatergic transmission may have a role in learning and memory, central pain transduction, and alterations in the activities of key enzymes of glutamate metabolism that signify modulation in the turnover rates of glutamate in the brain; implicative of the pathophysiology of neuronal death after brain injury.[44,45]

With regards to the lipid profile, there was a significant decrease in serum levels of HDL-C, whilst a significant increase in the levels of TChol., LDL-C and triglyceride (TG) were observed in this study when the animals were administered only tramadol. This observation is in line with those of Ezzeldin,[46] Solin[47] and Barbosa.[48] These results suggests that tramadol administration caused disturbances in lipid metabolic pathways associated with pain and inflammation

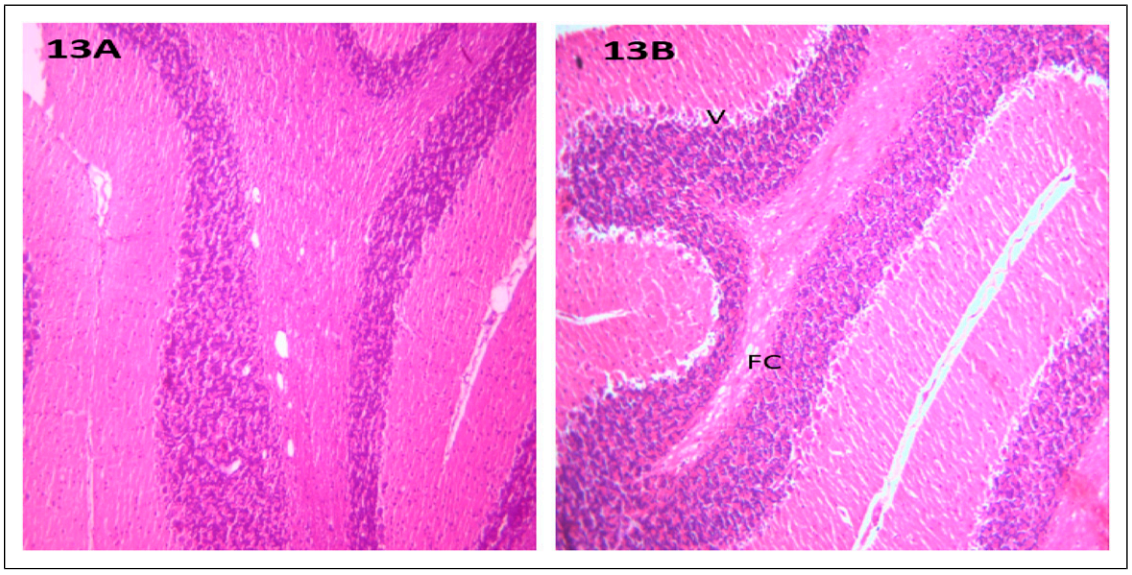

Figure 13. Photomicrograph of the brain of group 7 administered only 5 mL/Kg b.wt. CPSO (x100/400) (H/E). Mild vacuolation (V) and mild fatty change (FC) in observed in 13B.

consistent with the involvement of lipid in the synthesis of prostaglandins and modulation of other pain and inflammatory responses.[49–51] Atherosclerosis has been associated with inflammatory recruitment of modified lipids in the walls of the arteries.[52] Lipid peroxidation is a leading cause of the modification of lipids.[53] ROS-induced lipid peroxidation has been shown to be associated with modified LDL-C and glycation of HDL-C.[54,55] Thus, increasing the serum levels of cholesterol and other lipids of atherogenic tendencies.

CPSO treatment significantly decreased the activity of glutamine dehydrogenase and significantly increased the activity of glutaminase towards the value found in the normal control when compared to the group that received tramadol only without treatment. This result is consistent with various studies showing the potential use of herbs in the prevention of drug induced neurodegenerative disorders.[56] The results obtained from the lipid profile showed a significant increase in the serum HDL-C levels as well as a significant decrease in the LDL-C, TAG and TChol. levels in all the treated groups as compared to the group that received tramadol alone. Studies have shown a positive relationship between the generation of free radicals and lipids accumulation and most herbs with antioxidant potentials reduce the oxidization of LDL-C, increase the HDL-C levels by increasing the activities of the Lecithin Cholesterol Acyl Transferase (LCAT) enzyme and block the biosynthesis of cholesterol by inhibiting the HMG-COA reductase enzyme.[55,57–59] This result is supported by several studies indicating the anti-atherogenic potential of nutraceuticals and herbs as reported by Abuelgassim,[60] Cicero[61], and Qin.[62] These effects of the CPSO may be attributed to their antioxidant activity in scavenging the various reactive oxygen species including peroxynitrate radicals generated by tramadol toxicity. The antioxidant potential may be due to the presence of α-tocopherol, selenium, and other antioxidative phyto-compounds found in the oil.[63]

Microscopic examination of brain sections of control rats showed a normal brain with molecular layer (ML), granular layer (GL), and well-outlined Purkinje cells within the Purkinje layer when compared to the group that received tramadol only. The neurons of tramadol-administered animals showed extensive neuronal damage by severe degeneration of neurons with severe fatty change (FC) (steatosis), severe vacuolation (V), and necrosis (N) within the molecular layer and granular layer and moderate vacuolation in the tramadol-treated animals when compared to the control animals which showed normal brain upon microscopic examinations. These results agree with the finding of Bekheet[64] and Cannon,[65] whose studies have shown reduced Purkinje cell proliferation, cell differentiation, and increased Purkinje cell death as well as cerebellar-damaging effects of opioids including tramadol. Neurodegenerative features as seen in tramadol toxicity could be caused by the overproduction of mitochondrial reactive oxygen species causing oxidative stress on the neurons. Neurotoxic effects of opioids that caused cell death could be the result of mitochondrial damage in the brain. Another possible mechanism of opioids that could be responsible for the alteration of the Purkinje cells might be blockage of neuronal activity, causing the neurons to receive internal signals to self-destruct.[66] Administration of CPSO showed moderate healing with mild fatty change (FC) (steatosis), mild vacuolation within the molecular layer and granular layer together with mild vacuolation in the group that received tramadol alone. Generally, the histological results revealed that administration of CPSO protects the brain against tramadol-induced neurological disorders and this could be a result of some bioactive compounds such as hexadecenoic

acid in the oil which possesses anti-apoptotic and antioxidant properties.

Limitation of the Study

This study's limitations include a lack of crude oil extract pharmacokinetics and a cognition test on the animal model.

Conclusion

Following the results of the lipid profile, neuronal function enzymes, and microscopic examination of the histology of the brain obtained in this study, administration of *Cucurbita pepo* seed oil showed great potential in the prevention of dyslipidemia and neuronal dysfunction associated with tramadol-induced toxicity.

Acknowledgments

The authors are grateful for the efforts of the research assistants from the laboratory in which the work was performed.

Author Contributions

This research is collaborative work. Each of the outlined authors has made meaningful contributions as follows: **Conceptualization**: Aja, Patrick Maduabuchi, Ekpono Ezebuilo Ugbala, and Ibiam Udu Ama **Methodology**: Aja, Patrick Maduabuchi, Agu, Peter Chinedu, Ekpono Ezebuilo Ugbala **Validation**: Ifie Eseoghene Josiah, Ejike Daniel Eze. **Formal analysis**: Afodun Adam Moyosore; Okoye Osita Gabriel. **Investigation**: Ejike Daniel Eze, Ekpono Ezebuilo Ugbala, Ayomide Victor Atoki **Resources**: Aja, Patrick Maduabuchi, Ekpono Ezebuilo Ugbala, Agu, Peter C, Ifie Eseoghene Josiah, Ejike Daniel Eze, Okoye Osita Gabriel and Afodun Adam Moyosore. **Data Curation**: Aja, Patrick Maduabuchi, and Ekpono Ezebuilo Ugbala. **Writing - Original Draft**: Ekpono Ezebuilo Ugbala and Okoye Osita Gabriel. **Writing - Review & Editing**: Aja, Patrick Maduabuchi, Ayomide Victor Atoki, and Ifie Eseoghene Josiah. **Supervision**: Aja, Patrick Maduabuchi, Ibiam Udu Ama. **Project administration**: Aja, Patrick Maduabuchi and Ekpono, Ezebuilo Ugbala.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iDs

Patrick Maduabuchi Aja https://orcid.org/0000-0001-7805-9027
Ejike Daniel Eze https://orcid.org/0000-0002-9119-8870
Ayomide Victor Atoki https://orcid.org/0000-0003-1914-973X

Data Availability Statement

Data is available on request from the authors.

References

1. Pergolizzi JV, Taylor R, Raffa RB. Extended-release formulations of tramadol in the treatment of chronic pain. *Expet Opin Pharmacother*. 2011;12(11):1757-1768.
2. Pathan H, Williams J. Basic opioid pharmacology: an update. *Br J Pain*. 2012;6(1):11-16.
3. Bravo García L, Llorca Torralba M, Berrocoso Domínguez EM, Micó Segura JA. Monoamines as drug targets in chronic pain: Focusing on neuropathic pain. *Front Neurosci*. 2019;13:1268.
4. Raj K, Chawla P, Singh S. Neurobehavioral consequences associated with long term tramadol utilization and pathological mechanisms. *CNS Neurol Disord: Drug Targets*. 2019;18(10):758-768.
5. Moulis F, Rousseau V, Abadie D, et al. Serious adverse drug reactions with tramadol reported to the French pharmacovigilance database between 2011 and 2015. *Therapie*. 2017;72(6):615-624.
6. Arafa MH, Atteia HH. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6) are associated with long term tramadol treatment-induced oxidative damage and hepatotoxicity. *Toxicol Appl Pharmacol*. 2018;346:37-44.
7. Ibrahim MA, Ibrahim HM, Mohamed AA, Tammam HG. Vitamin E supplementation ameliorates the hepatotoxicity induced by Tramadol: toxicological, histological and immunohistochemical study. *Toxicol Mech Methods*. 2020;30(3):177-188.
8. Hansen CA, Ernst MT, Stougaard M, Abrahamsen B. Tramadol prescribed use in general and chronic noncancer pain: a nationwide register-based cohort study of all patients above 16 years. *Scand J Pain*. 2020;20(1):109-124.
9. Hansen CA, Inacio MCS, Pratt NL, Roughead EE, Graves SE. Chronic use of opioids before and after total knee arthroplasty: a retrospective cohort study. *J Arthroplasty*. 2017;32(3):811-817.
10. Peprah P, Agyemang-Duah W, Appiah-Brempong E, Akwasi AG, Morgan AK. "With tramadol, I ride like a Jaguar": a qualitative study of motivations for non-medical purpose tramadol use among commercial vehicle operators in Kumasi, Ghana. *Subst Abuse Treat Prev Pol*. 2020;15:49.
11. Wise RA, Robble MA. Dopamine and addiction. *Annu Rev Psychol*. 2020;71(1):79-106.
12. Zeng XS, Geng WS, Wang ZQ, Jia JJ. Morphine addiction and oxidative stress: the potential effects of thioredoxin-1. *Front Pharmacol*. 2020;11:82.
13. Dikalov S, Itani H, Richmond B, et al. Vascular Biology and Microcirculation: tobacco smoking induces cardiovascular mitochondrial oxidative stress, promotes endothelial dysfunction, and enhances hypertension. *Am J Physiol Heart Circ Physiol*. 2019;316(3):H639-H646.
14. Musich S, Wang SS, Schaeffer JA, Slindee L, Kraemer S, Yeh CS. Safety events associated with tramadol use among older adults with osteoarthritis. *Popul Health Manag*. 2021;24(1):122-132.

15. Konukoglu D, Uzun H. Endothelial dysfunction and hypertension. In: *Hypertension: From Basic Research to Clinical Practice*. Cham: Springer; 2017:511-540.
16. Chen Q, Wang Q, Zhu J, Xiao Q, Zhang L. Reactive oxygen species: key regulators in vascular health and diseases. *Br J Pharmacol*. 2018;175(8):1279-1292.
17. Alhayaza R, Haque E, Karbasiafshar C, Sellke FW, Abid MR. The relationship between reactive oxygen species and endothelial cell metabolism. *Front Chem*. 2020;8:592688.
18. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol*. 2014;4:177.
19. Fjær EL, Landet ER, McNamara CL, Eikemo TA. The use of complementary and alternative medicine (CAM) in Europe. *BMC Complement Med Ther*. 2020;20:108-109.
20. Krimer-Malešević V. Pumpkin seeds: phenolic acids in pumpkin seed (*Cucurbita pepo* L.). In: *Nuts and Seeds in Health and Disease Prevention*. Amsterdam, Netherlands: Elsevier; 2020:533-542.
21. Kulczyński B, Sidor A, Gramza-Michałowska A. Antioxidant potential of phytochemicals in pumpkin varieties belonging to *Cucurbita moschata* and *Cucurbita pepo* species. *CyTA-Journal of Food*. 2020;18(1):472-484.
22. Kulczyński B, Gramza-Michałowska A. The profile of carotenoids and other bioactive molecules in various pumpkin fruits (*Cucurbita maxima* Duchesne) cultivars. *Molecules*. 2019; 24(18):3212.
23. Dotto JM, Chacha JS. The potential of pumpkin seeds as a functional food ingredient: a review. *Sci Afr*. 2020;10:e00575.
24. El-Mosallamy AEMK, Sleem AA, Abdel-Salam OME, Shaffie N, Kenawy SA. Antihypertensive and cardioprotective effects of pumpkin seed oil. *J Med Food*. 2012;15(2):180-189.
25. Batool M, Ranjha MMAN, Roobab U, et al. Nutritional value, phytochemical potential, and therapeutic benefits of pumpkin (*Cucurbita sp*.). *Plants*. 2022;11(11):1394.
26. Mathangi S. A study on extraction of oil from Pumpkin seed using sun drying and hot air oven drying. *Int J Food Sci Nutr*. 2018;3(1):34-36.
27. Nwozo SO, Ukeje MO, Ifie JE. The radio-protective effects of n-Hexane extracts of *Telfairia occidentalis* Hook. f. and *Cucumeropsis mannii* Naud. seed oils on the liver of irradiated male Wistar rats. *Plant Sci Today*. 2020;7(3):432-440.
28. Agu PC, Aja PM, Ugbala EE et al. *Cucumeropsis mannii* seed oil (CMSO) attenuates alterations in testicular biochemistry and histology against Bisphenol-induced toxicity in male Wister albino rats. *Heliyon*. 2022;8(2022):e09162.
29. Aja PM, Chiadikaobi CD, Agu PC et al. *Cucumeropsis mannii* seed oil ameliorates Bisphenol-A-induced adipokines dysfunctions and dyslipidemia. *Food Sci Nutr*. 2023;11(6): 2642-2653.
30. 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.
31. 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.
32. Tamaddonfard E, Farshid AA, Asri-Rezaee S, et al. Crocin improved learning and memory impairments in streptozotocin-induced diabetic rats. *Iran J Basic Med Sci*. 2013;16(1):91-100.
33. Bandegi AR, Rashidy-Pour A, Vafaei AA, Ghadrdoost B. Protective effects of *Crocus sativus* L. extract and crocin against chronic-stress induced oxidative damage of brain, liver and kidneys in rats. *Adv Pharmaceut Bull*. 2014;4(Suppl 2):493-499.
34. Chetan PS, Sangeetha R, Mohan PM, Rajendra W. Alterations in glutamate metabolism in rat brain by tramadol analgesia during non-induction of pain. *Saudi J Med Pharm Sci* 2015;1:26-36.
35. Abell LL, Levy BB, Brodie BB, Kendall FE. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J Biol Chem*. 1952;195(1):357-366.
36. Sardesai VM, Manning JA. The determination of triglycerides in plasma and tissues. *Clin Chem*. 1968;14(2):156-161.
37. Albers JJ, Warnick GR, Chenng MC. Quantitation of high density lipoproteins. *Lipids*. 1978;13(12):926-932.
38. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972; 18(6):499-502.
39. Bancroft JD, Gamble M. *Theory and Practice of Histological Techniques*. Amsterdam, Netherlands: Elsevier Health Sciences; 2008.
40. Zhou Y, Danbolt NC. Glutamate as a neurotransmitter in the healthy brain. *J Neural Transm*. 2014;121:799-817.
41. Kosten TR, George TP. The neurobiology of opioid dependence: implications for treatment. *Sci Pract Perspect*. 2002;1(1):13-20.
42. Chen YF, Lee MM, Fang HL, Yang JG, Chen YC, Tsai HY. Paeoniflorin inhibits excitatory amino acid agonist-and high-dose morphine-induced nociceptive behavior in mice via modulation of N-methyl-D-aspartate receptors. *BMC Compl Alternative Med*. 2016;16:240.
43. Singh AK. Alcohol interaction with cocaine, methamphetamine, opioids, nicotine, cannabis, and γ-hydroxybutyric acid. *Biomedicines*. 2019;7(1):16.
44. Crupi R, Impellizzeri D, Cuzzocrea S. Role of metabotropic glutamate receptors in neurological disorders. *Front Mol Neurosci*. 2019;12:20.
45. Zhang Y, Chu JMT, Wong GTC. Cerebral glutamate regulation and receptor changes in perioperative neuroinflammation and cognitive dysfunction. *Biomolecules*. 2022;12(4):597.
46. Ezzeldin E, Souror WAH, El-Nahhas T, Soudi ANMM, Shahat AA. Biochemical and neurotransmitters changes associated with tramadol in streptozotocin-induced diabetes in rats. *BioMed Res Int*. 2014;2014(1):238780.
47. Solin AV, Lyashev AY, Lyashev YD. Effects of opioid peptides on changes in lipid metabolism in rats subjected to swimming stress. *Bull Exp Biol Med*. 2017;162:313-315.
48. Barbosa J, Faria J, Garcez F, et al. Repeated administration of clinical doses of tramadol and tapentadol causes hepato-and nephrotoxic effects in Wistar rats. *Pharmaceuticals*. 2020;13(7):149.

49. Piomelli D, Hohmann AG, Seybold V, Hammock BD. A lipid gate for the peripheral control of pain. *J Neurosci*. 2014;34(46): 15184-15191.
50. Chiurchiù V, Leuti A, Maccarrone M. Bioactive lipids and chronic inflammation: managing the fire within. *Front Immunol*. 2018;9:38.
51. Cas MD, Roda G, Li F, Secundo F. Functional lipids in autoimmune inflammatory diseases. *Int J Mol Sci*. 2020;21(9):3074.
52. Lorey MB, Öörni K, Kovanen PT. Modified lipoproteins induce arterial wall inflammation during atherogenesis. *Front Cardiovasc Med*. 2022;9:841545.
53. Shao B, Heinecke JW. HDL, lipid peroxidation, and atherosclerosis. *J Lipid Res*. 2009;50(4):599-601.
54. Hedrick CC, Thorpe SR, Fu MX, et al. Glycation impairs high-density lipoprotein function. *Diabetologia*. 2000;43:312-320.
55. Quiles JL, Mesa MD, Ramírez-Tortosa CL, et al. *Curcuma longa* extract supplementation reduces oxidative stress and attenuates aortic fatty streak development in rabbits. *Arterioscler Thromb Vasc Biol*. 2002;22(7):1225-1231.
56. Bagheri SM, Rezvani ME, Vahidi AR, Esmaili M. Anticonvulsant effect of *Ferula assa-foetida* oleo gum resin on chemical and amygdala-kindled rats. *N Am J Med Sci*. 2014; 6(8):408-412.
57. Galle M, Kladniew BR, Castro MA, et al. Modulation by geraniol of gene expression involved in lipid metabolism leading to a reduction of serum-cholesterol and triglyceride levels. *Phytomedicine*. 2015;22(7-8):696-704.
58. Ganjali S, Blesso CN, Banach M, Pirro M, Majeed M, Sahebkar A. Effects of curcumin on HDL functionality. *Pharmacol Res*. 2017;119:208-218.
59. Shi K, Gao Z, Shi TQ, et al. Reactive oxygen species-mediated cellular stress response and lipid accumulation in oleaginous microorganisms: the state of the art and future perspectives. *Front Microbiol*. 2017;8:793.
60. Abuelgassim AO, Al-Showayman SIA. The effect of pumpkin (*Cucurbita Pepo* L) seeds and L-arginine supplementation on serum lipid concentrations in atherogenic rats. *Afr J Tradit, Complementary Altern Med*. 2012;9(1):131-137.
61. Cicero AFG, Colletti A, Bajraktari G, et al. Lipid-lowering nutraceuticals in clinical practice: position paper from an International Lipid Expert Panel. *Nutr Rev.* 2017;75(9):731-767.
62. Qin S, Huang L, Gong J, et al. Efficacy and safety of turmeric and curcumin in lowering blood lipid levels in patients with cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Nutr J*. 2017;16:68.
63. Rouag M, Berrouague S, Djaber N, et al. Pumpkin seed oil alleviates oxidative stress and liver damage induced by sodium nitrate in adult rats: biochemical and histological approach. *Afr Health Sci*. 2020;20(1):413-425.
64. Bekheet SH, Saker SA, Abdel-Kader AM, Younis AEA. Histopathological and biochemical changes of morphine sulphate administration on the cerebellum of albino rats. *Tissue Cell*. 2010;42(3):165-175.
65. Cannon CZ, Kissling GE, Hoenerhoff MJ, King-Herbert AP, Blankenship-Paris T. Evaluation of dosages and routes of administration of tramadol analgesia in rats using hot-plate and tail-flick tests. *Lab Anim (NY)*. 2010;39(11):342-351.
66. Farber NB, Olney JW. Drugs of abuse that cause developing neurons to commit suicide. *Brain Res Dev Brain Res*. 2003; 147(1-2):37-45.