Original Research Article

Assessments of Neuro-Protective Effects of*Curcuma longa* AND *Zingiber officinale*-*supplemented feed* inTramadol-Exposed Male Wistar Rats.

ABSTRACT

Aim: This study aimed to investigate the protective effects of *Zingiber officinale* (ginger) and *Curcuma longa* (turmeric) on tramadol-induced neurotoxicity.

Study design: Twenty male Wistar rats, with an average weight of 130g, were randomly divided into four groups (n=5 per group). Group 1 served as the control, Group 2 received tramadol hydrochloride at a dose of 25mg/kg body weight, Group 3 was treated with tramadol plus *Z. officinale*(10% w/w supplemented feed), and Group 4 received tramadol plus *C. longa* (10% w/w supplemented feed). Treatments were administered daily for 90 days.

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

Methodology: At the end of the treatment period, animals were sacrificed, 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 colorimetric method. Additionally, the levels of 8-oxodeoxyguanosine (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. Statistical analysis was performed using GraphPad Prism 5 software.

Results: The results indicated a significant decrease ($P < 0.5$) in SOD activity and GSH concentration, coupled with a significant increase ($P < 0.5$) in MDA concentration, nitric oxide, 8-OHdG, dopamine levels, MAO, and AChE activities in the tramadol-treated group compared to the control group. Additionally, there was a significant depletion in GABA levels in the tramadol group. Tramadol administration significantly increased oxidative stress markers, including elevated MDA, NO, and 8-OHdG levels, along with increased AChE and MAO activities and decreased GABA levels. Histological analysis revealed degenerative changes in the neurons of tramadol-treated rats.

However, supplementation with *Z. officinale* and *C. longa* significantly reduced these oxidative stress markers and enzymatic activities, preserving neuronal structure and function

Conclusion: The study demonstrates that Zingiber *officinale* and Curcuma *longa* offer neuroprotection against tramadol-induced neurotoxicity, likely due to their 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.

INTRODUCTION

Food can be broadly defined as any material necessary for sustaining life, supplying essential nutrients, supporting metabolic activities, energy production, and maintaining the body. Over 2,000 years ago, Hippocrates aptly stated, "Let food be your medicine and medicine be your food." This statement underscores the concept that food can serve not only as nourishment but also as a form of medicine, leading to the emergence of what are known as functional foods. Functional foods are generally considered to be any food that resembles regular meals in appearance and is consumed as part of a normal diet but offers additional physiological benefits beyond basic nourishment, such as promoting health or preventing disease. These foods can be derived from plants, animals, or marine sources [1].

The application of herbs and spices, particularly those derived from plants, has been extensively studied. Spices and culinary herbs have been added to food since ancient times to enhance flavor and improve its sensory qualities. Beyond their culinary uses, herbs and spices have long been recognized for their medicinal properties and preservative effects. 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 [2,3]. Previous studies have demonstrated that spices such as cinnamon, garlic, and curry leaves possess hepatoprotective, nephroprotective, and neuroprotective properties [4,5,6].

In Nigeria, *Curcuma *longa** (*C. longa*) is commonly used as a condiment to flavor and preserve food. In Pakistan, it is traditionally taken as folk medicine for conditions such as acne and wound healing [8]. Known locally as Yung-ba, *C. longa* is used in Bhutanese 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. Assamese tribal women apply fresh rhizome paste to their skin to treat infections and improve skin appearance. 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 coloring agent in cheese, butter, mustard sauce, and chips [7].

*Zingiber *officinale** (ginger) is another spice 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 when combined with lime juice and rock salt. It has been reported to treat a variety of conditions, including dyspepsia, anorexia, arthritis, bleeding, cancer, chest congestion, chickenpox, cholera, chronic bronchitis, cold extremities, colic, colitis, common cold, cough, cystic fibrosis, diarrhea, breathing difficulties, dropsy, fever, flatulence, indigestion, gallbladder disorders, morning sickness, nausea, rheumatism, sore throat, throat pain, stomach ache, and vomiting [9].

Given the significant challenge of drug abuse, particularly with tramadol, which is widely misused in many countries, the role of food spices in mitigating such toxicity becomes crucial. While pharmaceutical interventions alone may not suffice in addressing this issue, commonly used food

spices, aside from enhancing the palatability of food, may offer protective effects against tramadol toxicity.

2.0 MATERIALS AND METHODS

2.1 Drug: The tablets of tramadol used in this study were purchased

2.2 Collection and Preparation of Rhizomes

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 drugs were suspended in distilled water and administered orally to the rats using an oral cannula. Curcuma *longa* was washed under running water, cut into smaller pieces, air-dried to a constant weight, and then pulverized into a fine powder using a blender. The same process was applied to Zingiber *officinale*.

2.2.1 Feed formulation for the study

*Z. officinale*powder (10% w/w) [32] and *C. longa* powder 10%w/w [31] were incorporated into different animal feed as shown in (tables 1 and 2) below;

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

Composition	**Quantity in %w/w**
Maize	40.0
Wheat offale	10.0
Soya meal	12.0
Corn bron	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 offale	10.0
Soya meal	12.0
Corn bron	11.2
GNC	8.0
GPKC	8.0
Salt	0.2
Premix broiler	0.2
Lysine	0.2
Methionine	0.2
C. longa	10.0

2.3 Chemicals and Reagents Used

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

2.4 Experimental Animals

Twenty male Wistar albino rats, each weighing between 120g and 150g, 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 prior to the start of the experiment. The study adhered to the "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985).

2.5 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 (Control), Group 2 (Tramadol only), Group 3 (Tramadol + 10% w/w Z. *officinale*-supplemented feed), and Group 4 (Tramadol + 10% w/w C. *longa*-supplemented feed).

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 labeled 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.

2.7 Determination of oxidative status

The activities of antioxidant enzymes, including Superoxide Dismutase (SOD), were measured as described by [11, 12]. Concentrations of reduced glutathione (GSH) were assessed following the method outlined by [11]. Malondialdehyde (MDA) levels were determined according to [13]. Nitric oxide (NO) was measured based on the protocol provided by [14]. 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, including unknowns and standards, was assayed in duplicate or triplicate. A volume of 2.50 µL of each sample (H□O□ standard, control, or sample) was added to individual microtiter plate wells. For wells containing H□O□ 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. $\text{MAO Activity}\left(\frac{\text{Units}}{\text{L}}\right) = \frac{(\text{H}_2\text{O}_2\text{ generated})}{\text{Reaction time (minutes)}} \times \text{Sample dilution}$

2.9 Determination of dopamine level

The wells for diluted standards, blanks, and samples were identified. 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. Note: A microplate washer can be used for this and other washing steps. Ensure the tested strips are used immediately after washing and avoid allowing wells to dry.

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 color, 50 µL of Stop Solution was added to each well. If color 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 color resulting from the reaction of thiocholine with DTNB. The change in absorbance per minute was then calculated using the formula:

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

AChE activity = U/ml.

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.

Statistical analysis

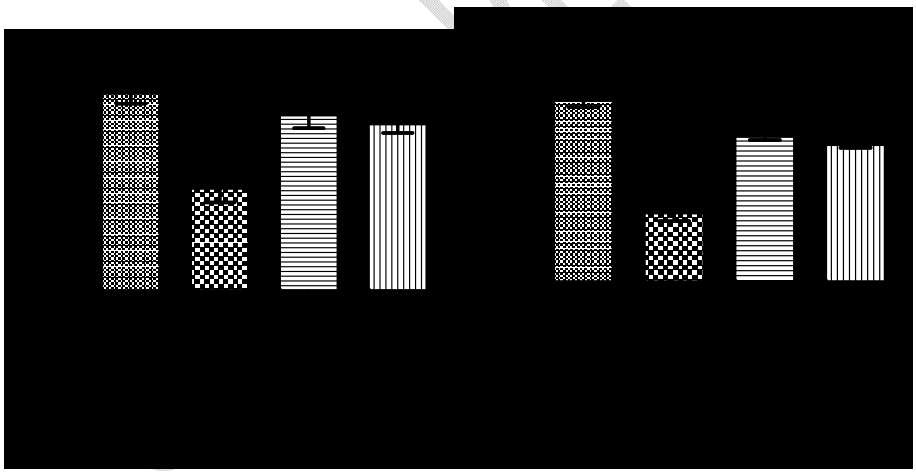
All data presented were presented as mean ± SEM. The mean of different treatment groups tested for significance using one-way analysis of variance (ANOVA) and were compared using the Tukey test. Differences were considered significant at $P<.05$.

3. RESULTS AND DISCUSSION

3.1 Effects of *Zingiber *officinale and *Curcuma *longa** on Redox Status of Experimental Rats Exposed to Tramadol**

To assess the impact of *Zingiber *officinale** and *Curcuma *longa** on the redox status of rats exposed to tramadol, several oxidative stress biomarkers were evaluated, as presented in Figure 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 *Zingiber *officinale** and *Curcuma *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-oxodeoxyguanosine (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 *Zingiber *officinale** and *Curcuma *longa** supplementation, further highlighting the antioxidative properties of these compounds in mitigating the oxidative damage induced by tramadol.



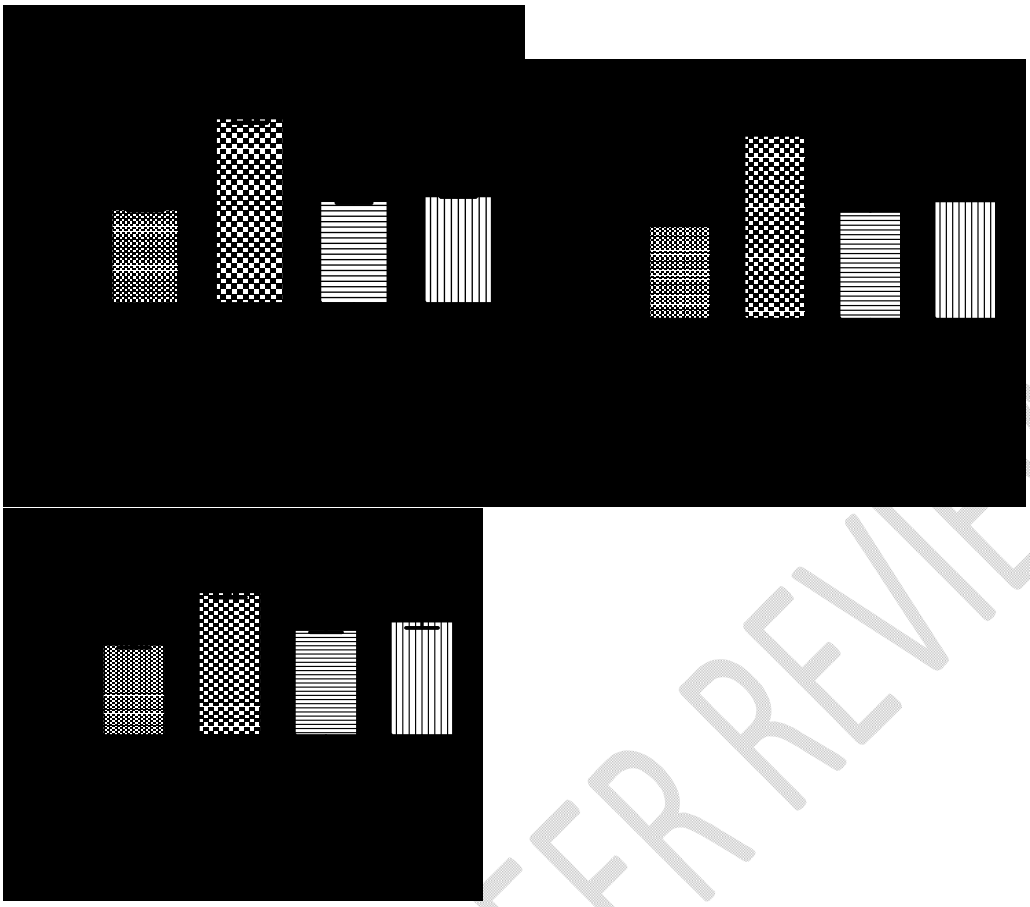


Figure 1: Effects of *Z. officinale* and *C. longa* on redox status of the experimental rats exposed to tramadol. Values are Mean ±SEM (n=5). ^{abc} mean values with different superscript letters are significantly different, whereas means with the same superscript letter are not significantly different.

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 [10].

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 [15, 16]. However, supplementation with *Curcuma *longa** and *Zingiber *officinale** attenuated MDA levels, suggesting that these supplements enhanced the antioxidant defenses, as observed in similar research [17].

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 [18]. However, supplementation with *Z. *officinale** and *C. *longa** reduced NO levels, likely due to their antioxidant properties [17, 19].

The oxidative effects of tramadol in the brain were further evidenced by the increased levels of the oxidative DNA product 8-oxodeoxyguanosine (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 [16, 18]. 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.

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 *Zingiber *officinale** or *Curcuma *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 supplemented with *Z. *officinale** or *C. *longa**.

Table 3: Effects of *Z. officinale* and *C. longa* on acetylcholinesterase, monoamine oxidase activities, dopamine level, 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). ^{abd}mean values with different superscript letters are significantly different, $P<.001$; whereas means with the same superscript letter are not significantly different.

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 [27]. 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 *Zingiber *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 [20] that reported upregulation of AChE activity in the diabetic cerebellum, which was dramatically reduced following treatment with *Z. *officinale** ($P < .001$).

Similarly, *Curcuma *longa** supplementation also decreased AChE activity in tramadol-treated rats, supporting previous research [30]. 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 [21].

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 [28], 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 [22]. 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 [29] 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.

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 [27]. However, supplementation with *Z. *officinale** reduced dopamine levels, consistent with findings from related research [22]. Similarly, *C. *longa** supplementation also reduced dopamine levels, likely due to its antioxidant properties [23, 24].

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 [26]. 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 [25].

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 *Zingiber *officinale** or *Curcuma *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.

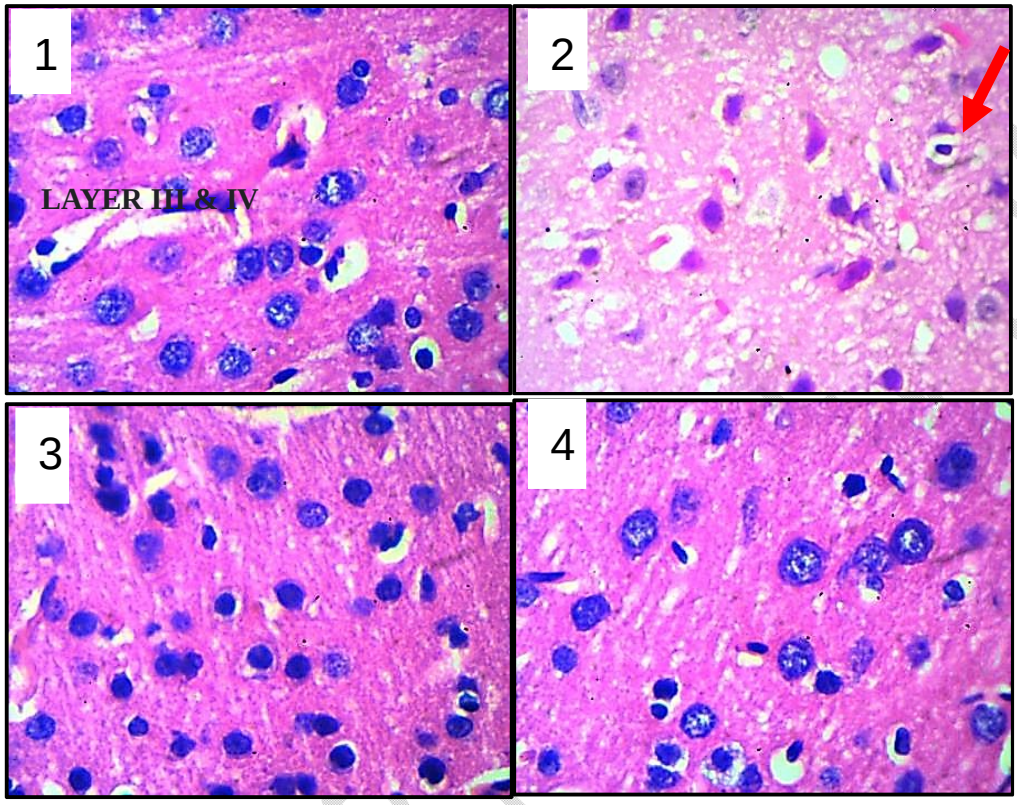


Plate 1: Photomicrographs showing layer three (iii) and four (iv) of the prefrontal cortex micromorphological presentations in Wistar rats across the study groups. H&E stain (*X400*) of 1(normal control), 2 (Tramadol 25mg/kg body weight only), 3 (Tramadol(25mg/kg) + *Z. officinale* 10%w/w), 4 (Tramadol(25mg/kg)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

Histological examination of the brains of tramadol-treated rats revealed degenerative changes and structural alterations in the neurons, consistent with previous studies [34]. However, the supplementation of *Zingiber *officinale** and *Curcuma *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 with *Zingiber officinale* and *Curcuma longa*offers protection against these harmful effects, likely due to their antioxidant properties.

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