Asian Journal of Food Research and Nutrition

Volume 3, Issue 4, Page 943-955, 2024; Article no.AJFRN.123544

Assessments of Neuro-protective and Antioxidant Activities of *Curcuma longa* (Linn) and *Zingiber officinale* (Roscoe)-Supplemented Feed in Tramadol-exposed Male Wistar Rats

Alice Boluwatife Oyelude [a], Temitope Deborah Olaniyi [a*] and Adewale Adetutu [a]

[a] Department of Biochemistry, Natural Products and Phytomedicine Unit, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Oyo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/123544

Original Research Article

Received: 25/07/2024
Accepted: 28/09/2024
Published: 08/10/2024

ABSTRACT

Aim: This study aimed to investigate the protective effects of *Zingiber officinale Roscoe* (ginger) and *Curcuma longa* Linn(turmeric) on tramadol-induced neurotoxicity.
Study Design: Twenty male Wistar rats, with an average weight of 130 g, were randomly divided into four groups (n=5 per group). Group 1 served as the control and was fed normal feed, Group 2 received tramadol hydrochloride at a dose of 25mg/kg body weight and fed normal feed while

**Corresponding author: E-mail: tdolaniyi90@lautech.edu.ng;*

Cite as: *Oyelude, Alice Boluwatife, Temitope Deborah Olaniyi, and Adewale Adetutu. 2024. "Assessments of Neuro-Protective and Antioxidant Activities of Curcuma Longa (Linn) and Zingiber Officinale (Roscoe)-Supplemented Feed in Tramadol-Exposed Male Wistar Rats". Asian Journal of Food Research and Nutrition 3 (4):943-55. https://www.journalajfrn.com/index.php/AJFRN/article/view/187.*

Groups 3 and 4 were treated with tramadol and fed *Z. officinale* and *C. longa* (10% w/w supplemented feed) respectively. Treatments were administered daily for 90 days.

Place and Duration of Study: The study was conducted in the Laboratory of the Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, from March 2022 to January 2023.

Methodology: At the end of the treatment period, animals were sacrificed by cervical dislocation and blood samples were collected via cardiac puncture. The brains were harvested for biochemical assays and histological analysis. Superoxide dismutase (SOD) activity, reduced glutathione (GSH), and malondialdehyde (MDA) concentrations were determined by spectrophotometry, while nitric oxide concentration was measured using a colourimetric method. Additionally, the levels of 8-hydroxyl deoxyguanosine (8-OHdG) and dopamine were quantified by ELISA, and monoamine oxidase (MAO), gamma-aminobutyric acid (GABA), and acetylcholinesterase (AChE) activities were measured spectrophotometrically. Histological examination of the brain tissue was conducted using Hematoxylin and Eosin (H&E) staining. Data were subjected to one-way analysis of variance (ANOVA) using GraphPad Prism 5 statistical software to compare the difference among the groups.

Results: The results indicated a significant decrease (P<0.5) in SOD activity (.69±.037 to .37±0.51 U/mol) and GSH concentration (.76±.029 to .29±.035 mM), coupled with a significant increase (P<0.5) in levels of MDA (29.24±.60 to 57.23±1.64 µM), nitric oxide (from 8.25±.47 to 16.25±.75 µM), 8-OHdG (9.20±.37 to 14.47±.61 ng/ml), dopamine (55.74±0.57 to 72.90±2.94 pg/ml), MAO (1.59±0.02 to 4.90±0.27 U/g protein), and AChE (.29±0.04 to 1.50±0.29 U/ml) activities in the tramadol-treated group compared to the control group. Additionally, there was a significant depletion in GABA levels (73.90±2.54 to 40.13±1.51 pg/ml) in the tramadol group. Histological analysis revealed degenerative changes in the neurons of tramadol-treated rats. However, supplementation of feed with *Z. officinale* and *C. longa* significantly prevented these damaging biochemical changes, preserving neuronal structure and function.

Conclusion: The study demonstrated that *Z. officinale* and *C. longa* offer neuroprotection against tramadol toxicity by preserving key neural biochemistry involving acetylcholine esterase, monoamine oxidase, dopamine and gamma-aminobutyric acid via potent antioxidant properties. These findings suggest potential therapeutic benefits of these natural supplements in mitigating the adverse effects of tramadol on the brain.

Keywords: Brain; Zingiber officinale; curcuma longa; 8-OHDG; dopamine; tramadol.

1. INTRODUCTION

Food is any material necessary for sustaining life, supplying essential nutrients, supporting metabolic activities, energy production, and maintaining the body. Beyond this, some food substances contain ingredients that confer additional benefits, especially relating to health. Such food is classified as functional food. Simply put, functional foods are foods that are specially formulated to contain precisely safe amounts of health-preserving substances [1]. Due to the health benefits of spices, they are being focused on in the development of novel functional foods. Although not consumed alone as foods, spices are an important part of many dishes globally, where they perform functions such as improving flavour, add colour and aiding digestion [2]. Beyond their culinary uses, herbs and spices have long been recognized for their medicinal properties and preservative effects [3]. Due to the potent antioxidant activity of several spices and their beneficial effects on human health, there has been significant research interest in these natural products across various countries [4,5]. Previous studies have demonstrated that spices such as cinnamon, garlic, and curry leaves possess hepatoprotective, nephroprotective, and neuroprotective properties [6,7,8]. In Nigeria, *Curcuma longa* Linn (*C. longa*) and *Zingiber officinale* Roscoe (*Z. officinale*), commonly known as turmeric and ginger respectively, both belonging to the family Zingiberaceae, are among the widely-used spices both as food and as medicine. In Pakistan, *C. longa* is traditionally taken as a folk medicine for conditions such as acne and wound healing [9]. *C. longa* is used in traditional medicine as a preservative, tonic, and antiseptic. It is believed to aid in the healing of birth canal lesions when its poultice is applied to the perineum and is also used to treat digestive problems, including ulcers, acid reflux, and upper abdominal pain. Chinese women apply fresh rhizome paste to their skin to treat infections and improve skin appearance [10]. Additionally, C.

longa rhizome is used in animal husbandry to treat cattle with loose stools. It serves as a colorant, antibacterial, and anti-inflammatory agent, and is added to various products such as curries, tea, cosmetics, and beverages. Moreover, it is utilized as a preservative and colouring agent in cheese, butter, mustard sauce, and chips [11]. *Z. officinale* is also widely recognized for its medicinal properties. It is commonly used as a stimulant and to treat fevers, malaria, and dyspepsia. Ginger stimulates the secretion of gastric juice and enhances appetite [12]. It has been reported to treat a variety of conditions, including, anorexia, arthritis, bleeding, cancer, chest congestion, chickenpox, cholera, chronic bronchitis, colic, colitis, common cold, cough, cystic fibrosis, diarrhoea, breathing difficulties, dropsy, flatulence, indigestion, gallbladder disorders, nausea, rheumatism, sore throat, stomach ache, bloating, vomiting, hypertension and dementia [13,14].

Given that the major pharmacological benefit of ginger and turmeric lies in their antioxidant capacities, they are of great potential as antidotes or adjuvants in the management or treatment of free-radical-mediated conditions [15]. One major biochemical path through which excess free radicals are generated is drug metabolism and drug abusers stand a risk of degenerative organ damage. Tramadol, an opioid pain killer is one of the second most abused drug in Nigeria, after cannabis [16]. Increase in abuse of tramadol have been reported in many regions of Africa [17]. With evidence to prove that tramadol exerts neurotoxicity via excessive generation of free radicals [18], We postulate that the inclusion of antioxidant-rich substances in the diet may offer some protection against tramadol-induced damage. Owing to the strong antioxidant capacity of *C. longa* and *Z. officinale,* this study investigated the protective roles of *C. longa* and *Z. officinale* inclusion in daily diet on the brain of male Wister rats using selected antioxidants parameters as well as some brain function indices.

2. MATERIALS AND METHODS

2.1 Place and Duration of Study

The study was conducted in the Laboratory of the Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria from March 2022 to January 2023.

2.2 Collection and Preparation of Rhizomes

Rhizomes of *Curcuma longa* and *Zingiber officinale* were purchased from an open market in Ogbomoso North Local Government and were authenticated by a botanist at the Department of Pure and Applied Biology, LAUTECH, Ogbomoso. The spices were washed under running water to remove sand and other debris, cut into smaller pieces, air-dried to a constant weight, and then pulverized into a fine powder using a blender.

2.3 Formulation of feed for the study

The powder form of *Z. officinale* and *C. longa* were added to separate feed formulae up to 10%w/w as presented in Tables 1 and 2 respectively [19,20].

Table 1. Proportion of the rat feed components with *Z. officinale*

Composition	Quantity in %w/w
Maize	40.0
Wheat offal	10.0
Soya meal	12.0
Corn bran	11.2
Groundnut cake	8.0
GPKC	8.0
Salt	0.2
Premix broiler	0.2
Lysine	0.2
Methionine	0.2
Z. officinale	10.0

Table 2. Proportion of the rat feed components with *C. longa*

Composition	Quantity in %w/w
Maize	40.0
Wheat offal	10.0
Soya meal	12.0
Corn bran	11.2
Groundnut cake	8.0
GPKC	8.0
Salt	0.2
Premix broiler	0.2
Lysine	0.2
Methionine	0.2
C. longa	10.0

2.4 Chemicals and Reagents Used

Kits for reduced glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), nitric oxide (NO), 8-hydroxyl deoxyguanosine (8-OHdG), acetylcholine esterase (ACHE), mono anime oxidase (MAO), dopamine, and gamma-aminobutyric acid (GABA) were sourced from Sigma USA and Chemelex Barcelona, Spain. Other reagents used were of analytical grade.

2.5 Experimental Animals

Twenty male Wistar albino rats, each weighing between 120 g and 150 g, were sourced from a commercial breeder in Ogbomoso. The animals were housed in plastic cages within the Biochemistry Department at Ladoke Akintola University, Ogbomoso, under controlled environmental conditions. They were provided with animal pellets and distilled water and were maintained on a 12-hour light and 12-hour dark cycle. The rats were allowed to acclimatize for two weeks before the start of the experiment. The study adhered to the "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985).

Experimental design: Twenty male Wistar rats, each with an average weight of 130 g, were randomly assigned to four groups of five rats each and housed together in cages. The rats received tramadol hydrochloride at a dose of 25 mg/kg of body weight daily for 90 days. The groups were treated as follows: Group 1 (rats were fed a feed that was not supplemented with either of the two spices, did not receive tramadol and was designated as Control), Group 2 (rats were fed a feed that was not supplemented with either of the two spices, received tramadol at 25 mg/Kg body weight), Group 3 (rats were feed 10% w/w Z. *officinale*-supplemented feed and received tramadol at 25 mg/Kg body weight), and Group 4 (rats were fed 10% w/w C. *longa*-supplemented feed and received tramadol at 25 mg/Kg body weight). Tramadol was administered orally for 90 days.

2.6 Sample Collection and Preparation

At the end of 90 days of tramadol administration, rats were sacrificed by cervical dislocation after an overnight fast of 10-12 hours. Dissection was performed using a dissecting blade, and blood samples were collected directly from the heart via cardiac puncture. The blood was transferred into appropriately labelled plain sample bottles and centrifuged at 4000×g for 10 minutes to obtain serum. The brain was quickly removed, rinsed with phosphate-buffered saline (4°C, pH 7.4, 0.1M) to remove any residual blood, and then stored at -20°C until further analysis. Brain homogenate was prepared as described by Olaniyi et al. (2021), A portion of the brain (0.5 g) was homogenized in 5 mL of phosphate buffer saline (PBS) using a tissue homogenizer. The homogenate was centrifuged at 4°C using cold centrifuge at 3000 rpm for 15 minutes. The supernatants were collected and used for biochemical assays [21].

2.7 Determination of Oxidative Status

The activities of antioxidant enzymes, including Superoxide Dismutase (SOD), were measured as described by [22,23]. Concentrations of reduced glutathione (GSH) were assessed following the method outlined by [22]. Malondialdehyde (MDA) levels were determined according to [24]. Nitric oxide (NO) was measured based on the protocol provided by [25]. The levels of 8-hydroxy-2-deoxyguanosine (8-OHdG) were quantified using the ELISA method.

2.8 Estimation of Monoamine Oxidase

All reagents were prepared and mixed thoroughly before use. Each sample was assayed in triplicate. A volume of 2.50 µL of each sample (H_2O_2 standard, control, or sample) was added to individual microtiter plate wells. For wells containing H_2O_2 standards and samples without inhibitor, 5 µL of Assay Buffer was also added. The contents of each well were mixed thoroughly by pipetting or using a horizontal shaker and incubated for 30 minutes at room temperature to allow the inhibitor to react with the enzyme.

Following the incubation, 50 µL of Assay Working Solution was added to each well. The contents were mixed thoroughly and incubated for 45-60 minutes at room temperature, protected from light. After incubation, the plate was read using a spectrophotometric microplate reader set to the 540-570 nm range [26].

$$\text{MAO Activity } \left(\frac{\text{Units}}{\text{L}}\right) = \frac{(\text{H}_2\text{O}_2 \text{ generated})}{\text{Reaction time (minutes)}} \times \text{Sample dilution}$$

2.9 Determination of Dopamine Level

The wells for diluted standards, blanks, and samples were labelled. To each appropriate well, 100 µL of the diluted standard, blank, or sample was added, with all samples and standards assayed in duplicate. The plate was covered with the provided sealer and incubated for 90 minutes at 37°C. After incubation, the liquid from each well was decanted without washing.

Next, 100 µL of Biotinylated Detection Antibody working solution was immediately added to each well. The plate was covered with a new sealer and incubated for 1 hour at 37°C. The solution from each well was then decanted, and 350 µL of wash buffer was added to each well. The wells were soaked for 1 minute, then aspirated or decanted, and patted dry against clean absorbent paper. This washing step was repeated three times.

Following the wash steps, 100 µL of HRP Conjugate working solution was added to each well. The plate was covered with a new sealer and incubated for 30 minutes at 37°C. After incubation, the solution from each well was decanted, and the wash process was repeated five times as described previously. Substrate reagent was then added to each well, the plate was covered with a new sealer, and incubated for about 15 minutes at 37°C, protected from light.

Before optical density (OD) measurement, the microplate reader was preheated for approximately 15 minutes. 50 µL of Stop Solution was added to each well in the same order as the substrate solution. The OD of each well was then determined immediately using a microplate reader set to 450 nm.

2.10 Estimation of Gamma-aminobutyric Acid Concentration

All reagents and samples were equilibrated to room temperature before use. Samples, controls, and standards were assayed in duplicate. An assay layout sheet was consulted to determine the number of wells required. The remaining wells, along with the desiccant, were returned to their pouch, which was then sealed in a Ziploc bag, and unused wells were stored at 2-8°C.

A blank well was prepared with Standard Diluent Buffer. To each well, 50 µL of the standard or sample was added, followed by 50 µL of Biotinylated Conjugate (1x). The contents were mixed thoroughly, covered with the provided adhesive films, and incubated for 1 hour at 37°C. After incubation, each well was aspirated and washed three times with Wash Buffer (200 µL per well) using a squirt bottle. Excess Wash Buffer was removed by aspiration or decanting, and the plate was inverted and blotted against clean paper towels.

Next, 100 µL of Streptavidin-HRP (1x) was added to each well, which was then covered with adhesive films and incubated for an additional hour at 37°C. After incubation, wells were aspirated and washed five times as described previously. The remaining Wash Buffer was removed, and the plate was inverted and blotted against clean paper towels.

Following this, 90 µL of Substrate Solution was added to each well and incubated for 20 minutes at 37°C, avoiding drafts and direct light. When the first four wells with the highest standard concentrations developed an obvious blue colour, 50 µL of Stop Solution was added to each well. If colour development was uneven, the plate was gently tapped to ensure thorough mixing. Optical density was measured within 5 minutes using a microplate reader set to 450 nm. To correct for optical imperfections, readings at 540 nm were subtracted from those at 450 nm.

2.11 Estimation of Acetylcholinesterase

To measure acetylcholinesterase (AChE) activity in brain homogenate, 0.4 ml of the sample was combined with 2.6 ml of phosphate buffer (0.1 M, pH 7.4), 0.1 ml of Ellman's reagent (DTNB), and 0.1 ml of acetylcholine iodide solution. The absorbance was measured at 412 nm using a spectrophotometer, with readings taken every 2 minutes for a total of 10 minutes. The molar extinction coefficient was 1.361 x 10^3 mmol^-1 x mm^-1. AChE activity was determined by monitoring the increase in yellow colour resulting from the reaction of thiocholine with DTNB [27]. The change in absorbance per minute was then calculated using the formula:

$$\text{AChE activity} = \frac{\Delta \text{ A X Total reaction vol X 1}}{\text{Time X sample Vol X extinction}}$$

AChE activity = U/ml.

2.12 Histological Examination

The brain section was stained with Harris hematoxylin for 5 minutes and then rinsed in water. Following this, the section was briefly differentiated in 1% acid alcohol, then further rinsed under tap water for 10 minutes. It was counterstained in 1% aqueous eosin for 3 minutes, rinsed again in water, and dehydrated through ascending grades of alcohol (70%, 80%, 90%, and absolute). The dehydrated section was cleared in xylene and subsequently mounted with a DPX mountant. Lesions in the brain were observed under a light microscope and interpreted by an expert.

2.13 Statistical Analysis

All data presented were presented as mean ± SEM. The mean of different treatment groups tested for significance using one-way analysis of variance (ANOVA) and were compared using the Tukey test. Differences were considered significant at P <.05.

3. RESULTS AND DISCUSSION

3.1 Effects of *Z. officinale* and *C. longa* on Redox Status of Rats Exposed to Tramadol

To assess the impact of *Z. officinale* and *C. longa* on the redox status of rats exposed to tramadol, several oxidative stress biomarkers were evaluated, as presented in Fig. 1. The administration of tramadol resulted in a significant reduction in the activity of superoxide dismutase (SOD) and the concentration of reduced glutathione (GSH) in the brain homogenates. However, in the groups that were fed with *Z. officinale* and *C. longa*-supplemented feed, both SOD activity and GSH concentration were significantly increased, suggesting a protective effect of these supplements against tramadol-induced oxidative stress.

Conversely, levels of malondialdehyde (MDA), nitric oxide, and 8-hydroxyl deoxyguanosine (8-OHdG) were significantly elevated in the tramadol-treated group compared to the control group, indicating heightened oxidative stress. In contrast, these markers were significantly reduced in the groups that received *Z. officinale* and *C. longa* supplementation, further highlighting the antioxidative properties of these compounds in mitigating the oxidative damage induced by tramadol.

Tramadol is a potent analgesic widely prescribed for managing both acute and chronic pain. In the present study, tramadol exposure significantly reduced the activity of the antioxidant enzyme superoxide dismutase (SOD) and the concentration of reduced glutathione (GSH) in the brain. This reduction may be attributed to the depletion of these antioxidants due to the oxidative stress induced by tramadol. The decrease in GSH concentration could also be related to the impaired ability of cells to replenish GSH during heightened oxidative stress [28,29]. Malondialdehyde (MDA) is a marker of lipid peroxidation and an indicator of oxidative stress. Our study demonstrated increased MDA levels in the brain, which aligns with tramadol-induced oxidative damage reported in previous studies [30,31]. However, supplementation with *C. longa* and *Z. officinale* maintained MDA levels, suggesting that these supplements enhanced the antioxidant defences, as observed in similar research [32]. Nitric oxide (NO) can exert cytotoxic effects depending on the redox status of the cells. Under conditions of oxidative stress, NO can react with superoxide radicals to form peroxynitrite, a potent oxidant that can damage proteins and DNA [33]. In this study, we observed a significant increase in NO levels in the brain homogenate of tramadol-treated rats, consistent with its role in oxidative stress-mediated damage [34]. However, supplementation with *Z. officinale* and *C. longa* reduced NO levels, likely due to their antioxidant properties [35,36].

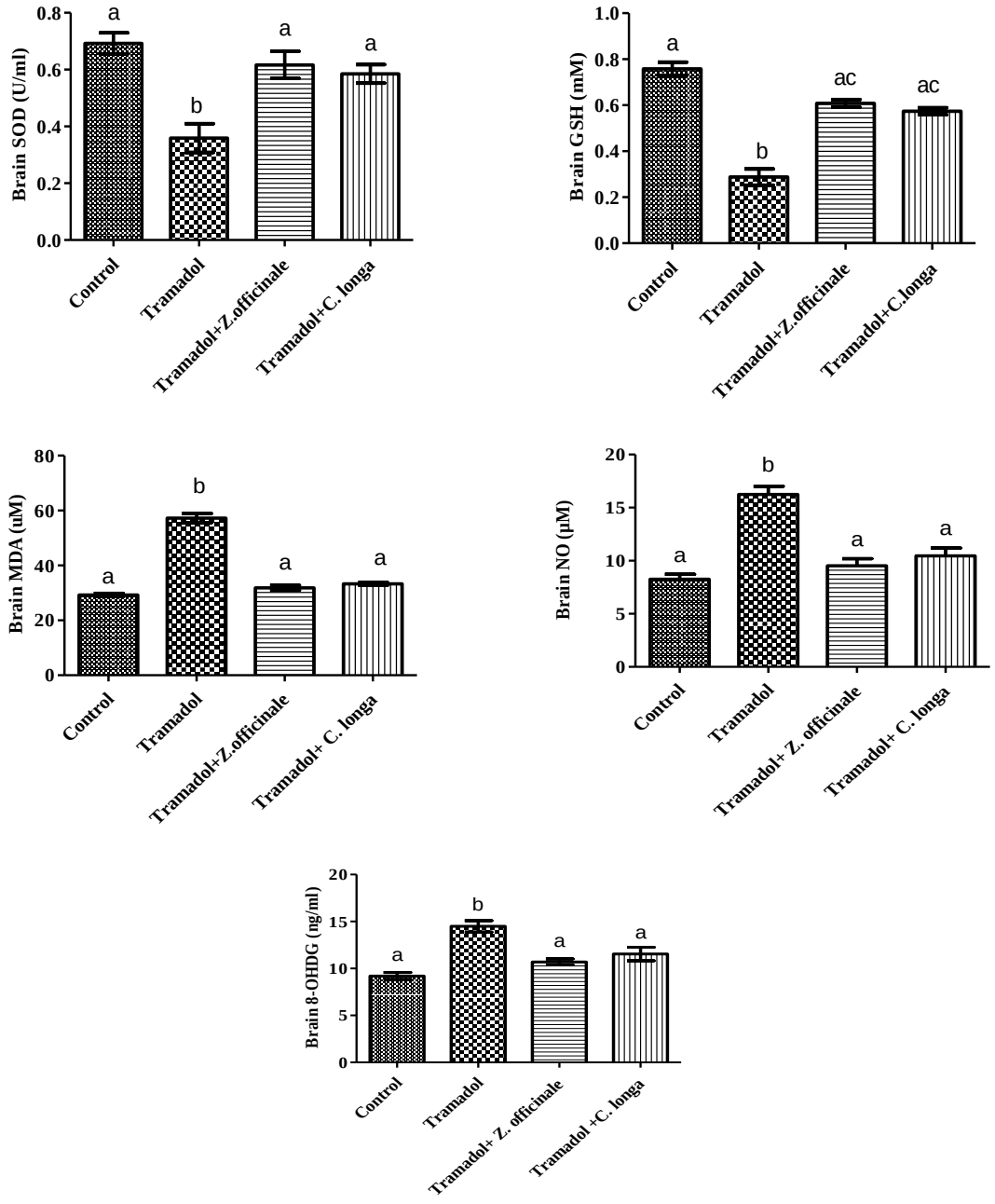

Fig. 1. Effects of *Z. officinale* and *C. longa* on redox status of the experimental rats exposed to tramadol. Values are Mean ±SEM (n=5). Values with different superscript letters are significantly different, whereas means with the same superscript letter are not significantly different

The oxidative effects of tramadol in the brain were further evidenced by the increased levels of the oxidative DNA product 8-hydroxyl deoxyguanosine (8-OHdG). Our results demonstrated that tramadol induced oxidative DNA damage in the brain, as indicated by significantly elevated 8-OHdG levels. These findings are consistent with previous studies suggesting that tramadol-induced brain oxidative DNA damage may be due to excessive ROS generation [34]. Notably, the inclusion of *Z. officinale* and *C. longa* significantly lowered the elevated levels of 8-OHdG, indicating their potential to mitigate oxidative DNA damage [37,38].

3.2 Effects of *Z. officinale* and *C. longa* on Acetylcholinesterase, Monoamine Oxidase Activities, Dopamine Level, and Gamma-aminobutyric Acid Concentration of the Exposed Rats to Tramadol

The administration of tramadol led to a significant increase in acetylcholinesterase (ACHE) activity, monoamine oxidase (MAO) activity, and dopamine levels in the rats that received tramadol only, compared to the control group. However, these parameters were significantly reduced in the groups that received tramadol along with *Z. officinale* or *C*. *longa* supplementation. Conversely, the concentration of gamma-aminobutyric acid (GABA) was significantly reduced in the tramadol-only group compared to the control, but it was significantly elevated in the groups that received tramadol feed supplemented with Z. *officinale* or C. *longa* (Table 3).

Our study revealed a significant increase (*P*<0.5) in acetylcholinesterase (AChE) activity in the brain homogenates of tramadol-treated rats, consistent with findings from previous research [39,40]. AChE activity is essential for normal brain function, and alterations in its activity are indicative of neurotoxicity. Since cholinergic neurotransmission in the brain is crucial for cognitive functions such as learning and memory, the observed increase in AChE activity suggests tramadol-induced neurotoxicity. However, supplementation with *Z*. *officinale* in the feed significantly decreased AChE activity in the tramadol-treated rats. This reduction could be attributed to the protective role of *Z*. *officinale* against oxidative damage in the brain. Our findings align with previous studies [41] that reported upregulation of AChE activity in the diabetic cerebellum, which was dramatically reduced following treatment with *Z*. *officinale* which is also in line with [27]. Similarly, *C*. *longa* supplementation also decreased AChE activity in tramadol-treated rats, supporting previous research [42]. In studies assessing the protective effects of curcumin (derived from *C*. *longa*) in combination with donepezil, improvements in learning and memory were observed, along with reductions in AChE, butyrylcholinesterase, and adenosine deaminase activities. The protective effect of *C. *longa** in our study may be attributed to its antioxidant properties [43].

Monoamine oxidase (MAO) activity was significantly increased ($P < .05$) in the brain homogenates of the tramadol-treated group, likely due to the elevated levels of free radicals generated by prolonged tramadol use. This finding is consistent with previous studies [44], which demonstrated a significant increase in plasma MAO levels in both low and high-dose tramadol groups compared to normal controls ($P < .05$). The inclusion of Z. *officinale* significantly reduced MAO activity, consistent with findings that the ethyl acetate fraction of Z. *officinale* extract significantly decreased MAO activity under oxidative stress conditions [45]. The protective effect of Z. *officinale* may be attributed to its free radical-scavenging activity. *C*. *longa* supplementation also significantly reduced MAO activity in this study, supporting earlier research [46] that showed curcumin from *C*. *longa* and ellagic acid inhibit MAO activity and may be considered potential MAO inhibitors for treating Parkinson's and other neurological disorders.

Table 3. Effects of *Z. officinale* and *C. longa* on acetylcholinesterase, monoamine oxidase activities, dopamine level, and gamma-aminobutyric acid concentration of the exposed rats to tramadol

Group	Control	Tramadol	Tramadol+ *Z. officinale*	Tramadol+ *C. longa*
ACHE(U/ml)	0.29±0.04^{a}	1.50±0.29^{d}	0.52±0.05^{ab}	0.49±0.05^{ab}
MAO (u/gProt)	1.59±0.02^{a}	4.90±0.27^{d}	2.16±0.39^{ab}	2.42±0.32^{ab}
DOPAMINE (Pg/ml)	55.74±0.57^{a}	72.90±2.94^{b}	67.67± 1.12^{a}	67.04± 1.45^{a}
GABA (Pg/ml)	73.90±2.54^{a}	40.13±1.51^{b}	68.96±2.07^{a}	66.88±2.41^{a}

Values are Mean ±SEM (n=5). Values with different superscript letters are significantly different, P<.001; whereas means with the same superscript letter are not significantly different

The dopamine levels in brain homogenates were significantly increased in this study, likely due to the high levels of free radicals generated, as reported in previous studies [38]. However, supplementation with *Z*. *officinale* reduced dopamine levels, consistent with findings from related research [45]. Similarly, *C*. *longa* supplementation also reduced dopamine levels, likely due to its antioxidant properties [47,48].

Gamma-aminobutyric acid (GABA) levels were decreased in the tramadol-treated rats. Reduced GABA levels have been associated with cognitive impairments, high impulsivity, and weakened memory. Supplementation with *Z*. *officinale* and *C*. *longa* improved GABA levels. This improvement could be due to *C*. *longa* stimulating glutamic acid decarboxylase, the enzyme responsible for converting glutamic acid to GABA, as supported by similar studies [49]. Additionally, *Z*. *officinale* may exert its effects through anti-5-hydroxytryptamine 3-receptor (anti-5HT3-receptor) properties, as 5-HT3-receptor stimulation modulates the secretion of several neurotransmitters, including GABA [50].

3.3 Effects of *Z. officinale* and *C. longa* on the Histopathological of Livers of Rats Exposed to Tramadol

Plate 1 below shows the magnified cytoarchitecture of the cerebral cortex in Wistar rats. In the control group, as well as the groups that received tramadol with *Z*. *officinale* or *C*. *longa** supplementation, normal histological features of the cortex were observed. The perineural spaces surrounding these cells were intact, with preserved nuclear and cytoplasmic content. The staining intensity of the cells in these groups remained consistent, with no observable signs of pyknosis. In contrast, the group that received tramadol without feed supplementation exhibited conspicuous degenerative changes in the cortex, characterized by clustered pyknotic pyramidal neurons. The perineural spaces surrounding these degenerating neurons (indicated by the red arrow) were noticeably reduced, with axons and dendrites scarcely visible. Additionally, there was a clear loss of nuclear and cytoplasmic material in this group.

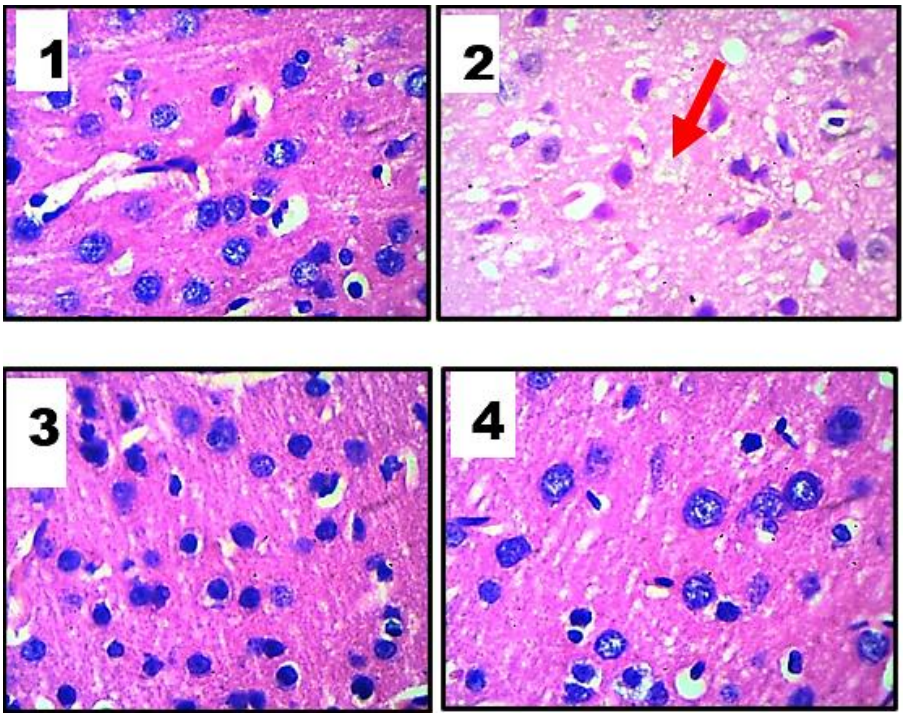

Plate 1. Photomicrographs showing layers three (iii) and four (iv) of the prefrontal cortex micromorphological presentations in Wistar rats across the study groups. H&E stain (*X400*) of 1(normal control), 2 (Tramadol 25mg/kg body weight only), 3 (Tramadol(25mg/kg) + *Z. officinale* 10%w/w), 4 (Tramadol(25mg/kg) + *C. longa* 10% w/w). The External pyramidal layer of pyramidale neurons (III) and internal granular layer of granular neurons (IV) are all demonstrated across study groups 1-4. Group with marked alteration is indicated by the red arrow

Histological examination of the brains of tramadol-treated rats revealed degenerative changes and structural alterations in the neurons, consistent with previous studies [51]. However, the supplementation of *Z. officinale* and *C, longa* in the feed mitigated the effects of tramadol, likely due to their antioxidant properties.

4. CONCLUSION

The findings from this study suggest that tramadol induces neurotoxicity through mechanisms involving oxidative stress and DNA damage. However, supplementation of daily feed intake with *Zingiber officinale* and *Curcuma longa* offers neuroprotection against these harmful effects of tramadol, by preserving key neural biochemistry involving acetylcholine esterase, monoamine oxidase, dopamine and gamma-aminobutyric acid likely due to their antioxidant properties. The significance of the study lies in its contribution to understanding the potential therapeutic benefits of these natural supplements in mitigating the adverse effects of tramadol on the brain.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENTS

The authors would like to express their deep gratitude to Prof. G. E. Adeleke for the professional advice and support provided during the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Temple NJ. A rational definition for functional foods: A perspective. Frontiers in Nutrition**.** 2022;9:957516.
DOI: 10.3389/fnut.2022.957516
2. Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Alvarez JA. Spices as functional foods. Critical Reviews in Food Science and Nutrition. 2011;51(1):13–28.
Available:https://doi.org/10.1080/10408390903044271
3. Pop A, Muste S, Păucean A, Chis S, Man S, Salanță L, Marc R, Muresan A, Marțiș G. Herbs and spices in terms of food preservation and shelf life. Hop and Medicinal Plants. 2019;27:1-2.
4. Patra K, Jana K, Mandal DP, Bhattacharjee S. Evaluation of the antioxidant activity of extracts and active principles of commonly consumed Indian spices. Journal of Environmental Pathology, Toxicology and Oncology. 2016;35(4):299–315.
5. Ene-Obong HN, Onuoha NC, Aburime LC, Mbah OC. Chemical composition and antioxidant activities of some indigenous spices consumed in Nigeria. Food Chemistry; 2017.
6. Ho WY, Beh BK, Lim KL, Mohamad NE, Yusof HM, Ky H, Yeap SK. Antioxidant and hepatoprotective effects of the food seasoning curry leaves *Murraya koenigii (L*.) Spreng. (Rutaceae). RSC Advances. 2015;5(122):100589–100597.
7. Wani AA, Sikdar M, Zuber SM. Nephroprotective action of taurine and garlic extract against copper sulfate induced renal toxicity in *Clarias gariepinus*. International Journal of Pharmaceutical and Biological Sciences. 2018;8(1):2321–2372.
8. Ogbunugafor HA, Ugochukwu CG, Kyrian-Ogbonna AE. The role of spices in nutrition and health: A review of three popular spices used in Southern Nigeria. Food Quality and Safety. 2017;1(3):171–185.
9. Fuloria S, Mehta J, Chandel A, Sekar M, Rani NNIM, Begum MY, Subramaniyan V, Chidambaram K, Thangavelu L, Nordin A. A comprehensive review on the therapeutic potential of Curcuma longa Linn. In relation to its major active constituent curcumin. Frontiers in Pharmacology. 2022;13: 820806.
10. Jayasinghe AMK, Kirindage KGIS, Kim SH, Lee S, Kim KN, Kim EA, Ahn G. Leaves and pseudostems extract of Curcuma longa attenuates immunoglobulin E/bovine serum albumin-stimulated bone marrow-derived cultured mast cell activation and passive cutaneous anaphylaxis in BALB/c mice. Journal of Ethnopharmacology. 2024;321:117529.
11. Ayati Z, Ramezani M, Amiri MS, Moghadam AT, Rahimi H, Abdollahzade A,

Sahebkar A, Emami SA. Ethnobotany, phytochemistry, and traditional uses of *Curcuma spp*. and pharmacological profile of two important species (C. longa and C. zedoaria): A review. Current Pharmaceutical Design. 2019;25(8):871–935

12. Dissanayake KGC, Waliwita WALC, Liyanage RP. A review on medicinal uses of Zingiber officinale (ginger). International Journal of Health Sciences and Research. 2020;10(6):142-148.
13. Sathi AS. An overview on chemical constituents and biological activities of *Zingiber officinale*. International Journal of Herbal Medicine. 2022;10:14–19.
14. Rahmawati N, Sholikhah IYM, Subositi D, Mustofa FI, Haryanti S, Widodo H, Widiyastuti Y. Traditional uses of ginger (*Zingiber officinale Roscoe*) based on ethno-medicine study in 254 Indonesian ethnic groups. Indian Journal of Traditional Knowledge (IJTK). 2024;23(5):464-472.
15. Ballester P, Cerdá B, Arcusa R, García-Muñoz AM, Marhuenda J, Zafrilla P. Antioxidant activity in extracts from Zingiberaceae Family: Cardamom, Turmeric, and Ginger. Molecules (Basel, Switzerland). 2023;28(10):4024. Available:https://doi.org/10.3390/molecules 28104024
16. Zwawua O, Ismail R, Mohd Azhar M, Mohd Noor N, Iorvaa T. Development and psychometric validation of a scale for measurement of tramadol abuse. African Journal of Drug and Alcohol Studies. 2021;20(2). Available:https://dx.doi.org/10.4314/ajdas.v 20i2.2
17. Herrnsdorf EA, Holmstedt A, Håkansson A. Tramadol misuse in treatment-seeking adolescents and young adults with problematic substance use – Prediction of treatment retention. Addictive Behaviors Reports. 2022;19:100446
18. Mohammadnejad L, Soltaninejad K. Tramadol-induced organ toxicity via oxidative stress: A review study. International Journal of Medical Toxicology and Forensic Medicine. 2022;12(1): 35430. Available:https://doi.org/10.32598/ijmtfm. v12i1.35430.
19. Elmeged LSMA, Alshehri KM. The beneficial effects of turmeric plant on biochemical changes in rats injected with carbon tetrachloride (CCl4). International Journal of Pharmaceutical Research and Allied Sciences. 2021;10(1):67–76. Available:https://doi.org/10.51847/-mgwfyc
20. Ezeuko VC, Nwokocha CR, Mounmbegna PE, Nriagu CC. Effects of *Zingiber officinale* on liver function of mercuric chloride-induced hepatotoxicity in adult Wistar rats. Electronic Journal of Biomedical. 2007;3:40–45.
21. Olaniyi TD, Awodugba TM, Adetutu A. Ethnobotanical survey and evaluation of Anti Salmonella potentials of commonly used plants for typhoid treatment in Ogbomoso, Oyo State, Nigeria. Journal of Complementary and Alternative Medical Research. 2021;15(1):1-15,
22. Magnani L, Gaydou EM, Hubaud JC. Spectrophotometric measurement of antioxidant properties of flavones and flavonols against superoxide anion. Analytica Chimica Acta. 2000;411(2):209–216.
23. Ellman GL. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics. 1959;82(1):70–77.
24. Montilla-López P, Muñoz-Águeda MC, Feijóo López M, Muñoz-Castañeda JR, Bujalance-Arenas I, Túnez-Fiñana I. Comparison of melatonin versus vitamin C on oxidative stress and antioxidant enzyme activity in Alzheimer's disease induced by okadaic acid in neuroblastoma cells. European Journal of Pharmacology. 2002; 451(1):237–243.
25. Green LC, Wagna DA, Glokowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analytical Biochemistry. 1982;126:131–138.
26. Votyakova TV, Reynolds IJ. Neurochemistry. 2001;79:266.
27. Talebi M, Ilgün S, Ebrahimi V, Talebi M, Farkhondeh T, Ebrahimi H, Samarghandian S. Zingiber officinale ameliorates Alzheimer's disease and cognitive impairments: Lessons from preclinical studies. Biomedicine and Pharmacotherapy. 2021;133:111088.
28. Adwas AA, Elsayed A, Azab AE, Quwaydir FA. Oxidative stress and antioxidant mechanisms in the human body. Journal of Applied Biotechnology and Bioengineering. 2019;6:43–47.
29. Adegbola PI, Aborisade AB, Olaniyi TD, Adetutu A. Evaluation of long-term effects of nickel and benzo [a] anthracene contaminated diets in rats' kidney; Mimicking human exposure from food.

International Journal of Biochemistry and Molecular Biology. 2024;15(1):8.
30. Aboulhoda BE, Hassan SS. Effect of prenatal tramadol on postnatal cerebellar development: Role of oxidative stress. Journal of Chemical Neuroanatomy. 2018; 94:102–118.
31. Ali HA, Afifi M, Saber TM, Makki AA, Keshta A, Baeshen M, Al-Farga A. Neurotoxic, hepatotoxic, and nephrotoxic effects of tramadol administration in rats. Journal of Molecular Neuroscience. 2020; 70:1934–1942.
32. Hadree DH, Farhan AA, Fadhil RM. Evaluation of the antioxidant activity of *Zingiber officinale* alcoholic extract and vitamin E on liver damage induced by paracetamol drug in males of New Zealand rabbits. Iraqi Journal of Veterinary Sciences. 2022;36:1-5.
33. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiological Reviews. 2007; 87(1):315–424.
34. Mohammed M, Aboulhoda B, Mahmoud R. Vitamin D attenuates gentamicin-induced acute renal damage via prevention of oxidative stress and DNA damage. Human and Experimental Toxicology. 2019;38(3): 321–335.
35. Hosseini A, Hosseinzadeh H. Antidotal or protective effects of Curcuma longa (turmeric) and its active ingredient, curcumin, against natural and chemical toxicities: A review. Biomedicine and Pharmacotherapy. 2018;99:411–421.
36. Ballester P, Cerdá B, Arcusa R, García-Muñoz AM, Marhuenda J, Zafrilla P. Antioxidant activity in extracts from Zingiberaceae family: Cardamom, turmeric, and ginger. Molecules. 2023; 28(10):4024.
37. Longobardi C, Damiano S, Andretta E, Prisco F, Russo V, Pagnini F, Ciarcia R. Curcumin modulates nitrosative stress, inflammation, and DNA damage and protects against ochratoxin A-induced hepatotoxicity and nephrotoxicity in rats. Antioxidants. 2021;10(8):1239.
38. Abd El-Monem DD, Elwakeel SHB. Radioprotective efficacy of ginger (*Zingiber officinale*) extract against gamma-ray radiation-induced genotoxicity in rats. International Journal of Radiation Research 2020;18(1):43-55.
39. Elwy AEHM, Tabl G. Impact of tramadol and morphine abuse on the activities of acetylcholine esterase, Na+/K+-ATPase, and related parameters in cerebral cortices of male adult rats. Electronic Physician. 2017;9(3):4027.
40. Hussein K, Ahmed E. The interaction effect of abamectin and tramadol on brain neurotransmitters in rats. World Journal of Advanced Research and Reviews. 2020; 7(1):263-272.
41. El-Akabawy G, El-Kholy W. Neuroprotective effect of ginger in the brain of Streptozotocin-induced diabetic rats. Annals of Anatomy - Anatomischer Anzeiger. 2014;196(2–3):119–128.
42. Akinyemi AJ, Oboh G, Fadaka AO, Olatunji BP, Akomolafe S. Curcumin administration suppresses acetylcholinesterase gene expression in cadmium-treated rats. Neurotoxicology. 2017;62:75–79.
43. Farkhondeh T, Samarghandian S. The hepatoprotective effects of curcumin against drugs and toxic agents: An updated review. Toxin Reviews. 2016; 35(3–4):133–140.
44. El-Baky A, Hafez MM. NOS expression in oxidative stress, neurodegeneration, and male infertility induced by the abuse of tramadol. Biochemistry and Pharmacology (Los Angel). 2017;6(223):2167–0501.
45. Okesola MA, Ajiboye BO, Oyinloye BE, Ojo OA. Neuromodulatory effects of ethyl acetate fraction of *Zingiber officinale* Roscoe extract in rats with lead-induced oxidative stress. Journal of Integrative Medicine. 2019;17(2):125–131.
46. Khatri DK, Juvekar AR. Kinetics of inhibition of monoamine oxidase using curcumin and ellagic acid. Pharmacognosy Magazine. 2016;12(2):S116.
47. Badawy G, Atallah M, Sakr S. The ameliorative role of ginger administration against gabapentin-induced hepatotoxicity in rat fetuses. European Journal of Pharmaceutical and Medical Research. 2019;6:622–631.
48. Abd-Elhakim YM, Moselhy AA, Aldhahrani A, Beheiry RR, Mohamed WA, Soliman MM, Saffaf BA, El-Deib M. Protective effect of curcumin against sodium salicylate-induced oxidative kidney damage, nuclear factor-kappa dysregulation, and apoptotic consequences in rats. Antioxidants. 2021; 10(6):826.
49. Moghadam AR, Tutunchi S, Namvaran-Abbas-Abad A, Yazdi M, Bonyadi F, Mohajeri D, Mazani M, Marzban H, Łos MJ, Ghavami S. Pre-administration of

turmeric prevents methotrexate-induced liver toxicity and oxidative stress. BMC Complementary and Alternative Medicine. 2015;15:1–13.

50. Farag MR, Abou-EL Fotoh MF, EL-Sayed GG, EL-Sayed EW. Modulatory effect of ginger aqueous extract against imidacloprid-induced neurotoxicity in rats. Zagazig Veterinary Journal. 2019;47(4): 432–446.

51. Ragab IK, Mohamed HZ. Histological changes of the adult albino rat's entorhinal cortex under the effect of tramadol administration: Histological and morphometric study. Alexandria Journal of Medicine. 2017;53(2):123–133.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/123544