Tramadol abuse and addiction: effects on learning, memory, and organ damage

Loveday U. Zebedee[a], Moses W. Bariweni[b], Yibala I. Oboma[a], Ikhide G. Ilegbedion[a]

[a]Department of Medical Laboratory Sciences, Faculty of Basic Medical Science, College of Medicine, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria, [b]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

Correspondence to Moses W. Bariweni, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. Tel: +2348064246190; e-mails: mbariweni@ndu.edu.ng, mbariweni@yahoo.com

Received: 1 August 2021
Revised: 1 October 2021
Accepted: 1 December 2021
Published: 7 March 2022

Egyptian Pharmaceutical Journal 2022, 21:75–83

Background
Addiction to controlled or prescription drugs is an increasing health burden in most countries and Nigeria is not an exception. Tramadol abuse is a menace in Nigeria. There are few reports on the health consequences of tramadol addiction in the Niger Delta region of Nigeria.
Aim
The aim of the study was to determine and educate the public on the effects of tramadol addiction on learning, memory, and organ damage.
Materials and methods
Adult rats were randomly assigned into four groups, *n*=5. Group 1 received 5 ml/kg of 0.9% normal saline orally, while groups 2, 3, and 4 were administered 50, 100, and 200 mg/kg of tramadol daily for 28 days, respectively. Behavioral tests (Y-maze and Morris water maze) were conducted on the first and last weeks of the experiment. On the 29th day, the animals were sacrificed under halothane anesthesia and organs were excised for histological examination. The results were analyzed using one-way analysis of variance (GraphPad Prism 6).
Results
The behavioral assessments revealed dose-dependent aggression, anxiety, and spasms. Also, a reduction in escape latency in the Morris water maze and increased alternations in the Y-maze occurred to various degrees in the treated groups compared with the control. Different grades of histological abnormalities occurred in the brain, liver, and kidneys of treated rats.
Conclusion
Tramadol misuse can lead to learning and memory impairment in addition to long-term organ damage involving the brain, liver, and kidneys.

Keywords:
addiction, behavior, brain, histology, memory, Purkinje cells, tramadol

Egypt Pharmaceut J 21:75–83

1687-4315

Introduction

Substance misuse, abuse, and addiction are thriving phenomena in societies with poor medicine security. These vices are often of high incidence in regions of poor security and high crime rate as they are fueling factors for perpetration of crime [1]. Addiction to controlled or prescription drugs is an emerging health burden in most countries and Nigeria is not an exception. Opioid analgesics are a class of drugs frequently abused with resultant addiction. Tramadol is a centrally acting analgesic used worldwide. It is considered an alternative to traditional opioids because it has better adverse-event profile [2]. Death, injury, and addiction are new trends increasingly arrogated to tramadol use in Nigeria [3,4]. In addition to the rise in tramadol-induced health effects, there is also a sharp rise in criminal activities linked with persons addicted to unlicensed use of tramadol in the Niger Delta region of Nigeria. There are few reports on the health consequences of addiction to tramadol in the Niger Delta region of Nigeria. This study aimed at educating the public on the organ-specific effects of tramadol addiction.

Materials and methods

Experimental animals

Adult rats weighing 150–200 g were obtained from the animal breeding and research unit of the institution. The rats were housed in four (4, *n*=5) cages (40 cm×40 cm padded with wood shaving that was changed daily) labeled accurately in a ventilated room with normal room temperature for the two-week period of acclimatization. The rats were fed with standard rat chow (Vital Feeds Ltd. Edo State)

and water (ad libitum). Ethical approval (NDU/PHARM/PCO/AEC/36) dated February 12, 2020, was obtained from the institutional Animal Ethics Committee. All animals were handled in accordance with EU directive (2010/63/EU) for animals.

Drugs

Tramadol HCl (Nkoyo Pharm, Nigeria). All other reagents and chemicals used were of analytical grade and were obtained from reputable companies.

Experimental protocol

Adult rats were assigned into four groups (n=5). Group 1 (placebo control) was administered 5 ml/kg of 0.9% normal saline orally daily for 28 days. Groups 2, 3, and 4 were administered 50, 100, and 200 mg/kg of tramadol for 28 days, respectively.

Evaluation of learning and memory

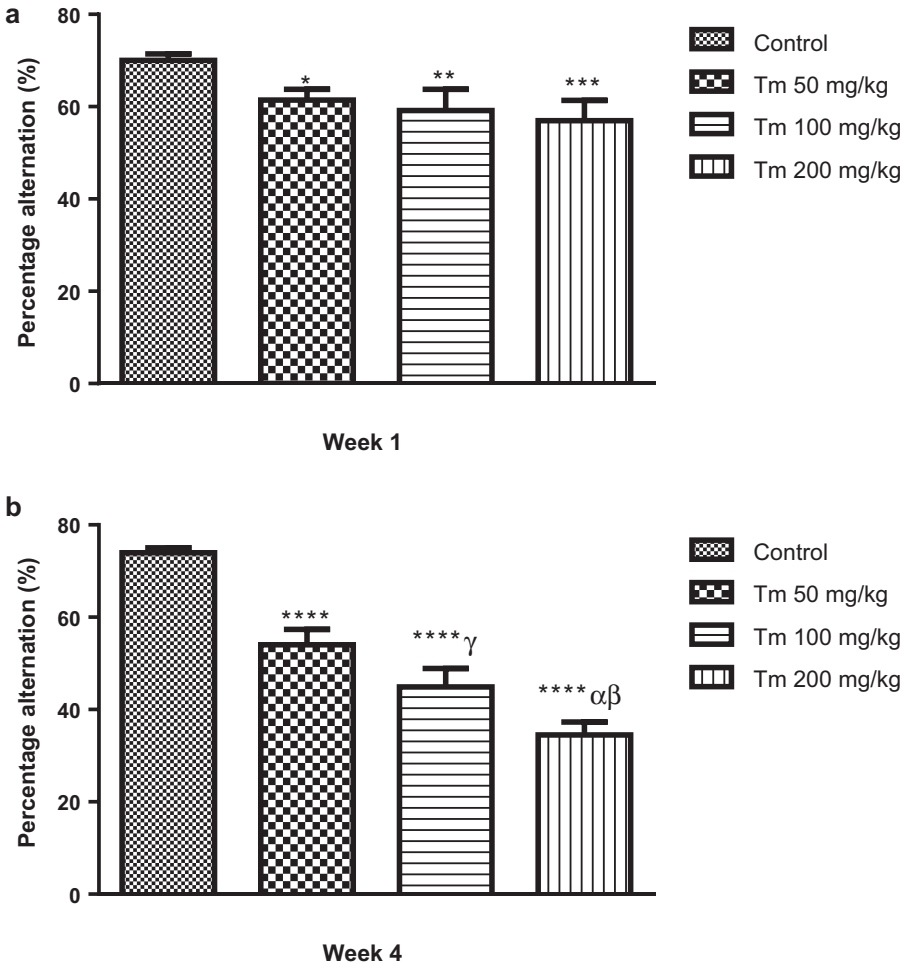
Y-maze

Spontaneous alternation was tested as described previously by Holcomb *et al.* [5] using the Y-maze (Stoelting, Wood Dale, Illinois, USA). Thirty minutes post treatment, each rat was placed in the center of the Y-maze and was allowed to explore freely through the maze during an 8-min session. Arm-entry sequence and time on each arm was recorded. An arm entry is recorded when the hind paws of the rat are placed in the arm. Percentage alternation is the total number of arm entries divided by the maximum possible alternations (the total number of arms entered minus 2)×100.

Morris water maze

The rats were trained in the standard Morris water maze (MWM) with a hidden platform (Stoelting, Wood Dale, Illinois, USA). A nontoxic white dye (starch solution) was added to the water to conceal the platform. Visual cues (images on white walls) were placed around the room and remained constant throughout testing. The rats were placed in the water and subjected to two training sessions that were 20 min apart and the time taken for them to find the platform was recorded; the rats were allowed to

Figure 1



a Percentage alternation of rats on the Y-maze following 1-week treatment with tramadol. *P=0.041; **P=0.006; ***P=0.0008 compared with control. b Percentage alternation of rats on the Y-maze following 4 weeks of administration of tramadol. ****P=0.00009 compared with control. $^{\gamma}P$=0.009; $^{\alpha}P$=0.00006 compared with 50 mg/kg treated group; $^{\beta}P$=0.008 compared with 100 mg/kg treated group.

stay on the platform for 10 s after locating. The animals that could not locate the platform after 120 s were placed on the platform for 10 s before removing it from the pool. All animals were toweled dry after each trial and returned to their home cage. This was repeated once daily for 4 days [6].

Apart from the behavioral tests conducted, the observed physical activities and behaviors of the rats were also recorded.

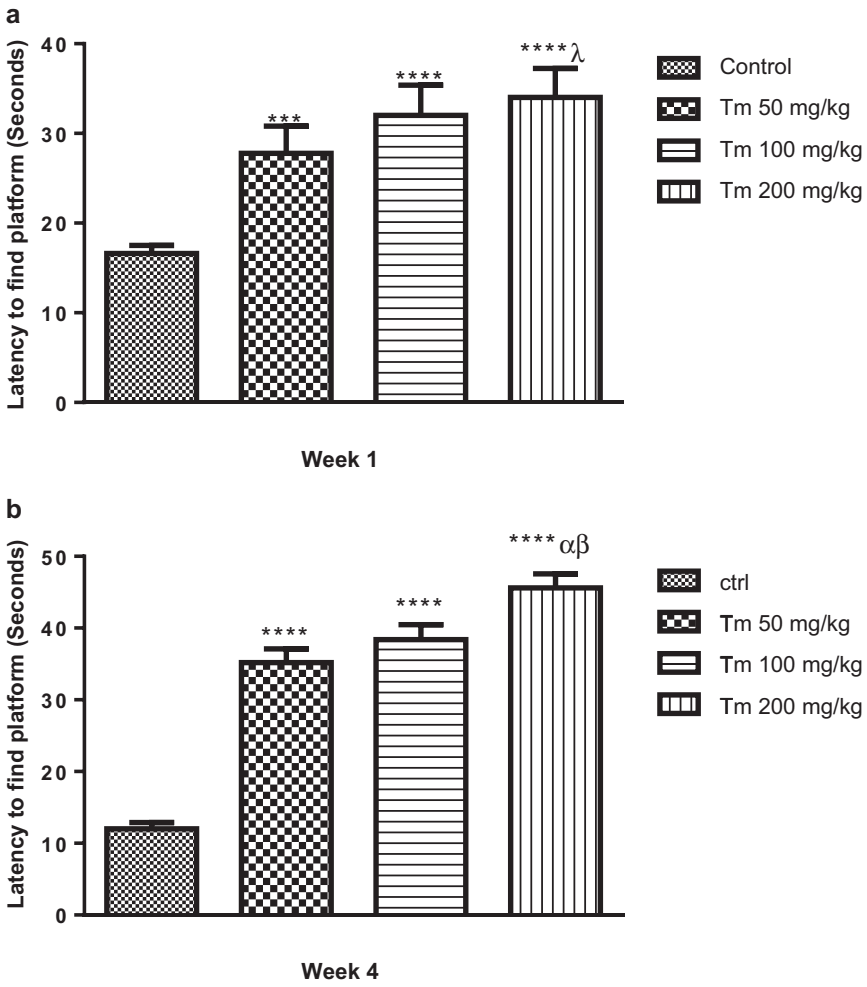
Histological assessments

On the 29th day, the animals were sacrificed under halothane anesthesia and organs were excised, rinsed in normal saline, and blotted with Whatmann's number-1 filter paper. The organs were observed for visible injury, weighed, and fixed in 10% buffered formal saline for a minimum of 48 h. The tissues were subsequently trimmed (Leica TP 1020), dehydrated in 4 grades of alcohol (70%, 80%, 90%, and absolute alcohol), cleared in 3 grades of xylene, and embedded (Leica EG 1160) in molten wax. On solidifying, the blocks were sectioned, 5-µm thick with a rotary microtome (Leica RM 2125), floated in a water bath, and incubated at 60°C for 30 min. The sectioned tissues were cleared in xylene and hydrated in alcohol (90, 80, and 70%). The sectioned tissues were stained (hematoxylin), blued (ammonium chloride), differentiated (1% acid alcohol), counterstained (eosin), and mounted in bibutylphthalate polystyrene xylene. Photomicrographs were produced with a Motic^{TM} 9.0-megapixel microscope camera at ×400 magnifications [7].

Statistical analysis

The results are presented as mean±standard error of mean (SEM) and '*n*' represents the number of animals per group. Statistical analysis was done using one-way analysis of variance followed by Dunnet's post hoc test for multiple comparisons (GraphPad Prism 6

Figure 2



a Effect of 1-week treatment with tramadol on escape latency of rats in the Morris water-maze test. ****P=0.0001 compared with control. $^{\lambda}P$=0.038 compared with 50 mg/kg group. b Escape latency of rats on the Morris water-maze test following 4 weeks of treatment with tramadol. ****P=0.00009 compared with control. $^{\alpha}P$=0.00008 compared with 50 mg/kg treated group; $^{\beta}P$=0.0004 compared with 100 mg/kg treated group.

Software, San Diego, California, USA). Statistical differences between compared data were considered significant at *P* less than 0.05.

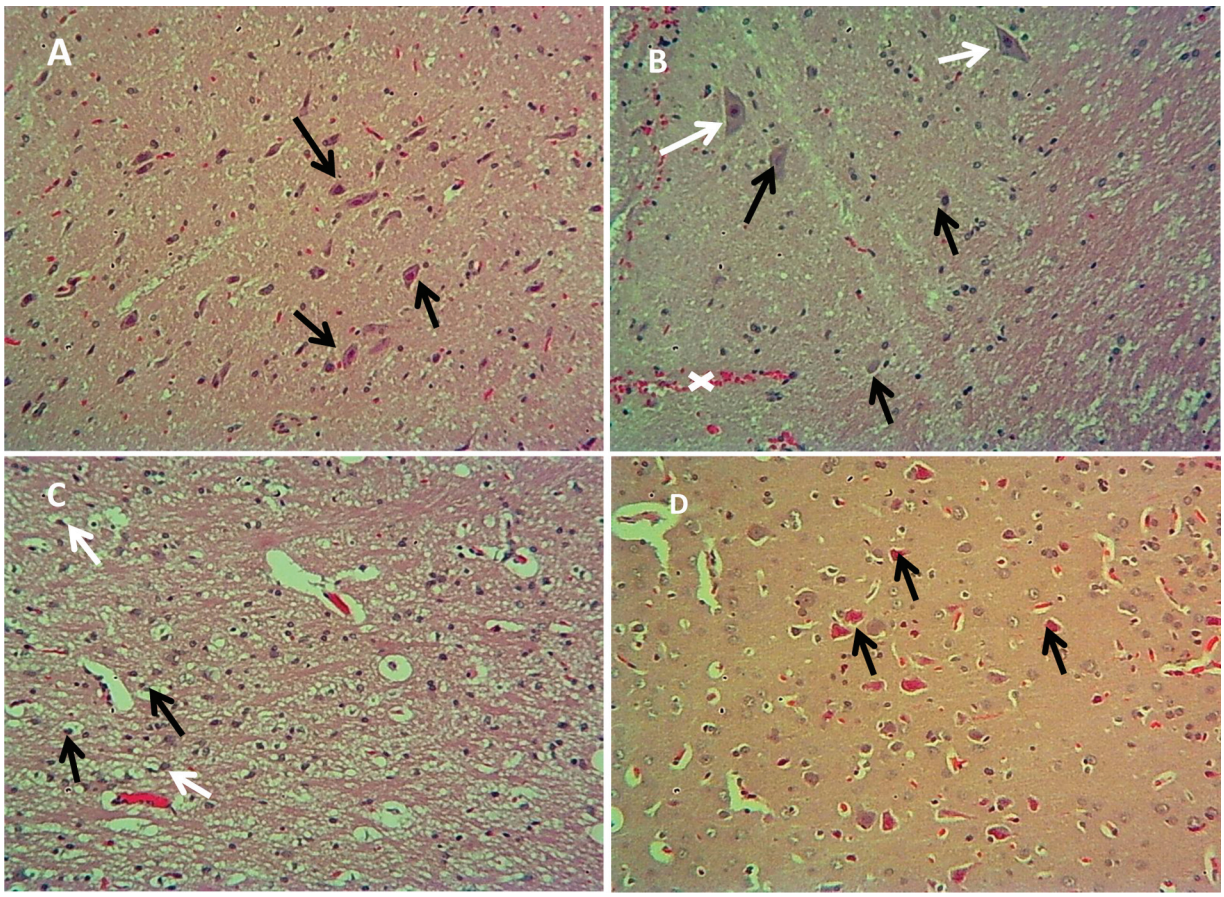
Results and discussion

Tramadol, an opioid analgesic licensed to treat moderate-to-severe pain [8], binds to μ-opioid receptors found on several central nervous system pathways, including noradrenergic, GABAergic, and serotonergic systems, modulating neurotransmitter effects on these systems, leading to analgesia [9,10]. Like many other opioids, tramadol is not bereft of adverse effects; however, these effects are less severe with tramadol than most opioids [11]. In comparison with other frequently abused opioid analgesics, tramadol has a lower tendency for addiction following chronic use [12,13]. However, there are rising reports of addiction to tramadol, leading to injury and death in Nigeria [14,15], with little research on the long-term effects of tramadol abuse in the Niger Delta region.

Evaluation of learning and memory

During the first week, all the animals behaved normally, except for sedation and calmness, which occurred in the tramadol-treated groups. In the second week, the treated animals became restless, irritable, and some had tremors (100 and 200 mg/kg group) one to two hours before dosing, after which they became calm. In the third week, the behavior in the 50 mg/kg treated group remained unchanged. The rats in groups 3 and 4 became more irritable, some of them developed aggressiveness, yet the manifestations alleviated shortly after the dose, their feeding habit also reduced drastically. At week 4, the rats in groups 3 and 4 developed spasm and convulsions. Group-4 rats were drooling continuously with difficulty in breathing. The behavioral assessment of treated animals revealed aggression, anxiety, and spasms following chronic

Figure 3



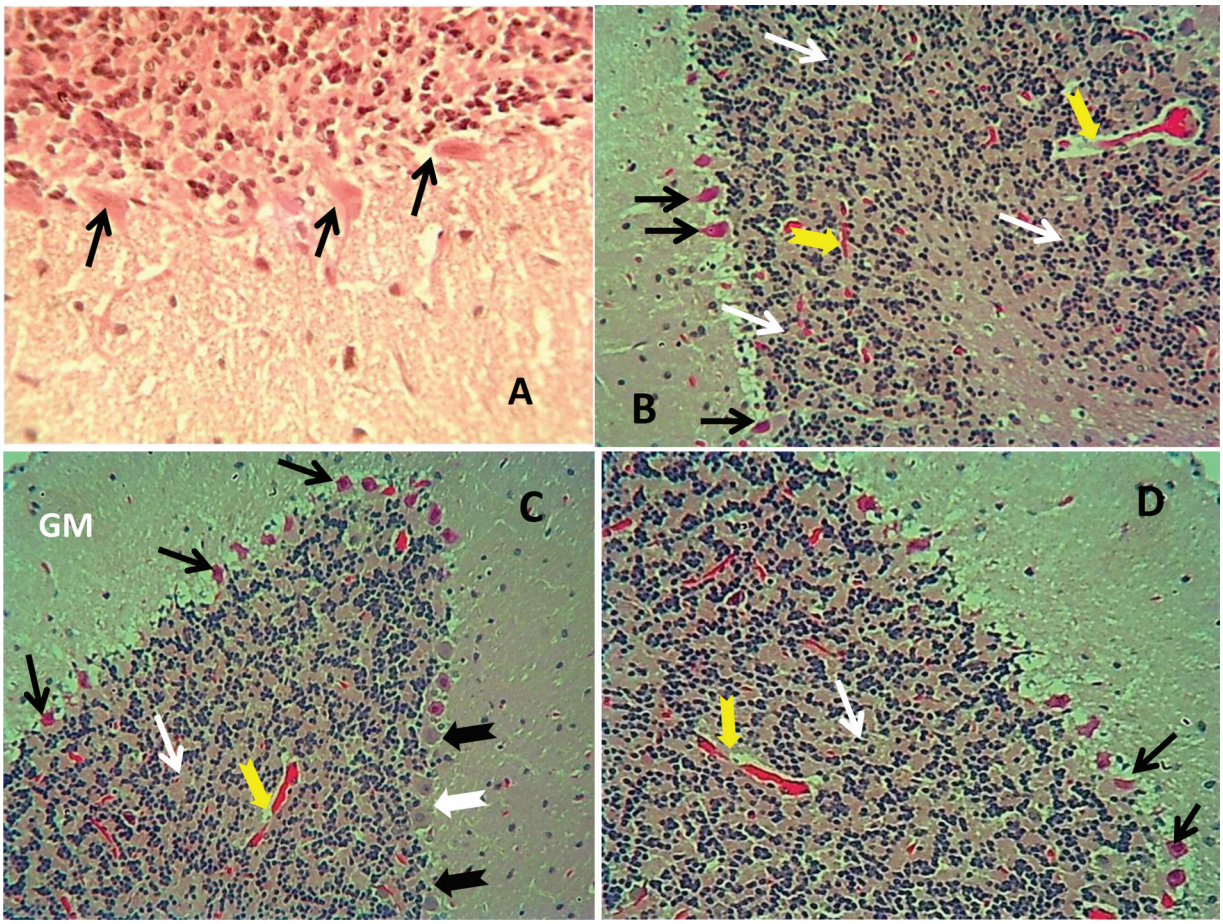
Photomicrograph of rat cerebrum (pyramidal cell layer). Plate A: the control group showing normal cellular and parenchymal architecture. Arrows show normal pyramidal cells. Plate B: pyramidal layer of the 50 mg/kg treated group showing locally extensive areas of cerebral hemorrhage and mild vacuolation (white x) of the neuropil. A few ghost pyramidal cells are present (black arrows), together with normal cells (white arrows). Plate C: pyramidal cell layer of 100 mg/kg treated group showing multifocal intramyelinic edema (black arrows), neuronal pyknosis (white arrows), moderate, multifocal gliosis, and widespread vacuolation (spongiosis) of the neuropil. Plate D: pyramidal cell layer of 200 mg/kg group showing eosinophilic necrosis of several pyramidal cells (arrows). H&E ×400.

administration. The aggressive behavior correlates with that seen in tramadol users, often leading to crime. Anxiety and aggressive behavior that resolves with drug administration is a classical sign of addiction to the administered drug [16]. Our study shows that continuous utilization of tramadol for as short as 2 weeks can result in addiction. We also established that respiratory failure may result from massive doses of tramadol as shown by the drooling and difficulty in breathing experienced by the animals during the third week.

Repeated administration of tramadol-induced memory impairment in the Y-maze and MWM tests

In the Y-maze test, the percentage alternation was significantly reduced in the treated animals compared with the saline group for weeks 1 (Fig. 1a) and 4 (Fig. 1b). During the first week, changes in percentage alternation between treatments were insignificant (Fig. 1a). However, by the fourth week, there was a significant reduction in percentage alternation between the treated groups (Fig. 1b). In the MWM experiment, the escape latency was elevated in all drug-treated groups for both the first (Fig. 2a) and fourth (Fig. 2b) week. The escape latency was higher in the 200 mg/kg treated group compared with the 50 mg/kg treated group in the first week (Fig. 2a). The MWM and Y-maze tests are common behavioral tasks used to determine deficits in learning and memory in animal models [17]. The reduction in escape latency in the MWM and increased alternations in the Y-maze depict memory loss and reduced learning capabilities in the affected rats [18]. Hosseini-Sharifabad *et al.* [19] reported memory impairment at therapeutic doses. Tramadol increases acetylcholinesterase activity in the cerebral cortex and

Figure 4



Photomicrograph of rat cerebrum (Purkinje cell layer). Plate A: control group showing intact Purkinje cell layer and Purkinje cells (black arrows). Plate B: section of Purkinje cell layer in the 50 mg/kg group showing multifocal loss of cells in the Purkinje cell layer. A few evident Purkinje cells show eosinophilic necrosis (black arrows), pyknotic granular cells (white arrows), and capillary congestion (notched yellow arrows). Plate C: 100 mg/kg treated group showing loss of cells in the Purkinje cell layer. Several Purkinje cells show eosinophilic necrosis (black arrows), while some ghost cells are present (notched black arrows). One relatively normal Purkinje cell is evident (notched white arrow). Several pyknotic granular cells are also present (white arrow), accompanied by capillary congestion (yellow notched arrow). Plate D: 200 mg/kg group showing loss of cells in the Purkinje cell layer. Eosinophilic necrosis of several Purkinje cells (black arrows), pyknotic granular cells (white arrow), and capillary congestion (yellow notched arrows) are seen. H&E ×400.

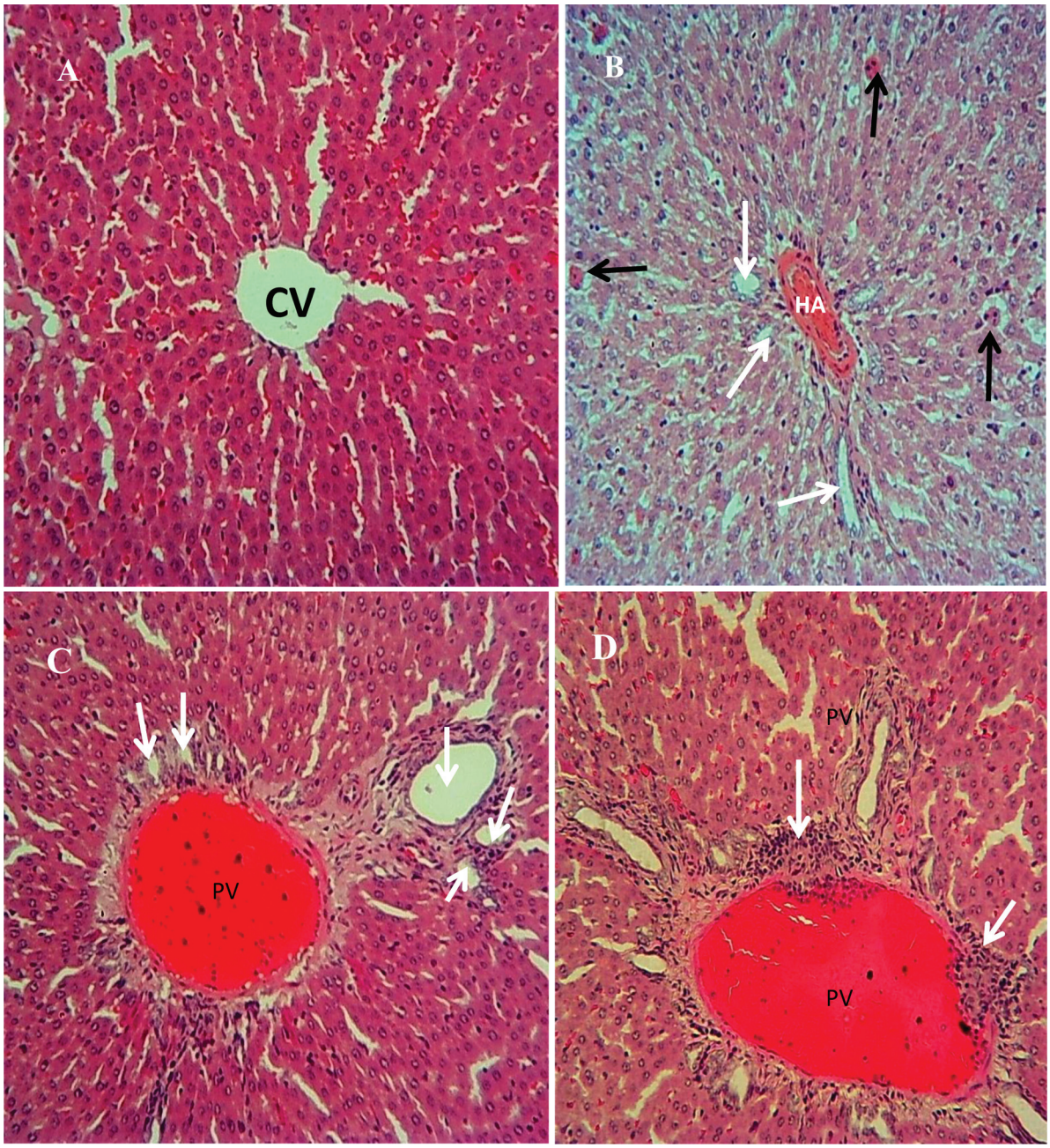
also modulates neurotransmitter activity in the central nervous system of rats [9,10], these effects may contribute to the cognitive deficits exhibited by treated rats.

Chronic administration of tramadol causes alteration of histological parameters in rats

The effects of clinically used doses of tramadol on specific organs, including histological distortions in rat-brain section such as vascular dilatation, congestion, hemorrhage, and edema, are mentioned by other researchers [20–22]. However, the authors investigated doses commonly used by tramadol addicts (victims) and abusers for this study. Sections of the brain, liver, and kidney excised from the control group showed normal parenchymal architecture.

Our findings in the rat-brain histology (Figs 3 and 4) include degenerated, vacuolated neurons with pyknotic and vacuolated nuclei, with heterogeneous

Figure 5

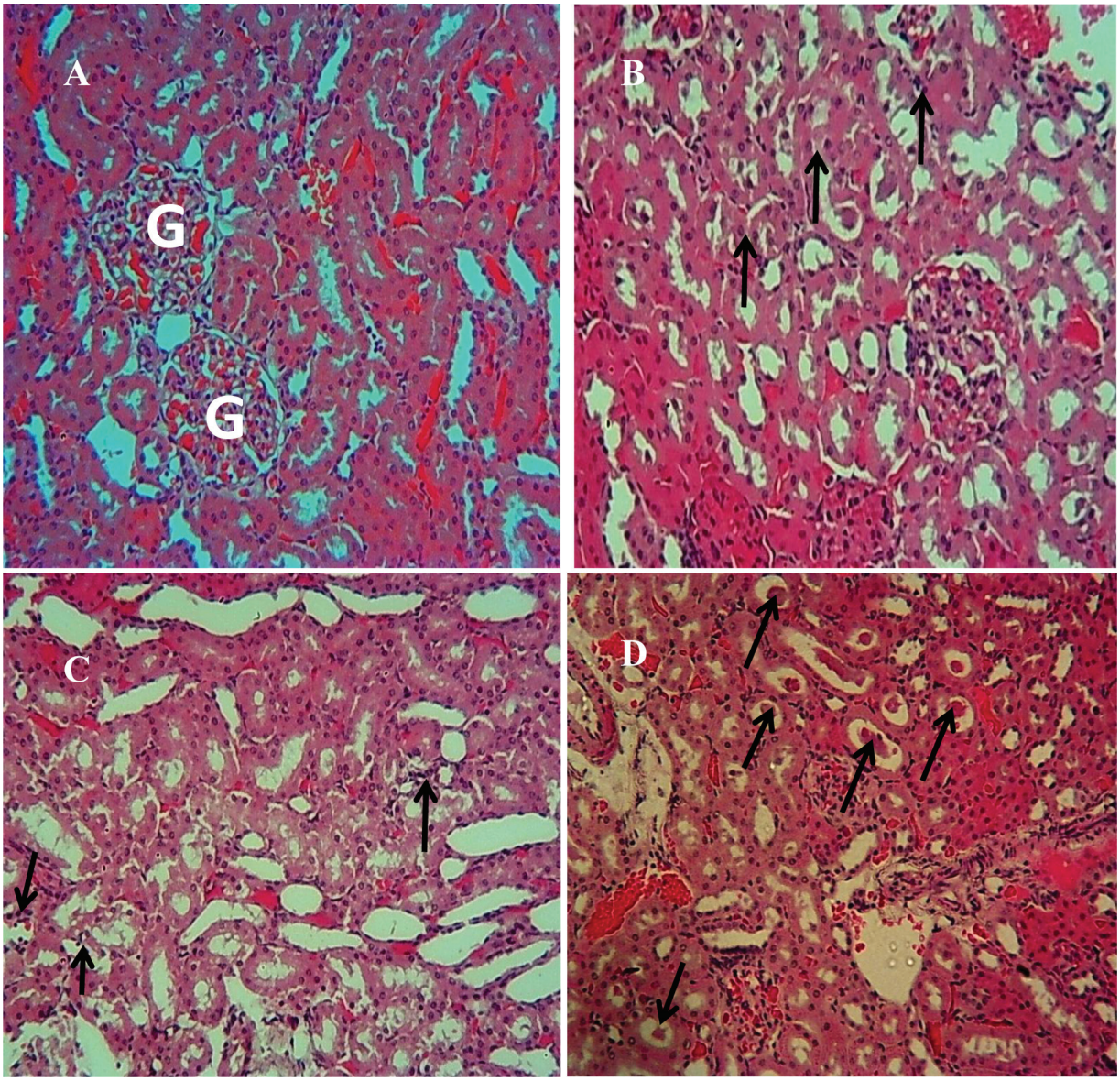


Photomicrographs of rat liver. Plate A: liver of rat (control group) showing a relatively normal hepatic parenchymal architecture, central vein (CV). Plate B: liver of rat in the 50 mg/kg group showing widespread hepatocellular degeneration and multifocal necrosis, accompanied by several apoptotic hepatocytes (black arrows). Hepatic artery (HA), bile ductules (white arrows). Plate C: section of the liver of rat in 100 mg/kg group showing hepatocellular degeneration, bile-duct hyperplasia (arrows), portal fibrosis, and congested portal vein (PV). Plate D: section of the liver of rat in the 200 mg/kg group showing dissociated hepatic cords and foci of dense inflammatory cellular infiltration (arrows), and congested portal vein (PV). H&E ×400.

pigmentation and evident signs of apoptosis. The 50 mg/kg group (plate B) shows cerebral hemorrhage, vacuolation of the neuropil, ghost pyramidal cells, loss of Purkinje cells, pyknosis of granular cells, and congestion of the gray matter. Multifocal intramyelinic edema, neuronal pyknosis, moderate, multifocal gliosis, and spongiosis of the neuropil in addition to the features present in the 50 mg/kg group occurred in groups 3 and 4 to varying degrees. These findings are in consonance with [23,24] and may also contribute to the impaired learning and memory exhibited by the treated rats.

The liver and kidneys are predisposed to toxic injury due to their role in tramadol metabolism and excretion [24]. Sections of the liver and kidneys extracted from the control group showed normal parenchymal architecture. The liver histology showed variable distorted features that are in consonant with the findings of Youseff and Azza [25]. The liver of treated groups (Fig. 5) showed hepatocellular degeneration and multifocal necrosis, accompanied by several apoptotic hepatocytes (plate B), hepatocellular hypertrophy, karyomegaly, multifocal coagulative necrosis, multifocal inflammatory-cell proliferation in the parenchyma and portal area (plate C), bile-duct hyperplasia, portal fibrosis, and congestion of portal vessels accompanied with inflammatory-cell infiltration (plate D). Photomicrographs of rat kidneys (Fig. 6) show tubular epithelial degeneration and sloughing in the

Figure 6



Photomicrographs of rat kidney. Plate A: kidney of rat in the control group showing normal histologic architecture. Glomerulus (G). Plate B: kidney of a rat in the 50 mg/kg group showing tubular intraluminal casts (arrows). Plate C: kidney of a rat in the 100 m/kgg group showing widespread tubular epithelial degeneration and sloughing. There is moderate multifocal inflammatory cellular infiltration (arrows). Plate D: kidney of a rat in the 200 mg/kg group showing several tubular intraluminal casts (arrows), necrotic tubules, and multifocal hemorrhage. H&E ×400.

50 mg/kg treated group (plate B), the 100 mg/kg treated group (plate C) shows tubular epithelial degeneration and necrosis, while the 200 mg/kg group (plate D) shows several tubular intraluminal casts, necrotic tubules, and multifocal hemorrhages. The effects of tramadol on the kidney histology of the treated rats are dose. Oxidative stress, glutathione-peroxidase depletion, and subsequent potentiation of lipid peroxidation, apoptosis, and renal impairment have been linked with tramadol administration in rats [26,27]. The authors did exploit oxidative-stress parameters; however, the distortions in the liver and kidney histology may have arisen from oxidative stress and accumulation of toxic metabolites as reported in the literature.

Conclusion

Tramadol misuse, abuse, and addiction are on the increase and have attained epidemic proportions in Nigeria. There are no reports on prevalence but verbal and other unofficial reports exist about the trend and the resultant criminal activities. There are no age or sex limits to misuse of tramadol; however, young people are mostly involved and are common victims of addiction. Most of the victims are driven by the immediate reward they feel and have no idea about the long-term health effects of tramadol and the huge economic implications to them, their families, and the society at large. There is a need for population-based studies to determine the true prevalence of tramadol misuse in our setting and the country.

The authors conclude that tramadol misuse can lead to learning and memory impairment in addition to long-term organ damage involving the brain, liver, and kidneys. We recommend that intervention programs using health education and information be used to address the menace and health institutions strengthened with rehabilitation centers for victims of addiction.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Rafaiee R, Olyaee S, Sargolzaiee A. The relationship between the type of crime and drugs in addicted prisoners in Zahedan Central Prison. Int J High Risk Behav Addict 2013; 2:139–140.

2 Barbosa J, Faria J, Garcez F, Leal S, Afonso LP, Nascimento AV, *et al.* Repeated administration of clinically relevant doses of the prescription opioids tramadol and tapentadol causes lung, cardiac, and brain toxicity in Wistar rats. Pharmaceuticals (Basel). 2021; 14:97.

3 Ibrahim AW, Yerima MM, Pindar SK, Onyencho VC, Ahmed HK, Machina BK, *et al.* Tramadol abuse among patients attending an addiction clinic in North-Eastern Nigeria: outcome of a four year retrospective study. Adv Psychol Neurosci 2017; 2:31–37.

4 Chikezie UE, Ebuenyi ID. Tramadol misuse in the Niger Delta: a review of cases presenting within a year. J Substance Use 2019; 24:487–491.

5 Holcomb LA, Gordon MN, Jantzen P, Hsiao K, Duff K, Morgan D. Behavioral changes in transgenic mice expressing both amyloid precursor protein and presenilin-1 mutations: lack of association with amyloid deposits. Behav Genet 1999; 29:177–185.

6 Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984; 11:47–60.

7 Miotto K, Cho AK, Khalil MA, Blanco K, Sasaki JD, Rawson R. Trends in tramadol: pharmacology, metabolism, and misuse. Anesth Analg. 2017; 124:44–51.

8 Bloms-Funke P, Dremencov E, Cremers TI, Tzschentke TM. Tramadol increases extracellular levels of serotonin and noradrenaline as measured by in vivo microdialysis in the ventral hippocampus of freely-moving rats. Neurosci Lett 2011; 490:191–195.

9 Kimura M, Obata H, Saito S. Antihypersensitivity effects of tramadol hydrochloride in a rat model of postoperative pain. Anesth Analg 2012; 115:443–449.

10 Rehab MS, Naira FM, Tahany MS. Acute toxicity and dependence of tramadol in albino rats: relationship of nestin and Notch 1 as stem cell markers. J Am Sci 2012; 8:313–327.

11 Dobscha SK, Morasco BJ, Duckart JP, Macey T, Deyo RA. Correlates of prescription opioid initiation and long-term opioid use in veterans with persistent pain. Clin J Pain 2013; 29:102–108.

12 Makunts T, Andrew U, Atayee RS, Abagyan R. Retrospective analysis reveals significant association of hypoglycemia with tramadol and methadone in contrast to other opioids. Sci Rep 2019; 9:12490.

13 Duru C, Oluoha U, Okafor C, Diwe K, Iwu A. Socio-demographic determinants of psychoactive substance use among students of tertiary institutions in Imo State, Nigeria. J Addict Res Ther 2017; 8:345.

14 Yunusa U, Bello UL, Idris M, Haddad MM, Adamu D. Determinants of substance abuse among commercial bus drivers in Kano Metropolis, Kano State, Nigeria. Am J Nurs 2017; 6:125–130.

15 Nicole MA, Pedro R, Bartley GH. Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. Neurosci Biobehav Rev 2008; 32:20–39.

16 Sibille EL, Pavlides C, Benke D, Toth M. Genetic inactivation of the serotonin1A receptor in mice results in downregulation of major GABAA receptor α subunits, reduction of GABAA receptor binding, and benzodiazepine-resistant anxiety. J Neurosci 2000; 20:2758–2765.

17 El-Hamid MEA, Tabi G. Impact of tramadol and morphine abuse on the activities of acetylcholine esterase, Na+/K+-ATPase and related parameters in cerebral cortices of male adult rats. Electron Physician 2017; 9:4027–4034.

18 Hosseini-Sharifabad A, Rabbani M, Sharifzadeh M, Bagheri N. Acute and chronic tramadol administration impair spatial memory in rat. Res Pharm Sci 2016; 11:49–57.

19 Ragab IK, Mohamed HZE. Histological changes of the adult albino rats entorhinal cortex under the effect of tramadol administration: histological and morphometric study. Alexandria J Med 2016; 53:123–133.

20 Baghishani F, Mohammadipour A, Hosseinzadeh H, Hosseini M, Ebrahimzadeh-Bideskan A. The effects of tramadol administration on hippocampal cell apoptosis, learning and memory in adult rats and neuroprotective effects of crocin. Metab Brain Dis 2018; 33:907–916.

21 Ali HA, Afifi M, Saber TM, Makki AA, Keshta AT, Baeshen M, Al-Farga A. Neurotoxic, hepatotoxic and nephrotoxic effects of tramadol administration in rats. J Mol Neurosci 2020; 70:1934–1942.

22 Nafea OE, ElKhishin IA, Awad OA, Mohamed DA. A study of the neurotoxic effects of tramadol and cannabis in adolescent male albino rats. Int J Sci Rep 2016; 2:143–154.

23 Awadalla EA, Salah-Eldin AE. Molecular and histological changes in cerebral cortex and lung tissues under the effect of tramadol treatment. Biomed Pharmacother 2016; 82:269–280.

24 Pirmohamed M. Acceptance of biomarker-based tests for application in clinical practice: criteria and obstacles. Clin Pharmacol Ther 2010; 88:862–866.

25 Youssef HS, Azza ZHM. Histopathological and biochemical effects of acute & chronic tramadol drug toxicity on liver, kidney and testicular function in adult male albino rats. Forensic Res Criminol Int J 2016; 2:00060.

26 Abdel-Zaher AO, Abdel-Rahman MS, Elwasei FM. Protective effect of Nigella sativa oil against tramadol-induced tolerance and dependence in mice: role of nitric oxide and oxidative stress. Neurotoxicology 2011; 32:725–733.

27 Owoade AO, Adetutu A, Olorunnisola O. Hematological and biochemical changes in blood, liver and kidney tissues under the effect of tramadol treatment. J Alcohol Drug Depend 2019; 7:1–7.