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Original Article

Tramadol Induces Alterations in the Cognitive Function and Histoarchitectural Features of the Hippocampal Formation in Adult Wistar Rats

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Abstract

BACKGROUND AND AIM: Tramadol abuse has a substantial risk of altering the histoarchitecture and causing cognitive decline. Therefore, this study investigated the effects of tramadol on cognitive function and histopathological changes in the structures of the hippocampal formation.
METHODOLOGY: Twelve apparently healthy Wistar rats (120-150g) were used for this study. They were divided into two groups with six (n=6) rats in each group. The control group was given 2ml/kg of distilled water while 50 mg/kg of tramadol was given to group 2 orally for 21 days. The Morris Water Maze (MWM) was utilized to assess spatial learning and memory over the last six days of the experiment. The rats were euthanized and brains harvested, fixed in 10% formol saline, and processed routinely using haematoxylin & eosin (H&E) for general histoarchitecture.
RESULTS: The result of MWM showed a prolonged time taken and longer distance covered to locate the escape platform in the acquisition phase while the probe test showed less time spent in the target quadrant in which the platform was previously located in the tramadol-treated group. Histologically, there were several neuronal degenerative changes presenting as pyknotic nuclei, dark neurons, vacuolations, congested blood vessels, karyorrhexis, necrotic cells, and infiltration of cells in the various layers of the structures of the hippocampal formation.
CONCLUSION: findings from this study revealed changes in spatial learning and memory, and hippocampal formation histopathology.

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hippocampal formation, histopathology, memory impairment, morris water maze neurodegeneration

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INTRODUCTION

Tramadol, an artificially produced opioid sedative, is used to treat moderate to severe pain. Despite this, it has been abused in Nigeria due to its euphoric effects and ease of access. Tramadol abuse is a significant public health concern in Nigeria, with a reported incidence rate of 54.4% and the drug is mostly sourced without prescription (Ibrahim *et al*., 2017). Misuse of tramadol can cause respiratory depression, convulsions, death, and addiction in addition to other mental health and physical problems (Nakhaee *et al*., 2021). It has been resolved that there is an issue with tramadol abuse in Nigeria. Nigerian officials banned the distribution and importation of high-dose tramadol, making it a prohibited substance (Nelson *et al*., 2023). Public health programs have been established countrywide to further educate the public on the dangers of tramadol use and the importance of receiving treatment for addiction. Despite these efforts, tramadol use is still rising in Nigeria. This may be due to the market's convenience of low-dose tramadol and the lack of execution of existing guidelines (Nakhaee *et al*., 2021).

Tramadol has an impact on the central nervous system (CNS), more especially the -opioid receptors. The hippocampal formation which includes a number of regions such as the entorhinal cortex, dentate gyrus, cornu ammonis, and subiculum has apparently been impacted by tramadol. These areas are essential for spatial navigation and associative memory, the creation of new

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memories, memory consolidation and recall, and spatial and episodic memory. Several studies have found that these areas exhibit decreased neuronal numbers and dendritic complexity, which in turn affects their abilities to perform certain tasks following incessant tramadol administration in rat models (Arslan *et al*., 2014; Ebrahimpoor *et al*., 2017; Mohammadi *et al*., 2018; Kermani *et al*., 2019). The medial temporal lobe of the brain houses the intricate hippocampal formation which is essential for memory, learning, and spatial navigation. Declarative and episodic memories, or experiences that can be intentionally recalled and spoken verbally, are memories that can be retrieved from the hippocampal formation (Eichenbaum, 2001). According to studies, damage to hippocampal formation can cause serious memory loss as well as difficulties with spatial navigation. For instance, individuals with impaired hippocampal formation brought on by conditions like Alzheimer's disease or stroke can experience serious memory loss and have trouble navigating their surroundings (Scoville and Milner, 1957).

Numerous neurological and psychological issues have also been connected to hippocampal formation. For instance, those with post-traumatic stress, depression, and anxiety disorder have aberrant hippocampal formations (Karatsoreos *et al*., 2011). Additionally, epilepsy, a neurological ailment characterized by recurring seizures, has been connected to hippocampal formation (Engel *et al*., 2003). The purpose of this study is to investigate the neurotoxic effects of tramadol on the hippocampal formation, as previous studies have examined the neurotoxic effects of tramadol on various other areas of the brain, but the impact on the hippocampal formation remains unknown. This study aims to address this gap in the current understanding and offer new insights into how tramadol may influence the hippocampal formation, a critical brain structure involved in various cognitive and neurological processes.

MATERIALS AND METHODS

Ethical approval

Ethical approval for this study was granted by the Ahmadu Bello University Ethics Committee on Animal Use and Care, with approval code ABUCAUC/2022/031.

Drug and chemical procurement

Tramadol hydrochloride (50 mg capsules), produced by VADIS PHARM. LTD. Plot RD-14-Trans-Ekulu, Enugu, Nigeria was procured and used as the reference drug for the study. Ketamine (50 mg/ml ketamine hydrochloride injection USP) was utilized as the anesthetic. It was procured from Swiss Parenterals PVT Ltd. In Gusssjarat, India.

Experimental design and treatment of animals

Twelve (12) male adult Wistar rats weighing 150–190 g was acquired from the Animal House of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU) in Zaria, Nigeria. Prior to the start of the tests, they were moved and kept in wired cages at the Department of Human Anatomy, Faculty of Basic Medical Sciences of the same institution. They were acclimatized for two weeks before the experimentation. All animals were given pelletized feed (Grand Cereals and Oil Mills Limited, Plateau State, Nigeria) and water *ad libitum*.

The twelve (12) male Wistar rats were allocated into two groups of six (6) each at random. Control group (2 ml/kg of water) and tramadol-treated group (50 mg/kg) (Ibrahim & Hala, 2016; Bekjarovski *et al*., 2012; Ekpo *et al*., 2023). All administrations were carried out orally, once daily for 21 days. The dosage of the medications was estimated based on the weight of the animals and administered. It was suspended in a normal saline solution and freshly given to the rats by feeding them through a needle.

Morris Water Maze Test

The Morris water maze (MWM) is a test for spatial learning for rodents that requires them to move from their starting points around the edge of an open swimming pool in search of a submerged escape platform using distal cues. For this test, a black circular pool with a depth of 30 cm, a height of 60 cm, and a temperature range of 23-25^{0}C was placed in the middle of a small room. In the center of the northeast quadrant of the pool, about 2 cm below the water's surface, was a circular platform with dimensions of 10 cm in diameter and 28 cm in height. There were also consistent visual cues outside the MWM in various places across the space, such as laptops, and posters. The rats performed a trial on each of the five consecutive days. The rat was introduced into the pool and released at one of the four quadrants, facing the walls. North, East, South, and West were designated as the four quadrants inside the apparatus's borders. Throughout the trial, the released location was kept at a predefined, random place. The animals were free to swim during each trial until they located the platform and stayed there for 20 seconds. The rats that could not discover the platform were led there after the 60 seconds had passed. For the next 20 seconds, the rats were allowed on the platform. After which, the rats were taken out of the pool and dried after the experiments. A video tracking system kept track of the time and distance traveled to get to the platform. The platform was taken out on day six. The time spent and the distance traveled in the target quadrant were compared between the groups after the rats were given 60 seconds to swim (Sadeeq *et al*., 2013; Huang *et al*., 2016; Mohammadipour *et al*., 2016; Ekpo *et al*., 2023).

Animal Euthanization

After completion of the experiment, the animals were anesthetized with 75 mg/kg ketamine intraperitoneally (Kurdi *et al*., 2014), and perfused transcardiacally with normal saline and 10 % formol saline. The brains of the rats were carefully removed from their skulls after perfusion and cleaned with normal saline. The fixed brains were taken to the Histology Unit, Department of Human Anatomy, Ahmadu Bello University, Zaria, for tissue processing and staining.

Histopathological studies

Slices of fixed brain tissues were processed using histological methods, with a focus on hippocampal formation, utilizing the Rats Brain Atlas as a reference (IVAN Study Investigators *et al*., 2012). The Department of Human Anatomy's Histology Unit, ABU, Zaria produced, processed, and stained histological sections using hematoxylin and eosin (H & E) stains to display the histoarchitecture of the structures of the hippocampal formation (entorhinal cortex, dentate gyrus, CA1, CA3, and subiculum). An optical microscope (HM-LUX, Leitz Wetzlar, Germany) and a digital microscopic camera (MA 500 AmScope®, USA) were utilized in the Laboratory for Microscopy and Stereology Research of the same institution to conduct microscopy and micrography.

DATA ANALYSIS

Data were shown as mean and SEM (standard error of the mean). Student t-test was employed to see the mean difference between the groups and two-way split ANOVA for differences within the groups. Statistically significant values were those with $P < 0.05$. Data analysis was done using statistical product and service solutions (IBM SPSS 26).

RESULTS

Morris Water Maze test for spatial learning and memory

The Morris Water Maze test was used to evaluate the Wistar rats' spatial learning and memory. The Wistar rats' swimming distance and mean latency time were used to assess their level of learning as they navigated from the start quadrant to the escape platform. There was a significant ($p<0.05$) increase in mean latency time taken and distance covered for the Wistar rats to locate the escape platform among the tramadol-treated group when compared to the control group in the acquisition phase (Figure 1). During the probe test, a significant ($p<0.05$) decrease in the time spent by the Wistar rats in the quadrant where the escape platform was initially located was observed in the tramadol-treated group compared to the control (Figure 1).

Histological effects of tramadol on the entorhinal cortex

Histological examination of the entorhinal cortex reveals neuronal cells arranged in layers I, II III, IV, V, and VI. Layer I is poorly cellular, layer II contains islands of rounded cells with nuclei having large nucleoli, III contains medium pyramidal cells with large nucleoli in their nuclei, layer IV has lamina dissecans with small pyramidal cells and stellate cells, layer V contains mainly large pyramidal cells, and layer VI containing cells that have various sizes and shapes in the control group (Figure 2). The tramadol-treated group shows layers I, II, and III having many degenerated pyramidal cells that appear condensed with pyknotic nuclei, dark neurons, congested blood vessels, and infiltration of cells while layers IV, V, and VI manifesting several neurodegenerative changes such as vacuolation, dark neurons, karyorrhexis, congested blood vessel and necrotic cell (Figure 3).

Effects of tramadol on the dentate gyrus

Histological examination of the dentate gyrus shows a distinct molecular layer that contains some glial cells, a granular cell layer with densely packed rounded to oval-shaped granular cells, and a polymorphic layer with some glial cells. The tramadol-treated group has many degenerated granular cells that appear condensed with pyknotic nuclei, karyorrhexis, and cytoplasmic vacuolation (Figure 4).

Effects of tramadol on the CA1 and CA3 regions

Histological examination of the CA1 and CA3 regions of the control group reveals a molecular layer, pyramidal cell layer, and polymorphic layer with normal pyramidal cells, granular cells, and some glial cells. Section of the CA1 and CA3 regions of the tramadol-treated group reveal degenerated neuronal cells presenting as karyorrhexis, cytoplasmic vacuolation, and perineural vacuolation (Figures 5 & 6).

Effects of tramadol on the subiculum

Histological examination of the subiculum region of the control group reveals the molecular layer, the granular cell layer, and the polymorphic layer of the subiculum having normal medium-sized pyramidal cells, and deeply stained nuclei of glial cells with normal blood capillaries. The tramadol-treated group shows many degenerated pyramidal cells that appear condensed with pyknotic nuclei, karyorrhexis, and congested blood vessels (Figure 7).

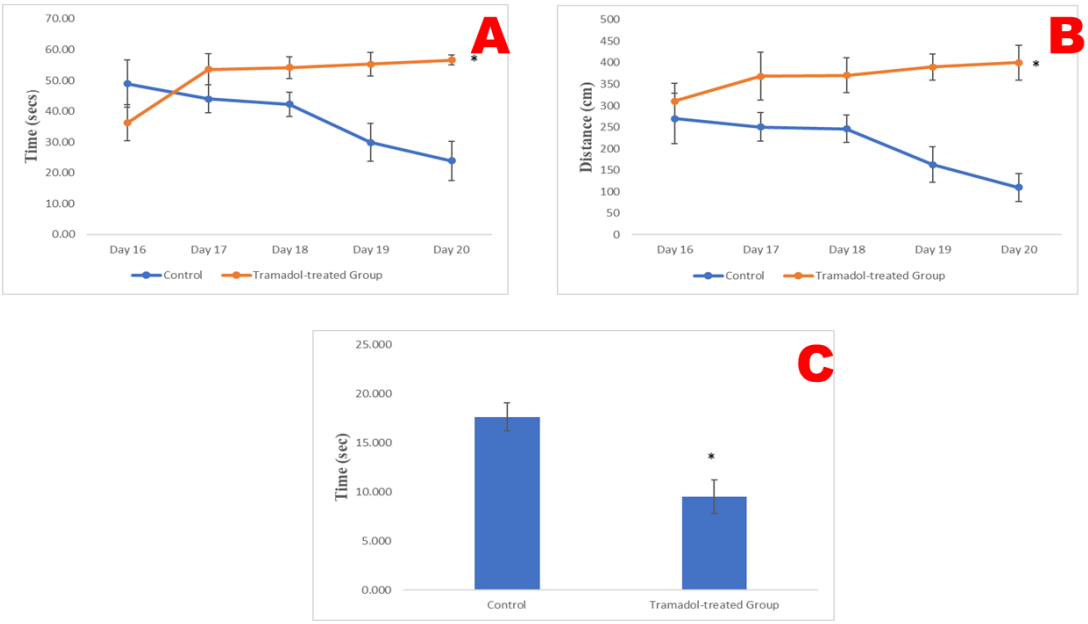

Figure 1: (A) Morris water maze acquisition time for Wistar rats following oral administration of tramadol. n=6; mean ± SEM, two-ways split-plot ANOVA, *Tukey post-hoc test*, *=p<0.05 when tramadol group was compared to control group. Morris water maze acquisition distance of Wistar rats following oral administration of tramadol. n=6; mean ± SEM, two-ways split-plot ANOVA, *Tukey post-hoc test*, when compared to control. (C) Morris water maze probe time for Wistar rats following oral administration of tramadol and Z. officinale. n=6; mean ± SEM, one-way ANOVA, Tukey post-hoc test, *=p<0.05 when compared to control group. Control = 2 ml/kg of distilled water, Tram = 50 mg/kg of Tramadol.

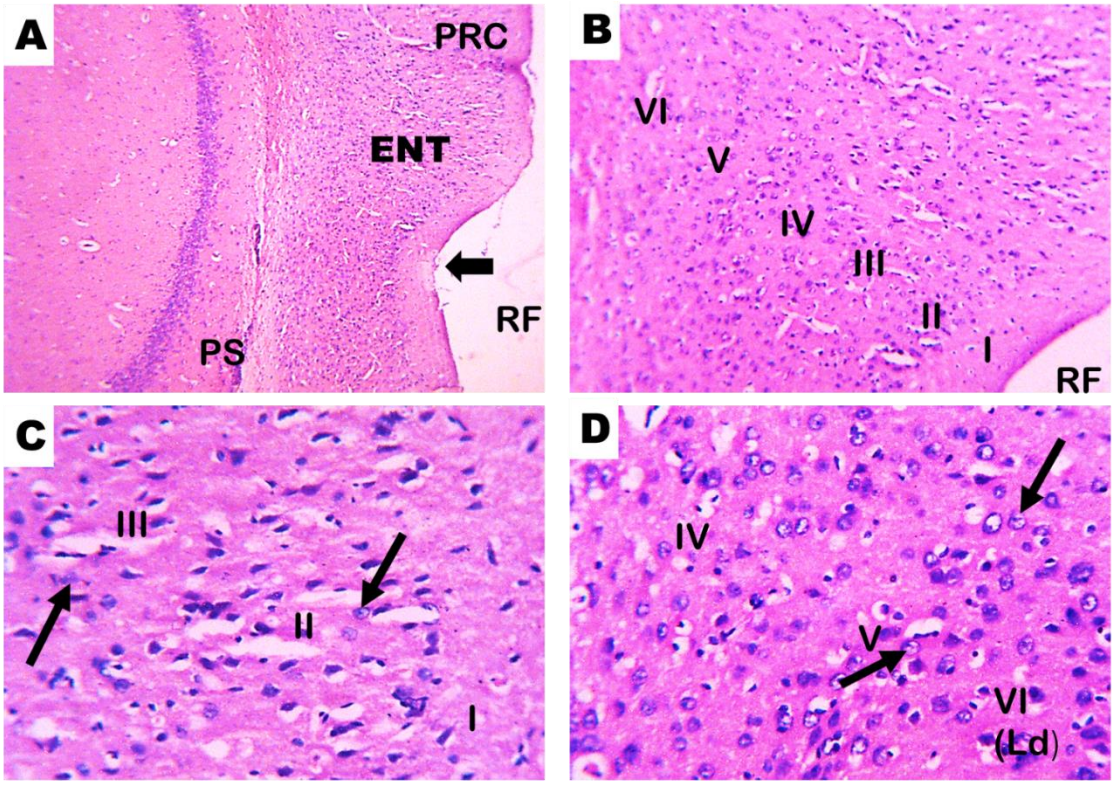

Figure 2: Photomicrographs of the entorhinal cortex (ENT) region. (A) Coronal section of adult Wistar rat cerebral hemisphere of control group showing the location of the entorhinal cortex, bordered medially by the parasubiculum (PS) and laterally by perirhinal cortex (PRC). A black arrow pointing to rhinal fissure (RF) H&E; 40x. (B) Coronal section of the entorhinal cortex of the control group showing neuronal cells arranged in layers I, II III, IV, V, and VI. H&E; 100x. (C) Section of the entorhinal cortex of the control group showing layer I which is poorly cellular, layer II which contains islands of rounded cells with nuclei having large nucleoli (arrows), layer III contains medium pyramidal cells with large nucleoli in their nuclei. H&E; 250x. (D) Section of the entorhinal cortex of the control group showing layer IV pointing to lamina dissecans with small pyramidal cells and stellate cells, layer V containing mainly large pyramidal cells (arrow), and layer VI containing cells that have various sizes and shapes. H&E; 250x.

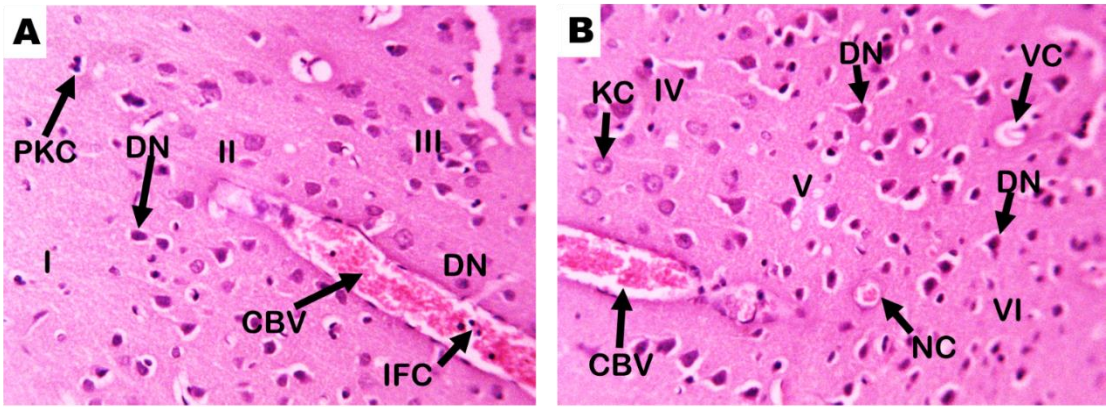

Figure 3: Photomicrographs of Wistar rat entorhinal cortex treated with tramadol. (A) section of the entorhinal cortex of tramadol treated group showing layers I, II, and III having many degenerated pyramidal cells that appear condensed with pyknotic nuclei (PKC), dark neurons (DN), congested blood vessel (CBV) and infiltration of cells (IFC). H&E; 250x. (B) Section of the entorhinal cortex of tramadol treated group showing layers IV, V, and VI manifesting several neurodegenerative changes such as vacuolation (VC), dark neurons (DN), karyorrhexis (KC), congested blood vessel (CBV) and necrotic cell (NC). H&E; 250x.

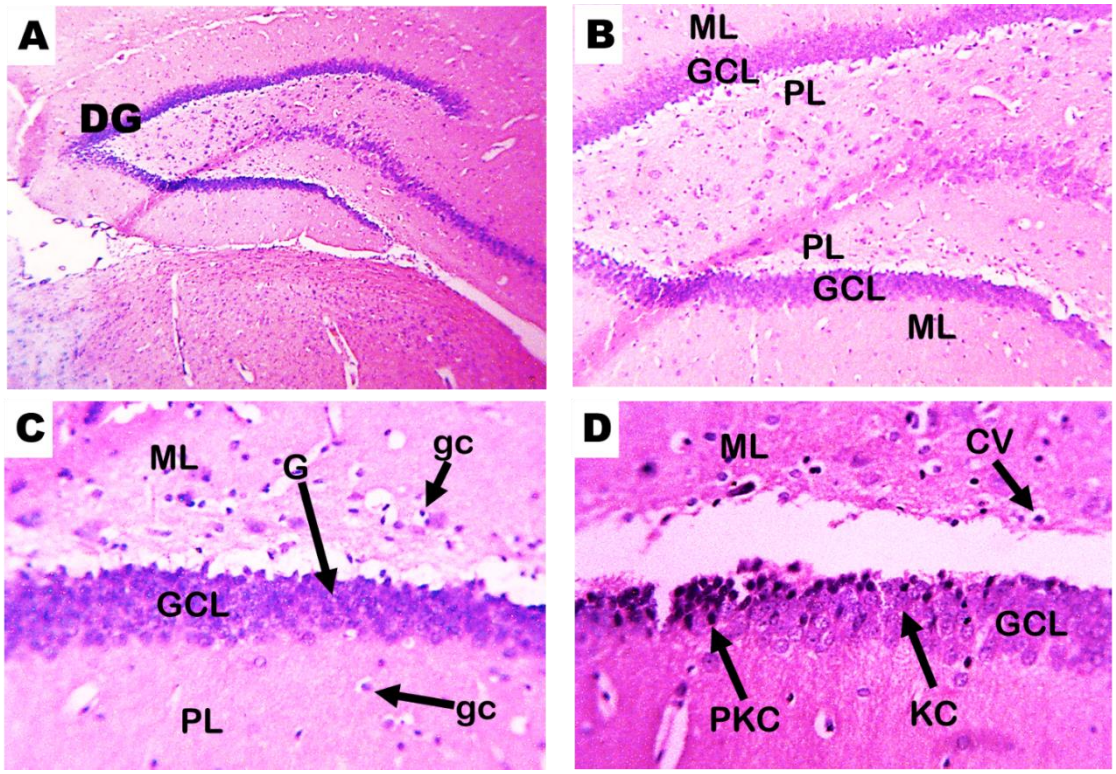

Figure 4: Photomicrographs of the dentate gyrus (DG) region. (A) The control group showing the location of the dentate gyrus (DG). H&E; 40x. (B) The control group presenting three layers: the molecular layer (ML), the granular cell layer (GCL), and the polymorphic layer (PL). H&E; 100x. (C) The control group showing a distinct molecular layer (ML) which contains some glial cells, a granular cell layer (GCL) with densely packed rounded to oval-shaped granular cells (GC), and a polymorphic layer (PL) with some glial cells (gc). H&E; 250x. (D) The tramadol-treated group having many degenerated granular cells that appear condensed with pyknotic nuclei (PKC), karyorrhexis (KC), and cytoplasmic vacuolation (CV). H&E; 250x.

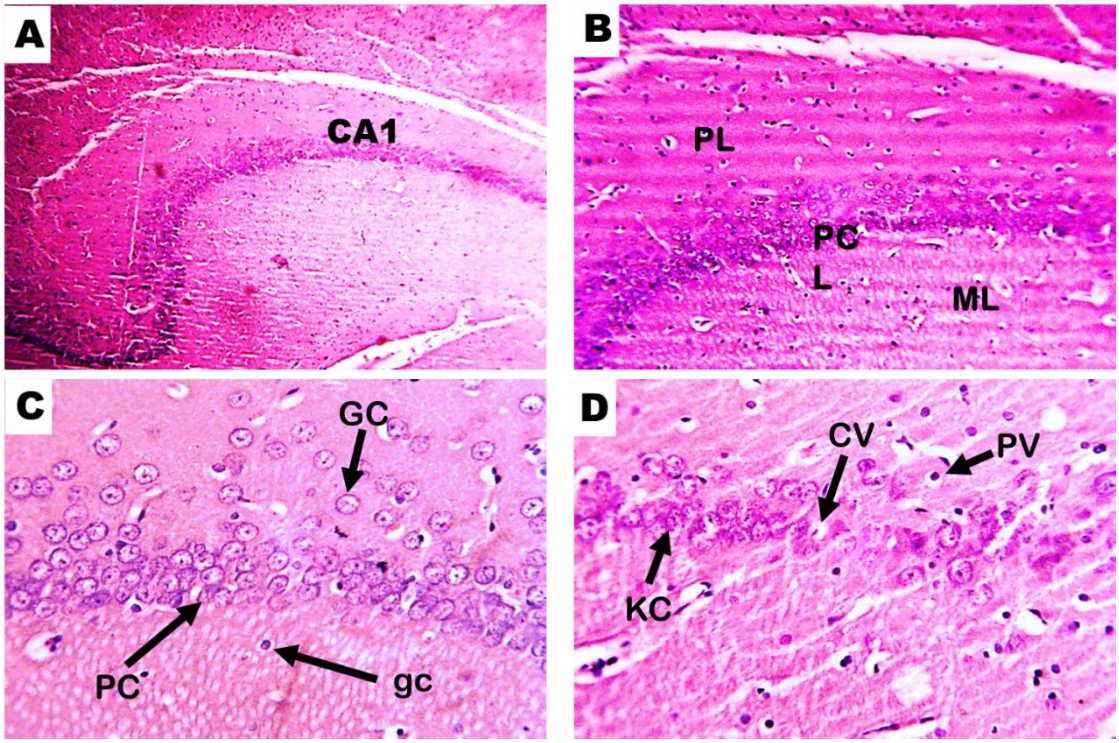

Figure 5: Photomicrographs of the CA1 region of the hippocampus. (A) Section of Wistar rat hippocampal region of control group showing the location of CA1. H&E; 40x. (B) Section of CA1 region of the control group showing molecular layer (ML), pyramidal cell layer (PCL), and polymorphic layer. H&E; 100x. (C) Section of CA1 region of control group having normal pyramidal cells (PC), granular cells (GC), and some glial cells (gc). H&E; 250x. (D) section of the CA1 region of tramadol-treated group revealing degenerated neuronal cells presenting as karyorrhexis (KC), cytoplasmic vacuolation (CV), and perineural vacuolation (PV). H&E; 250x.

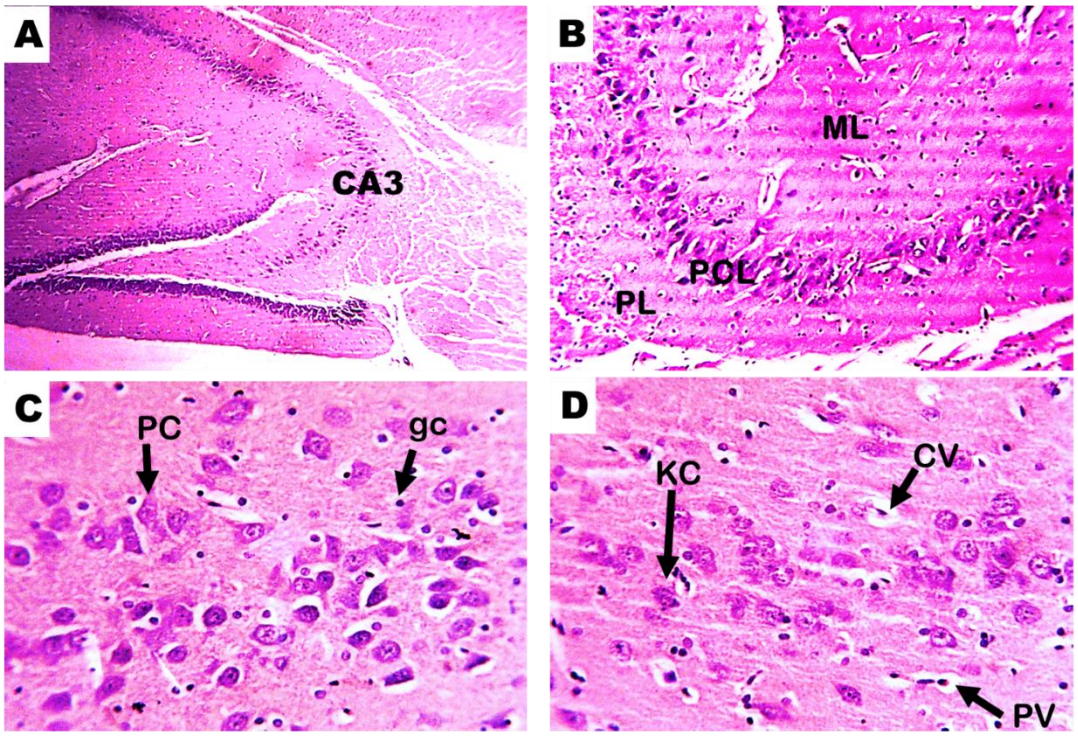

Figure 6: Photomicrographs of the CA3 region of the hippocampus. (A) Section of Wistar rat hippocampal region of control group showing the location of CA3. H&E; 40x. (B) Section of CA3 region of control group showing molecular layer (ML), pyramidal cell layer (PCL), and polymorphic layer. H&E; 100x. (C) Section of CA3 region of control group having normal pyramidal cells (PC), and some glial cells (gc). H&E; 250x. (D) section of the CA3 region of tramadol-treated group revealing degenerated neuronal cells presenting as karyorrhexis (KC), cytoplasmic vacuolation (CV), and perineural vacuolation (PV). H&E; 250x.

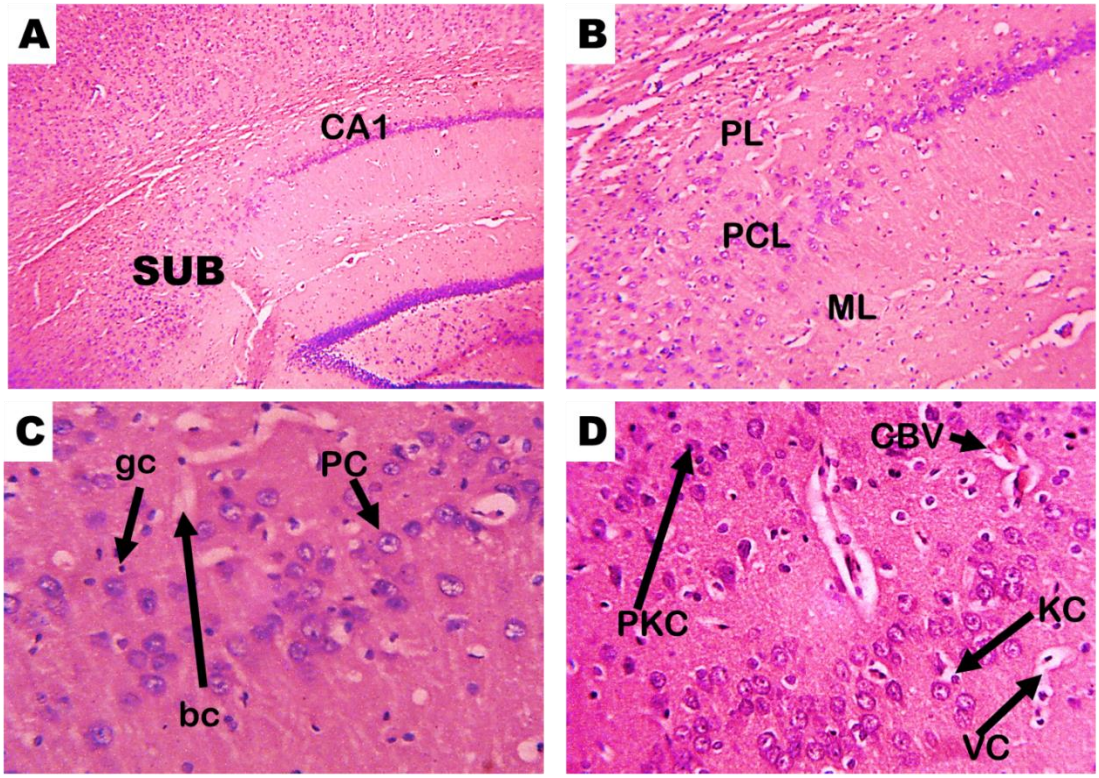

Figure 7: Photomicrographs of the subiculum (SUB) region. (A) The control group showing the location of the subiculum. H&E; 40x. (B) The control group showing the molecular layer (ML), the granular cell layer (PCL), and the polymorphic layer (PL) of the subiculum. H&E; 100x. (C) The control group showing normal medium-sized pyramidal cells, and deeply stained nuclei of glial cells with normal blood capillaries. H&E; 250x. (D) The tramadol-treated group having many degenerated pyramidal cells that appear condensed with pyknotic nuclei (PKC), karyorrhexis (KC) with congested blood vessels (CBV). H&E; 250x.

DISCUSSION

Tramadol has suddenly become a global problem in underdeveloped nations like Nigeria, which may be due to the drug's low cost and easy availability (United Nations Office on Drugs & Crime, 2018). Additionally, tramadol is being used more frequently across the globe since it is thought to have fewer negative effects than other opioids (Zhuo *et al*., 2012). Because it accelerates neurodegeneration in numerous areas of the brain, tramadol, a synthetic counterpart of codeine can cause a variety of behavioral deficits and histopathological changes. Additionally, when taken orally, it might be swiftly absorbed 9Groud & Sablotzki, 2004), and it has the potential to quickly cross the blood-brain barrier, having a variety of impacts on the central nervous system (CNS) (Hosseini-Sharifabad *et al*., 2016)

The hippocampal formation is a complex structure located in the medial temporal lobe of the brain. It is believed to be involved in memory, spatial navigation, and attention management. In all mammals, the hippocampal formation's neuronal architecture and circuits are remarkably similar (Anderson *et al*., 2007). One of the parts of the brain that is particularly prone to damage is hippocampal formation. Significant cognitive abnormalities, such as memory impairment and problems with spatial navigation, can result from damage to this region (Smith *et al*., 2003). As a result, the effects of tramadol on the hippocampal formation's structural components, spatial learning, and memory were investigated. In the present study, continuous administration of tramadol to Wistar rats resulted in prolonged time taken and longer distance covered to locate the escape platform during the five days of the acquisition phase, suggesting the inability of the rats to learn while the probe test showed memory deficit presented as less time spent in the target quadrant in which the platform was previously located. The results of this study are consistent with the findings of a vast number of other investigations, which found that opioid agonists impair many types of memory (Dubrovina and Llyutchennok, 1996; Ghamati *et al*., 2014; Hasanein and Ghaferi-Vahed, 2016; Khatmi *et al*., 2022; Ekpo *et al*., 2023). After receiving 50 mg/kg of tramadol daily for 28 days, Baghishani *et al*. (2018) noticed that the rats were unable to recall where the hidden platform was during the probe test of MWM. Using the Morris Water Maze, Nafea *et al*. (2016) also reported impairment in spatial memory in rats given tramadol at doses of 42, 84, and 168 mg/kg/day throughout the first, second, and third ten days of the research, respectively. Similarly, a reduction in learning and memory after giving rats 60 mg/kg body weight of tramadol for 30 days was observed using the Morris water maze, passive avoidance test, and novel object recognition test (Adekomi *et al*., 2019). In the same vain, Ekpo *et al*. (2023) reported memory impairment following administration of 50 mg/kg tramadol to laboratory rats for twenty-one days. This was

marked by their inability to locate the escape platform early in Morris Water Maze test.

Tramadol's ability to impair memory is not entirely understood, however, there are a number of possibilities that have been put out. One explanation for how tramadol works is through its effects on neurotransmitters, particularly serotonin, and norepinephrine. By inhibiting their reuptake, tramadol stimulates the mu-opioid receptors and increases the availability of serotonin and norepinephrine (Khezri *et al*., 2015). However, excessive stimulation of these receptors may cause cognitive impairment, including memory loss (Smith *et al*., 2019). Numerous studies show that tramadol inhibits the GABA system while increasing glutamate release, which could alter the balance of neurotransmitters in the brain (Hara *et al*., 2005; Rehni *et al*., 2018; Abdel-Zaher *et al*., 2011; Hassanian-Moghaddam, 2013). Studies show that learning, memory, neuronal death, and neurotoxicity depend on glutamate and GABA neurotransmitters and their receptors (Zeng *et al*., 2007; Costa *et al*., 2016). The overactivity of NMDA receptors is correlated with the neurotoxic effects of glutamate (Kawasaki *et al*., 1997). Tramadol also has an impact on the hippocampus, a region of the brain crucial for memory consolidation. Decreased neurogenesis, synaptic plasticity, and long-term potentiation have all been associated with morphological and functional changes in the hippocampus when opioid use is chronic (Chen *et al*., 2021). These changes may limit the hippocampus' ability to retain and retrieve memories.

Tramadol has been linked to histological abnormalities such as increased apoptosis in rat cerebral cortex linked to oxidative stress after chronic treatment (Ghoneim *et al*., 2014). Additionally, research demonstrates that tramadol increases oxidative stress in a variety of organs, including the brain (Abdel-Zaher *et al*., 2011). Hippocampal, amygdala, and cerebellar granule cells have reportedly been found to be the most vulnerable to oxidative stress in several investigations (Wang & Michaelis, 2010). They are allegedly the first to experience functional deterioration. Additionally, harmful agents and chemicals are known to alter Nissl bodies and change their metabolic activity (Davis & Robertson, 1991). Microscopic studies using haematoxylin and eosin revealed a normal histoarchitecture having distinct layers and cells in the entorhinal cortex, dentate gyrus, CA1, CA3, and the subiculum of the hippocampal formation in the control group while the tramadol-treated group revealed several neuronal degenerative changes presenting as pyknotic nuclei, dark neurons, vacuolations, congested blood vessel, karyorrhexis, necrotic cell and infiltration of cells in the various layers of these structures.

Tramadol has been shown to change the histoarchitecture of the brain when given continuously to experimental animals at different doses (Ghoneim *et al*., 2014; Badawy *et al*., 2014). Similar to our study, Ragab and Mohamed, (2017) found that administering 50 mg/kg of tramadol intraperitoneally for twenty-eight days caused degenerative neuronal changes like multinuclear cells, absence of the nuclei, dilated congested blood capillary, diffuse chromatolysis of nuclear chromatin and absence of nucleoli, degenerative vacuolization, and intercellular oedema among other things using toluidine blue stain. According to another study, rats given 50 mg/kg/day of tramadol intraperitoneally for four weeks developed irregular, darkly stained pyramidal cells with pyknotic nuclei and haloes, markedly shrunken cytoplasmic vacuolization, faintly stained cytoplasm and nuclei of some pyramidal cells, and the presence of dilated congested blood vessels with inflammatory cells and red neurons (Ghoneim *et al*., 2014). Also, according to Awadalla and Salah-Eldin (2016), the brains of rats treated with tramadol showed striking histomorphological changes in the cerebral cortex and hippocampus.

Liu *et al*. (2013), reported that numerous opioid effects on neuronal structure (cytoskeleton) are viewed as indicators of neuronal damage brought on by long-term morphine and other opioid use. Tramadol's neurotoxic effects have occasionally been linked to its negative impacts on antioxidants like glutathione and its reduction of glutathione peroxidase activity. Tramadol use has also been linked to increased levels of malondialdehyde, excessive nitric oxide production, and oxidative damage to brain cells. Tramadol use has also been linked to increased levels of malondialdehyde, excessive nitric oxide production, and oxidative damage to brain cells (Abdel-Zaher *et al*., 2011). Furthermore, oxidative changes have been linked to reduced enzyme activity and decreased function (Butterfield *et al*., 2007). Increased lipid peroxidation, which can be used as a marker of reactive oxygen species (ROS)-induced cell damage, may help to explain why tramadol has toxic effects on cells (Popovic *et al*., 2009). This may explain the increase in time and the distance taken to locate the escape platform observed in the Morris water maze leading to alteration in spatial learning and memory. In conclusion this study's findings demonstrated the negative impacts of tramadol administration over time. The study observed cognitive impairment and histopathological changes in the hippocampal formation following tramadol administration, and as such, people who abuse this substance should be discouraged from doing so.

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Authors' contributions

Conceptualization: UUE, UEU, AAS, OPG Data acquisition: UUE, OPG, SS, IBI, Data analysis or interpretation: UUE, OPG, BOO, KBR. Drafting of the manuscript: UUE, SS, IBI. Critical

revision of the manuscript: UEU, AAS, OPG, BOO, KBR, Approval of the final version of the manuscript: all authors.

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