Received: 9 March 2025 | Accepted: 17 July 2025 Nig J Neurosci 16(3):98-115.2025

Nigerian Journal of Neuroscience

https://njn.neurosociety.org.ng

DOI: 10.47081/njn2025.16.3/004

Original Article https://ojshostng.com/index.php/NJN Open Access

Pentazocine Exposure Elicits Motor-social, Neurochemical, and Neuromorphological Deviations in the Cerebellum of Juvenile Male BALB/c Mice

Damilare A. Adekomi[1], Dorcas I. Ogungbemi[1], Ruqayyah Y. Ibiyeye[2], Omowumi O. Adewale[3], Adesina O. Adekeye[4], Ebenezer I.O. Ajayi[3], Linus A. Enye[4], Edem E. Edem[4], Adejoke Y. Onaolapo[5], Temidayo D. Adeniyi[6], Adewale V. Aderemi[7], Opeyemi S. Osuntokun[8], Tajudeen O. Ajadi[1], Gbenga J. Adedayo[9], Akinola O. Ajeleti[10]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, Osun State University, Osogbo, Nigeria; [2]Department of Anatomy, Kwara State University, Malete, Nigeria; [3]Department of Biochemistry, Faculty of Basic and Applied Sciences, Osun State University, Osogbo, Nigeria; [4]Department of Anatomy, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Nigeria; [5]Department of Anatomy, Ladoke Akintola University of Technology, Ogbomoso, Nigeria; [6]Department of Medical Laboratory Science, University of Ilorin, Ilorin, Nigeria; [7]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Osun State University, Osogbo, Nigeria; [8]Department of Health and Social Care, Mont Rose College of Management and Sciences- Buckinghamshire New University, East London, United Kingdom; [9]Department of Anatomy, University of Ilorin, Ilorin, Nigeria; [10]Department of Anatomy, Bowen University, Iwo, Nigeria

ABSTRACT
Pentazocine is an opioid used as an analgesic for treating moderate or severe pain. Its abusive use can trigger numerous deleterious effects. We examined the impact of pentazocine administration on the cerebellum of juvenile male mice, focusing on motor-social behaviours, lipid peroxidation, reduced glutathione, superoxide dismutase, catalase, myeloperoxidase, nitric oxide, cyclooxygenase-2, tumour necrosis factor alpha, and sodium-potassium pump (Na^+/K^+-ATPase), serotonin, dopamine, acetylcholinesterase, and butyrylcholinesterase, as well as mitochondrial functions in the cerebellum of juvenile male mice. The mice were administered 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg of pentazocine dissolved in sterile water for injection s.c. twice daily for 14 days. The results of this study revealed that exposure to pentazocine triggers upregulated activities of increased respective levels of lipid peroxidation, reduced glutathione, superoxide dismutase, catalase, myeloperoxidase, nitric oxide, cyclooxygenase-2, and tumour necrosis factor-alpha, respectively. Our data also showed that exposure to graded doses of pentazocine altered the respective levels of serotonin, dopamine, acetylcholinesterase, and butyrylcholinesterase, with a reduction in the activity of Na^+/K^+-ATPase. Our experiment further showed that exposure to pentazocine is linked to mitochondrial dysfunctions as well as neuronal degeneration in the cerebellum. Conclusively, our study reveals the significant neurotoxic effects of pentazocine, particularly on motor and social behaviour, along with notable biochemical and neuropathological changes in the cerebellar cortex. These findings suggest that the abusive use of pentazocine causes critical cellular and molecular alterations that contribute to neurotoxicity.

Keywords
Central nervous system, Addiction, Lifestyle, Socio-medical economy, Development, Opioid

Correspondence: Damilare A. Adekomi, PhD. Neuroscience Unit, Department of Anatomy, Faculty of Basic Medical Sciences, Osun State University, Osogbo, Nigeria. E-mail: adedayo.Adekomi@uniosun.edu.ng; Phone Number:+2348038441671; ORCID: 0000-0002-7674-8168
Dorcas I. Ogungbemi - gazellecrown92@gmail.com, 0009-0009-5287-5267; Ruqayyah Y. Ibiyeye - ruqayyah.ibiyeye@kwasu.edu.ng, 0000-0002-0628-1507; Omowumi O. Adewale - omowumi.adewale@uniosun.edu.ng, 0000-0003-0387-585X; Adesina O. Adekeye - adekeyeao@abuad.edu.ng, 0000-0003-0691-149X; Ebenezer I.O. Ajayi - ebenezer.ajayi@uniosun.edu.ng, 0000-0001-6348-5840; Linus A. Enye - enyelinus@abuad.edu.ng, 0000-0002-9336-0384; Edem E. Edem - ee.edem@abuad.edu.ng, 0000-0001-5921-5311; Adejoke Y. Onaolapo - ayonaolapo@lautech.edu.ng, 0000-0001-7126-7050; Temidayo D. Adeniyi - adeniyi.td@unilorin.edu.ng, 0000-0002-8482-9136; Adewale V. Aderemi - adewale.aderemi@uniosun.edu.ng, 0000-0001-6388-012X; Opeyemi S. Osuntokun - o.osuntokun@mrcollege.ac.uk, 0000-0002-1363-1843, Tajudeen O. Ajadi - ajaditajudeen8@gmail.com, 0009-0003-7130-7229; Gbenga J. Adedayo - adedayo-ayobami043319@gmail.com, 0009-0005-3188-6339), Akinola O. Ajeleti - segun.ajeleti@bowen.edu.ng, 0000-0001-5101-4072

Cite as: Adekomi, *D.A., Ogungbemi, D.I., Ibiyeye, R.Y., Adewale, O.O., Adekeye, O.O., Ajayi, E.I.O., Enye, L.A.., Edem, E.E., Onaolapo, A.Y., Adeniyi, T.D., Aderemi, A.V., Osuntokun, O.S., Ajadi, T.O., Adedayo, G.J., and Ajeleti, A.O. (2025). Pentazocine exposure elicits motor-social, neurochemical, and neuromorphological deviations in the cerebellum of juvenile male BALB/c mice. Nig. J. Neurosci. 16(3), 98-115. doi: 10.47081/njn2025.16.3/004*

Received: 9 March 2025 | Accepted: 17 July 2025 Nig J Neurosci 16(3):98-115.2025

Published by Neuroscience Society of Nigeria. This work is an open access article under the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). Copyright © 2025 by authors.

INTRODUCTION

The unprescribed and recreational use of opioids is often associated with brain injuries and cognitive deficits, with corresponding impaired regulation of impulses as well as problem-solving abilities, which may increase drug use and risk-taking (Winstanley *et al*., 2021). According to Falade *et al*. (2023), with reference to clinical pharmacology, pentazocine, a known benzodiazepine derivative, is an opioid that has the clinical features of both an agonist and a partial or weak antagonist, and its uncontrolled use can result in marked addiction and withdrawal symptoms.

Inflammation and oxidation are important in the multi-systemic toxicity seen in substance use disorders, as noted by Bachi *et al*. (2017). Reactive oxygen species (ROS) and reactive nitrogen species are aggravated during oxygen catabolism and play pivotal roles in several functional processes in the cell (Ye *et al*., 2015). While important in regulatory processes, their balance is sustained by antioxidant enzymes like superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px), which inhibit oxidative dysfunction (Zhang *et al*., 2011).

Addiction to opioids disturbs the functional integrity of the mitochondria and calcium signalling, resulting in adverse effects on neuroadaptation. This detrimental disturbance alters energy production and aggravates oxidative stress, which impairs neuronal functions. Mitochondria are important for energy generation, the release of neurotransmitters, and sustaining calcium balance. The disturbances in the functional integrity of mitochondrial kinetics can provoke cellular dysfunctions, and the imbalances are connected to diseases with increased neuronal cell death (Howarth *et al*., 2012; Manji *et al*., 2012; Baranov *et al*., 2019).

Mitophagy involves mechanisms connected with the release of cytochrome c, which triggers apoptotic pathways, mitochondrial dysfunction, and impaired signalling. Accumulated ROS can destroy the morphology and functional integrity of the mitochondrial components, activating mitophagy, while mutations in mitochondrial DNA (mtDNA) can hinder ATP production (Xu *et al*., 2025).

The administration of opioids disturbs mitochondrial function and increases oxidative stress, which impacts neuronal signalling. Mitochondria are crucial for generating ATP, and their dysfunction adversely influences energy-demanding dopaminergic neurons (Norat *et al*., 2020; Gao *et al*., 2022; Trigo *et al*., 2023).

The cerebellar cortex consists of more neurons than the rest of the brain (Huang and Ricklefs, 2013), plays a pivotal role in opioid addiction but is often neglected due to its association with the control and regulation of motor functions and its involvement in reward pathways (Moulton *et al*., 2014; Cheng *et al*., 2015; Miquel *et al*., 2016; Sun *et al*., 2017; De Pirro *et al*., 2018). Wang and Lan (2023) emphasised the importance of understanding the complexities of drug abuse and the non-motor functions of the cerebellum. The effect of exposure to graded doses of pentazocine on cerebellar-associated behaviour, oxidative stress, inflammation, apoptosis, and mitochondrial function in juvenile models remains unknown.

Neurons are the basic units of the nervous system, communicating via neurotransmitters like dopamine. When triggered, a neuron releases neurotransmitters into a synapse, which links the presynaptic neuron to the postsynaptic neuron. These neurotransmitters fuse to the receptors on the postsynaptic neuron, giving rise to heterogeneous responses, which could either excite or inhibit the firing of signal responses. This binding can exacerbate several complex biochemical changes and alter cellular signalling and metabolism. Several drugs interact with the neurotransmitter systems and affect intracellular molecules, serving as "second messengers" that promote cellular processes, including gene expression (Wang *et al*., 2024).

Neurons also control the levels of neurotransmitters through reuptake, where neurotransmitters are reabsorbed by the presynaptic neuron via transporters (Teleanu *et al*., 2022). This mechanism prevents overstimulation of postsynaptic neurons. The release of neurotransmitters is linked to calcium ion (Ca^{2+}) influx, which triggers release while inhibiting ATPase activity (Südhof, 2012). Some agents can enhance or suppress this process, which illustrates complex signalling dynamics. Enkephalins, a class of opioids, may inhibit neurotransmitter release by hyperpolarising the neuronal membrane. Studies have also explored the effects of opioids on the sodium-potassium pump (Na^+/K^+-ATPase), with contradictory reports (Masocha *et al*., 2002; Masocha *et al*., 2016; El-Hamid Mohamed Elwy and Tabl, 2017). According to Reeves *et al*. (2022), morphine can provoke Na^+/K^+-ATPase activity *in vivo*, suggesting a complex role in neurotransmission (Reeves *et al*., 2022).

With the rising concerns over pentazocine misuse, understanding its impact on the cerebellum is important for having a better understanding of its effect on the cerebellum during juvenile life, hence, this study examines the effects of exposure to graded doses of pentazocine on the cerebellar cortex of juvenile male BALB/c mice.

MATERIALS AND METHODS

Ethical Approval

This study was performed according to the protocols described in the 1996 (No.80-23) regulations of the National Institute of Health (NIH). The Osun State University Animal Care and Use Ethical Committee approved the application (UNIOSUNAC/2023/0133) for the experimental treatment of mice based on the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guideline. The experimental methods were ethically approved and adhered to ARRIVE standards.

Experimental Animal
This study utilised 40 F1 juvenile BALB/c mice (5 weeks old, 15–20 g) for the investigation. The mice were obtained from Dem-Fayo Research Animal's Breeding Facility in Ede (Osun State, Nigeria). During the 7-day acclimatisation and experimental phases, the mice were maintained under standard temperature (22 ± 2°C) and humidity (45-55%), with a natural light and dark cycle in the rodent colony facility of the Animal House of the Faculty of Basic Medical Sciences, Osun State University (Osogbo, Nigeria), with unlimited access to rodent food and clean water.

Animal Groupings, Dosage, and Drug Administration
The mice (N = 40) were randomly divided into four groups (n = 10): the control group (sterile water-injected) and three other groups exposed to intraperitoneal injections of pentazocine 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg s.c. The dosage of pentazocine used in this study was picked from an unpublished behavioural study performed on mice exposed to pentazocine and a published report from our laboratory (Adekomi *et al*., 2017). The graded doses of pentazocine were administered to the mice twice daily (6 hours apart) for 14 days. The pentazocine injection (2 mg/ml ampoule) was purchased from Tazowin (New Delhi, India). The pentazocine injection was reconstituted with 2 mL of sterile water for injection.

Behavioural Assays
The mice in each experimental group underwent a series of behavioural assessments, including the three-chamber test, the open-field test, and the rotarod test. The first behavioural test we performed was the 3-chamber test; it was performed for two consecutive days (days 7 and 8 of the study). This behavioural test allows researchers to evaluate the animals' general sociability and interest in social novelty. Following this, the open-field test was conducted on days 10 and 11, and it revealed the mice's anxiety-related behaviours by measuring their willingness to explore the open chamber. Lastly, on days 13 and 14, the Rotarod test was administered, a task designed to assess motor coordination in mice. Each of the behavioural paradigms was completed 30 min after administering the respective doses of the experimental drug (Paul *et al*., 2021).

3-chamber Sociability Test
A three-chamber sociability test was conducted to evaluate general sociability and interest in social novelty (Shoji and Miyakawa, 2019; Bian *et al*., 2022). The test was performed according to the methods described in the study of Zhang *et al*. (2022). Briefly, the testing equipment was a black box segmented into three chambers. Each chamber measuring about 33 × 22 × 30 cm^3 had an opening measuring 5 × 3 cm^2 in the partitioned wall, which permitted the mice free access to all the chambers. During the test, the subject mouse was placed in the central portion of the chamber and permitted to explore freely for 5 min. Subsequently, for the social interaction test, one cylindrical iron cage was randomly placed in each of the two side chambers. One cage was empty (E), and the other contained a sex- and age-matched stranger mouse (S1). The subject mouse was again placed in the central chamber and allowed to freely explore the three chambers and interact either with the empty cage or the S1 mouse for an additional 5 min. The time spent sniffing during this period was recorded to calculate the social interaction index, which was determined as follows:
Total sniffing time = time spent sniffing the cage containing S1 + time spent sniffing the empty cage
Social interaction index = [(time spent sniffing the cage containing S1) - (time spent sniffing the empty cage)] / total sniffing time × 100%.

To measure interest in social novelty, a second stranger mouse (S2) was exposed to the empty cage. The total sniffing time and the social novelty preference index were calculated as follows:
Total sniffing time = time spent sniffing the cage containing S1 + time spent sniffing the cage containing S2
Social novelty preference index = [(time spent sniffing the cage containing S2) - (time spent sniffing the cage containing S1)] / total sniffing time × 100%.
Throughout these tests, the empty cage and the cages containing the stranger mice (S1 and S2) were randomly orientated.

Open-Field Test
The open-field assay was conducted following the procedure outlined by Alcott *et al*. (2020). The mice were acclimatised to a room with illumination set to 200 lux and a background ambient white noise level of 60 dB. Each mouse from the experimental groups was placed in the centre of the open field chamber measuring 40 cm x 40 cm x 30 cm. This chamber was equipped with photobeams programmed to automatically record movement. Each mouse was tested for 15 min, during which activity was recorded using Fusion software (Accuscan Instruments). The standard movement segmentation settings were applied, and the data collected and statistically analysed included movement time, the total distance travelled, the number of horizontal movements, and the number of vertical movements during the 15-min testing period.

Rotarod Test
A rotarod treadmill (Ugo Basile, Varese, Italy) was used to assess motor coordination in mice (Pyatha *et al*., 2023). On day 13 of the study, the mice in each of the experimental groups participated in a training session on the apparatus, becoming familiar with the equipment and the tasks required before the actual trial began on day 14. The rotarod increased speed from 4 to 40 rotations per minute over 10 s, with a total test duration of 300 s. Mice were placed on the rod, and the time taken for each to fall off was recorded. Each trial was conducted three times, and the mean time from all trials was calculated.

Collection of Cerebellum for Histological and Neurochemical Assays and the Evaluation of Mitochondrial Functions
Twenty-four hours after the administration of the last respective doses, the mice were sacrificed by cervical dislo-

cation under 2% halothane anaesthesia. The heads were quickly removed from the body, the skulls were cracked open with forceps, and the intact brains were removed. The brains were sectioned, and the cerebellum was excised. The cerebellar tissues were rapidly rinsed in ice-cold phosphate-buffered formalin. The cerebella of the mice in the respective experimental groups were homogenised in freshly prepared 0.1 M phosphate buffer (1:10 w/v; pH 7.4) and centrifuged at 3,000 rpm. The obtained clear supernatants were used for neurochemical assays. For histological investigation of the cerebellum, cerebellar tissues were fixed in freshly prepared 10% neutral buffered formalin.

Neurochemical Assays

Levels of Malondialdehyde

The level of malondialdehyde (MDA) in the cerebellum of mice from the respective experimental groups was determined using an MDA ELISA kit (MAK085 Sigma-Aldrich). The procedure followed was a competitive ELISA, and the manufacturer's detailed specifications were adhered to. Briefly, the cerebellar tissue samples were washed, weighed, and then homogenised in phosphate-buffered saline at a ratio of 1:9 using a glass homogeniser on ice. The homogenates were subsequently centrifuged for 5 min at 5,000× g to obtain the supernatant. This supernatant was used to analyse MDA levels, which were measured using a microplate reader (BIOTEK EL310, Burlington, VT) at a wavelength of 450 nm. The results for MDA levels were expressed in nanomoles of MDA per gram of wet tissue.

Levels of Reduced GSH

The method described by Beutler *et al*. (1963) was employed to measure the levels of GSH in the cerebellar tissue homogenates of mice from the respective experimental groups. An equal volume of 4% sulfosalicylic acid was added to an aliquot of the tissue, which was then centrifuged at 4,000 rpm for 5 min to deproteinise the sample. The resulting cerebellar supernatant was mixed with 4.5 ml of Ellman's reagent in a 0.5 ml to 4.5 ml ratio. A blank sample was prepared using 0.5 ml of the diluted precipitating agent and 4.5 ml of Ellman's reagent. GSH absorbs at 412 nm, which was measured using a microplate reader. The results were expressed as micrograms of GSH per milligram of protein.

Activities of SOD

SOD activity was measured in cerebellar homogenates from mice using the method of Oberley and Spitz (1984). The assay relies on the ability of SOD to prevent the reduction of nitro blue tetrazolium dye by phenazine methosulfate. In the procedure, a 0.02 mL sample was mixed with 0.2 mL of an experimental solution (50 mM/L phosphate buffer, pH 8.5, 1 mM/L) and a nitro blue tetrazolium solution (1 mM/L NADH) in a 1:10:1 mL ratio. To initiate the reaction, 0.02 ml of the enzyme working solution was added to the mixture. Twenty minutes after incubating the plates at 37°C, the absorption spectrum was recorded at 450 nm using a microplate reader, and the results obtained were expressed as units per milligram of protein (U/mg protein).

Activities of Catalase

Catalase activity was measured in the cerebellar tissue homogenate of mice in the respective experimental groups. The activity of catalase was examined by observing the degradation of hydrogen peroxide into water and oxygen in compliance with the method described in the study of Maehly and Chance (1954). Briefly, the assay mixture was composed of 0.30 mL of hydrogen peroxide in 50 mL of 0.05 M sodium phosphate buffer, pH 7.0. A 20 μL aliquot of 10% (w/v) homogenate was added to 980 μL of this substrate mixture. The initial absorbance was documented after 1 min, and the final absorbance was quantified after 6 minutes. The reactions were observed at a wavelength of 230 nm. To establish a standard curve, purified catalase (Sigma-Aldrich) was used under identical conditions. All samples were diluted with 0.1 mmol/L sodium phosphate buffer (pH 7.0) to achieve a 50% inhibition of the diluent rate (i.e., the uninhibited reaction). The results were expressed as units of catalase activity per milligram of protein (U/mg protein).

Activities of Myeloperoxidase

The activity of myeloperoxidase (MPO) was measured in cerebellar tissue samples from the mice in each experimental group using the MPO ELISA Kit (EC 1.11.2.2) purchased from Cusabio Life Sciences, Wuhan, China. MPO activity was assessed following the manufacturer's protocol for the assay kit. The enzymatic activity was measured at a wavelength of 450 nm using a microplate reader. The results were expressed as units per milligram of protein (U/mg protein).

Activities of Nitric Oxide

The activity of nitric oxide was measured in cerebellar tissue homogenates from the mice in each experimental group, using the nitric oxide assay kit (MAK454, Sigma-Aldrich). Following the manufacturer's protocol for the assay kit, nitric oxide activity was assessed, and the absorbance was read at 540 nm with a microplate reader. The results were expressed as micromoles of nitrite content per milligram of protein (μmol nitrite/mg protein).

Activities of Cyclooxygenase-2

The activity of cyclooxygenase-2 (COX-2) was measured in the cerebellar tissue homogenates from the mice in each experimental group using the COX-2 assay ELISA kit (CSB-E12910m, Cusabio Life Sciences, Wuhan, China). Following the manufacturer's protocol for the assay, COX-2 activity was assessed, and the absorbance was measured at 450 nm with a microplate reader. The results were expressed in picograms per millilitre (pg/mL).

Activities of Tumour Necrosis Factor-Alpha (TNF-α)

The activity of TNF-α was measured in the cerebellar tissue homogenates from the mice in each experimental group using the TNF-α assay ELISA kit (CSB-E04741m, Cusabio Life Sciences, Wuhan, China). Following the

manufacturer's protocol for the assay, TNF-α activity was assessed, and the absorbance was measured at 450 nm with a microplate reader. The results were expressed in picograms per millilitre (pg/mL).

Levels of Serotonin

The serotonin levels were measured in the cerebellar tissue homogenate of the mice from each experimental group. The cerebellar tissues were first weighed and then homogenised with appropriate volumes of phenylmethylsulfonylfluoride (P7626, Sigma-Aldrich), which is a general inhibitor of serine proteases. The concentration of serotonin was determined using an ELISA kit (KA2518, Abnova, Taipei, Taiwan), following the manufacturer's protocols. The results were presented in ng/ml.

Levels of Dopamine

The dopamine levels in the cerebellar tissue of the mice from each experimental group were determined through rapid dissection of the cerebellar samples on ice. This analysis utilised the mouse dopamine ELISA kit (CSB-E08661m, Cusabio Life Sciences, Wuhan, China), according to the manufacturer's instructions. The absorbance of each well was measured at a wavelength of 450 nm using a microplate reader, and the results were presented in µg/g tissue.

Levels of Acetylcholinesterase (AChE)

The level of AChE enzyme in the cerebellar tissue homogenate of the mice from each experimental group was measured using a quantification ELISA kit (CSB-E17521m, Cusabio Life Sciences, Wuhan, China). The analysis of the level of AChE was performed following adherence to the method substantiated in the study of Ellman *et al*. (1961), and the results were presented in µmol/min/mg protein.

Level of Butyrylcholinesterase (BChE)

The level of BChE in the cerebellar tissue of the experimental mice was quantified using the RayBio® Mouse BChE ELISA kit (ELM-BCHE-1, China), in strict adherence to the method described in the study of Ellman *et al*. (1961), and the results were expressed in µmol/min/mg protein.

Levels of Na^+/K^+-ATPase

The determination of the Na^+/K^+-ATPase assay was performed according to the method described by Akagawa and Tsukada (1979) and reported in the experiment by Imam-Fulani *et al*. (2016). The reaction mixture, totalling 1 mL, contained 200 mM NaCl, 40 mM KCl, 60 mM Tris (pH 7.4), 80 mM $MgCl_2 \cdot 6H_2O$, 20 mM ethylene glycol-bis (2-aminoethyl ether), tetra-acetic acid, an enzyme source, and 8 mM ATP. This mixture was incubated for 1 h at 37°C. To stop the reaction, 5% ice-cold sodium lauryl sulphate was added. The medium was thereafter spun at 1000 x g for 10 minutes. Inorganic phosphate produced in the supernatant was measured using the method of Fiske and Subbarow (1925), as cited in Imam-Fulani *et al*. (2016).

Isolation of Cerebellar Mitochondria and the Evaluation of Mitochondrial Functions

Mitochondria were isolated from the cerebellum using a glass handheld homogeniser followed by differential centrifugation, as earlier documented in the published report of Ghazi-Khansari *et al*. (2006) and Taghizadeh *et al*. (2020). The suspensions of the final mitochondrial pellets in Tris buffer (0.05 M Tris–HCl, 0.25 M sucrose, 20 mM KCl, 2.0 mMMgCl2, and 1.0 mM Na2HPO4 (pH 7.4) at 4°C, except for the mitochondria that were used to determine swelling, ROS level, and mitochondrial membrane potential (MMP), which were incubated in swelling buffer (5 mM succinate, 70 mM sucrose, 1 µM of rotenone, 230 mM mannitol, 2 mM Tris–phosphate, and 3 mM HEPES), respiration buffer (50 µM EGTA, 0.32 mM sucrose, 0.5 mM MgCl2, 0.1 mM KH2PO4 10 mM Tris, 20 mM Mops, and 5 mM sodium succinate), and MMP assay buffer (220 mM sucrose, 5 mM sodium succinate, 68 mM D-mannitol, 10 mM KCl, 2 µM rotenone, 5 mM KH2PO4, 50 µM EGTA, 2 mM MgCl2, 10 mM HEPES), respectively. Protein concentrations were determined using the Coomassie blue method and bovine serum albumin as the standard (Bradford, 1976). 500 µg protein/ml mitochondrial samples were consistently employed for both normalisation and standardisation of experimental conditions (Shaki *et al*. 2013). Protein concentration was measured using the Coomassie blue method with bovine serum albumin (BSA) as the reference standard (Bradford, 1976). Briefly, a total of 100 mg of Coomassie Brilliant Blue (document number 1) was dissolved in 100 mL of 85% (v/v) phosphoric acid and 50 mL of 95% (v/v) ethanol. Once the dye was fully dissolved, the solution's volume was adjusted to 1 L with water. Next, a Bovine Serum Albumin (BSA) protein standard was prepared at a concentration of 1 mg/mL in duplicate. This protein standard was subsequently diluted in a volume of 20 µL to produce five different concentrations, spanning 10 to 50 µg of protein. For the study, 20 µL of the protein solution was added to 1 mL of the dye reagent, and the combination was rigorously mixed and incubated for 120 seconds at room temperature. Finally, the absorbance was measured using a spectrophotometer at a wavelength of 595 nm. Protein/mL mitochondrial samples (500 µg) were consistently used across the experiments for normalisation and standardisation purposes (Shaki *et al*., 2013).

Cerebellar Mitochondrial Swelling

The analysis of mitochondrial swelling in the cerebellum was carried out using isolated mitochondria at a concentration of 0.5 mg protein/mL. This was accessed by observing differences in light scattering spectrophotometrically at 540 nm and at a temperature of 30°C, using the method outlined by Hosseini *et al*. (2013). To prepare the samples, the isolated mitochondria were suspended in a swelling buffer consisting of 70 mM sucrose, 230 mM mannitol, 3 mM HEPES, 2 mM trisphosphate, 5 mM succinate, and 1 µM rotenone. Absorbance was calculated at 549 nm at 10-minute intervals using a Biotek ELISA reader (model ELX-800). A decrease in absorbance indicated an increase in mitochondrial swelling, as shown by Shaki *et al*. (2013) and Taghizadeh *et al*. (2020).

Cerebellar Mitochondrial ROS Level
The cerebellar mitochondrial ROS level was determined using a Shimadzu RF5000U fluorescence spectrophotometer (excitation: 500 nm, emission), upon adding DCFH-DA (final concentration: 10 μM) to the mitochondrial fractions in respiration buffer. DCFH-DA is sequentially hydrolysed to DCFH, which ultimately forms highly fluorescent DCF upon reaction with ROS. The ROS level is directly proportional to DCF fluorescence as measured using a spectrophotometer according to Eskandari *et al*. (2012) and Taghizadeh *et al*. (2020).

Cerebellar Mitochondrial Membrane Potential
Isolated brain mitochondria were incubated with Rh 123, a cationic fluorescent dye, in MMP assay buffer. The Shimadzu RF5000U fluorescence spectrophotometer was used at 490 nm excitation and 535 nm emission wavelengths to measure mitochondrial Rh 123 uptake in accordance with Shaki *et al*. (2013) and Taghizadeh *et al*. (2020). Due to a high negative charge on the mitochondrial inner membrane (-180 mV), positively charged Rh 123 can pass through it. In intact mitochondria, the maximum Rh 123 uptake takes place, resulting in the least Rh 123 presence in the buffer and the minimum dye fluorescence.

Cerebellar Mitochondrial Outer Membrane Damage
The cytochrome C oxidase assay kit (Sigma, Taufkirchen, Germany) was employed to assess cerebellar mitochondrial outer membrane damage. The colorimetric assay measures the reduced absorbance of ferrocytochrome c at 550 nm, resulting from its oxidation to ferricytochrome C by cytochrome C oxidase. The experiment was carried out according to the manufacturer's instructions, using 20 μL of the freshly isolated mitochondrial fraction (at a concentration of 1000 mg/ml) for each reaction and performing duplicates for each assay. The mitochondrial fraction was diluted and incubated with 1 mM n-dodecyl β-d-maltoside in the enzyme dilution buffer (10 mM Tris–HC, pH 7, containing 250 mM sucrose) on ice for 30 min for measuring the total mitochondrial cytochrome c oxidase activity. 0.22 mM ferrocytochrome C substrate solution was added to initiate the reaction. The oxidation of ferrocytochrome C by cytochrome C oxidase results in a decrease in absorbance at 550 nm. Cytochrome C oxidase activity was normalised per protein amount in the reaction. Mitochondrial cytochrome *c* oxidase activity was measured to determine outer membrane integrity, with and without the addition of n-dodecyl β-d-maltoside detergent. The activity ratio of cytochrome C oxidase, measured with and without detergent, was used to determine the damage to the mitochondrial outer membrane (Taghizadeh *et al*., 2020).

Cytochrome *C* Release from Cerebellar Mitochondria
The cytochrome C ELISA kit (M., R & D Systems, Abingdon, UK) was employed to measure the amount of cytochrome *c* released from the cerebellar mitochondria into the incubation buffer, according to the manufacturer's guidelines. A monoclonal antibody that recognises rat/mouse cytochrome *C* was bonded to the microplate. 75 μL of conjugate and 50 μL of standard and positive control were dispensed into respective microplate wells. 1.0 μg of protein from each supernatant was added to the sample wells. Into two wells of the microplate were added all the standards, controls, and samples. 100 μl of substrate solution was added to each well after a 2-h incubation and further incubated for 30 min. 100 μl of the stop solution was pipetted into each of the wells, and the microplate spectrophotometer recorded the optical density at 450 nm. The standard curve, made with the provided kit standards, was used for the quantification of cytochrome C (Taghizadeh *et al*., 2020).

Histological Investigation
Samples of the cerebellum from the experimental mice underwent histopathological evaluation. The cerebellum was routinely processed, then fixed with 10% neutral buffered formalin and embedded in paraffin wax. 5 μm-thick cerebellar sections were obtained and stained with haematoxylin and eosin (H&E) on glass slides. 400× (scale bar of 20 μm) magnified images of the cerebellum were captured using a light microscope. The International Harmonization of Nomenclature and Diagnostic Criteria for Lesions (INHAND) Terms (Bradley *et al*., 2020) were employed to measure the cerebellar pathology seen in the H&E-stained slides.

Statistical Analysis
The statistical values for each group were calculated by averaging the results from ten mice, providing a definitive and representative measure for analysis. Data were presented as mean ± standard deviation (SD). The differences between groups were compared using a one-way analysis of variance (ANOVA) followed by the Tukey *post-hoc* test. The p-value of ≥ 0.05 was considered statistically significant.

RESULTS

Social Behaviour is Inhibited in the Mice Exposed to Graded Doses of Pentazocine
To investigate whether exposure to graded doses of pentazocine could trigger dysfunctional social interactions and affect social novelty preference in experimental mice, we conducted social interaction and social novelty preference tests. The results of a one-way ANOVA revealed that exposure to graded doses of pentazocine significantly impaired the social interaction index in the sociability test. Post hoc analysis indicated that the social interaction index was significantly lower in a dose-dependent manner compared to the control group ([$F(3, 16) = 1209$, $p < 0.0001$], Fig. 1a). Additionally, a one-way ANOVA demonstrated that graded doses of pentazocine significantly affected total sniffing time. The post hoc analysis showed a significant difference in total sniffing time between the control group and the groups treated with 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg of pentazocine ([$F(3, 16) = 1575$, $p < 0.0001$], Fig. 1b). Furthermore, the one-way ANOVA indicated that exposure to graded doses of pentazocine significantly affected both the social novelty preference index

and total sniffing time in the social novelty preference test ([F (3, 16) = 730.2, p < 0.0001], Fig. 1c). Mice in the 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg pentazocine-treated groups exhibited significantly longer total sniffing durations than those in the control group ([F (3, 16) = 539.6, p < 0.0001], Fig. 1d).

Motor Coordination Tests Revealed Significant Deficits in the Mice Following Pentazocine Exposure

Using behavioural assays, we assessed the motor functions of experimental mice with cerebellar dysfunctions caused by pentazocine exposure. Considering that modifications in cerebellar circuit functions could impact ambulatory behaviour in an open field, we assessed the ambulatory activities (Fig. 2a-d) of mice treated with pentazocine at doses of 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg compared to those in the control group.

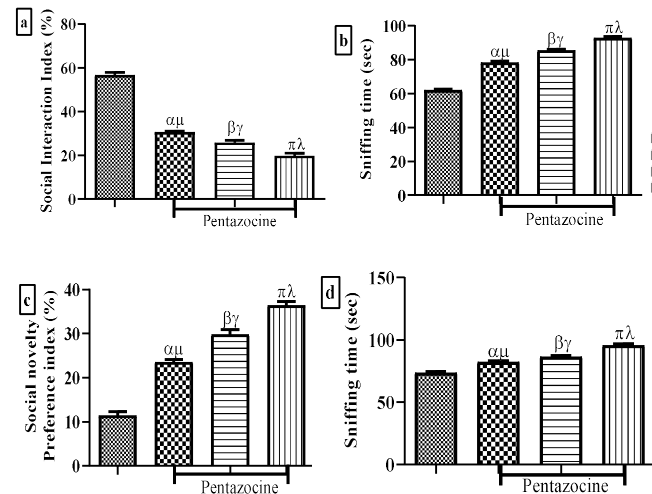

Fig. 1: The effects of pentazocine exposure on the mice in the treated groups compared to those in the control group during the 3-chamber socialisation test. (a) Social interaction index in the social interaction test (b), Total sniffing time in the social interaction test (c), Social novelty preference index in the social novelty preference test, and (d) Total sniffing time in the social novelty preference test. The data is presented as mean ± SD (n = 10 per group). α = Significant difference (p<0.05) between control and 0.25 mL/kg pentazocine-treated; β = Significant difference (p<0.05) between control and 0.5 mL/kg pentazocine-treated, π = Significant difference (p<0.05) between control and 0.75 mL/kg pentazocine-treated; μ = Significant difference (p<0.05) between 0.25 mL/kg pentazocine-treated and 0.5 mL pentazocine-treated; λ = Significant difference (p<0.05) between 0.25 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated; and γ = Significant difference (p<0.01) between 0.5 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated.

We observed that the groups of mice exposed to 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg of pentazocine had significantly reduced movement time [F (3, 16) = 3516, p<0.0001] (Fig. 2a), significantly reduced total distance travelled [F (3, 16) = 49294, p<0.0001] (Fig. 2b), and a significantly reduced number of horizontal [F (3, 16) = 2575906, p<0.0001] and vertical [F (3, 16) = 13451, p<0.0001] activities (Fig. 2c and 2d) compared to the control group. The open field arena revealed significantly diminished spontaneous motor activities in response to graded doses of pentazocine by the mice in 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg compared with those in the control.

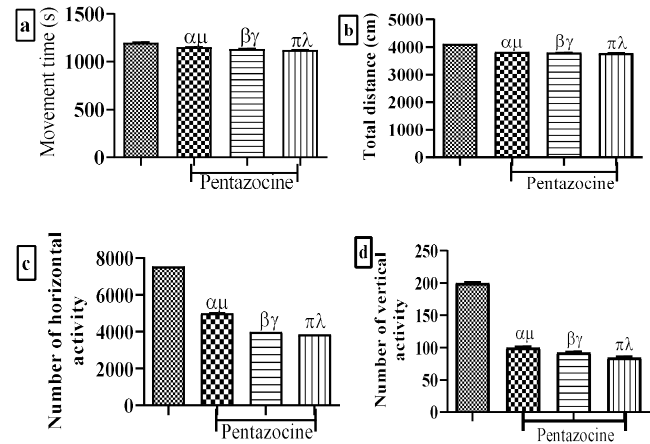

Fig. 2: The open field test activities of the mice in the control and the pentazocine-treated groups. (a) movement time, (b) total distance, (c) number of horizontal activities, and (d) number of vertical activities between control and 0.25 mL/kg pentazocine-treated, 0.5 mL/kg pentazocine-treated, and 0.75 mL/kg pentazocine-treated, respectively. The data is presented as mean ± SD (n = 10 per group). α = Significant difference (p<0.01) between control and 0.25 mL/kg pentazocine-treated, β = Significant difference (p<0.01) between control and 0.5 mL/kg pentazocine-treated, π = Significant difference (p<0.05) between control and 0.75 mL/kg pentazocine-treated; μ = Significant difference (p<0.01) between 0.25 mL/kg pentazocine-treated and 0.5 mL/kg pentazocine-treated; λ = Significant difference (p<0.01) between 0.25 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated; and γ = Significant difference (p<0.05) between 0.5 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated.

To test motor performance and learning differences between pentazocine-treated (i.e., 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg) and control mice, we used the latency to fall on an accelerating rotarod as an indicator of cerebellar dysfunction. In Figure 3, mice in 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg display significant differences [F (3, 16) = 1777, p < 0.0001] in motor performance and motor learning compared to the mice in control.

Neurochemical Evaluations

Exposure to Graded Doses of Pentazocine Impaired the Antioxidant Status in the Cerebellum of the Mice

From the statistical analysis of the data obtained as shown in Figure 4, we show the effect of 14 days of exposure of the mice in 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg to the doses of pentazocine on the levels of MDA (Fig. 4a), GSH (Fig. 4b), SOD (Fig. 4c), and catalase (Fig. 4d) in the cerebellum of the experimental mice compared to the mice in control.

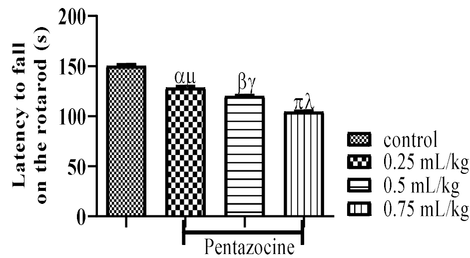

Fig. 3: The effect of pentazocine-treated mice on the latency to fall off the accelerating rotarod. The data is presented as mean ± SD (n = 10 per group). α = Significant difference (p<0.05) between control and 0.25 mL/kg pentazocine-treated; β = Significant difference (p<0.05) between control and 0.5 mL/kg pentazocine-treated; π = Significant difference (p<0.05) between control and 0.75 mL/kg pentazocine-treated**;** μ = Significant difference (p<0.01) between 0.25 mL/kg pentazocine-treated and 0.5 mL/kg pentazocine-treated; λ = Significant difference (p<0.01) between 0.25 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated; and γ = Significant difference (p<0.01) between 0.5 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated.

We observed that exposure of the mice in 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg to the doses of pentazocine significantly reduced the levels of GSH [F(3, 16) = 647.1, p < 0.0001], SOD [F(3, 16) = 1847, p < 0.0001], and catalase [F(3, 16) = 2993, p < 0.0001] in a dose-dependent pattern compared to the mice in control. Conversely, exposure to graded doses of pentazocine significantly elevated [F(3, 16) = 2173, p < 0.0001] the levels of MDA in a dose-dependent manner in the cerebellum of the experimental mice relative to the control mice.

Pentazocine Triggered Neuroinflammation in the Cerebellum

The effects of graded doses of pentazocine on the levels of MPO, nitric oxide, COX-2, and TNF-α are respectively presented in Figures 5a-d. Exposure to graded doses of pentazocine elevated the levels of MPO [F (3, 16) = 79.42, p < 0.0001], NO [F (3, 16) = 672.1, p < 0.0001], COX-2 [F (3, 20) = 578.5, p < 0.0001], and TNF-α [F (3, 16) = 1256, p < 0.0001] in the cerebellum of the mice in 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg relative to the mice in control.

Pentazocine Modulates the Activities of Serotonin, Dopamine, AChE, and BChE

Figure 6a-d shows the respective activities of serotonin, dopamine, AChE, and BChE. From the analysis of the data obtained, we observed that the activities of serotonin (Fig. 6a) were significantly downregulated [F (3, 16) = 125.3, p < 0.0001] in the cerebellum of the mice exposed to graded doses of pentazocine in a dose-dependent manner in 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg relative to control. On the other hand, the activity of dopamine (Fig. 6b) was significantly upregulated [F (3, 16) = 885.0, p < 0.0001] in a dose-dependent manner in the cerebellum of the mice in 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg compared with the mice in control. Compared to the mice in control, the respective activities of AChE [F (3, 16) = 694.0, p < 0.0001, Fig. 6c] and BChE [F (3, 16) = 437.2, p < 0.0001, Fig. 6d] in the cerebellum of the mice in 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg were significantly reduced in a dose-dependent manner.

The Effect of Pentazocine on Membrane-Bound ATPases in the Cerebellum

The alteration in the activity of the ATPase enzyme in the cerebellum of the mice in control, 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg was presented in Figure 7. Relative to the control, exposure to graded doses of pentazocine in the mice at 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg significantly decreased [F (3, 16) = 3175, p < 0.0001] the activity of the ATPase enzyme in a dose-dependent pattern in the cerebellum.

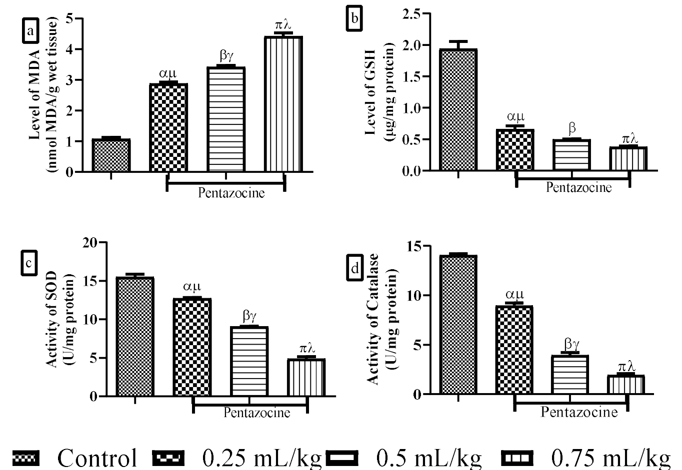

Fig. 4: The levels of MDA and reduced GSH and the activities of SOD and CAT in the cerebellar tissues of the control and the pentazocine-treated groups. (a) MDA, (b) GSH, (c) SOD, and (d) CAT activities between the control group and the pentazocine-treated groups, respectively. The data is presented as mean ± SD (n = 10 per group). α = Significant difference (p<0.05) between control and 0.25 mL/kg pentazocine-treated, β = Significant difference (p<0.05) between control and 0.5 mL/kg pentazocine-treated, π = Significant difference (p<0.05) between control and 0.75 mL/kg pentazocine-treated, μ = Significant difference (p<0.05) between 0.25 mL/kg pentazocine-treated and 0.5 mL/kg pentazocine-treated, λ = Significant difference (p<0.05) between 0.25 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated, and γ = Significant difference (p<0.05) between 0.5 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated. CAT - Catalase, SOD - superoxide dismutase

The Effect of Pentazocine on Cerebellar Mitochondrial Functions

Respectively, in mice in groups of 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg that were exposed to graded doses of pentazocine, a one-way statistical analysis revealed a dose-dependent increase in mitochondrial swelling in cerebellar mitochondria (Fig. 8). This The increase was significantly greater [F (3, 16) = 2324, p < 0.0001] compared to the mice in the control group (Fig. 8a). observation suggests that cerebellar mitochondria in mice in 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg are with increased swelling. In Figure 8b, it was shown that exposure to graded doses of pentazocine significantly elevated [F (3, 16) = 46537267, p < 0.0001] cerebellar mitochondrial ROS levels in mice of

0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg compared to those in the control. The study found that exposure to graded doses of pentazocine is associated with significantly increased [$F(3, 16) = 3980643$, $p < 0.0001$] mitochondrial membrane potential in the cerebellar mitochondria of mice in 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg compared to those in control (Fig. 8c). Furthermore, as indicated in Figure 8d, mice exposed to graded doses of pentazocine (0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg) experienced significantly higher [$F(3, 16) = 5683$, $p<0.0001$] mitochondrial outer membrane damage compared to the control mice. Additionally, the release of cytochrome c was significantly higher [$F(3, 16) = 25013$, $p < 0.0001$] in the cerebellar mitochondria of mice in 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg following exposure to graded doses of pentazocine compared to the mice in the control (Fig. 8e).

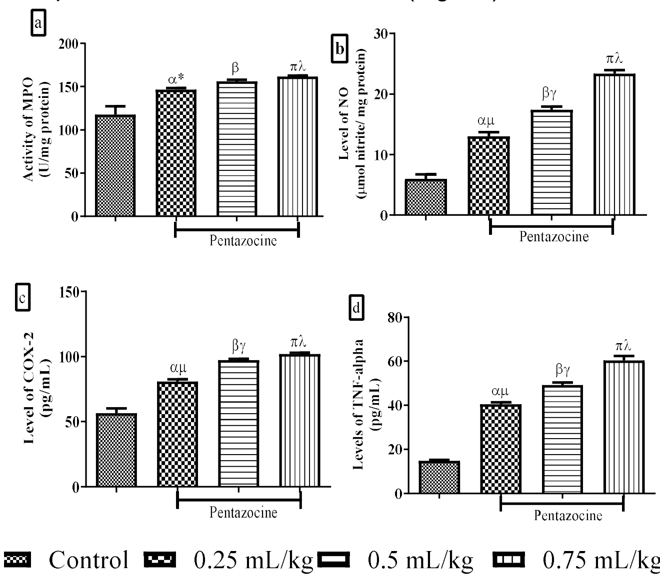

Fig. 5: The effect of graded doses of pentazocine on the activity of MPO and the levels of nitric oxide, COX-2, and TNF-α relative to the control. The data is presented as mean ± SD (n = 10 per group). (a) MPO, (b) NO, (c) COX-2, and (d) TNF-alpha in the cerebellar tissues of the control and the pentazocine-treated groups. α = Significant difference ($p<0.05$) between control and 0.25 mL/kg pentazocine-treated, β = Significant difference ($p<0.05$) between control and 0.5 mL/kg pentazocine-treated, π = Significant difference ($p<0.05$) between control and 0.75 mL/kg pentazocine-treated, μ = Significant difference ($p<0.05$) between 0.25 mL/kg pentazocine-treated and 0.5 mL/kg pentazocine-treated, λ = Significant difference ($p<0.05$) between 0.25 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated, and γ = Significant difference ($p<0.05$) between 0.5 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated. COX-2 - cyclooxygenase-2, MPO – myeloperoxidase, TNF-α - tumour necrosis factor-alpha

Neurohistology of the Cerebellum

The neurohistological section of the cerebellar tissue from the representative mice from each of the experimental groups is presented in Figure 9. In the control group, Purkinje cells (indicated by yellow arrows) display eosinophilic cytoplasm and lightly stained, round to oval, centrally placed nuclei. These Purkinje neurons have intact dendrites (shown by black arrows). Additionally, the granular layer contains numerous neuronal nuclei that are centrally located. Conversely, the cerebellar tissue of mice exposed to 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg of pentazocine shows vacuolation in the overlying molecular cell layer (marked by golden arrows), which suggests swelling and/or degeneration of the Purkinje neuron dendrites. The Purkinje neurons in the cerebellum of these mice demonstrate characteristics such as nuclear oedema and cellular shrinkage (indicated by red arrows). Some of these Purkinje cells have been displaced from the Purkinje layer, and others appear spindle-shaped. Furthermore, the cerebellar sections from mice exposed to 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg of pentazocine also displayed neuropil vacuolation.

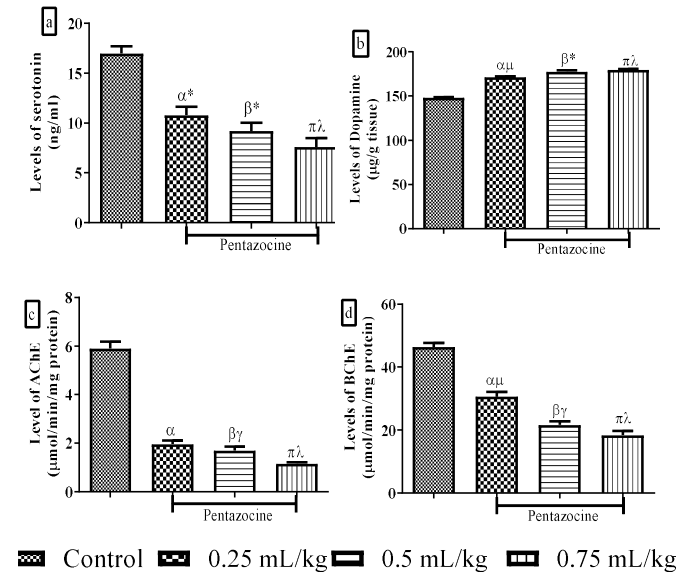

Fig. 6: The effect of pentazocine on the cerebellar level of neurotransmitters relative to the control. (a) serotonin, (b) dopamine, (c) AChE, and (d) BChE in the cerebellar tissues of the control and the pentazocine-treated groups. The data is presented as mean ± SD (n = 10 per group). α = Significant difference ($p<0.05$) between control and 0.25 mL/kg pentazocine-treated, β = Significant difference ($p<0.05$) between control and 0.5 mL/kg pentazocine-treated, π = Significant difference ($p<0.05$) between control and 0.75 mL/kg pentazocine-treated, μ = Significant difference ($p<0.05$) between 0.25 mL/kg pentazocine-treated and 0.5 mL/kg pentazocine-treated, λ = Significant difference ($p<0.05$) between 0.25 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated, and γ = Significant difference ($p<0.01$) between 0.5 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated.

DISCUSSION

The behavioural assays used in this research evaluated neural effects during a difficult-to-assess juvenile phase. The examined behaviours were unbiased, non-invasive, and did not require any specialised equipment (Chan and Leung, 2022). In comparison to the control group, mice exposed to graded doses of pentazocine showed impairment in the three-chamber, open-field, and rotarod behavioural tests. These findings indicate that the abusive use of pentazocine can lead to a decline in motor-social behav-

iours with worsened neurological deficits and circuit malfunctions, which may alter the physical strength of the mice. However, at the time of this study, the specific mechanism behind these behavioural observations was unknown. The observed alteration in the behaviour of the experimental mice is inconsonant with the reports of Fujii *et al*. (2019), who reported that chronic exposure to fentanyl modifies behavioural responses in experimental animals, as well as Paul *et al*. (2021), who published that exposure to morphine diminished motor behaviours in Sprague–Dawley rats.

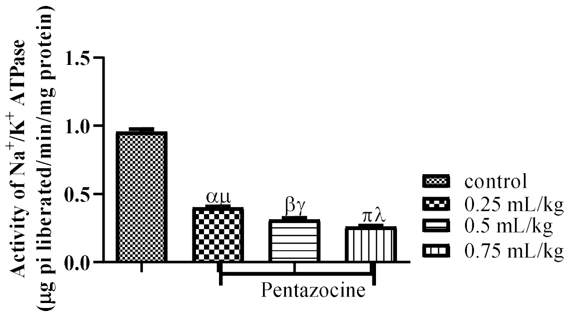

Fig. 7: The effect of graded doses of pentazocine on the activity of membrane-bound ATPases relative to the control. The data is presented as mean ± SD (n = 10 per group). α = Significant difference (p<0.05) between control and 0.25 mL/kg pentazocine-treated, β = Significant difference (p<0.05) between control and 0.5 mL/kg pentazocine-treated, π = Significant difference (p<0.05) between control and 0.75 mL/kg pentazocine-treated, μ = Significant difference (p<0.01) between 0.25 mL/kg pentazocine-treated and 0.5 mL/kg pentazocine-treated, λ = Significant difference (p<0.05) between 0.25 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated, and γ = Significant difference (p<0.05) between 0.5 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated.

The evident impaired sociability, demonstrated by a dose-dependent reduced social interaction index and the exaggerated social preference index, may be an early sign of neurological conditions connected with neurodevelopmental and neurodegenerative disorders, probably reflecting deficiency in social cognition and memory, aggravated social anxiety, or behavioural restriction. The observed reduction in interest in the social interaction test can be explained by the lens of anxiety-like behaviours and social anhedonia. Social anhedonia is defined by a reduced capability to derive pleasure or a sense of reward from taking part in social activities (Wu *et al*., 2025). A dose-dependent analysis has revealed a significant decrease in social interaction indices alongside an increase in social preference indices, suggesting that individuals may experience a disconnection from typical social rewards. This phenomenon has been documented in various studies, particularly animal models that exhibit behaviours indicative of schizophrenia and other psychiatric disorders (McKibben *et al*., 2014; Shirenova *et al*., 2023). These conditions often arise following exposure to opioid abuse, pointing to a disturbing link between the non-prescribed use of opioids and the deterioration of social functioning. The evidence suggests that the harmful effects of substance misuse adversely impact the central nervous system, resulting in notable impairments in social behaviour. These deficiencies may become evident alongside numerous neurological and mental health disorders, accentuating the general involvement of opioid abuse in mental health.

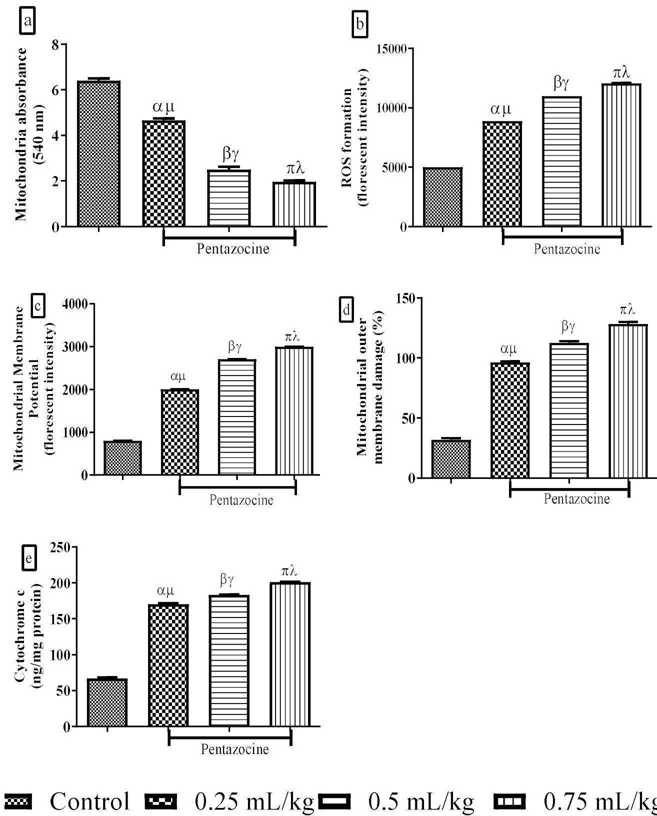

Fig. 8: The effect of exposure to graded doses of pentazocine on different specifications of brain mitochondrial functions, including mitochondrial swelling relative to the control. The data is presented as mean ± SD (n = 10 per group). α = Significant difference (p<0.01) between control and 0.25 mL/kg pentazocine-treated, β = significant difference (p<0.01) between control and 0.5 mL/kg pentazocine-treated, π = significant difference (p<0.05) between control and 0.75 mL/kg pentazocine-treated, μ = Significant difference (p<0.01) between 0.25 mL/kg pentazocine-treated and 0.5 mL/kg pentazocine-treated; λ = Significant difference (p<0.01) between 0.25 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated; and γ = Significant difference (p<0.05) between 0.5 mL/kg pentazocine-treated and 0.75 mL/kg pentazocine-treated.

Furthermore, the evaluation of an individual's preference for social novelty is intrinsically connected to cognitive functions associated with social recognition and memory formation. A noted impairment in this preference indicates potential deficits in processing social information and in the retrieval of social memories (Chan *et al*., 2022). The observation from the social behaviour as seen in the 3-chamber test provides substantive data suggesting a progressive decline in social behaviours among experimental mice relative to the control. This decline is reflected in a measurable reduction of the social interaction index, paralleled by an increase in the social preference index. Such results underscore the complexity of social behaviours and

the potential impact of both physiological and psychological factors in substance abuse.

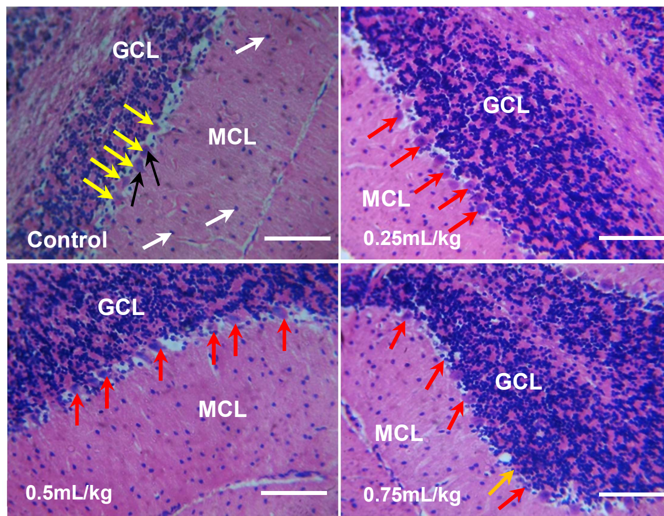

Fig. 9: Cerebellar photomicrographs of experimental mice in various treatment groups examined at a 20 µm magnification and stained with H&E. The cerebellum in the control group displayed normal cellular morphology, with yellow arrows indicating intact Purkinje neurons and black arrows showing the dendrites of these neurons. In contrast, the cerebellar tissue of mice exposed to 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg of pentazocine exhibited marked degeneration of the cerebellar cortex, characterised by distorted Purkinje neurons (indicated by red arrows) and swollen and vacuolized neurons (marked by golden arrows) within the molecular layer.

Social interactions are enjoyable occurrences for both humans and rodents, and they activate the brain reward circuits (Borland *et al*., 2025). The prolonged sniffing time seen in mice exposed to pentazocine indicates a sense of aversion. Therefore, exposure to graded doses of pentazocine may impair social interactions by affecting the social seat in the cerebellum.

According to a study conducted by Zahmatkesh *et al*. (2017), exposure to opioids reduces the functional integrity of the brain's antioxidant defence mechanisms, resulting in oxidative damage to cellular components. During oxidative stress, redundant production of free radicals reduces the levels of antioxidant enzymes, such as SOD and catalase (Birben *et al*., 2012). The involvement of these enzymes in the brain and related areas is pivotal for regulating the levels of ROS (Kaygusuzoglu *et al*., 2018). Ojo *et al*. (2023) reported that when ROS in the central nervous system biosocialise with lipids, carbohydrates, proteins, and DNA, they impede the physiology of the brain. In the cerebellum of the mice exposed to graded doses of pentazocine for 14 days, the respective levels of GSH and activities of SOD and catalase were simultaneously diminished due to enhanced products of lipid peroxidation and impeded functions of the antioxidant enzymes. The depletion of the GSH pool and the observed decrease in the levels of antioxidant enzymes in the cerebellum, as seen in this study, may be due to the action of pentazocine and its electrophilic metabolites, which could have inhibited the activities and levels and the bio-effectiveness of the antioxidant enzymes. The decreased levels of GSH (Asatiani *et al*., 2025), SOD (Sadat-Shirazi *et al*., 2020), and catalase (Safarinejad *et al*., 2013) have been reported as a consequence of acute exposure to opioids. Studies have indicated an increase in MDA (Atici *et al*., 2005) and a decrease in GSH levels in the brain (Guzmán *et al*., 2006; Ozmen *et al*., 2007) after long-term administration of morphine in rats and rabbits, respectively.

MPO is an enzyme known as the neutrophil infiltration index because it serves as a marker for neutrophil accumulation in inflamed tissues. MPO is a secretion of activated inflammatory cells that is mainly manufactured by monocytes, macrophages, microglia, and neutrophils (Bradley *et al*., 1982; Bilge *et al*., 2021). According to Chen *et al*. (2020), oxidative stress-induced neuroinflammation can be identified using MPO as a therapeutic marker. In human neutrophils, MPO acts as part of the body's defence against microbes, primarily generating hypochlorous acid, a potent oxidant (Hoy *et al*., 2002; Bilge *et al*., 2021). Among different neurotoxic oxidants in the brain, hypochlorous acid is stable, highly reactive, and dominant. This acid plays a role in several neurodegenerative disorders (Ray and Katyal, 2016). In mice exposed to graded doses of pentazocine, the cerebellum revealed down-regulated antioxidant levels, heightened oxidative stress, and elevated levels of MPO, nitric oxide, COX-2, and TNF-α, thereby suggesting that pentazocine is capable of escalating neuroinflammation in the cerebellum. Our result is in tandem with the report of Salarian *et al*. (2018), who reported increased oxidative stress and inflammation in patients with opioid use disorder.

Nitric oxide is a functional free radical that controls several physiological functions in the brain (Leong *et al*., 2002). A significant level of nitric oxide activates vasodilation, hinders apoptosis, and performs a crucial role in memory processes. However, overproduction of nitric oxide can be deleterious, especially under oxidative stress conditions, because of the oxidation and nitrotyrosination of functional proteins (Guix *et al*., 2005). Our results suggested that exposure to graded doses of pentazocine gave rise to elevated levels of nitric oxide in the cerebellum of the mice. Our results indicated that exposure to varying doses of pentazocine resulted in elevated levels of nitric oxide in the cerebellum of the mice. Nitric oxide is among the reactive nitrogen species and can be readily converted into peroxynitrite anion, which is highly reactive and can initiate cytotoxic radical chain reactions (Zhang *et al*., 2008). Neurodegenerative process is associated with oxidative stress generated by nitric oxide (Guix *et al*., 2005). Furthermore, in addition to oxidative stress, overproduction and accumulation of nitric oxide can trigger apoptosis, which is also linked to nitric oxide-induced neurotoxicity (Wei *et al*., 2000; Dash *et al*., 2025).

COX-2 is found in the perinuclear, dendritic, and axonal regions of glutamatergic neurons, particularly in the cortex, hippocampus, and amygdala (Hewett *et al*., 2006). Under normal conditions, COX-2 is present and contributes to important brain functions such as synaptic activity, memory consolidation, and functional hyperaemia (Minghetti,

2004). The increase in COX-2 generated by oxidative stress intensifies prostaglandin E2's (PGE2) role in stimulating the release of glutamate and causing oxidative damage through the production of reactive oxidant species (Minghetti, 2004; Tian *et al*., 2008). In mice, the level of COX-2 in the cerebellum was found to increase dose-dependently upon exposure to pentazocine. Persistent COX-2 expression in the CNS may result in neurodegenerative processes through the promotion of chronic inflammation. With the elevated level of COX-2 in the cerebellum as observed in this study, there are concerns about the possible consequences of pentazocine on locomotion. In the cerebellum of experimental mice, exposure to graded doses of pentazocine leads to a notable COX-2 expression, indicating that inflammation contributes to its neurotoxicity.

Misuse of opioids in numerous healthcare facilities has led to imbalances between pro-inflammatory and anti-inflammatory cytokines due to non-prescribed use and abuse (Zhang *et al*., 2020). These imbalances require the involvement of pro-inflammatory cytokines like interleukin-6, TNF-α, and interferon gamma to elicit inflammatory responses (Chen *et al*., 2018). Significantly increased TNF-α levels ($p < 0.05$) were detected in the cerebellum of pentazocine-exposed mice. The release of the MOR-PKCε-Akt-ERK1/2 signalling pathway, mediated by TNF-α, is linked to morphine tolerance (Wang *et al*., 2022). Findings from animal experiments show that inhibiting TNF-α signalling could hinder the development of morphine tolerance (Tu *et al*., 2021). Our results in this study revealed that exposure to pentazocine enhances irregular alterations in cytokine levels in a dose-dependent pattern. These observations are interconnected with the up-regulation of TNF-α levels and are closely related to previously reported findings on the misuse of opioids. Activated glia cells have also been shown to stimulate pain transmission and antagonise the analgesic properties of opioids (Raghavendra *et al*., 2003; Hutchinson *et al*., 2010; Eidson and Murphy, 2019). Opioids activate glial cells by binding to the glycoprotein myeloid differentiation factor-2 (MD-2) on the innate immune receptor TLR4 (Lewis *et al*., 2010), influencing increased production and release of TNF-α, (interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) pro-inflammatory cytokines and neuro-excitotoxic free radicals such as nitric oxide, NOS, and iNOS, and these signalling factors are connected with opioid tolerance (DeLeo *et al*., 2004; Wang *et al*., 2012; Kaushik *et al*., 2020). The binding of TNF-α to their target receptors on astrocytes and microglia results in further release of IL-1β, IL-6, and TNF-α pro-inflammatory cytokines, thereby creating a vicious cycle of neuro-inflammation (Watkins *et al*., 2005; Watkins *et al*., 2009). The upregulated level of TNF-α seen in this study could be a synergistic effect of pentazocine, its metabolites and neuroinflammation.

Serotonin (5-HT) is crucial for learning and memory in the central nervous system (Afshar *et al*., 2023). In the hypothalamus, midbrain, and limbic system of the brain, where serotonin is comparably distributed, tryptophan hydroxylase plays an important function in the synthesis of serotonin (Ney *et al*., 2017). In our study, mice that were exposed to graded doses of pentazocine showed significantly heightened levels of serotonin in their various cerebella. Based on this finding, we suggest that exposure to pentazocine may adversely interfere with the reuptake of serotonin by impeding the functional integrity of the serotonin transporter, leading to exaggerated concentrations of serotonin that trigger the serotonin receptors in the synaptic cleft. According to Baldo and Rose (2020), opioids that strongly inhibit serotonin transporters are frequently associated with serotonin toxicity. Our results are in tandem with those of Tzschentke *et al*. (2007) and Bloms-Funke *et al*. (2011), who reported that tramadol and tapentadol augment the levels of serotonin and noradrenaline, respectively, in the rat brain via in vivo microdialysis.

We found that administering graded doses of pentazocine to mice led to a dose-dependent reduction in dopamine levels. Low dopamine levels in the central nervous system, caused by oxidative damage, could result in harmful changes to its neurochemistry. The respective levels of dopamine in the cerebellum of the groups of mice exposed to 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg of pentazocine decreased in a dose-dependent manner in response to pentazocine compared to the control. It is possible that the reported observation could be due to altered dopamine receptors arising from decreased dopamine receptor expression and impaired μ-opioid receptor function in the cerebellum (Teicher *et al*., 1995; Tarazi *et al*., 1998, 1999, 2000). To determine the effects of pentazocine on the cerebellum, it is essential to investigate the activities of enzymes such as AChE and BChE. According to Tsakiris *et al*. (2000), a decrease in the activity of AChE is connected to the unbounded presence of free radicals and successive acetylcholine upregulation, which eventually leads to cholinergic hyperactivity and neurological anomalies, such as convulsions and status epilepticus. Opioid-induced CNS injury could be recognised by the depletion of AChE levels in the brain owing to its influence on behavioural processes. The inhibition of AChE and BChE varies in the cerebellum of the mice, suggesting the build-up of acetylcholine and butyrylcholine due to disturbed cholinergic transmission by pentazocine. Anatomical evidence shows an overlay between AChE and opioid receptors in the striatum during rat development, which continues into and is primarily involved with μ-opioid receptors (Tien *et al*., 2004). This topological interaction between AChE and opioid receptors is linked to their functional interactions. For example, morphine has been discovered to increase acetylcholine levels in the central nervous system, and this modification is region-specific (Green *et al*., 1976). Our results show that the level of AChE was reduced in the cerebellum of mice exposed to pentazocine, indicating a deleterious action triggered by an imbalance in acetylcholine and butyrylcholine release through μ-opioid receptors in the cerebellum. Indirectly, pentazocine could impair cholinesterase metabolism with its neurotoxic effect, causing a decline in the respective levels of AChE and BChE in the cerebellum.The formation of cellular energy, the absorption and release of catecholamines and serotonin, and neural excitability are all controlled by the Na^+/K^+-ATPase (Bogdanski *et al*., 1968; Mata *et al*., 1980). 14 days of exposure to

graded doses of pentazocine potentiated and aggravated the inhibition of the activity of Na^+/K^+-ATPase. Pentazocine inhibits Na^+/K^+-ATPase activity, possibly by modifying intracellular cyclic adenosine monophosphate (cAMP) concentration, thereby inducing changes in Na^+/K^+-ATPase basal phosphorylation via the cAMP/protein kinase A (PKA) signalling pathway (Wu *et al*., 2006). Previous observations suggest that phosphorylation of Na^+/K^+-ATPase, which occurs in neurons and is concentrated in the mammalian brain, inhibits the enzyme activity and is mediated by the activation of opioid receptors and accumulated intracellular cAMP (Wu *et al*., 2006). It restores the Na^+ and K^+ gradient across the cell membrane after periods of activity, contributing to the resting membrane potential of the cell. This process similarly affects monovalent cation gradient-related processes, including Na^+-Ca^{2+} exchangers, as discussed by Blaustein and Lederer (1999). Due to the damage of the Na^+/K^+ pump, intracellular K^+ levels decrease, intracellular Na^+ levels increase, and both membrane depolarisation and an increase in intracellular free Ca^{2+} occur through the activation of voltage-gated Ca^{2+} channels and the reversed operation of the Na^+-Ca^{2+} exchanger (Blaustein and Lederer, 1999). Na^+/K^+-ATPase participates in synaptic plasticity and learning/memory processes, according to Wyse *et al*. (2004). Suppression of field excitatory postsynaptic potentials in rat hippocampal CA1 cells by dihydroouabain results in depotentiation of long-term potentiation (LTP), a neuronal mechanism implicated in learning and memory (Reich *et al*., 2004). Long-term opiate use is known to affect hippocampal long-term potentiation (Pu *et al*., 2002). Opioids may cause maladaptive changes in hippocampal LTP by diminishing Na^+/K^+-ATPase activity through up-regulation of the cAMP/PKA signalling pathway.In our study, we noticed heightened formation of mitochondrial ROS in the cerebellum of the mice treated with pentazocine in groups of 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg (Figure 8a-e). The observed distortion in the development of mitochondrial ROS, mitochondrial swelling, disintegration of MMP, impairment of mitochondrial outer membrane, and the liberation of cytochrome c following exposure to graded doses of pentazocine could arise from differences in the molecular activities of mitochondrial electron transport chain complexes. In 2018, Bameri *et al*. stated that a single intravenous injection of tramadol at a dose of 25 mg/kg led to a considerable increase in mitochondrial ROS production, protein carbonyl, and lipid peroxidation, along with diminished mitochondrial GSH levels in the brains of mice. These observations indicated that opioid-induced oxidative stress is associated with mitochondrial dysfunction. The enhanced formation of mitochondrial ROS can bring about peroxidation of cell membrane lipids, culminating in the subsequent damage of DNA and proteins, bringing about neuronal cell death in the cerebellum. ROS can also give rise to inflammation by stimulating the redox-sensitive transcription factor NF-κB and subsequently giving rise to the release of multiple inflammatory mediators (Mohamed and Mahmoud, 2019).In this study, exposure to graded doses of pentazocine produced numerous neurodegenerative changes in the cerebellum of mice exposed in comparison to the control group. The cerebellum of the control group showed a normal histoarchitecture, with well-preserved layers of molecular cells, granular cells, and Purkinje cells. The folia of the cerebellar cortex appeared normal, well-preserved, and well-separated in the control group, with narrow grooves. On the other hand, the cerebellar histology of the mice exposed to 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg of pentazocine exhibited deviations in the histoarchitecture of the Purkinje cells, which showed irregular and distorted features. The granular and molecular cells exposed to 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg of pentazocine exhibited several perinuclear spaces. The Purkinje cells in 0.25 mL/kg, 0.5 mL/kg, and 0.75 mL/kg were deformed and shrunken, losing their characteristic shape. These findings align with a previous report by Hauser *et al*. (1994), who stated that morphine prevents the survival and differentiation of Purkinje cells in mouse cerebellar cultures. The authors reiterated that extended exposure or higher doses of opioids may give rise to neurotoxicity in Purkinje cells, possibly leading to cell loss through apoptosis or necrosis.

The interplay between the impaired motor-social behaviour, increased level of oxidative stress markers, upregulated levels of the studied markers of neuroinflammatory and apoptotic cytokines, and mitochondrial dysfunction in the cerebellum of the juvenile mice is a complex, interconnected pathway that significantly impairs the functional integrity of the cerebellum in abusive consumption of pentazocine and can contribute to the development of neurological disorders. Mitochondrial dysfunction is a primary source of ROS, which could trigger deleterious levels of oxidative stress, which in turn damages cellular components and disrupts energy production (Kowalczyk *et al*., 2021). The brain's immune response during neuroinflammation can be triggered by oxidative stress or mitochondrial dysfunction. Pro-inflammatory cytokines, expressed during neuroinflammation, can promote oxidative stress, producing a feedback loop. Inflammation could also interrupt neuronal circuitry and undermine motor and social behaviours. In addition, neuroinflammation can provoke oxidative stress, further worsening the functional integrity of mitochondria. This cycle can bring about a cascade of neuronal damage, which can adversely affect motor and social behaviours. The intricate interplay between these factors can directly influence motor skills, social cognition, and emotional responses. Oxidative stress, neuroinflammation, and apoptosis are believed to play significant roles in the neurological processes related to pentazocine abuse and its accompanying behavioural changes. This suggests that alterations in the biochemical and physiological balance of oxidative substances, along with compromised mitochondrial function resulting from pentazocine exposure, may contribute not only to the toxic effects of the drug but also to the development of neurochemical and molecular abnormalities. This association highlights the intricate interplay between neurochemical modifications in the cerebellum and the neurological and physiological consequences of pentazocine misuse. ConclusionIn conclusion, this study demonstrates that the misuse of pentazocine enhances impaired motor and social behaviour;

elevated oxidative dysfunction, neuroinflammation, apoptosis, decreased levels of antioxidants, and altered neurotransmitters in the cerebellar cortex. Pentazocine elevated the respective levels of lipid peroxidation, myeloperoxidase, nitric oxide, COX-2, and TNF-α, and diminished levels of GSH, SOD, and catalase. Additionally, there was a reduction in the levels of serotonin, AChE, and butyrylcholine, with a corresponding increase in the levels of dopamine in the cerebellum of the mice. Exposure to graded doses of pentazocine further triggered a reduction in the activity of Na^+/K^+-ATPase. Regarding the effect of pentazocine on cerebellar mitochondrial functions, exposure to graded doses of pentazocine significantly impaired mitochondrial absorbance and led to an increase in the generation of reactive oxygen species, heightened mitochondrial membrane potential, increased damage to the outer mitochondrial membrane, and elevated levels of cytochrome c. In addition, considering the significance of the studied pathway in pentazocine misuse, modifying the dynamics and functional integrity of the pathway may be a beneficial target for the pharmaco-therapeutic interference that can inhibit pentazocine neurotoxicity. The research reveals that exposure to pentazocine is connected with increased oxidative stress, as shown by the elevated levels of lipid peroxidation, a process that impairs the biochemical, morphological, and physiological capability of the cell membranes, and myeloperoxidase, an enzyme connected to inflammation. Furthermore, there is a significant increase in nitric oxide and COX-2 levels, both of which are markers of inflammatory processes. TNF-α, a cytokine concerned with systemic inflammation, is also significantly elevated, corroborating the idea that pentazocine enhances the neuroinflammatory environment. Conversely, the study highlights a substantial decline in the levels of key antioxidants, including reduced GSH, SOD, and catalase. These antioxidants play crucial roles in counteracting reactive oxygen species (ROS) and protecting neuronal cells from oxidative damage. The reduction of these protective agents suggests that the balance between oxidative stress and antioxidant defence is disturbed in the presence of pentazocine. Furthermore, there are noticeable changes in the dynamics of the studied neurotransmitters within the cerebellum of the experimental mice. The research revealed a decline in serotonin, AChE, and butyrylcholine levels, which are vital for the regulation of mood and cognitive functions. In contrast, an increase in dopamine levels was detected, which may be connected to the drug's euphoric effects and a potential long-term neurochemical imbalances. The study also reveals that graded doses of pentazocine significantly impair the activity of the Na^+/K^+-ATPase enzyme, required for maintaining neuronal excitability and homeostasis. A reduction in the activity of this enzyme can result in adversely modulated cellular ion balance, further inhibiting normal cellular function. In terms of mitochondrial function, the results indicate that exposure to pentazocine down-regulates mitochondrial absorbance, an indication of impaired mitochondrial respiration and energy production. This impairment is coupled with increased formation of reactive oxygen species, which disrupts mitochondrial components and contributes to heightened mitochondrial membrane potential and outer membrane damage. The release of cytochrome c from the mitochondria is a primary event indicating the activation of apoptotic pathways, signifying further neuronal loss and dysfunction. Given these insights, the study suggests that targeting the identified pathways may provide a therapeutic route for mitigating neurotoxicity associated with pentazocine. By modifying the dynamics and functional integrity of these pathways, it may be possible to develop therapeutic procedures that prevent or alleviate the adverse neurochemical effects induced by pentazocine, thus enhancing neuronal health and functional recovery. Overall, this analysis provides information on the mechanisms underlying pentazocine-induced neurotoxicity, providing the groundwork for future research aimed at therapeutic interventions or attenuating neurotoxicity triggered by pentazocine.

DECLARATION

Acknowledgement
None.

Grants and Financial Support
None declared.

Conflict of Interest
None declared.

Ethical Approval
The experimental study received ethical approval from the Osun State University Animal Care and Use Ethical Committee. This approval is supported by the reference number UNIOSUNAC/2023/0133, ensuring that all necessary ethical considerations regarding animal welfare were considered before the commencement of the research.

Consent to Participate and Publish Data
Not Applicable.

Authors Contributions
All authors contributed to the conception and design of the study. Material preparation, data collection, and analysis were conducted by DAA, DAO, and RYI. AOA, LAE, and EEE were responsible for the care and husbandry of the animals. The behavioural assays were performed by AYO and OSS, while the biochemical assays were conducted by OOA, AVA, and EIOA. Histopathological analysis of the cerebellar tissue was carried out by TDA and GJA. Statistical analysis of the collected data was performed by TOA and AOA. The manuscript draft was written by DAA, with all authors providing comments on the write-up. All authors have read and approved the final manuscript.

REFERENCES

Adekomi, D. A., Adewole, O. S., Dare, B. J., Olaniyan, O. O., Ojebisi, R. M., and Ajao, M. S. (2017). Alcohol-pentazocine combination: implication on the cytoarchitectural profile of the medial prefrontal cortex and cerebellum

of juvenile male rats. Anat J Africa 6, 1080–1091. doi: 10.4314/aja.v6i3.163517

Afshar, S., Shahidi, S., Baooshi, H., Hoseini, M., Esmaeili, M., Hashemi-Firouzi, N., *et al*. (2023). The role of hippocampal 5-HT1D and 5-HT1F receptors on learning and memory in rats. Naunyn Schmiedebergs Arch Pharmacol 396(7), 1451-1460. doi: 10.1007/s00210-023-02411-x

Akagawa, K., and Tsukada, Y. (1979). Presence and characteristics of catecholamine-sensitive Na-K ATPase in rat striatum. J Neurochem 32(1), 269-71. doi: 10.1111/j.1471-4159.1979.tb04544.x.

Alcott, C. E., Yalamanchili, H. K., Ji, P., van der Heijden, M. E., Saltzman, A., Elrod, N., *et al*. (2020). Partial loss of CFIm25 causes learning deficits and aberrant neuronal alternative polyadenylation. Elife 9, e50895. doi: 10.7554/eLife.50895.

Asatiani, N., Sapojnikova, N., Kartvelishvili, T., Asanishvili, L., Sichinava, N., and Chikovani, Z. (2025). Blood catalase, superoxide dismutase, and glutathione peroxidase activities in alcohol- and opioid-addicted patients. Medicina (Kaunas) 61(2), 204. doi: 10.3390/medicina61020204.

Atici, S., Cinel, I., Cinel, L., Doruk, N., Eskandari, G., and Oral, U. (2005). Liver and kidney toxicity in chronic use of opioids: an experimental long term treatment model. J Biosci 30(2), 245-52. doi: 10.1007/BF02703705.

Bachi, K., Sierra, S., Volkow, N. D., Goldstein R. Z., and Alia-Klein N. (2017). Is biological aging accelerated in drug addiction? Curr Opin Behav Sci 13, 34-39. doi: 10.1016/j.cobeha.2016.09.007

Baldo, B. A., and Rose, M. A. (2020). The anaesthetist, opioid analgesic drugs, and serotonin toxicity: a mechanistic and clinical review. Br J Anaesth 124(1), 44-62.

Bameri, B., Shaki, F., Ahangar, N., Ataee, R., Samadi, M., and Mohammadi, H. (2018). Evidence for the involvement of the dopaminergic system in seizure and oxidative damage induced by tramadol. Int J Toxicol 37(2), 164-170. doi: 10.1177/1091581817753607

Baranov, S. V., Baranova, O. V., Yablonska, S., Suofu, Y., Vazquez, A. L., Kozai, T. D. Y., *et al*. (2019). Mitochondria modulate programmed neuritic retraction. Proc Natl Acad Sci 116(2), 650–659. doi: 10.1073/pnas.1811021116.

Barkus, E., Badcock, J. C. (2019). A Transdiagnostic Perspective on Social Anhedonia. Front Psych 10, 216.

Beutler, E., Duron, O., and Kelly, B. M. (1963). Improved method for the determination of blood glutathione. J Lab Clin Med 61, 882-888.

Bian, W. J., Brewer, C. L., Kauer, J. A., and de Lecea, L. (2022). Adolescent sleep shapes social novelty preference in mice. Nat Neurosci 25(7), 912-923.

Bilge, N., Yevgi, R., Kızıldağ, N., and Kızıltunç, A. (2021). Low serum myeloperoxidase levels in multiple sclerosis patients. New Trends Med Sci 2(1), 63–68.

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., and Kalayci, O. (2012). Oxidative stress and antioxidant defense. World Allergy Organ J 5(1), 9-19.

Blaustein, M. P., and Lederer, W. J. (1999). Sodium/calcium exchange: its physiological implications. Physiol Rev 79(3), 763-854

Bloms-Funke, P., Dremencov, E., Cremers, T. I., and Tzschentke, T. M. (2011). Tramadol increases extracellular levels of serotonin and noradrenaline as measured by in vivo microdialysis in the ventral hippocampus of freely-moving rats. Neurosci Lett 490(3), 191-195.

Bogdanski, D. F., Tissuri, A., and Brodie, B. B. (1968). Role of sodium, potassium, ouabain and reserpine in uptake, storage and metabolism of biogenic amines in synaptosomes. Life Sci 7, 419-428.

Borland, J. M. (2025). A review of the effects of different types of social behaviors on the recruitment of neuropeptides and neurotransmitters in the nucleus accumbens. Front Neuroendocrinol 77, 101175. doi: 10.1016/j.yfrne.2025.101175.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72(1–2), 248–254.

Bradley, A. E., Bolon, B., Butt, M. T., Cramer, S. D., Czasch, S., Garman, R. H., *et al* (2020). Proliferative and nonproliferative lesions of the rat and mouse central and peripheral nervous systems: New and Revised INHAND Terms. Toxicol Pathol 48(7), 827–844. doi: 10.1177/0192623320951154

Bradley, P. P., Christensen, R. D., and Rothstein, G. (1982). Cellular and extracellular myeloperoxidase in pyogenic inflammation. Blood 60(3), 618–622.

Chan, J. K. Y., and Leung, P. W. L. (2022). Common outcome, different pathways: Social information-processing deficits in autism spectrum disorder and attention-deficit/hyperactivity disorder. World J Psych 12(2), 286–297. https://doi.org/10.5498/wjp.v12.i2.286

Chen, S., Chen, H., Du, Q., and Shen, J. (2020). Targeting myeloperoxidase (MPO) mediated oxidative stress and inflammation for reducing brain ischemia injury: Potential application of natural compounds. Front Physiol 11, 433.

Chen, S., Li, Q., Shi, H., Li, F., Duan, Y., and Guo, Q. (2024). New insights into the role of mitochondrial dynamics in oxidative stress-induced diseases. Biomed Pharmacotherapie 178,117084.doi: 10.1016/j.biopha.2024.117084

Dash, U. C., Bhol, N. K., Swain, S. K., Samal, R. R., Nayak, P. K., Raina, V., *et al*. (2025). Oxidative stress and inflammation in the pathogenesis of neurological disorders: Mechanisms and implications. Acta Pharm Sin B. 15(1), 15-34. doi: 10.1016/j.apsb.2024.10.004.

DeLeo, J. A., Tanga, F. Y., and Tawfik, V. L. (2004). Neuroimmune activation and neuroinflammation in chronic pain and opioid tolerance/hyperalgesia. Neurosci 10(1), 40–52.

Eidson, L. N., and Murphy, A. Z. (2013). Blockade of Toll-like receptor 4 attenuates morphine tolerance and facilitates the pain relieving properties of morphine. J Neurosci 33(40), 15952-15963.

El-Hamid Mohamed Elwy, A., and Tabl, G. (2017). 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. Elect Phys 9(3), 4027–4034. doi: 10.19082/4027

Ellman, G. L., Courtney, K. D., Andres, V., and Feather-Stone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7(2), 88–95.

Eskandari, M. R., Fard, J. K., Hosseini, M-J., and Pourahmad, J. (2012). Glutathione mediated reductive activation and mitochondrial dysfunction play key roles in lithium induced oxidative stress and cytotoxicity in liver. Biometals 25(5), 863–873.
Falade, J., Osho, P. O., Ajayi, A. A., Ogungbemi, A. O., Dare, R. F., Akinnuoye, A. A., *et al*. (2023). Anti-craving property of chlorpromazine in pentazocine addiction among sickle cell disease patients: A case series. Babcock University Medical Journal 6(2), 62–65.
Fiske, C. H., and Subbarow, Y. (1925). The colorimetric determination of phosphorus. J Biol Chem 66, 375.
Fujii, K., Koshidaka, Y., Adachi, M., and Takao, K. (2019). Effects of chronic fentanyl administration on behavioral characteristics of mice. Neuropsychopharmacol Rep 39(1), 17-35
Gao, X. Y., Yang, T., Gu, Y., and Sun, X. H. (2022). Mitochondrial dysfunction in Parkinson's disease: From mechanistic insights to therapy. Front Aging Neurosci 20;14, 885500.
Ghazi-Khansari, M., Mohammadi-Bardbori, A., and Hosseini, M. J. (2006). Using Janus green B to study paraquat toxicity in rat liver mitochondria: role of ACE inhibitors (thiol and nonthiol ACEi). Ann N Y Acad Sci 1090(1), 98–107
Gonzalez, L. G., Masocha, W., Sánchez-Fernández, C., Agil, A., Ocaña, M., Del Pozo, E., *et al* (2012). Changes in morphine-induced activation of cerebral Na(+),K(+)-ATPase during morphine tolerance: biochemical and behavioral consequences. Biochemical Pharmacol 83(11), 1572-1581. doi: 10.1016/j.bcp.2012.02.023
Green, J. P., Glick, S. D., Crane, A. M., and Szilagyi, P. I. (1976). Acute effects of morphine on regional brain levels of acetylcholine in mice and rats. Eur J Pharmacol 39(1), 91-99.
Guix, F. X., Uribesalgo, I., Coma, M., and Muñoz, F. J. (2005). The physiology and pathophysiology of nitric oxide in the brain. Prog Neurobiol 76(2):126-52.
Guzmán, D. C., Vázquez, I. E., Brizuela, N. O., Alvarez, R. G., Mejía, G. B., García, E. H. (2006). Assessment of oxidative damage induced by acute doses of morphine sulfate in postnatal and adult rat brain. Neurochem Res 31(4):549-54.
Hauser, K. F., Gurwell, J. A., and Turbek, C. S. (1994). Morphine inhibits Purkinje cell survival and dendritic differentiation in organotypic cultures of the mouse cerebellum. Exp Neurol 130(1), 95-105.
Hewett, S. J., Silakova, J. M., and Hewett, J. A. (2006). Oral Treatment with Rofecoxib Reduces Hippocampal Excitotoxic Neurodegeneration. J. Pharmacol. Exp. Ther 319, 1219-1224.
Howarth, C., Gleeson, P., and Attwell, D. (2012). Updated energy budgets for neural computation in the neocortex and cerebellum. J Cereb Blood Flow Metab 32(7), 1222–1232.
Hoy, A., Leininger-Muller, B., Kutter, D., Siest, G., and Visvikis, S. (2002). Growing significance of myeloperoxidase in non-infectious diseases. Clin Chem Lab Med 40(1), 2-8.
Huang, C., and Ricklefs, R. E. (2013). Cerebellum and Human Evolution: A Comparative and Information Theory Perspective. In: Manto, M., Schmahmann, J. D., Rossi, F., Gruol, D. L., Koibuchi, N. (eds) Handbook of the Cerebellum and Cerebellar Disorders. Springer, Dordrecht. doi: 10.1007/978-94-007-1333-8_59
Hutchinson, M. R., Lewis, S. S., Coats, B. D., Rezvani, N., Zhang, Y., Wieseler, J.L., *et al*. (2010). Possible involvement of toll-like receptor 4/myeloid differentiation factor-2 activity of opioid inactive isomers causes spinal proinflammation and related behavioral consequences. Neurosci 167, 880–893.
Imam-Fulani, A. O., Bamikole, O. K., and Owoyele, B. V. (2016). Effects of caffeine administration on brain sodium-potassium ATPase activity in healthy and streptozotocin-induced diabetic female Wistar rats. J Caffeine Res 6(3), 117-125.
Kaushik, A. S., Strath, L. J., and Sorge, R. E. (2020). Dietary interventions for treatment of chronic pain: oxidative stress and inflammation. Pain Ther 9(2), 487–498.
Kaygusuzoglu, E., Caglayan, C., Kandemir, F. M., Yıldırım, S., Kucukler, S., Kılınc, M. A., *et al*. (2018). Zingerone ameliorates cisplatin-induced ovarian and uterine toxicity via suppression of sex hormone imbalances, oxidative stress, inflammation and apoptosis in female Wistar rats. Biomed Pharmacother 102, 517–530.
Kowalczyk, P., Sulejczak, D., Kleczkowska, P., Bukowska-Ośko, I., Kucia, M., Popiel, M., *et al*. (2021). Mitochondrial oxidative stress-a causative factor and therapeutic target in many diseases. Int J Mol Sci 22(24), 13384. doi: 10.3390/ijms222413384
Leong, S. K., Ruan, R. S., and Zhang, Z. (2002). A critical assessment of the neurodestructive and neuroprotective effects of nitric oxide. Ann N Y Acad Sci 962, 161-181.
Leri, M., and Vasarri, M. (2025). Advancing neuropharmacology and neurodegenerative disease therapy: Bridging gaps and paving new pathways. Pharmaceuticals 18(5), 606. doi: 10.3390/ph18050606
Lewis, S. S., Hutchinson, M. R., Rezvani, N., Loram, L. C., Zhang, Y., Maier, S. F., *et al*. (2010). Evidence that intrathecal morphine-3-glucuronide may cause pain enhancement via toll-like receptor 4/MD-2 and interleukin-1beta. Neurosci 165, 569-583.
Maehly, A. C., and Chance, B. (1954). The assay of catalases and peroxidases. Methods Biochem Anal 1, 357-424.
Manji, H., Kato, T., Di Prospero, N. A., Ness, S., Beal, M. F., Krams, M., *et al*. (2012). Impaired mitochondrial function in psychiatric disorders. Nat Rev Neurosci 13(5), 293–307.
Masocha, W., González, L. G., and Agil, A. (2016). Distinguishing subgroups among μ-opioid receptor agonists using Na(+),K(+)-ATPase as an effector mechanism. Eur J Pharm 774, 43–49. doi: 10.1016/j.ejphar.2016.01.010
Masocha, W., González, L. G., Baeyens, J. M., and Agil, A. (2002). Mechanisms involved in morphine-induced activation of synaptosomal Na+, K+-ATPase. Brain Res 957(2), 311–319. doi: 10.1016/s0006-8993(02)03663-6
Mata, M., Fink, D. J., Gainer, H., Smith, C. B., Davidsen, L., Savakis, H., *et al*. (1980). Activity-dependent energy metabolism in rat posterior pituitary, primarily reflects sodium pump activity. J Neurochem 34, 214–215.

McKibben, C. E., Reynolds, G. P., and Jenkins, T. A. (2014). Analysis of sociability and preference for social novelty in the acute and subchronic phencyclidine rat. J Psychopharmacol 28(10), 955-963.
Minghetti, L. (2004). Cyclooxygenase-2 (COX-2) in inflammatory and degenerative brain diseases. J Neuropathol Exp Neurol 63, 901-910.
Mohamed, H. M., and Mahmoud, A. M. (2019). Chronic exposure to the opioid tramadol induces oxidative damage, inflammation and apoptosis, and alters cerebral monoamine neurotransmitters in rats. Biomed Pharmacother 110, 239-247.
Ney, D. M., Murali, S. G., Stroup, B. M., Nair, N., Sawin, E. A., Rohr, F., *et al*. (2017) Metabolomic changes demonstrate reduced bioavailability of tyrosine and altered metabolism of tryptophan via the kynurenine pathway with ingestion of medical foods in phenylketonuria. Mol Genet Metab 121(2), 96-103.
Norat, P., Soldozy, S., Sokolowski, J. D., Gorick, C. M., Kumar, J. S., Chae, Y., *et al*. (2020). Mitochondrial dysfunction in neurological disorders: Exploring mitochondrial transplantation. NPJ Regen Med 5(1), 22.
Oberley, L. W., and Spitz, D. R. (1984). Assay of superoxide dismutase activity in tumor tissue. Methods Enzymol, 105, 457–464. doi: 10.1016/s0076-6879(84)05064-3
Ojo, O. A., Rotimi, D. E., Ojo, A. B., Ogunlakin, A. D., and Ajiboye, B. O. (2023). Gallic acid abates cadmium chloride toxicity via alteration of neurotransmitters and modulation of inflammatory markers in Wistar rats. Sci Rep 13(1), 1577.
Ozmen, I., Naziroğlu, M., Alici, H. A., Sahin, F., and Cengiz, M., Eren, I. (2007). Spinal morphine administration reduces the fatty acid contents in spinal cord and brain by increasing oxidative stress. Neurochem Res 32(1), 19-25.
Paul, A. K., Gueven, N., and Dietis, N. (2021). Profiling the effects of repetitive morphine administration on motor behavior in rats. Molecules 26(14), 4355.
Pu, L., Bao, G. B., Xu, N. J., Ma, L., and Pei, G. (2002). Hippocampal long-term potentiation is reduced by chronic opiate treatment and can be restored by re-exposure to opiates. J Neurosci 22(5), 1914-1921.
Pyatha, S., Kim, H., Lee, D., and Kim, K. (2023). Co-exposure to lead, mercury, and cadmium induces neurobehavioral impairments in mice by interfering with dopaminergic and serotonergic neurotransmission in the striatum. Front Public Health, 11, 1265864. doi: 10.3389/fpubh.2023.1265864
Raghavendra, V., Tanga, F., Rutkowski, M. D., and DeLeo, J. A. (2003). Anti-hyperalgesic and morphine-sparing actions of propentofylline following peripheral nerve injury in rats: mechanistic implications of spinal glia and proinflammatory cytokines. Pain 104, 655–664
Ray, R. S., and Katyal, A. (2016). Myeloperoxidase: Bridging the gap in neurodegeneration. Neurosci Biobehav Rev 68, 611-620.
Reeves, K. C., Shah, N., Muñoz, B., and Atwood, B. K. (2022). Opioid Receptor-Mediated Regulation of Neurotransmission in the Brain. Front Mol Neurosci 15, 919773. doi: 10.3389/fnmol.2022.919773
Reich, C. G., Mason, S. E., and Alger, B. E. (2004). Novel form of LTD induced by transient, partial inhibition of the Na,K-pump in rat hippocampal CA1 cells. J Neurophysiol 91(1), 239-247. doi: 10.1152/jn.00722.2003.
Sadat-Shirazi, M. S. Zarrindast, M. R., and Ashabi, G. (2020). Oxidative stress enzymes are changed in opioid abusers and multidrug abusers. J Clin Neurosci 72, 365–369.
Safarinejad, M. R., Asgari, S. A., Farshi, A., Ghaedi, G., Kolahi, A. A., Iravani, S., *et al*. (2013). The effects of opiate consumption on serum reproductive hormone levels, sperm parameters, seminal plasma antioxidant capacity and sperm DNA integrity. Reprod Toxicol 36, 18-23.
Salarian, A., Kadkhodaee, M., Zahmatkesh, M., Seifi, B., Bakhshi, E., Akhondzadeh, S., *et al*. (2018). Opioid use disorder induces oxidative stress and inflammation: the attenuating effect of methadone maintenance treatment. Iranian J Psych 13(1), 46.
Shaki, F., Hosseini, M. J., Ghazi-Khansari, M., and Pourahmad, J. (2013). Depleted uranium induces disruption of energy homeostasis and oxidative stress in isolated rat brain mitochondria. Metallomics 5(6), 736–744
Shirenova, S. D., Khlebnikova, N. N., and Krupina, N. A. (2023). Changes in sociability and preference for social novelty in female rats in prolonged social isolation. Neurosci Behav Physiol 53(1), 103-118.
Shoji, H., and Miyakawa, T. (2019). Age-related behavioral changes from young to old age in male mice of a C57BL/6J strain maintained under a genetic stability program. Neuropsychopharmacol Rep 39(2), 100-118.
Südhof, T. C. (2012). Calcium control of neurotransmitter release. Cold Spring Harbor Persp Biol 4(1), a011353. doi: 10.1101/cshperspect.a011353
Taghizadeh, G., Mehdizadeh, H., Lavasani, H., Hosseinzadeh, A.Y., Foroumadi, A., Halvaei, K. Z., *et al*. (2020). Dose concentration and spatial memory and brain mitochondrial function association after 3,4-methylenedioxymethamphetamine (MDMA) administration in rats. Arch Toxicol 94(3), 911-925.
Tarazi, F. I., Campbell, A., Yeghiayan, S. K., and Baldessarini, R. J. (1998). Localization of dopamine receptor subtypes in corpus striatum and nucleus accumbens septi of rat brain: comparison of D1-, D2-, and D4-like receptors. Neurosci 83, 169–176.
Tarazi, F. I., Tomasini, E. C., and Baldessarini, R. J. (1999). Postnatal development of dopamine D1-like receptors in rat cortical and striatolimbic brain regions: an autoradiographic study. Dev Neurosci 21, 43–49.
Tarazi, F. I., Zhang, K., and Baldessarini, R. J. (2000). Effects of nigrostriatal dopamine denervation on ionotropic glutamate receptors in rat caudate-putamen. Brain Res 881, 69–72.
Teicher, M. H., Andersen, S. L., and Hostetter, J, C. (1995). Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. Brain Res Dev Brain Res 89, 167–172
Teleanu, R. I., Niculescu, A. G., Roza, E., Vladâcenco, O., Grumezescu, A. M., Teleanu, D. M. (2022). Neurotransmitters-Key Factors in Neurological and Neurodegenerative

Disorders of the Central Nervous System. Int J Mol Sci 23(11), 5954. doi: 10.3390/ijms23115954

Tian, J., Kim, S. F., Hester, L., and Snyder, S. H. (2008). S-nitrosylation/activation of COX-2 mediates NMDA neurotoxicity. Proc Natl Acad Sci USA 105(30), 10537-10540.

Tien, L. T., Fan, L. W., Sogawa, C., Ma, T., Loh, H. H., and Ho, I. K. (2004). Changes in acetylcholinesterase activity and muscarinic receptor bindings in mu-opioid receptor knockout mice. Brain Res Mol Brain Res 126(1), 38-44.

Trigo, D., Nadais, A., Carvalho, A., Morgado, B., Santos, F., Nóbrega-Pereira, S., *et al*. (2023). Mitochondria dysfunction and impaired response to oxidative stress promotes proteostasis disruption in aged human cells. Mitochondrion 69, 1-9.

Tsakiris, S., Angelogianni, P., Schulpis, K. H., and Stavridis, J. C. (2000). Protective effect of L-phenylalanine on rat brain acetylcholinesterase inhibition induced by free radicals. Clin Biochem 33(2), 103-106.

Tu, H., Chu, H., Guan, S., Hao, F., Xu, N., Zhao, Z., *et al*. (2021). The role of the M1/M2 microglia in the process from cancer pain to morphine tolerance. Tiss Cell 68, 101438. doi: 10.1016/j.tice.2020.101438.

Tzschentke, T. M., Christoph, T., and Kogel, B. (2007). A novel μ-opioid receptor agonist/norepinephrine reuptake inhibitor with broad-spectrum analgesic properties. J Pharmacol Exp Ther 323(1), 265-276.

Wang, H., Luo, J., Chen, X., Hu, H., Li, S., Zhang, Y., *et al*. (2022). Clinical observation of the effects of oral opioid on inflammatory cytokines and gut microbiota in patients with moderate to severe cancer pain: A retrospective cohort study. Pain Ther 11(2), 667-681.

Wang, S., Guo, S., Guo, J., Du, Q., Wu, C., Wu, Y *et al* (2024). Cell death pathways: molecular mechanisms and therapeutic targets for cancer. MedComm 5(9), e693. doi: 10.1002/mco2.69

Wang, X., Loram, L. C., Ramos, K., de Jesus, A. J., Thomas, J., Cheng, K., *et al*. (2012). Morphine activates neuroinflammation in a manner parallel to endotoxin. Proc Natl Acad Sci USA 109(16), 6325-6330.

Watkins, L. R., Hutchinson, M. R., Johnston, I. N., and Maier, S. F. (2005). Glia: novel counter-regulators of opioid analgesia. Trends Neurosci 28(12), 661–669

Watkins, L. R., Hutchinson, M. R., Rice, K. C., and Maier, S. F. (2009). The "toll" of opioid-induced glial activation: improving the clinical efficacy of opioids by targeting glia. Trends Pharmacol Sci 30(11), 581–591.

Wei, T., Chen, C., Hou, J., Xin, W., and Mori, A. (2000). Nitric oxide induces oxidative stress and apoptosis in neuronal cells. Biochim Biophys Acta 1498(1), 72-79.

Winstanley, E. L., Mahoney, J. J. 3rd, Castillo, F., and Comer, S. D. (2021). Neurocognitive impairments and brain abnormalities resulting from opioid-related overdoses: A systematic review. Drug Alcohol Depend 226, 108838.

Wu, C., Mu, Q., Gao, W., and Lu, S. (2025). The characteristics of anhedonia in depression: a review from a clinically oriented perspective. Trans psych 15(1), 90. doi: 10.1038/s41398-025-03310-w

Wu, X. C., Yu, B. T., Hou, A. L., Hu, T. T., and Lu, G. Q. (2006). Study on stability of gallic acid. Med J Natl Def Forces Southwest China 16, 484–485.

Wyse, A. T., Bavaresco, C. S., Reis, E. A., Zugno, A. I., Tagliari, B., Calcagnotto T, *et al*. (2004). Training in inhibitory avoidance causes a reduction of Na^{+}-K^{+}-ATPase activity in rat hippocampus. Physiol Behav 80(4), 475-479.

Ye, Z. W., Zhang, J., Townsend, D. M., and Tew, K. D. (2015). Oxidative stress, redox regulation and diseases of cellular differentiation. Biochim Biophys Acta 1850(8), 1607-1621.

Zahmatkesh, M., Kadkhodaee, M., Salarian, A., Seifi, B., and Adeli, S. (2017). Impact of opioids on oxidative status and related signaling pathways: An integrated view. J Opioid Manag 13(4), 241-251.

Zhang, H., Cui, M., Cao, J. L., and Han, M. H. (2022). The role of beta-adrenergic receptors in depression and resilience. Biomed 10(10), 2378. doi: 10.3390/biomedicines 10102378

Zhang, J., Xie, Z., Dong, Y., Wang, S., Liu, C., and Zou, M. H. (2008). Identification of nitric oxide as an endogenous activator of the AMP-activated protein kinase in vascular endothelial cells. J Biol Chem 283(41), 27452-27461.

Zhang, Y., Du, Y., Le, W., Wang, K., Kieffer, N., and Zhang, J. (2011). Redox control of the survival of healthy and diseased cells. Antioxid Redox Signal 15(11), 2867-2908.

Zhang, Z., Li, Y., Feng, H., Li, S., Qin, Z., Li, J., *et al*. (2024). Effects of postweaning cadmium exposure on socioemotional behaviors in adolescent male mice. Ecotoxicol Environ Safety 272, 116089.

Published by Neuroscience Society of Nigeria

Copyright © 2025 by author(s).*This* work *is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).*