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Original Article

Tramadol and Coffee Synergy Triggered Oxidative Stress, Altered Cerebellar Histomorphology and Behaviour in Rats

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ABSTRACT

Tramadol intoxication with other substances has recently raised neurological concerns especially among youths in the society. The effects of tramadol and coffee on the cerebellum of Wistar rats were studied. Thirty adult Wistar rats (average weight of 180 g) were randomly assigned into six groups (n=6) as follows: Control, coffee (72.46 mg/kg), coffee (108.69 mg/kg), tramadol (2.86 mg/kg), tramadol (5.71 mg/kg) and coffee (72.46 mg/kg) + tramadol (2.86 mg/kg). The administrations were orally, and done once daily for twenty-eight consecutive days. On day 29, spontaneous alternation in the T-maze was carried out, and the rats anaesthetized with ketamine hydrochloride (50 mg/kg intraperitoneally). Their blood were assayed for antioxidant, while the 10 % buffered formalin perfused brains were routinely processed with haematoxylin and eosin technique. Behavioural study showed significantly ($p<0.05$) increased spontaneous alternation in coffee (108.69 mg/kg), tramadol (5.71 mg/kg), and coffee (72.46 mg/kg) + tramadol (2.86 mg/kg) groups compared to the control. Catalase, superoxide dismutase and reduced glutathione levels were not different ($p>0.05$), while the malondialdehyde level was significantly ($p<0.05$) higher in the coffee (72.46 mg/kg) + tramadol (2.86 mg/kg) group compared to the control. Histological observations showed cerebellar cytoarchitectural alterations, including atrophy of the Purkinje cells in the coffee (108.69 mg/kg), tramadol (5.71 mg/kg), and coffee (72.46 mg/kg) + tramadol (2.86 mg/kg) groups compared to the control. In conclusion, co-administration of tramadol and coffee triggered free radicals, damaged the cerebellar tissues and caused a more deleterious effect on memory function than individual doses.

Keywords
Histology, Cerebellum, Oxidative stress, Behaviour, Tramadol, Coffee

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INTRODUCTION

Tramadol is a synthetically produced opioid with a specific chemical formula C_{16}-H_{25}-NO_2 (Shah et al. 2013; Carrillo-Munguía et al. 2015). It is an "atypical" opioid because both an opioid and non-opioid component have been demonstrated in their mechanism of action. Tramadol is also an analgesic used as a racemic mixture of two synergistic enantiomers: Its positive component binds to the opioid receptor primarily responsible for pain modulation, as well as generates serotonin reuptake inhibition enhancing serotonin release. In contrast, its negative component preferentially inhibits noradrenaline reuptake (Shah et al. 2013). The effects of its neurotoxicity have been reported, while continuous administration resulted in rats brain weight loss (Tashakori and Afshari 2010; Zhou et al. 2012; Hosseini-Sharifabad et al. 2016; Miotto et al. 2017).

Tramadol overdose can cause life-threatening seizures, respiratory depression, coma, nausea, vomiting, hypotension, and serotonin syndrome (Boostani and Derakhshan 2012; Stassinos et al. 2019; Faria et al. 2018; Mohammadipour et al. 2019). In the brain, chronic administration of tramadol is associated with oxidative stress and cerebral cortex apoptosis (Abdel-Zaher et al. 2011; Ghoneim et al. 2014). Atrophy of the cerebellum, microgliosis, neuro-inflammation and apoptosis has also been reported (Ezi et al. 2021).

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Coffee (*Coffea arabica*) is the most regularly consumed caffeine-containing beverage (Reyes and Cornelius 2018). It contains a complex mixture of chemicals that provide important amounts of chlorogenic acid and caffeine (Drewnowski and Rehm 2016). The properties of coffee have been certified to potentially have additive or synergistic effects due to its bioactive constituents including caffeine (methylxanthine), chlorogenic acids (polyphenol), diterpenes, and other phenolics (Pourshahidi et al. 2016). Contrary to its health benefits, coffee (caffeinated) does not always offer protective benefits (Umoh and Jimmy 2017). Also, heavy coffee consumption is associated with a higher death risk (Marventano et al. 2016).

Caffeine intake by sulking pups altered the composition of fatty acids in the cerebellum, affecting the rapid period of brain growth (Yazdani et al. 2004). The cerebellum is a heterogeneous structure and has been anatomically divided into vermal and hemispheric sub-regions designated from I–X (Manto et al. 2012). In healthy controls, anatomical and functional neuroimaging studies have confirmed that the cerebellum has a distinct functional motor and cognitive module linked to the cerebral cortex through the basal ganglia and the thalamus, respectively (Middleton and Strick 2001; Duan et al. 2015), as well as emotional functions (Manto et al. 2012; Koziol et al. 2014).

Excess reactive oxygen species (ROS) are detoxified by antioxidants (enzymatic and non-enzymatic) and in reactions catalysed by superoxide dismutase, glutathione peroxidase and catalase (Chen et al. 2011). Imbalances in the rate of ROS generation and detoxification leads to oxidative stress and the consequent production of free radicals that can damage deoxyribonucleic acid (DNA), proteins and lipids, and reports suggest that high levels of ROS are intimately linked to neuronal death. These include chronic diseases like Parkinson's and Alzheimer's (Guglielmotto et al. 2009), acute injuries to the brain like trauma and cerebral ischaemia (Valko et al. 2007; Chen et al. 2011) or psychiatric disorders such as autism, depression and schizophrenia (Michel et al. 2012). In this study, the effect of co-administration of tramadol and coffee on oxidative stress, histology of the cerebellum and behaviour were assessed.

MATERIALS AND METHODS

Animal Handling

Thirty adult male Wistar rats with an average weight of 180 g in weight were obtained from the Animal House, College of Health Sciences of the University of Uyo and allowed to acclimatise for two weeks under standard housing conditions. The rats were fed with standard rat chow and water allowed *ad libitum*.

Ethical Approval

Ethical approval was obtained from the Faculty of Pharmacy Ethics Committee on the Use of Laboratory Animals, University of Uyo, Uyo, Nigeria. All procedures were carried out following the National Academy of Science's Guide for Care and Use of laboratory animals (National Research Council 2011). Approval of tramadol use with number NDLEA/AKSC/77/VOL.IV/18, was obtained from the National Drug Law Enforcement Agency, Akwa Ibom State Command.

Preparation and Administration of Tramadol and Coffee

Tramadol tablets (100 g each) (Hexal AG, Germany with batch number EMEA/H/C/001182) were ground to powder and dissolved in 100 mL of physiological saline solution (Carrillo-Munguía et al. 2015) and appropriate doses were calculated.

Coffee (50 g, Nescafé ®, Gresik Limited, Indonesia) were dissolved in 500 mL of distilled water and appropriate doses calculated. Each 100 g of Nescafe contained 4.72 g of caffeine (Nmaju et al. 2014). Administrations were orally via orogastric tubes.

Experimental Design

The rats were randomly divided into six groups (five rats per group) as shown in Table 1.

Table 1: The experimental design

Groups	Description/Dosage	Duration of Administration (Days)
1	10 mL/kg of distilled water	28
2	Coffee (72.46 mg/kg)	28
3	Coffee (108.69 mg/kg)	28
4	Tramadol (2.86 mg/kg)	28
5	Tramadol (5.71 mg/kg)	28
6	Coffee (72.46 mg/kg) + Tramadol (2.86 mg/kg)	28

The administrations lasted for 28 days. Acute toxicity assessment was used to determine the doses for coffee only (Lorke 1983). The doses for tramadol were obtained following previous reports (Babalonis et al. 2013; Chikezie and Ebuenyi 2019). The animals were anaesthetized using 50 mg/kg of ketamine hydrochloride intraperitoneally.

Neurobehavioural Testing

T-Maze Spontaneous Alternation Test

This test was carried out on day 29 after the last administration. Three phases of the test were done: First phase was the habituation; where the animals were handled in the cage to slowly accustom them to touch, without picking them up. This was done severally within five minutes; the second phase involved allowing food ration overnight. Each animal was weighed and fed small chow pieces (1.5 g/mouse or 5 g/100 g rat) inside the cage. Small pieces were given to ensure that one animal cannot monopolize

the food. They were left for 5–10 min after taking them into the testing room.

The third phase was the habituation and trial phase. The animals were made to acclimatise with the reward (food pellets) an hour before the experiment began, and this is referred to as habituation. The maze was made up of the start area, reward arm (where the food pellet is), and the alternation arm. During the trial, if an animal stayed at the start area for 90 sec, the trial was cancelled and repeated. Each animal was placed in the start area until the selected criterion (whole body plus tail tip on goal arm, or other criterion) was reached. With the number of trials (six trials), a proportion correct per animal was calculated and the resulting data compared using an analysis of variance for multi-group comparisons, and the percentages (score/ expected score × 100) calculated afterwards. The time was adequately measured with a stop watch. (Robert et al. 2006).

Biochemical Analysis

Blood (3 mL) was obtained by intracardiac puncture and centrifuged at 1,500 resolution per minute (rpm) for 10 min. The obtained serum was subjected to antioxidant analysis using superoxide dismutase, reduced glutathione, catalase and malondialdehyde markers.

Superoxide Dismutase (SOD): Total superoxide dismutase (SOD) activity was measured at 480 nm by the method described by Xu et al. (2013). 1 mL of the test sample and 1 mL of a chloroform and ethanol mixture (3/5 v/v) were mixed in a centrifuge tube. The solution was centrifuged at 1580 g for 45 min to remove the precipitate. The assay solution consists of a sodium carbonate buffer (400 mM), 0.3 mM xanthine, 150 mol/L nitro blue tetrazolium (NBT), 0.6 mmol/L Na_2EDTA, 1 g/L bovine serum albumin, xanthine oxidase 167 U/L and sample was mixed in a cuvette. The activity was measured using xanthine and xanthine oxidase to generate superoxide radicals that reacted with NBT.

Reduced glutathione (GSH): The reduced glutathione (GSH) concentration in tissues was estimated as described by Rukkumani et al. (2004). Briefly, 10 % trichloroacetic acid (TCA) was added and centrifuged. 1.0 mL of supernatant was mixed with 0.5 mL of Ellman's reagent, 19.8 mg/kg of 5, 5'-dithiobis nitro benzoic acid-DTNB (Sigma Aldrich Inc., St. Louis, Mo, USA Batch D8130-5G) in 100 mL of 0. 1 % sodium nitrate and 3.0 mL of phosphate buffer (0.2M, pH8.0). The absorbance was read at 412 nm on the spectrophotometer. The concentration of GSH was expressed as µmol/mL.

Catalase (CAT): Catalase was assayed colorimetrically at 620 nm and expressed as mol of H2O2 consumed/min, as described in Rukkumani et al. (2004). Briefly, the reaction mixture of 1.5 mL contained 1.0 mL of 0.01M pH 7.0 phosphate buffer, 0.1 mL of homogenate and 0.4 mL of 2M H2O2. The reaction was stopped by the addition of 2.0 mL of dichromate-acetic acid reagent -5% potassium dichromate and glacial acetic acids were mixed in 1:3 ratios.

Malondialdehyde (MDA): Malondialdehyde (MDA) was measured using the thiobarbituric acid test to determine the concentrations of lipid peroxidation (nmol/L) (Placer et al. 1966). Oxidative damage 1 mL of the test sample and 2 mL of stock reagent (trichloroacetic acid and thiobarbituric acid) were mixed in a centrifuge tube. The solution was incubated in boiling water for 20 min. After cooling, the solution was centrifuged at 1,500 rpm for 5 min. to remove the precipitate and then the absorbance of the supernatant was spectro-photometrically measured at 532 nm against a blank on a spectrophotometer. The result was expressed as nmol/L.

Histological Analysis

The animals were euthanized, and phosphate buffered saline was used to perfuse them via cardiac puncture (left ventricle) for 2 min. and then with 4 % paraformaldehyde till tail stiffness was achieved in approximately 15 min. The brains were harvested, weighed and post-fixed in 4 % paraformaldehyde for 72 h. and after processed for light microscopy. Paraffinized cerebellum sectioned at 5 µm with the rotary microtome (Microtome Thermo Scientific – Microm HM 325, England) were routinely stained with haematoxylin and eosin. The photomicrographs were blindly assessed by three independent histopathologists, and the images were obtained via an Amscope digital camera (MU 1000, China) attached to a microscope (Olympus - CX31, Japan).

Statistical Analysis

Results were analysed using the GraphPad Prism 8.0 version. Data were reported as Mean ± SEM, and the mean effects of treatment groups were determined by the one-way analysis of variance (ANOVA), and multiple comparisons were done using the Bonferroni post-hoc tests. The significant difference was considered at $p < 0.05$.

RESULTS

Effect of Tramadol and Coffee on Neurobehaviour using T Maze Test

There was a significantly increased alternation in the group administered coffee (108.69 mg/kg) compared to the control at $p < 0.05$. There was also a significantly increased alternation in tramadol (5.71 mg/kg) compared to the control at $p < 0.01$. There were marked significant increase in alternation of the combination group coffee (72.46 mg/kg) + tramadol (2.86 mg/kg) compared to normal control at $p < 0.001$, respectively (Fig. 1).

Effect of Tramadol and Coffee on Antioxidant Enzymes

Tramadol and coffee had no significant effect on antioxidant enzymes. There was no significant difference in catalase, SOD and GSH between the administered groups and compared to the control (Table 2).

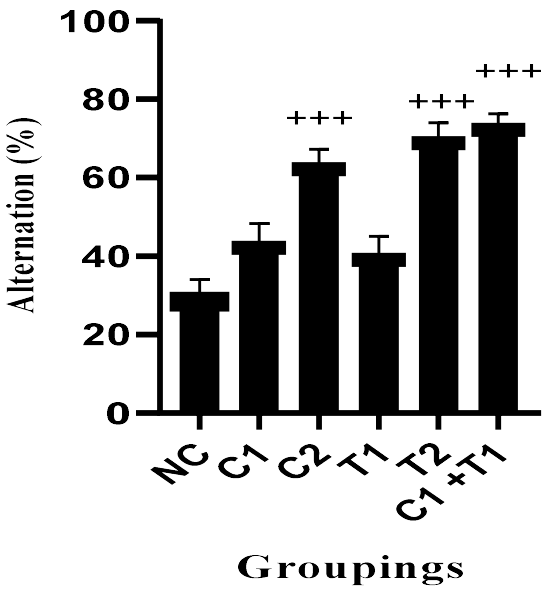

Fig. 1: Effect of tramadol and coffee co-administration on behaviour in the T Maze. +++ indicates significant increase compared to control at p<0.001; NC= Normal Control, C1= coffee (72.46 mg/kg), C2= coffee (108.69 mg/kg), T1= tramadol (2.86 mg/kg), T2= tramadol (5.71 mg/kg), C1 + T1= coffee (72.46 mg/kg) + tramadol (2.86 mg/kg)

Effect of Tramadol and Coffee on Malondialdehyde

The effects of tramadol and coffee on malondialdehyde (MDA) are shown in Table 2. The results showed that the combination group coffee (72.46 mg/kg) + tramadol (2.86 mg/kg) was significantly higher compared to the control and tramadol (2.86 mg/kg) group at $p < 0.001$ and $p < 0.01$, respectively. The result revealed a general increase in MDA levels in all the treatment groups compared to the control (Table 2).

Table 2: Effect of Tramadol and Coffee on Antioxidant Enzymes

Groups	SOD (U/mL)	CAT (U/mL)	GSH (µmol/mL)	MDA (µmol/L)
Control	92.63 ±0.75	508.8 ±8.02	26.07 ±0.72	31.86 ±0.51
Coffee (72.46 mg/kg)	104.90 ±3.82	524.2 ±3.62	27.10 ±1.93	40.37 ±1.12
Coffee (108.69 mg/kg)	108.20 ±5.76	466.1 ±14.98	26.91 ±1.50	40.83 ±2.16
Tramadol (2.86 mg/kg)	113.30 ±6.40	530.8 ±26.03	30.64 ±1.17	35.32 ±2.32
Tramadol (5.71 mg/kg)	104.00 ±4.23	506.8 ±4.50	28.02 ±2.10	41.18 ±1.66
Coffee (72.46 mg/kg) + Tramadol (2.86 mg/kg)	97.75 ±5.89	500.2 ±13.71	25.39 ±0.77	45.86 ±1.60***,a

Values are expressed in Mean±SEM
***,a Significant at p<0.001 and p<0.01 respectively, compared with Control, 'd' indicates significant increase compared with and tramadol (2.86 mg/kg) groups

Histopathological Effect of Tramadol and Coffee on Rats' Cerebellum

The control section of the cerebellar cortex of rats stained with haematoxylin and eosin revealed a normal cellular architecture of molecular layer consisting of the stellate and basket cells, Purkinje cell layer made up of the purkinje cells, granular layer made up of the granular cells and white matter. Coffee (72.46 mg/kg) administered group revealed atrophic Purkinje cells. Coffee (108.69 mg/kg) administered group showed atrophy of Purkinje cells and neuronal degenerations in the granular and Purkinje cell layers of the cerebellum (Fig. 2).

In tramadol (2.86 mg/kg/kg) administered group, the cerebellar cortex revealed atrophying Purkinje cells. The group administered with tramadol (5.71 mg/kg/kg) showed atrophy of the Purkinje cells and neuronal degenerations in the molecular and granular layers. The cerebellar cortex of rats in co-administered coffee+tramadol group (72.46 mg/kg) + (2.86 mg/kg/kg) revealed severe histological distortions with sparse cellular population, neuronal degenerations in molecular and Purkinje cell layer, atrophy of the Purkinje cells (Fig. 2).

DISCUSSION

Tramadol intoxication is reported as deleterious to the central nervous system (Faria et al. 2018; Mohammadi-pour et al. 2019; Raoofi et al. 2022), and thus, its combination with other drugs (analgesic or stimulant) could be more toxic to the brain. In the present study, the effect of co-administration of tramadol and coffee on behaviour, oxidative stress and histology of the cerebellum were assessed.

In neurobehavioural assessment using the T-maze spontaneous alternation test, rats administered coffee (72.46 mg/kg) and tramadol (2.86 mg/kg), had low alternation and thus, no deleterious effect on memory and cognitive functions: The higher the alternation, the higher the memory deficits, and vice versa (Galeano et al. 2014). Nmaju et al. (2014) reported that 50 and 100 mg doses of coffee had a stimulant effect on the central nervous system and thus, improved learning and memory. However, coffee (108.69 mg/kg), tramadol (5.71 mg/kg/kg) and their co-administration were significantly higher in alternation compared to the control. This depicts memory deficit exerted by the free radicals arising from the intoxication (Tashakori and Afshari 2010; Hosseini-Sharifabad et al. 2016).

Antioxidants appear to act against disease processes by increasing the level of endogenous antioxidant enzymes such as superoxide dismutase and catalase, and decreasing toxic by-products of lipid peroxidation such as malondialdehyde (Bansal et al. 2005). With catalase, no significant difference was observed in coffee and tramadol administered groups. This is contrary to previous reports that oxidants react directly with cellular macromolecules oxidising them (Ceconi et al. 2003; Giordano 2005; Sies et al. 2005; Verma et al. 2009). Superoxide dismutase and

reduced glutathione showed no significant difference in all the groups. This shows that there was a minimal effect of antioxidant enzymes on single doses and no effect on the combination of coffee and tramadol. More so, this is an indication that coffee and tramadol poses no antioxidative properties, and this supports previous studies (Abdel-Zaher et al. 2011; Ghoneim et al. 2014).

Lipid peroxidation was statistically insignificant in the single coffee and tramadol groups, but however, was significantly higher in rats co-administered tramadol and coffee. Therefore, this implies that the combination of tramadol and coffee exerted a high level of oxidation, and supports previous studies performed in different models (Guglielmotto et al. 2009; Chen et al. 2011). El-Gaafarawi (2006) also showed that administration of 80 mg of tramadol significantly induced the elevation of MDA at 10, 20 and 30 days of treatment. Concomitantly, Raoofi et al. (2022) reported that caffeine modulated increased MDA level caused by tramadol ingestion.

Ghoneim et al. (2014) reported that chronic administration of tramadol causes histological abnormalities including apoptosis in the rat's cerebral cortex. The present study revealed that rats administered coffee (72.46 mg/kg) and tramadol (2.86 mg/kg), respectively showed atrophic Purkinje cells on the cerebellar cytoarchitecture. However, coffee (108.69 mg/kg), tramadol (5.71 mg/kg) and combination, revealed such cellular abnormalities like atrophy of the Purkinje cells and neuronal degenerations in the granular and Purkinje cell layers of the rats' cerebellum. Studies reported that at high doses, caffeine can suppress activities of the central nervous system (Fisone et al. 2004; Wang et al. 2019; Yu et al. 2019; Houghton et al. 2020). Another report revealed that tramadol treated rats showed atrophy of the cerebellum, microgliosis, neuroinflammation and apoptotic biomarkers (Ezi et al. 2021). Tramadol administration during pregnancy caused profound structural abnormalities on the post-natal cerebellar cortex and was also associated with oxidative stress (Aboulhoda and Hassan 2018). Also, a recent study revealed that caffeine modulates apoptosis, oxidative stress, and inflammation damage induced by tramadol in cerebellum of male rats (Raoofi et al. 2022).

The neurotoxicity of tramadol and coffee combination in the present study was evident with increased spontaneous alternation behaviour, arising from increased MDA level and associated cerebella cellular atrophy.

Conclusion

The present study showed that coffee (72.46 mg/kg) and tramadol (2.86 mg/kg) caused no spontaneous alternation behaviour, no oxidative stress but histological alterations in the cerebellum. coffee (108.69 mg/kg) and tramadol (5.71 mg/kg) caused increased spontaneous alternation behaviour, no oxidative stress but histological alterations in the cerebellum. However, the co-administration of tramadol and coffee caused increased spontaneous alternation behaviour, oxidative stress and histological alterations in the cerebellum compared to individual doses.

Grants and Financial Support

No funding was received.

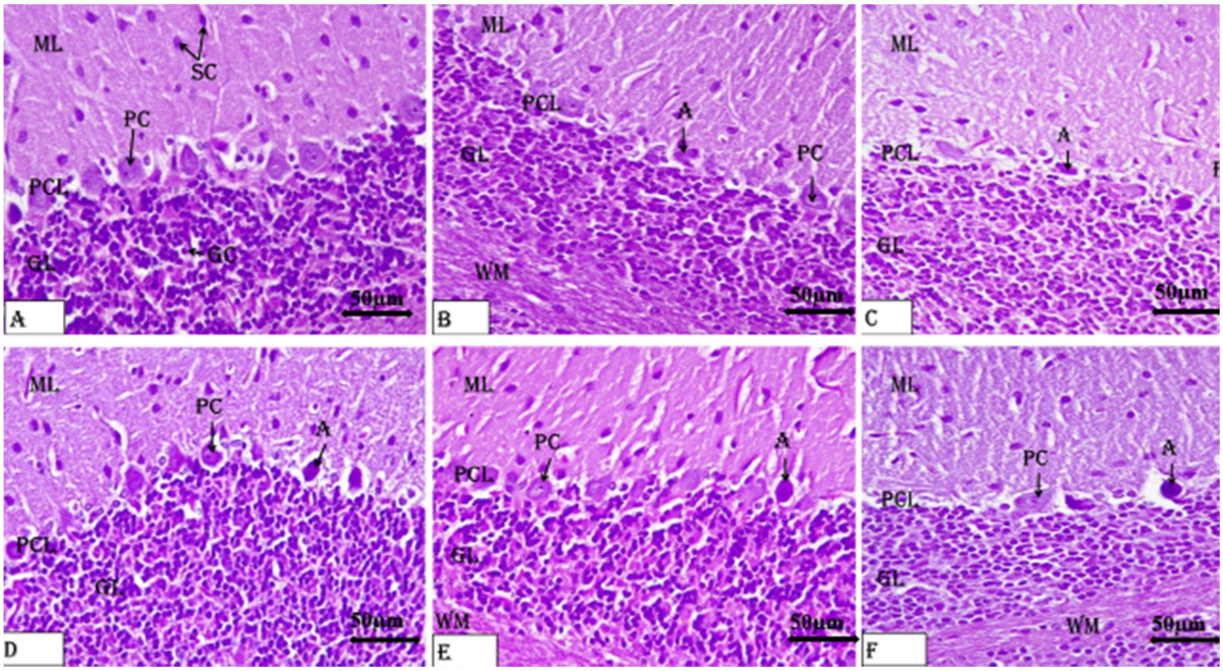

Fig. 2: Photomicrographs of the cerebellar cortex administered with coffee and tramadol showing molecular (ML), Purkinje cell (PCL), and granular (GL) layers. Purkinje cells (PC); A- Atrophy and neuronal degenerations. A= Control, B= coffee (72.46 mg/kg), C= coffee (108.69 mg/kg), D= tramadol (2.86 mg/kg), E tramadol (5.71 mg/kg), F= coffee (72.46 mg/kg) + tramadol (2.86 mg/kg). Haematoxylin and Eosin, × 400

Conflict of Interest
None declared.

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Authors' Contribution
SJU: Conceptualization, Data acquisition, Data analysis, Methodology, Visualization, Writing- Original draft, Writing- review and editing; IUU: Supervision, Validation, Visualization, Writing- review and editing; ANA: Supervision, Validation, Writing- review and editing.

REFERENCES

Abdel-Zaher, A.O., Abdel-Rahman, M.S. and Elwasei, F.M. (2011) Protective effect of Nigella sativa oil against tramadol-induced tolerance and dependence in mice: Role of nitric oxide and oxidative stress. Neurotoxicology. 32(6):725-733.

Aboulhoda, B.E. and Hassan, S.S. (2008) Effect of prenatal tramadol on postnatal cerebellar development: Role of oxidative stress. J Chem Neuroanat. 94:102-118

Babalonis, S., Lofwall, M.R., Nuzzo, P.A., Siegel, A.J. and Walsh, S.L. (2013) Abuse liability and reinforcing efficacy of oral tramadol in humans. Drug Alcohol Depend. 129(1-2):116-124.

Bansal, A.K., Bansal, M., Soni, G. and Bhatnagar, D. (2005) Protective role of Vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver. Chem Biol Interact. 156(2-3):101-111.

Boostani, R. and Derakhshan, S. (2012) Tramadol induced seizure: A 3-year study. Caspian J Intern Med. 3(3):484-487.

Carrillo-Munguía, N., González-Trujano, M.E., Huerta, M., Trujillo, X. and Diaz-Reval, M.I. (2015) Tramadol and tramadol+caffeine synergism in the rat formalin test are mediated by central opioid and serotonergic mechanisms. Biomed Res Int. 2015:686424.

Ceconi, C., Boraso, A., Cargnoni, A. and Ferrari, R. (2003) Oxidative stress in cardiovascular disease: Myth or fact? Arch Biochem Biophys. 420(2):217-221.

Chen, S.D., Yang, D.I., Lin, T.K., Shaw, F.Z. and Liou, C.W. and Chuang, Y.C. (2011) Roles of oxidative stress, apoptosis, PGC-1α and mitochondrial biogenesis in cerebral ischemia. Int J Mol Sci. 12(10):7199-7215.

Chikezie, U.E. and Ebuenyi, I.D. (2019) Tramadol misuse in the Niger delta; A review of cases presenting within a year. J Subst Use. 24(5):487-491.

Drewnowski, A. and Rehm, C.D. (2016) Sources of caffeine in diet of US children and Adults: Trends by beverage type and purchase location. Nutrients. 8(3):154.

Duan, M., Chen, X., He, H., Jiang, Y., Jiang, S., Xie, Q., et al. (2015) Altered basal ganglia network integration in schizophrenia. Front Human Neurosci. 9:561.

El-Gaafarawi, I. (2006) Biochemical toxicity induced by tramadol administration in male rats. Egypt J Hosp Med. 23(1):353-362.

Ezi, S., Boroujeni, M.E., Khatmi, A., Vakili, K., Fathi, M., Abdollahifar, M., et al. (2021) Chronic exposure to tramadol induces neurodegeneration in the cerebellum of adult wistar rats. Neurotox Res. 39:1134-1147.

Faria, J., Barbosa, J., Moreira, R., Queirós, O., Carvalho, F. and Dinis-Oliveira, R. (2018) Comparative pharmacology and toxicology of tramadol and tapentadol. Eur J Pain. 22(5):827-844

Fisone, G., Borgkvist, A. and Usiello, A. (2004) Caffeine as a psychomotor stimulant: mechanism of action. Cell Mol Life Sci. 61(7-8):857-872

Galeano, P., Leon, I. and Ponce, F.P. (2014) Spontaneous alternation in mice: a video tracking analysis. Behav Res Methods. 46(2):297-304.

Ghoneim, F.M., Khalaf, H.A., Elsamanoudy, A.Z. and Helaly, A.N. (2014) Effect of chronic usage of tramadol on motor cerebral cortex and testicular tissues of adult male albino rats and the effect of its withdrawal: histological, immunohistochemical and biochemical study. Int J Clin Exp Pathol. 7(11):7323-7341.

Giordano, F.J. (2005) Oxygen, oxidative stress, hypoxia and heart failure. J Clin Invest. 115(3):500-508.

Guglielmotto, M., Tamagno, E. and Danni, O. (2009) Oxidative stress and hypoxia contribute to Alzheimer's disease pathogenesis: two sides of the same coin. Sci World J. 9(1):781-791.

Hosseini-Sharifabad, A., Rabbani, M., Shaifzadeh, M. and Bagheri, N. (2016) Acute and chronic tramadol administration impair spatial memory in rat. Res Pharm Sci. 11(1):49-57.

Houghton, V., Pereez, A.D., Lefevre,-Arbogast, S., Lucia, D.C., Low, D.Y., Urpi-sarda, M., et al. (2020) Caffeine compromises proliferation of human hippocampal progenitor cells. Front Cell Dev Biol. 8:806.

Koziol, L.F., Budding, D., Andreasen, N., D'Arrigo, S., Bulgheroni, S., Imamizu, H., et al. (2014) Consensus paper: the cerebellum's role in movement and cognition. Cerebellum. 13:151-177.

Lorke, D. (1983) A new Approach to practical acute toxicity testing. Arch Toxicol. 54:275-280.

Manto, M., Bower, J.M., Conforto, A.B., Delgado-García, J.M., da Guarda, S.N., Gerwig, M., et al. (2012) Consensus paper: roles of the cerebellum in motor control--the diversity of ideas on cerebellar involvement in movement. Cerebellum. 11(2):457-487.

Marventano, S., Salomone, F., Godos, J., Pluchinotta, F. R., Rio, D.D., Mistretta, A., et al. (2016) Coffee and tea consumption in relation with non-alcoholic fatty liver and metabolic syndrome: A systematic review and meta-analysis of observational studies. Clin Nutr. 35(6):1269-1281.

Michel, T.M., Pulschen, D. and Thome, J. (2012) The role of oxidative stress in depressive disorders. Curr Pharm Des. 18(36):5890-5899.

Middleton, F.A. and Strick, P.L. (2001) Cerebellar projections to the prefrontal cortex of the primate. J Neurosci. 21:700-712.
Miotto, K., Cho, A.K., Khalil, M.A., Blanco, K., Sasaki, J.D. and Rawson, R. (2017) Trends in tramadol: pharmacology, metabolism, and misuse. Anesth Analg. 124(1):44-51.
Mohammadipour, A., Ashkezari, M.D., Farahmand, B. and Shokrzadeh, M. (2019) Demographic characteristics and functional performance of the kidneys and hearts of patients with acute tramadol toxicity. Drug Res (Stuttg). 69(4):207-210.
National Research Council (2011) Guide to the care and use of laboratory animals, 8th edition. The National Academics Press: Washington, DC, 240p.
Nmaju, A.U., Bisong, S.A., Nwankwo, A.A., Joshua, I.E. and Osim, E.E. (2014) Comparative effects of Garcina kola and coffee diets on learning and memory in mice. Br J Med Med Res. 4(2):731-748.
Placer, Z.A., Cushman, L.C. and Johnson, B.C. (1966) Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal Biochem. 16(2):359-364.
Pourshahidi, L.K., Navarini, L., Petracco, M. and Strain, J. (2016) A comprehensive overview of the risks and benefits of coffee consumption. Compr Rev Food Sci Food Saf. 15(4):671-684.
Raoofi, A., Delbari, A., Nasiry, D., Eslampour, R., Golmohammadi, R., Javadina, S.S., et al. (2022) Caffeine modulates apoptosis, oxidative stress, and inflammation damage induced by tramadol in cerebellum of male rats. J Chem Neuroanat. 123:102116.
Reyes, C.M. and Cornelius, M.C. (2018) Caffeine in the diet: Country-Level Consumption and Guidelines. Nutrients. 10(11):1772.
Robert, T., Dervins, D., Fabre, F. and Gangloff, S. (2006) Mrc1 and Srs2 are major actors in the regulation of spontaneous crossover. EMBO J. 25(12):2837-2846.
Rukkumani, R., Aruna, K., Varma, P.S. and Menon, V.P. (2004) Influence of ferulic acid on circulatory prooxidant-antioxidant status during alcohol and PUFA induced toxicity. J Physiol Pharmacol. 55(3):551-561.
Shah, A.V., Kumar, K.V.A. and Kumar, B.P.R. (2013) Comparative evaluation of pre-emptive analgesic efficacy of intramuscular ketorolac versus tramadol following third molar surgery. J Maxillofac Oral Surg. 12(2):197-202.
Sies, H., Stahl, W. and Sevanian, A. (2005) Nutritional, dietary and postprandial oxidative stress. J Nutr. 135:969-972.
Stassinos, G.L., Gonzales, L. and Klein-Schwartz, W. (2019) Characterising the toxicity and dose-effect profile of tramadol ingestions in children. Pediatr Emerg Care. 35((2):117–120.
Tashakori, A. and Afshari, R. (2010) Tramadol overdose as a cause of serotonin syndrome: a case series. Clin Toxicol (Phila). 48(4):337-341.
Umoh, I.U. and Jimmy, E.O. (2017) A Comparative histopathological effect of caffeinated and decaffeinated coffee on the histomorphology of the kidney of Adult male Albino Wistar rats. IOSR J Pharm Biol Sci. 12(3):62-66.
Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M. and Telser, J. (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 39(1):44-84.
Verma, A.R., Vijayakumar, M., Mathela, C.S. and Rao, C.V. (2009) In vitro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves. J Food Chem Toxicol. 47(9):2196-2201.
Wang, R., Xue, J., Meng, L., Lee, J.-W., Zhao, Z., Sun, P., et al. (2019) Caffeine improves the performance and thermal stability of perovskite solar cells. Joule. 3(6):1464-1477.
Xu, C., Liu, S., Liu, Z., Song, F. and Liu, S. (2013) Superoxide generated by pyrogallol reduces highly water soluble tetrazolium salt to produce a soluble formaan: a simple assay for measuring superoxide anion radical scavenging activities of biological and abiological samples. Anal Chem Acta. 2(793):53-60.
Yazdani, M., Ide, K., Asaditar, M., Gottshalk, S., Joseph, F. and Nakamoto, T. (2004) Effects of caffeine on the saturated and monounsaturated fatty acids of the newborn rats. Ann Nutr Metab. 48(2):79-83.
Yu, E.Y., Wesselius, A., van Osch, F., Stern, M.C., Jiang, X., Kellen, E., et al. (2019) The association between coffee consumption and bladder cancer in the bladder cancer epidemiology and nutritional determinants (BLEND): International pooled study. Cancer Causes Control. 30(8):859-870.
Zhou, H., Huang, L., Huang, H. and Cai, Z. (2012) Effects of chronic tramadol exposure on the zebrafish brain: A proteomic study. J Proteom. 75(11):3351-3364.