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Original Article

Zingiber officinale Exhibits Neuroprotective Properties against Tramadol-Induced Spatial Memory Impairment and Histopathological Changes

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ABSTRACT

Drug abuse has been on the increase in recent years. Tramadol is one of such drug commonly misused and reported to have neurotoxic effects. However, *Zingiber officinale* has been reported to have neuroprotective properties. This study aimed to evaluate the neuroprotective effects of *Z. officinale* on spatial memory deficits in tramadol-treated Wistar rats. Thirty adult male Wistar rats were divided into five groups, and respectively, orally administered the following for 21 days: the control group (2 ml/kg of distilled water), tramadol (Tram, 50 mg/kg), tramadol (50 mg/kg)+naltrexone (12.5 mg/kg), and tramadol (50 mg/kg)+*Z. officinale* (500 mg/kg+1000 mg/kg, respectively). A Morris water maze test was conducted for the assessment of spatial memory. The rats were euthanized by transcardiac perfusion, and the brains were harvested, fixed, and processed using haematoxylin and eosin stain for histology. A remarkable decrease in latency time and an increase in distance covered to locate the platform were observed in the tramadol-treated group compared to the control in the acquisition phase. The probe test showed a remarkable decrease in the time spent in the escape platform quadrant in the tramadol-treated group compared with the control. Neurodegenerative changes were observed in the hippocampus of the tramadol group, presenting as karyorrhexis, gliosis, and perineural vacuolation. These neurodegenerative changes were attenuated in *Z. officinale*-treated groups in a dose-dependent manner. *Z. officinale* improved the learning and memory of tramadol-treated rats. Findings from this study suggest that *Z. officinale* attenuated tramadol-induced neurotoxicity in Wistar rats.

Keywords
Neurobehavioural, Cognitive function, Histopathology, Cornnu ammonis, Morris water maze

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INTRODUCTION

Substance abuse, which has been a thorny public health concern throughout human history (Wing et al. 2020), is a global health and social problem with distinct conditions and problems that vary locally (World Health Organization 1987). Continued drug use has been reported to cause cognitive deficits, aggravating the difficulty of establishing sustained abstinence (Bassiony et al. 2017). The burden of drug abuse is increasing and becoming endemic in Nigeria (Abubakar et al. 2021), and it is emerging as a public health problem of serious concern despite the international and regional laws, policies, and agencies established by the government to prevent the menace (Ibrahim et al. 2018). It is reported that nearly 15% of the adult population, or 14.3 million people between the ages of 15 and 64, in Nigeria use a considerable level of psychoactive substances (United Nations Office on Drugs and Crime 2018).

Tramadol is used for the prevention and treatment of moderate to severe pains (Elkhateeb et al. 2015), and it has a high potential for misuse (Kertesz 2017). It is a synthetic, centrally acting analgesic opioid that exerts its analgesic effect by blocking the reuptake of norepinephrine and serotonin (Pothiawala and Ponampalam 2011). The neurotoxic effect of tramadol has been reported in patients receiving tramadol both at recommended and high dosage

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ranges in human studies (Bekjarovski et al. 2012). Studies have shown that long-term use of opioids is associated with addiction and physical and psychological dependence (Ghoneim et al. 2014). Baghishani et al. (2018) reported a memory function deficit in animal models acting through activation of μ-opioid receptors after tramadol administration.

On the other hand, chronic administration of tramadol results in such histological abnormalities as increased cerebral cortex apoptosis in the rat associated with oxidative stress (Ghoneim et al. 2014). Additionally, recent studies have shown that tramadol leads to increased oxidative stress in various tissues, such as the brain (Zhu et al. 2020).

One of the limbic system structures commonly affected by drugs is the hippocampus. The hippocampus is one of the areas involved in learning and memory in which both opioid peptides and opioid receptors are expressed (Motmedi et al. 2003). It is a plastic and vulnerable structure, so it is easily damaged by a variety of stimuli (Anand and Dhikav 2012). The hippocampus's high metabolic activity also makes it vulnerable to oxidative stress (Mohammadipour et al. 2016).

There is growing interest in the use of medicinal plants that have been relied upon for generations to treat various ailments. One such plant that has been documented and used in the treatment of many neurodegenerative diseases is *Zingiber officinale* (*Z. officinale)*, a member of the *Zingiberaceae* family. It is a flowering plant whose rhizome, ginger root, is widely used as a spice and folk medicine (National Center for Complementary and Integrated Health 2020). *Z. officinale* is reported to have a neuroprotective effect due to the phenolic and flavonoid compounds present in it (Ha et al. 2012). Studies have shown that *Z. officinale* exhibits neuroprotective properties by decreasing the levels of lipid peroxidation and increasing glutathione, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, quinone reductase, and the levels of presynaptic and postsynaptic proteins (Huh et al. 2018).

Although studies have evaluated the neurotoxic effects of tramadol on the hippocampus, there is a paucity of information on the ameliorating effects of *Z. officinale* on spatial memory deficits in rats treated with tramadol, hence, the need for this study.

MATERIALS AND METHODS

Ethical Approval

Ethical approval for this research was obtained from the Ahmadu Bello University Ethics Committee on Animal Use and Care with approval number **ABUCAUC/2022/031.**

Experimental Animals

Thirty adult male Wistar rats (150-190 g) were obtained from the Animal House of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. They were transferred and housed in wired cages in the Department of Human Anatomy, Faculty of Basic Medical Sciences of the same university, and allowed to acclimatise for two weeks before the commencement of the experiments. The rats were given pelletized feed (Grand Cereals and Oil Mills Limited, Nigeria) and water *ad libitum*.

Drug and Plant Collection and Identification

Tramadol hydrochloride (50 mg capsules, Vadis Pharm. Ltd. Nigeria) and naltrexone (50 mg tablets, (Healing Pharma India Pvt. Ltd. Nigeria), were obtained for this study. Fresh *Z. officinale* rhizomes were obtained at Samaru Ultra-Modern market, Zaria, and were identified and authenticated at the Herbarium unit of Botany Department, Faculty of Life Sciences, Ahmadu Bello University, Zaria, where a voucher number **ABU02261** was assigned.

Drug and Plant Preparation

The drugs (tramadol and naltrexone) solutions were prepared using the Organisation for Economic Co-operation and Development (OECD) guidelines on dosage calculation and stock solution preparation in experimental animal studies as described by Erhirhie *et al*. (2014). Tramadol (0.24 g) was dissolved in 2.4 mL of distilled water, 0.13 g of naltrexone was dissolved in 1.3 mL of distilled water; and 1.5 g of ethanol extract of *Z. officinale* (ginger) rhizome was dissolved in 15 mL of distilled water. The animals were weighed and given the calculated dose of the drugs and plant extract according to their body weight.

Preparation of Plant Extrac*t*

Fresh *Z. officinale* rhizomes were thoroughly washed with clean water to remove dirt, and 2,500 g of the fresh *Z. officinale* rhizomes were grinded and cold-macerated in two litres of 70% ethanol and intermittently stirred thoroughly. The mixture was left for 48 hours to allow the active ingredients to completely dissolve. The macerated pulp was first filtered by mesh cloth and then suction-filtered through Whatman No. 1 filter paper, and the resulting extract was concentrated using a rotary evaporator and evaporated to dryness on a water bath at 50°C (Evans 2009). The percentage yield was calculated to be 1.72%. The dried extracts of *Z. officinale* were properly stored in a container before further experiments and analysis.

Experimental Design and Treatment of Animals

The thirty male Wistar rats were randomly divided into five groups with six rats in each group. Control group (2 mL/kg of distilled water), tramadol (50 mg/kg), tramadol + naltrexone (12.5 mg/kg), tramadol (50 mg/kg) + 500 mg/kg of *Z. officinale,* and tramadol + 1,000 mg/kg of *Z. officinale*. For the co-administered groups, administrations were done one hour after tramadol administration. All the administrations were orally and done in the morning, once daily consecutively for 21 days. The animals were sacrificed twenty-four hours after the last administration.

Acute Toxicity Study of Z. officinale

The acute toxicity study (LD_{50}) for the ethanol extract of *Z. officinale* rhizome was carried out using the method of Lorke (1983). This method has two phases, 1 and 2.

Phase 1: This phase required nine animals. The nine animals were divided into three groups of three animals each. Each group of animals was administered different doses (10, 100, and 1,000 mg/kg) of e *Z. officinale* ethanol extract. The animals were placed under observation for 24 hours to monitor their behaviour as well as mortality.

Phase 2: This phase involved the use of three animals, which were distributed into three groups of one animal each. The animals were administered higher doses (1,600, 2,900, and 5,000 mg/kg) of ethanol extract of *Z. officinale* rhizome and then were observed for 24 h for behaviour as well as mortality.

The LD_{50} was calculated using the formula:

$LD_{50} = \sqrt{(D_0 X D_{100})}$

D_0 = highest dose that gave no mortality,

D_{100} = lowest dose that produced mortality

Morris Water Maze Test

Morris water maze (MWM) is a test of spatial learning for rodents that relies on distal cues to navigate from start locations around the perimeter of an open swimming arena to locate a submerged escape platform. For this test, a coloured circular pool (136 cm diameter, 60 cm high, and 30 cm depth), filled with water was set up in the centre of a small room. A circular platform (10 cm diameter and 28 cm high) was located in the pool and submerged about 2 cm beneath the water surface in the centre of the northeast quadrant. Outside the MWM, some steady visual cues such as desktop, table, and posters, were available in the room. The rats were placed in the pool and released facing the sidewall at the Northwest quadrant. The boundaries of the four quadrants and apparatus were divided into four quadrants Northwest, Northeast, Southwest and Southeast. The released location was maintained throughout the experiment. For every trial, the rats were allowed to swim until it finds and remains on the platform for 20 s. After the expiration of 60 s, the rats that could not find the platform were guided to it and allowed there for 20 s. At the end of four trials, the rats were removed from the pool and dried. The time spent and the travelled distance to reach the platform was recorded by a video tracking system. On the sixth day, the platform was removed. Then, the rats were allowed to swim for 60 s and the time spent and travelled distance in the target quadrant (Q1) was compared among the groups (Mohammadipour et al. 2016).

Morphological Studies

The body weight of the rats was taken at the beginning and at the end of the experiment. After the experiment, the data were analysed for body weight changes. The brain weight was measured immediately after dissection and was used in determining the organ-weight index.

Animal Sacrifice

After completion of the behavioural test, the animals were anesthetized with chloroform and perfused transcardially with normal saline and 10 % formal saline. After perfusion, the brains of the rats were carefully taken out from the skull and washed with normal saline. A mid-sagittal incision was made to open the skull and the brains were harvested and fixed in 10 % formal saline for 48 h. The fixed brains were processed for haematoxylin and eosin staining.

Data Analysis

All the results are expressed as mean ± SEM (standard error of the mean). The differences between and within the groups were analysed using analysis of variance (ANOVA) followed by the Tukey post hoc test. Values of $p < 0.05$ were considered statistically significant. Data were analysed using statistical product and service solutions (IBM SPSS 26). Graphs were plotted using GraphPad Prism 9.3.1.

RESULTS

Acute Toxicity Study of *Z. officinale* Ethanol Extract

The acute toxicity study (LD_{50}) of *Z. officinale* extract using Lorke's method was calculated to be above 5,000 mg/kg (Table 1). No behavioural changes were observed.

Table 1: Acute toxicity test results for ethanol extract of *Z. officinale* rhizome

Doses (mg/kg)	No. of animals	No. of deaths/groups
Phase 1		
10	3	0/3
100	3	0/3
1,000	3	0/3
Phase 2		
1,600	1	0/1
2,900	1	0/1
5,000	1	0/1

Body Weight

The body weights of the rats before and after the experiments were observed. There was a significant increase ($p < 0.05$) when the initial body weight was compared to the final body weight in control, tramadol, tramadol + 12.5 mg/kg of naltrexone, and tramadol + 1,000 mg/kg of *Z. officinale* (Fig. 1). There was no significant ($p > 0.05$) difference in the final body weight, and a percentage weight change was observed across the groups when compared to the control.

Morris Water Maze

Spatial memory in the Morris water maze showed a significant ($p<0.05$) decrease in latency and an increase in distance covered by the Wistar rats to locate the platform

in the tramadol-treated group compared to other groups. There was a significant ($p < 0.05$) decreased distance covered in locating the platform by the group treated with tramadol and naltrexone when compared to the control group. There was a significant ($p < 0.05$) decreased distance covered in locating the platform by the groups treated with tramadol + 12.5 mg/kg of naltrexone, tramadol + 500 mg/kg of *Z. officinale,* and tramadol + 1,000 mg/kg of *Z. officinale*, when compared to the tramadol-treated group. In the probe test, there was a significantly ($p<0.05$) decreased time spent in the escape platform quadrant in the group treated with tramadol + 12.5 mg/kg of naltrexone and tramadol + 500 mg/kg of *Z. officinale* when compared to the control group (Fig. 2).

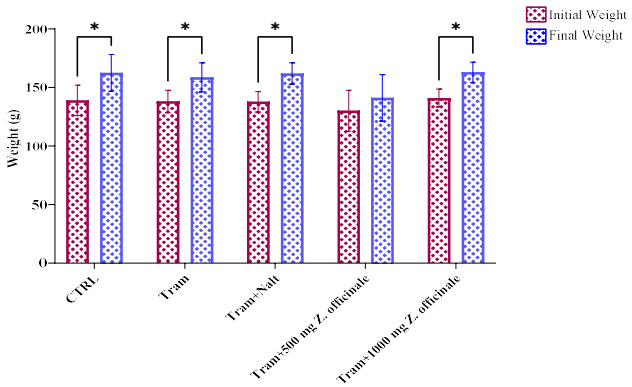

Fig. 1: Initial (day 1) and final (day 21) body weights comparison of Wistar rats following oral administration of tramadol and *Z. officinale*. n=6, mean ± SEM, paired sample t-test, *= $p < 0.05$ when initial and final weight was compared. CTRL = 2 ml/kg of distilled water, Tram = 50 mg/kg of tramadol, Nalt = 12.5 mg/kg of Naltrexone.

Histology studies

Histological examination of the Wistar rats` hippocampi (CA1 and CA3 regions) in the control group (2 mL/kg of distilled water) showed relatively normal histoarchitecture of these regions. It was the basic pattern of an ordered sheet of neurons (pyramidal and granule cells), whose cell bodies were all packed together. Large and sparse pyramidal cells in the CA3 region, and smaller and more closely packed pyramidal cells in the CA1 region. The Tramadol-treated group (Tram) demonstrated pathological changes in the CA1 and CA3 regions, such as karyorrhexis, cytoplasmic vacuolation, and perineural vacuolation. The group treated with tramadol (50 mg/kg) and naltrexone (12.5 mg/kg) (Tram+Nalt) revealed mild distortions such as karyorrhexis and cytoplasmic vacuolation in the histoarchitecture of the CA1 and CA3 regions. The group treated with tramadol (50 mg/kg) and a low dose of *Z. officinale* (500 mg/kg) (Tram+500 mg/kg EZO) showed moderate distortions in the histoarchitecture of the CA1 and CA3 regions, such as karyorrhexis and perineural vacuolation, and the group treated with tramadol (50 mg/kg) and a high dose of *Z. officinale* (1,000 mg/kg) (Tram+1,000 mg/kg EZO) showed improvement in the histoarchitecture in the CA1 and CA3 regions of the hippocampus (Fig. 3).

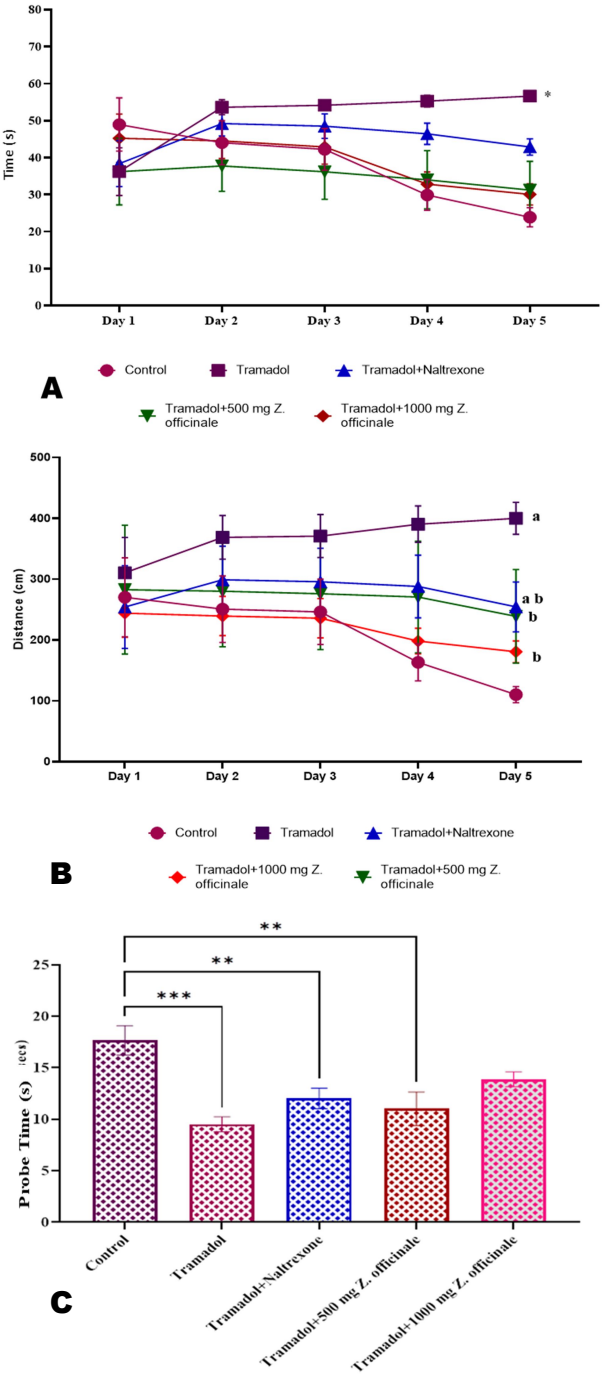

Fig. 2: Morris water maze (MWM) test: Comparison of the time spent, acquisition distance and probe time of Wistar rats following oral administration of tramadol and *Z. officinale*: A - Time spent in MWM; B - Acquisition distance in MWM; Two-way split-plot ANOVA. C - Probe time in MWM; Control = 2 mL/kg of distilled water, Tram = 50 mg/kg of tramadol, Nalt = 12.5 mg/kg of naltrexone. n=6; mean ± SEM, Tukey post-hoc test, *=$p<0.05$ when the tramadol group was compared to the control; a=$p<0.05$ when compared to control, and b=$p<0.05$ when compared to tramadol group; **=$p<0.05$ when control was compared to

Tram+Naltrexone, and Tram+500 mg *Z. officinale* group, ***=p<0.05 when control was compared to tramadol group

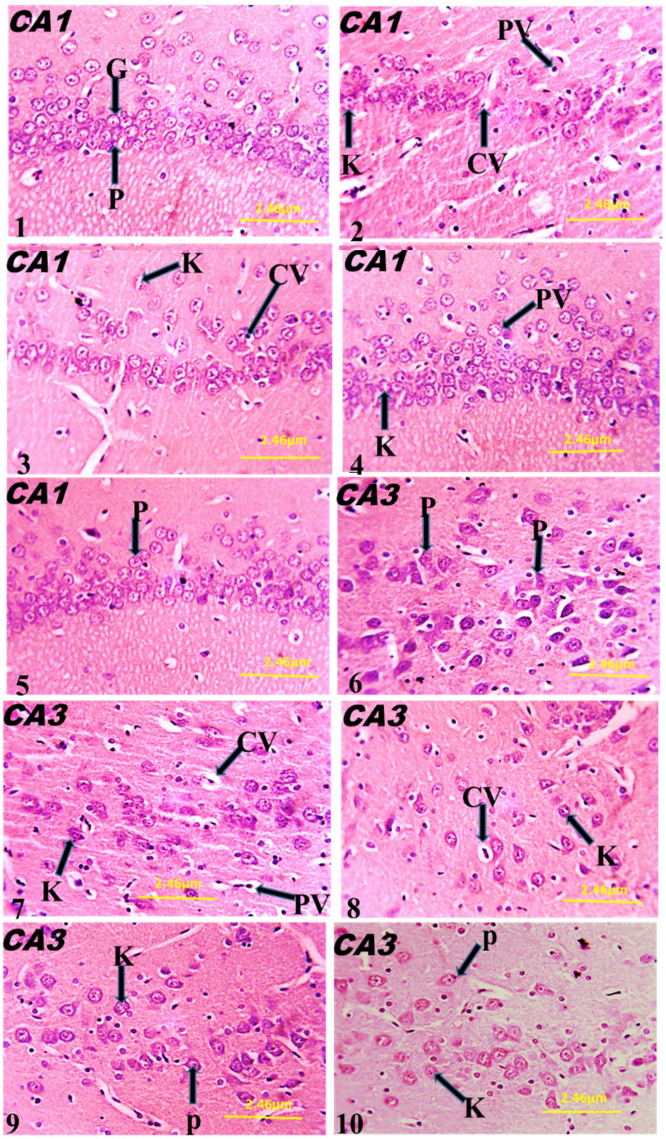

Fig. 3: Photomicrographs of the CA1 and CA3 regions of the hippocampus: 1 - CA1 region of control (2 mL/kg of distilled water) with normal histoarchitecture; 2 - CA1 of the tramadol (50 mg/kg) group showing distortions in the histoarchitecture; 3 – CA1 of the Tram + 12.5 mg/kg Nalt group showing mild distortion in the histoarchitecture; 4 - CA1 of the Tram + 500 mg/kg EZO group showing moderate distortion in the histoarchitecture; 5 - CA1 of the Tram + 1,000 mg/kg EZO group with improvement in the histoarchitecture; 6 – CA3 of the control (2 mL/kg of distilled water) with normal histoarchitecture. 7 - CA3 of the tramadol (50 mg/kg) group showing distortion in the histoarchitecture; 8 - CA3 of the Tram + 12.5 mg/kg of Tram + 500 mg/kg EZO group showing moderate distortion in the histoarchitecture; 10 - CA3 of the Tram + 1,000 mg/kg EZO group with improvement in the histoarchitecture. Pyramidal cells (P); granule cells (G); karyorrhexis (K); cytoplasmic vacuolation (CV); perineural vacuolation (PV). H & E, ×400.

DISCUSSION

There has been increased global use of tramadol over the years because it is believed to have fewer side effects than other opioids (Zhuo et al. 2012). Besides, tramadol is absorbed rapidly and almost entirely when administered orally (Grond and Sablotzki 2004). Many studies revealed that *Z. officinale* positively affects memory function and exhibits anti-neuroinflammatory activity, which might contribute to the management and prevention of neurodegenerative diseases (Huh et al. 2018). Hence, the effect of *Z. officinale* ethanol extract on tramadol-induced spatial memory deficits in adult Wistar rats was investigated.

A recent report indicated *Z. officinale* use as a natural remedy for various diseases is safe when administered at recommended therapeutic doses (Cunha 2021). In the present study, the lethal dose (LD_{50}) of *Z. officinale* ethanol extract (Lorke 1983) was above 5,000 mg/kg, indicating that the doses of *Z. officinale* ethanol extract used in the present experiment were safe, since they were below the lethal dose. This result contradicts many other works, including Nafiu et al. (2012), who observed the oral LD_{50} value of *Z. officinale* aqueous extract to be 4,525 mg/kg in rats. Abdulrazaq et al. (2012) reported the LD_{50} of *Z. officinale* ethyl acetate extract to be greater than 2,000 mg/kg in rats, while Anosike et al. (2009) calculated the LD_{50} of *Z. officinale* ethanol extract to be 1,000 mg/kg. The variations in the LD_{50} of the *Z. officinale* extract can be due to several factors. The composition of the plant extract can differ based on soil type, climate, and other environmental conditions (Kumar et al. 2013). Similarly, the genetic variability of the plant species across different regions and the extraction method can also affect the toxicity of the plant extract (Yao et al. 2016).

The effect of *Z. officinale* treatment on body weight was assessed in this study. There was a significant (p<0.05) increase in body weight of the tramadol-treated group, tramadol + 12.5 naltrexone, and tramadol + 1,000 mg/kg *Z. officinale* when the initial body weight of the Wistar rats was compared to the final body weight. There was also an increase in the tramadol + 500 mg/kg *Z. officinale* group, although it was not statistically significant. A non-significant reduction was observed in the final body weight of the tramadol-treated group when compared to the control. Mohamed et al. (2015) reported a reduction in body weight in groups treated with tramadol. In another study, a significant reduction in body weight at a later stage of the treatment period was observed with tramadol (0.71 mg/kg), which led to a suggestion of increased catabolism of lipids in the adipose tissue with tramadol treatment (Oyedeji 2020). Balogun et al. (2020) reported that a single-dose treatment of tramadol at 20 mg/kg reduced food consumption by rats when compared to the control and that the rats exposed to tramadol had low body weight.

On the other hand, there was an increase in body weight in the group treated with a high dose of the *Z. officinale* extract, which could be a result of the extract regulating the metabolism and reducing inflammation in the rats (Ebrahimzadeh et al. 2018). Ezeuko et al. (2007) reported

that an extract of *Z. officinale* was able to increase the mean body weight of rats exposed to mercury. In addition, Derbal and Kechrid (2019) observed that *Z. officinale* extract restored a near-normal body weight.

Tramadol has negative effects on memory and learning. However, the exact mechanism(s) resulting in these effects are still unknown. Some studies suggest that tramadol's negative impact on neuroplasticity, learning, and memory may be due to a decrease in the levels of intracellular signalling molecules such as cyclic adenosine monophosphate, cyclic guanosine monophosphate, protein kinase A, and protein kinase C. These molecules play important roles in regulating various cellular processes, and their decrease may contribute to the harmful effects of tramadol on the brain (Hosseini-Sharifabad et al. 2016).

Additionally, tramadol inhibits the M1 receptor, which may be a contributing factor to its negative effects on learning and memory. The M1 receptor is an acetylcholine-type receptor, a neurotransmitter that is important for cognitive function. Inhibition of this receptor by tramadol could therefore interfere with normal cognitive processes, leading to impaired learning and memory (Nakamura et al. 2005). Abdel-Salam et al. (2016) proposed that tramadol's negative central nervous system (CNS) impact could be due to its ability to inhibit the activity of butyrylcholinesterase and the paraoxonase-1 enzyme. These enzymes play a role in breaking down certain substances in the brain, and their inhibition by tramadol could result in the accumulation of these substances, leading to harmful effects on the CNS.

In the present Morris water maze test result, the tramadol-treated group had a significant decrease in the time it took them to locate the escape platform and a significant increase in the distance covered compared to the control group during the acquisition phase. This suggests that tramadol treatment elicits learning difficulties. Interestingly, the groups of Wistar rats that were treated with tramadol and either with 12.5 mg/kg naltrexone, 500 mg/kg *Z. officinale*, or 1,000 mg/kg *Z. officinale* showed a significant decrease in the distance it took them to locate the escape platform compared to the tramadol-treated group alone. This suggests that these rats were able to learn the location of the escape platform better than the rats treated with tramadol alone. Additionally, in the probe test, the rats treated with tramadol and either 12.5 mg/kg of naltrexone, 500 mg/kg of *Z. officinale*, or 1000 mg/kg of *Z. officinale* spent a significantly ($p<0.05$) decreased amount of time in the quadrant where the escape platform was located compared to the tramadol-treated group alone, indicating improvement in memory in these groups.

The present results are consistent with findings from several other studies. A large volume of studies demonstrated that opioid agonists will impair different kinds of memory (Hasanein and Ghafari-Vahed 2016). Baghishani et al. (2018) observed the inability of rats to remember the location of a hidden platform in the probe test of MWM following 28 days of tramadol (50 mg/kg) administration. In another study by Hosseini-Sharifabad et al. (2016), both acute and chronic administration of tramadol in rats was found to impair memory, as measured by an object recognition test. Interestingly, the study also found that a single dose of tramadol showed a more significant negative impact on memory than multiple doses of tramadol. Another study also reported impairment in spatial memory in rats treated with tramadol (42, 84, and 168 mg/kg/day) in the first, second, and third ten days of the study, respectively, using the Morris water maze (Nafea et al. 2016). Adekomi et al. (2019) also reported a decline in learning and memory following the administration of 60 mg/kg of tramadol for 30 days using the MWM, passive avoidance test, and novel object recognition test.

Studies suggest that tramadol can disturb the balance of neurotransmitters in the brain by increasing glutamate release while also having an inhibitory effect on the GABA system (Hassanian-Moghaddam et al. 2013). Glutamate and GABA neurotransmitters and their receptors have been frequently reported to play a major role in learning and memory, and they also have an important role in neuronal death and neurotoxicity (Costa et al. 2016). Glutamate acts as a neurotoxic agent, which is frequently attributed to the over activity of NMDA receptors (Kawasaki et al. 1997).

Khaliq *et al*. (2017) reported that a 6-week treatment of rats with *Z. officinale* aqueous extract (500 mg/kg) led to improved spatial and recognition memory, respectively, in MWM and novel object tests. Gomar et al. (2014) also reported that *Z. officinale* extract attenuated morphine-induced memory impairment in Wistar rats. *Z. officinale* has been reported to enhance the levels of norepinephrine, epinephrine, dopamine, and serotonin in the cerebral cortex and hippocampus (Waggas 2009). Moreover, this plant extract and its active components also inhibit cholinesterase activity, which in turn increases acetylcholine, a neurotransmitter that plays an important role in learning and memory (Ghayur et al. 2008).

The cognitive-enhancing effect of *Z. officinale* may be due to its ability to modulate both monoamine and cholinergic systems in various brain regions, including the prefrontal cortex and hippocampus. Additionally, the antioxidant properties of the extract, which are attributed to the presence of gingerol and shogaol, may also contribute to its cognitive-enhancing effects. By affecting these systems in the brain, *Z. officinale* extract may improve cognitive function and protect against oxidative stress-induced damage (Saenghong et al. 2012).

Chronic administration of tramadol has been reported to cause cerebral cortex-associated oxidative stress apoptosis in rats (Ghoneim et al. 2014). The hippocampus, amygdala, and cerebellar granule cells have been reported as the most susceptible to oxidative stress in some studies and consequently are purported to be the first to undergo functional decline (Wang and Michaelis 2010).

Microscopic studies using haematoxylin and eosin revealed normal histoarchitecture in the CA1 and CA3 regions of the hippocampus in the control group. Pathological changes in the histoarchitecture of the CA1 and CA3 regions of the hippocampus were observed in tramadol-treated groups, presenting as karyorrhexis, gliosis, and perineural vacuolation. There were

improvements in the histoarchitecture of the hippocampal neuronal cells of the extract-treated groups in a dose-dependent manner.

The neurotoxic effects of tramadol have sometimes been attributed to its adverse effects on antioxidants such as glutathione and inhibition of glutathione peroxidase activity. Tramadol administration has also been reported to cause overproduction of nitric oxide and brain tissue oxidative damage with a high level of malondialdehyde (Abdel-Zaher et al. 2011). The toxic effects of tramadol at the cellular level could be explained by increasing lipid peroxidation, which could be used as a marker of reactive oxygen species (ROS)-induced cell damage (Popovic et al. 2009).

Studies have shown that tramadol abuse confers deleterious effects on the functional integrities of the CNS (El-Bermawy and Salem 2015). In rats, tramadol preferentially gains access to the brain tissues compared to its active metabolite (Tao et al. 2002). It is postulated that tramadol induces neurotoxicity by decreasing the blood-brain barrier membrane fluidity as a consequence of the loss of unsaturation and fundamental changes in the structural concentrations of a number of fatty acids (Alici et al. 2003).

Continuous administration of tramadol at various doses to experimental animals has been delineated to result in alterations in the histoarchitecture of the brain. Ragab and Mohamed (2017) reported similar findings following a 28-day tramadol (50 mg/kg) administration, which resulted in multinuclear cells, absence of nuclei, dilated blood capillaries, diffuse chromatolysis of nuclear chromatin and absence of nucleoli, degenerative vacuolization, and intercellular oedema, amongst others. Ghoneim et al. (2014) reported that a 4-week administration of tramadol (50 mg/kg/day) resulted in irregular darkly stained pyramidal cells, with pyknotic nuclei surrounded by haloes, shrunken and marked cytoplasmic vacuolization, faintly stained cytoplasm and nuclei of some pyramidal cells, and the presence of dilated congested blood vessels with inflammatory cells in them and the red neurons.

The improvement observed in the extract-treated groups in the present study could be a result of its antioxidant properties. Several studies have found that *Z. officinale* has high antioxidant activity (Abolaji et al. 2017). This is mainly associated with their polyphenol contents (Li et al. 2012). *Z. officinale* is known to exhibit neuroprotective activity by activating nuclear factor erythroid 2-related factor 2 (Nrf2), scavenging free radicals, and elevating the levels of several phase II antioxidant molecules, such as NAD(P)H quinone dehydrogenase 1 (NQO1) and heme oxygenase-1 (HO-1), in neuron-like rat pheochromocytoma PC12 cells (Peng et al. 2015). 10-gingerol is delineated as being responsible for the strong anti-neuroinflammatory capacity of fresh *Z. officinale*. Another study reported that *Z. officinale* displayed a protective role in the brains of people with diabetes by reducing oxidative stress, inflammation, and apoptosis (El-Akabawy and El-Kholy 2014).

Several studies have reported the potential beneficial effects of *Z. officinale* on the brain and its structures. For example, El-Akabawy and El-Kholy (2014) found that *Z. officinale* extract improved the histological structure of the hippocampus in rats with Alzheimer's-like disease. Hussein et al. (2017) reported the neuroprotective effects of *Z. officinale* against monosodium glutamate-induced histological alterations and degeneration. Sangi et al. (2019) reported an ameliorative effect of *Z. officinale* extract on streptozotocin-induced brain damage in rats. Together, these studies suggest that *Z. officinale* may have potential neuroprotective effects and could be beneficial in preserving the structural integrity of the brain.

Conclusion

Findings from the present study suggest that *Z. officinale* ethanol extract attenuated the effects of tramadol-induced body weight, brain weight, spatial memory deficits, and histological alteration in the CA1 and CA3 regions in adult Wistar rats.

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Conflict of Interest

None declared.

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Authors' Contribution

Conceptualization: UUE, UEU, AAS. Data acquisition: UUE, YMS DK, GAT. Data analysis: UUE, YMS, GPO. Methodology: UEU, AAS, UUE. Supervision: UEU, AAS, GPO. Validation: UEU, AAS. Visualization: YMS, UUE, DK, GAT, GPO. Roles/Writing- draft: UUE, GPO. Writing - review and editing: UUE, UEU, AAS, YMS, GPO, GAT, DK.

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