

Behavioural, Neurochemical, and Brain Histomorphological Effects of Repeated Propofol Administration in Wistar Rats

Elijah Oluwaseun AJADI¹, Joshua Aduragbemi AJAO², Boluwatife Mary OLADIPUPO¹, Mary Olayemi AJADI³, Fausat Olabisi EYITAYO⁴, Adejoke Yetunde ONAOLAPO⁵, Olakunle James ONAOLAPO⁶

1. Department of Pharmacology and Therapeutics, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

2. Department of Pharmacology and Toxicology, Federal University Oye-Ekiti

3. Department of Forest Resource Management, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

4. Department of Mental Health and Psychiatric Nursing, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

5. Behavioural Neuroscience/Neurobiology Unit, Department of Anatomy, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. ayonaolapo@lautech.edu.ng ORCID: 0000-0001-7126-7050

6. Behavioural Neuroscience/Neuropharmacology Unit, Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria ojonaolapo@lautech.edu.ng ORCID: 0000-0003-2142-6046

Correspondence:

Elijah Oluwaseun Ajadi

Department of Pharmacology and Therapeutics,

Ladoke Akintola University of Technology, Ogbomoso, Oyo State.

Email: seunajadi80@gmail.com. **Tel:** +2348060826595.

Abstract

Propofol is a widely used intravenous anaesthetic whose principal central nervous system action involves potentiation of γ -aminobutyric acid type A (GABA_A) receptor-mediated inhibitory neurotransmission. Although propofol has recognised neuroprotective, antioxidant, and anti-inflammatory properties, repeated or prolonged exposure may produce dose- and context-dependent neural alterations. This study investigated the behavioural, circulating neurochemical, biochemical, and brain histomorphological effects of repeated propofol administration in Wistar rats. Forty Wistar rats, comprising 20 males and 20 females, were allocated to four groups (n = 10; 5 males and 5 females per group): control, 0.5, 1.0, and 1.5 mg/kg propofol groups. Behavioural assessments included the open-field test, elevated plus maze, and Y-maze, while body weight and feed intake were monitored. Serum dopamine, acetylcholine, serotonin, malondialdehyde (MDA), total antioxidant capacity (TAC), tumour necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β), and interleukin-10 (IL-10) were determined. The dentate gyrus and prefrontal cortex were examined histologically. Data were analysed using one-way analysis of variance followed by Tukey's multiple-comparison test. Repeated propofol exposure significantly altered feed intake, line crossing, rearing, elevated-plus-maze behaviour, and Y-maze performance, whereas body weight and self-grooming were not significantly altered. Serum dopamine decreased progressively, whereas acetylcholine increased at the medium and high doses. MDA increased significantly, accompanied by increased TAC at the highest dose. TNF- α and IL-10 were significantly reduced, whereas IL-1 β was unchanged. Histological examination demonstrated dose-related neuronal distortion and degenerative changes in the dentate gyrus and prefrontal cortex. Repeated propofol administration was associated with behavioural, circulating neurochemical, biochemical, and brain histomorphological alterations following repeated propofol exposure.

KEYWORDS: Neurobehavioural alterations; Neurochemical changes; Oxidative stress; Brain histomorphology

1. Introduction

Propofol, chemically known as 2,6-diisopropylphenol, is a short-acting intravenous hypnotic agent widely used for the induction and maintenance of general anaesthesia and procedural sedation [1, 2]. Its principal pharmacological action involves potentiation of γ -aminobutyric acid type A (GABA_A) receptor-mediated inhibitory neurotransmission, resulting in

dose-dependent suppression of neuronal excitability [2]. Beyond its effects on GABAergic signalling, propofol influences excitatory neurotransmission, calcium signalling, cerebral metabolism and inflammatory pathways, thereby producing a broad range of effects within the central nervous system [1-3]. The widespread clinical use and increasing abuse potential of propofol has prompted continued investigation of its biological

effects beyond the intended transient state of anaesthesia [1]. Experimental studies have demonstrated potentially beneficial antioxidant, anti-inflammatory and neuroprotective properties, particularly in models of cerebral ischaemia, traumatic brain injury and oxidative stress [5-7]. However, other experimental evidence suggests that prolonged or repeated exposure, particularly during periods of increased neuronal vulnerability, may be associated with neuronal injury, impaired neurogenesis and behavioural abnormalities [8-10]. These apparently contrasting observations indicate that the effects of propofol may be influenced by several factors, including dose, duration and pattern of exposure, developmental stage, pre-existing brain conditions and the experimental context.

The hippocampus and prefrontal cortex are important brain regions for investigating the potential consequences of repeated anaesthetic exposure because of their central roles in cognitive and behavioural functions. The hippocampus is critically involved in learning and memory, while the prefrontal cortex contributes to executive function, attention, behavioural regulation and working memory. Within the hippocampus, the dentate gyrus is a major site of adult neurogenesis and is therefore potentially sensitive to disturbances in neuronal homeostasis [11]. Alterations within these regions may consequently manifest as measurable changes in locomotor activity, exploratory behaviour, anxiety-related responses and spatial working memory.

Neurotransmitter systems provide important functional correlates of these behavioural and cognitive processes. Dopamine is involved in the regulation of locomotor activity, motivation, attention and cognitive processing, whereas acetylcholine plays important roles in learning, memory and attention. Serotonin contributes to the regulation of mood, anxiety, arousal and behavioural responses. Experimental evidence indicates that anaesthetic exposure can influence dopaminergic and cholinergic neurotransmission, although the direction and magnitude of these effects may vary according to the anaesthetic agent, dose, duration of exposure, brain region and experimental conditions [12-14]. Assessment of circulating dopamine, acetylcholine and serotonin may therefore provide complementary biochemical information alongside behavioural evaluation, although such measurements do not directly represent neurotransmitter activity within specific brain regions. Oxidative stress represents another potential pathway through which repeated anaesthetic exposure may influence neuronal function and morphology. The brain has a high metabolic demand and is particularly susceptible to oxidative injury because of its high oxygen consumption and abundance of polyunsaturated fatty acids. Malondialdehyde (MDA), a product of lipid peroxidation, is widely used as an indicator of oxidative damage, whereas total antioxidant capacity (TAC) provides an estimate of the overall antioxidant defence of a biological system.

Notably, propofol itself possesses antioxidant properties, partly attributable to its phenolic structure and free-radical-scavenging capacity [3-5]. Repeated exposure may therefore involve a complex balance between oxidative challenge and endogenous antioxidant responses, the net effect of which may depend on dose and experimental conditions.

Inflammatory signalling may also contribute to the biological effects associated with anaesthetic exposure. Tumour necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β) and interleukin-10 (IL-10) are important mediators of inflammatory regulation. Experimental studies indicate that propofol can suppress microglial activation and attenuate inflammatory signalling in several pathological models [4, 13]. Conversely, studies investigating propofol-associated developmental neurotoxicity have implicated neuroinflammatory responses and neuronal injury among the potential adverse consequences of prolonged exposure [7, 9]. These findings further highlight the context-dependent nature of propofol's effects and the need to examine both potentially protective and adverse biological responses.

The apparent divergence in the literature underscores the value of evaluating functional, biochemical and structural outcomes concurrently. Behavioural assessment can identify functional alterations that may not be apparent from biochemical measurements alone, while biochemical analyses can provide information about potential oxidative, inflammatory and neurochemical changes. Histological examination, in turn, may reveal structural alterations accompanying these functional and biochemical responses. An integrated assessment of these endpoints may therefore provide a broader characterisation of the consequences of repeated propofol exposure.

The present study therefore investigated the behavioural, circulating neurochemical, oxidative, inflammatory and brain histomorphological effects of repeated propofol administration in Wistar rats. Specifically, body weight, feed intake, locomotor and exploratory activity, anxiety-related behaviour and spatial working-memory performance were evaluated alongside serum dopamine, acetylcholine, serotonin, MDA, TAC, TNF- α , IL-1 β and IL-10. Histological examination of the dentate gyrus and prefrontal cortex was also performed to determine whether behavioural and biochemical alterations were accompanied by observable changes in brain tissue morphology.

2. Materials and Methods

2.1. Chemicals and Drugs

Propofol injection (Rusoma Laboratories, India), soybean oil (Golden Penny Ltd., Nigeria), interleukin-10 (IL-10) assay kit (BioVision Inc., Milpitas, CA, USA), tumour necrosis factor- α (TNF- α) assay kit (BioVision Inc., Milpitas, CA, USA), and total antioxidant capacity (TAC) assay kit (BioVision Inc., Milpitas, CA, USA) were used in the study. Assay kits for dopamine, acetylcholine, serotonin, malondialdehyde (MDA),

and interleukin-1 beta (IL-1 β) were procured from reliable vendors and used in this study

2.2. Experimental Animals

Forty healthy adult Wistar rats, weighing 120–150 g, were obtained from Empire Breeders, Osogbo, Osun State, Nigeria. The animals were housed in standard wooden cages measuring 20 × 10 × 12 inches and maintained under standard laboratory conditions at a temperature of 22 ± 2 °C with a 12:12-h light/dark cycle. Standard rat chow and clean drinking water were provided ad libitum throughout the experimental period.

The animals were acclimatised to the laboratory environment before commencement of the experimental procedures. All experimental procedures were approved by the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria, and were conducted in accordance with the provisions for the care and use of laboratory animals prescribed by European Council Directive 2010/63/EU for the protection of animals used for scientific purposes.

2.3. Experimental Design and Propofol Administration

Four experimental groups of animals (n = 10 per group) were randomly allocated. Group I served as the control, whereas Groups II, III, and IV were administered propofol at 0.5, 1.0, and 1.5 mg/kg, respectively. Subanesthetic doses were selected to evaluate the impact of repeated intermittent exposure instead of sustained anaesthesia [15]. Propofol was administered every four days over the 21-day experimental period, on days 1, 5, 9, 13, 17 and 21, resulting in a total six administrations per animal. The four-day interval was selected to allow recovery between successive administrations while providing repeated intermittent exposure over the experimental period. This dosing schedule was specific to the experimental protocol and was not intended to represent a standard clinical dosing regimen.

Propofol was obtained as a sterile 1% oil-in-water injectable emulsion containing 10 mg/mL propofol. According to the formulation information available for the preparation, the emulsion contained soybean oil, purified egg phosphatide (lecithin), glycerol and water for injection as excipients. Propofol was diluted with soybean oil immediately before administration and delivered intraperitoneally (i.p.) using a sterile 1-mL syringe fitted with a 26-gauge needle. The control animals received soybean oil at 2 ml/kg following the same treatment schedule. Feed intake and body weight were measured daily and weekly respectively following standard protocols. At the end of the experimental period animals were exposed to behavioural paradigms including the open field test, elevated plus maze and Y-maze paradigms. Twenty-four hours after the last behavioural tests animals in all groups were euthanised by cervical dislocation. Blood was taken via cardiac puncture for the assessment of oxidative stress markers (Lipid peroxidation and Total antioxidant activity and inflammatory cytokines). The brain was excised and either homogenised for assessment of

dopamine, serotonin, and acetylcholine levels or processed for general histology.

2.4. Body Weight and Feed Intake

Body weight and feed intake were monitored throughout the experimental period. Body weight was recorded weekly using a calibrated weighing balance, whereas feed intake was measured daily. Daily feed intake was determined from the difference between the quantity of feed provided and the quantity remaining as previously described [16, 17]. Percentage change in feed intake or change in body weight was calculated as follows:

$$\text{Percentage change} = \frac{\text{Final value} - \text{Initial value}}{\text{Initial value}} \times 100$$

2.5. Behavioural Assessment

Behavioural assessments were conducted 24 h after the final propofol administration. The open-field test, elevated plus maze and Y-maze were performed between 08:00 and 14:00 h. Apparatuses were cleaned with 70% ethanol between animals to minimise the influence of residual olfactory cues. The order of behavioural testing followed previously described protocols [18]

2.5.1. Open-Field Test

The open-field test was used to assess spontaneous locomotor and exploratory activity. Each rat was observed for 10 min in a rectangular open-field apparatus constructed of white-painted wood and measuring 36 × 36 × 26 cm. The floor was divided by permanent red markings into 16 equal-sized squares. Each rat was placed in the centre of the apparatus at the beginning of the test. Horizontal locomotion was assessed as the number of floor units entered with all four paws. Rearing frequency was recorded as the number of occasions on which the animal stood on its hind limbs, either with the forelimbs against the wall of the apparatus or freely in the air. Grooming frequency was recorded as the number of episodes of body-cleaning behaviour, including paw licking, body and pubic-area cleaning, and face washing, during the 10-min observation period as previously described [19].

2.5.2. Elevated Plus Maze

The elevated plus maze (EPM) was used to assess anxiety-like behaviour. The apparatus consisted of two opposing open arms measuring 50 × 10 cm and two opposing closed arms measuring 50 × 10 × 40 cm, elevated 50 cm above the floor. Each rat was placed on the central platform facing one of the open arms and allowed to explore the maze freely for 5 min. The number of entries into the open and closed arms and the time spent in each type of arm were recorded. The rat's exploration of the EPM paradigm followed previously described protocols. An arm entry was defined as placement of all four paws into the respective arm. Greater exploration of the open arms was interpreted as reduced anxiety-like behaviour, whereas reduced open-arm exploration was interpreted as increased anxiety-like behaviour [20]

2.5.3. Y-Maze Test

The Y-maze was used to assess spatial working memory based on the natural tendency of rodents to explore a previously unvisited arm. The apparatus consisted of three identical arms arranged at 120° angles to one another. Each arm measured approximately 40 cm in length, 10 cm in width and 15 cm in height. Each rat was placed at the end of one arm and allowed to explore the maze freely for 8 min. The sequence of arm entries was recorded manually as previously described [21].

Percentage spontaneous alternation was calculated as:

$$\% \text{ Alternation} = \frac{\text{Number of correct alternations}}{\text{Total Arm entries} - 2} \times 100$$

2.6. Blood Collection and Biochemical Analysis

Twenty-four hours after completion of the behavioural assessments, the animals were euthanised by cervical dislocation and blood samples were collected. The samples were processed to obtain serum for determination of dopamine, acetylcholine, serotonin, MDA, TAC, TNF- α , IL-1 β and IL-10. Of the 10 animals in each experimental group, six serum samples were ultimately suitable for biochemical analysis (n = 6 per group). The remaining samples were excluded because of haemolysis, inadequate serum volume or laboratory processing problems.

2.6.1 Determination of Oxidative Stress Parameters

Lipid peroxidation measured as malondialdehyde content (MDA) and antioxidant status measured a total antioxidant capacity (TAC) were determined using commercially available assay kits according to previously described protocols [22]. Malondialdehyde levels was assessed as an index of lipid peroxidation, while TAC was determined as an estimate of the overall antioxidant capacity of the biological system. Absorbance was measured at 450 nm, and concentrations were calculated from standard curves generated.

2.6.2 Determination of Inflammatory Cytokines

Serum concentrations of Tumour necrosis factor (TNF)- α , Interleukin (IL)-1 β and IL-10 were determined using rat-specific ELISA kits according to previously described protocols [32]. Standards and serum samples were added to antibody-coated wells, incubated and washed, followed by addition of enzyme-linked detection antibodies. After substrate development and termination of the reaction, absorbance was measured at 450 nm using an ELISA microplate reader. Cytokine concentrations were calculated from the respective standard curves and expressed in pg/mL.

2.7 Determination of Neurochemical Parameters

Whole brain homogenates were prepared following standard protocols as previously described. Whole brain concentrations of acetylcholine (ACh), serotonin (5-hydroxytryptamine; 5-HT) and dopamine (DA) were determined using commercially available rat-specific enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions.

Briefly, standards and homogenate samples were added to antibody-coated microplate wells and incubated. Following washing, enzyme-conjugated detection antibodies and substrate solution were added according to the respective assay protocols. The reactions were terminated using the appropriate stop solution, and absorbance was measured at 450 nm using an ELISA microplate reader. Concentrations were calculated from the corresponding standard calibration curves and expressed according to the manufacturers' recommendations.

2.8. Histological Examination

Following blood collection, the brains were carefully excised and fixed in 10% formol-calcium. The tissues were subsequently processed using standard histological procedures, embedded in paraffin and sectioned for microscopic examination. Sections containing the dentate gyrus and prefrontal cortex were stained with haematoxylin and eosin (H&E) and Cresyl Violet. Stained H and E sections were assessed for preservation of cytoarchitecture, cellular organisation, neuronal shrinkage, nuclear changes, pyknosis, vacuolation, neuropil alteration and other neuronal morphological abnormalities. Cresyl Violet staining was used to assess neuronal cell bodies and Nissl substance, including the presence of chromatolytic and other morphological changes. The available histological material comprised sections from the control, 0.5, 1.0 and 1.5 mg/kg propofol groups. Approximately two to three sections per animal were examined for each staining procedure, with approximately four to six sections available per experimental group. Sections were examined using a light microscope at $\times 100$ and $\times 400$ magnifications. A blinded observer assessed the sections to minimise observer bias. Particular attention was paid to cellular organisation, neuronal morphology, pyknosis, chromatolysis, vacuolation, apparent reduction in cellular profiles and other structural alterations within the dentate gyrus and prefrontal cortex.

2.9. Photomicrography and Histomorphometric Assessment

Representative photomicrographs were obtained using a digital microscope camera attached to the light microscope. Images were captured under consistent magnification and imaging conditions and used to illustrate the observed histomorphological changes.

2.10. Statistical Analysis

Data were analysed using Chris Rorden's ANOVA for Windows (version 0.98). For single end-point comparisons, one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc test was used to compare the experimental groups. Results were expressed as mean $\pm$ standard error of the mean (SEM), and $p < 0.05$ was considered statistically significant.

3. Result

3.1 Effects of Repeated Propofol Administration on Change in Body Weight and Feed Intake

Figures 1 and 2 show the effect of repeated propofol administration on change in body weight and change in feed intake respectively. Repeated propofol administration did not significantly [$F(3,36) = 1.35, p = 0.2746$] affect change in body weight among the experimental groups. Mean changes in body weight were 28.53 ± 3.57 g in the control group, 24.80 ± 1.47 g in the 0.5 mg/kg group, 22.26 ± 2.22 g in the 1.0 mg/kg group, and 18.14 ± 2.74 g in the 1.5 mg/kg group. Although mean body-weight gain showed a visual decline with increasing propofol dose, the overall difference among groups was not statistically significant. In contrast, feed intake differed significantly [$F(3,36) = 286, p < 0.000001$] among the experimental groups. Mean feed intake was 31.80 ± 0.49 g in the control group, 39.60 ± 1.72 g in the 0.5 mg/kg group, 26.60 ± 0.62 g in the 1.0 mg/kg group, and 21.20 ± 0.39 g in the 1.5 mg/kg group. Tukey's post-hoc analysis demonstrated significant differences among the experimental groups. The pattern of feed intake was non-linear, with the 0.5 mg/kg group showing the highest mean intake, whereas mean intake decreased at the 1.0 and 1.5 mg/kg doses. The lower body-weight gain observed at the higher propofol doses occurred concurrently with reduced feed intake; however, the absence of a statistically significant overall difference in body-weight change precludes attributing the observed numerical differences specifically to reduced feed intake.

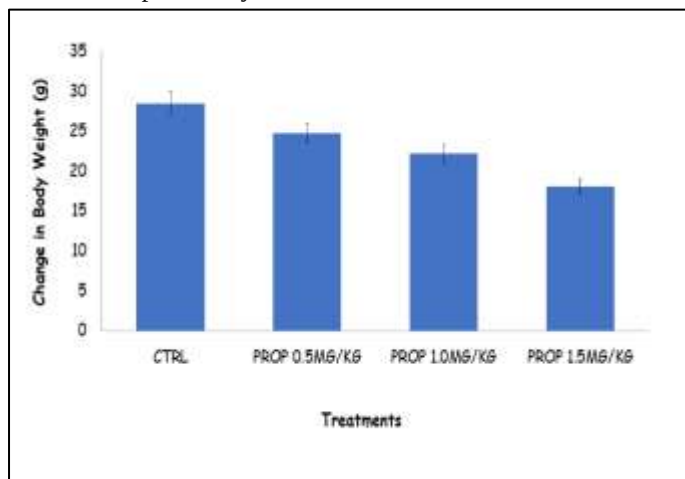


Figure 1. Effects of propofol on change in body weight in Wistar rats. Values are presented as mean $\pm$ SEM (n = 10 per group). CTRL: Control, PROP: Propofol

3.2 Effects of Repeated Propofol Administration on Open-Field Behaviour

Figures 3, 4 and 5 show the effect of propofol on horizontal locomotion, rearing and grooming. horizontal locomotor activity, assessed by line crossing, increased significantly [$F(3, 36) = 16.03, p = 0.001$] with repeated propofol

administration at 0.5 and decreased at 1.0 and 1.5 mg/kg respectively compared to control. The 0.5 mg/kg group exhibited the highest mean number of line crossings (106.00 ± 3.28), whereas lower mean values were observed in the 1.0 and 1.5 mg/kg groups (71.80 ± 14.01 and 61.00 ± 7.99 , respectively). Thus, locomotor activity showed a non-linear response across the propofol doses, with the highest dose associated with the lowest mean line-crossing activity. Rearing frequency (figure 4) also increased significantly [$F(3, 36) = 10.13, p = 0.001$] with repeated administration of propofol at 0.5 and a decrease at 1.0 and 1.5 mg/kg compared to control. Mean rearing frequency decreased progressively across the propofol dose range, from 66.00 ± 4.80 in the control group to 24.00 ± 4.33 in the 1.5 mg/kg group. In contrast, self-grooming behaviour did not significantly differ among the experimental groups [$F(3, 36) = 2.03, p = 0.1271$; Figure 5]. Mean grooming frequency ranged from 5.60 ± 1.91 in the control group to 7.80 ± 0.62 in the 1.5 mg/kg group. Although a numerical increase in grooming was observed with increasing propofol dose, this difference did not reach statistical significance.

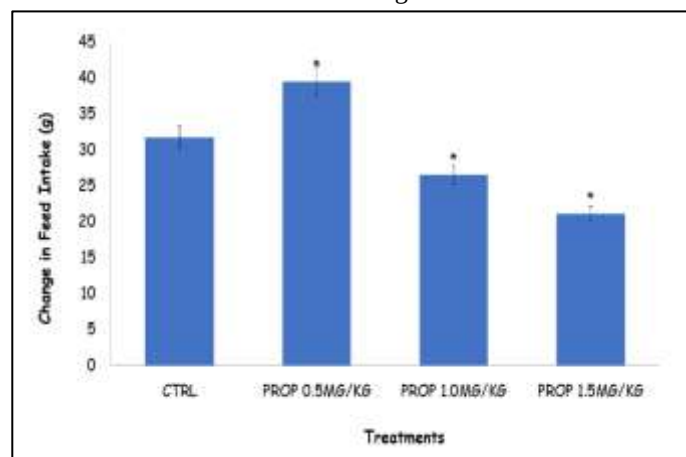


Figure 2. Effects of propofol on change in feed intake in Wistar rats. Values are presented as mean $\pm$ SEM (n = 10 per group). $P < 0.05$ versus the control group, CTRL: Control, PROP: Propofol.

3.3 Effects of Repeated Propofol Administration on Anxiety-Like Behaviour

Figure 6 shows the effect of repeated propofol administration on open-arm and closed arm exploration time in the elevated plus maze. Open arm time increased significantly [$F(3,36) = 6.17, p = 0.0017$] in groups administered propofol compared to control. Mean open-arm time increased from 19.00 ± 3.09 s in the control group to 51.67 ± 2.31 s in the 1.5 mg/kg propofol group. Closed-arm exploration time was also significantly decreased [$F(3,36) = 4.22, p = 0.0117$] across all propofol groups compared to control. Mean closed-arm time was 279.66 ± 2.81 s in the control group and decreased to approximately 245 s in the propofol-treated groups. Overall, repeated propofol administration was associated with increased open-arm

exploration and reduced closed-arm time in the elevated plus maze.

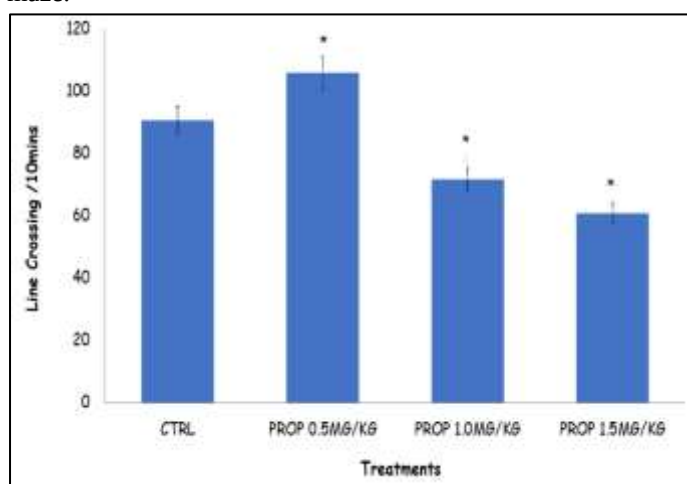


Figure 3. Effects of repeated propofol administration on horizontal locomotion. Values are presented as mean $\pm$ SEM. $P < 0.05$ versus the control group. CTRL: Control, PROP: Propofol

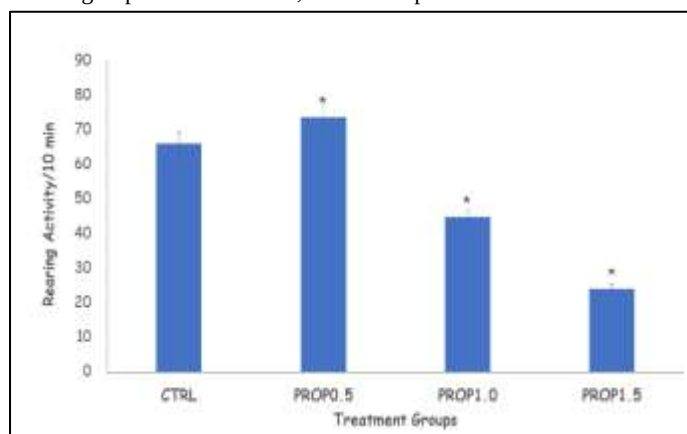


Figure 4. Effects of repeated propofol administration on rearing activity. Values are presented as mean $\pm$ SEM. $P < 0.05$ versus the control group. CTRL: Control, PROP: Propofol

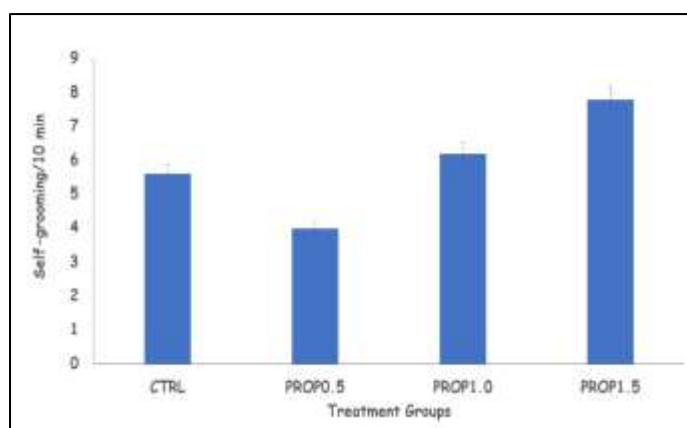


Figure 5. Effects of repeated propofol administration on self-grooming behaviour. Values are presented as mean $\pm$ SEM. $P < 0.05$ versus the control group. CTRL: Control, PROP: Propofol

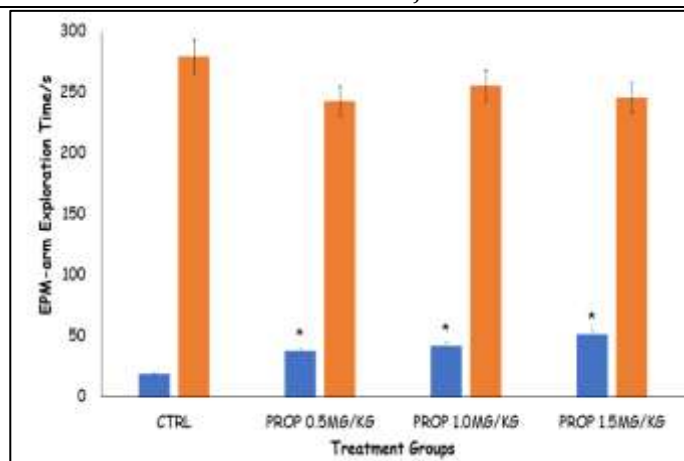


Figure 6. Effects of repeated propofol administration on time spent in the open and closed arms of the Elevated plus maze (EPM). Values are presented as mean $\pm$ SEM. $P < 0.05$ versus the control group. CTRL: Control, PROP: Propofol

3.4 Effects of Repeated Propofol Administration on Spatial Working Memory

Figure 7 shows the effect of repeated propofol administration on spatial working memory in the Y maze paradigm. Y-maze performance, assessed by spontaneous alternation increased significantly [$(F(3,36) = 6.54, p = 0.0012)$] with propofol at 0.5 and decreased with propofol at 1.0 and 1.5. The control group recorded a mean spontaneous alternation of $66.88 \pm 4.85\%$, whereas the 1.5 mg/kg propofol group exhibited the lowest mean value ($52.14 \pm 3.25\%$). These findings indicate an overall alteration in spatial working-memory performance following repeated propofol administration, with the greatest numerical reduction in spontaneous alternation observed at the highest propofol dose.

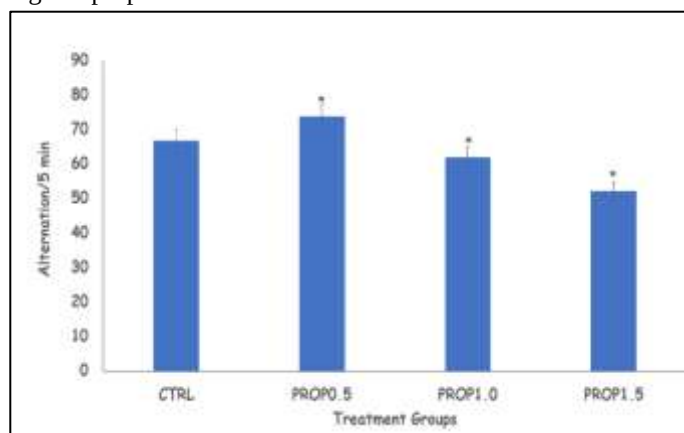


Figure 7. Effects of repeated propofol administration on Y maze performance measured as spontaneous alternation. Values are presented as mean $\pm$ SEM. $P < 0.05$ versus the control group. CTRL: Control, PROP: Propofol.

3.5 Effects of Repeated Propofol Administration on Neurotransmitter Concentrations

Table 1 shows the effect of repeated propofol on brain concentrations of dopamine, acetylcholine, and serotonin. Dopamine concentration decreased significantly [$F(3,20) = 9.93, p < 0.001$] with propofol at 0.5, 1.0 and 1.5 mg/kg compared to control respectively. Mean dopamine concentration decreased progressively with increasing propofol dose, from 71.19 ± 0.47 pg/mL in the control group to 62.45 ± 0.40 pg/mL, 50.87 ± 0.20 pg/mL, and 43.49 ± 0.37 pg/mL in the 0.5, 1.0, and 1.5 mg/kg propofol groups, respectively. The reported pairwise comparisons were statistically significant ($p < 0.0001$). This pattern indicates a progressive reduction in circulating dopamine concentration across the propofol dose range.

Acetylcholine concentration also increased significantly [$F(3,20) = 6.52, p = 0.003$] at 0.5, 1.0 and 1.5 mg/kg compared to control respectively. Mean concentrations were 0.67 ± 0.10 mM in the control group, 0.85 ± 0.04 mM in the 0.5 mg/kg group, 1.12 ± 0.10 mM in the 1.0 mg/kg group, and 1.11 ± 0.09 mM in the 1.5 mg/kg group. The highest mean acetylcholine concentration was observed in the 1.0 mg/kg group, with a comparable value in the 1.5 mg/kg group. Thus, the response was not strictly proportional to propofol dose.

Serotonin concentration decreased significantly [$F(3,20) = 4.83, p = 0.011$] with propofol at 0.5 and increased with propofol at 1.0 and 1.5 mg/kg compared to control. Mean serotonin concentrations were 1942.82 ± 201.01 ng/mL in the control group, 1585.25 ± 209.64 ng/mL in the 0.5 mg/kg group, 2136.05 ± 18.86 ng/mL in the 1.0 mg/kg group, and 1700.21 ± 78.40 ng/mL in the 1.5 mg/kg group. The highest mean concentration occurred in the 1.0 mg/kg group, whereas the lowest occurred in the 0.5 mg/kg group, indicating a non-linear response across the propofol doses.

Table 1. Effect of Repeated Propofol Administration on Neurotransmitter Concentrations

Group	Serotonin (ng/mL)	Acetylcholine (mM)	Dopamine (pg/mL)
Control	1942.82 ± 201.01	0.67 ± 0.10	71.19 ± 0.47
Propofol 0.5	$1585.25 \pm 209.64^*$	$0.85 \pm 0.04^*$	$62.45 \pm 0.40^*$
Propofol 1.0	$2136.05 \pm 18.86^*$	$1.12 \pm 0.10^*$	$50.87 \pm 0.20^*$
Propofol 1.5	$1700.21 \pm 78.40^*$	$1.11 \pm 0.09^*$	$43.49 \pm 0.37^*$

Data are presented as mean $\pm$ SEM (n = 10 rats per group). *p < 0.05 versus the control group.

3.6 Effects of Repeated Propofol Administration on Oxidative Stress and Antioxidant Status

Table 2 shows the effect of propofol on oxidative stress and antioxidant status. Serum malondialdehyde (MDA) concentration increased significantly [$F(3,20) = 9.47, p < 0.001$] with propofol at 1.5 compared to control. Mean MDA concentration was 0.62 ± 0.01 μ mol/L in the control group and increased to 0.65 ± 0.01 μ mol/L, 0.64 ± 0.01 μ mol/L, and 0.72 ± 0.03 μ mol/L in the 0.5, 1.0, and 1.5 mg/kg propofol groups, respectively. The highest mean MDA concentration was observed in the 1.5 mg/kg group.

Serum total antioxidant capacity (TAC) increased significantly [$F(3,20) = 13.3, p < 0.001$] with propofol at 1.5 compared to control. Mean TAC values were 10.65 ± 1.01 μ M in the control group, 11.97 ± 0.05 μ M in the 0.5 mg/kg group, 11.03 ± 0.28 μ M in the 1.0 mg/kg group, and 17.52 ± 1.41 μ M in the 1.5 mg/kg group. Post-hoc analysis indicated that the difference between the control and 1.5 mg/kg groups was statistically significant ($p = 0.0027$), whereas the control versus 0.5 mg/kg and control versus 1.0 mg/kg comparisons were not statistically significant. The highest TAC was recorded in the 1.5 mg/kg propofol group. Taken together, repeated propofol administration was associated with significant alterations in both MDA and TAC, with the greatest mean values for both parameters observed at the highest dose.

Table 2. Effect of Repeated Propofol Administration on Oxidative Stress and Antioxidant Status

Group	MDA (μ mol/L)	TAC (μ M)
Control	0.62 ± 0.01	10.65 ± 1.01
Propofol 0.5 mg/kg	$0.65 \pm 0.01^*$	11.97 ± 0.05
Propofol 1.0 mg/kg	$0.64 \pm 0.01^*$	11.03 ± 0.28
Propofol 1.5 mg/kg	$0.72 \pm 0.03^{\#}$	$17.52 \pm 1.41^{\#}$

Data presented as mean $\pm$ SEM (n = 10 rats per group). *p < 0.05 versus the control group.

3.7 Effects of Repeated Propofol Administration on Circulating Inflammatory Cytokines

Table 3 shows the effect of propofol on circulating levels of inflammatory cytokines. Serum TNF- α concentration decreased significantly [$F(3,20) = 16.2, p < 0.001$] with propofol at 0.5, 1.0 and 1.5 compared to control. Mean TNF- α concentrations were 40.73 ± 0.57 pg/mL in the control group, 19.82 ± 0.21 pg/mL in the 0.5 mg/kg group, 26.03 ± 0.88 pg/mL in the 1.0 mg/kg group, and 31.04 ± 4.25 pg/mL in the 1.5 mg/kg group. The lowest mean TNF- α concentration occurred in the 0.5 mg/kg group, while the control group recorded the highest mean concentration. The reported post-

hoc comparisons indicated significant differences for the 1.0 and 1.5 mg/kg groups relative to the control. Serum IL-10 concentration also decreased significantly [F(3,20) = 4.51, $p = 0.014$] with propofol at 1.0 and 1.5 compared to control. Mean IL-10 concentrations were 7.18 ± 2.25 pg/mL in the control group, 7.91 ± 2.65 pg/mL in the 0.5 mg/kg group, 1.10 ± 0.11 pg/mL in the 1.0 mg/kg group, and 1.21 ± 0.22 pg/mL in the 1.5 mg/kg group. The highest mean IL-10 concentration was observed in the 0.5 mg/kg group, whereas substantially lower concentrations were observed in the 1.0 and 1.5 mg/kg groups. According to the reported post-hoc comparisons, IL-10 concentrations in the 1.0 and 1.5 mg/kg groups were significantly lower than those of the control group. In contrast, serum IL-1 β concentration did not differ significantly [F(3,20) = 0.311, $p = 0.817$] among the experimental groups. Mean IL-1 β concentrations were 4.57 ± 0.50 ng/mL, 4.90 ± 0.19 ng/mL, 4.98 ± 0.54 ng/mL, and 5.04 ± 0.02 ng/mL in the control, 0.5, 1.0, and 1.5 mg/kg groups, respectively. Although a small numerical increase was observed across the propofol-treated groups, the overall difference was not statistically significant. Overall, repeated propofol administration significantly altered circulating TNF- α and IL-10 concentrations, whereas IL-1 β remained statistically unchanged.

Table 3. Effect of Repeated Propofol Administration on Circulating Inflammatory Cytokines

Group	TNF- α (pg/mL)	IL-10 (pg/mL)	IL-1 β (ng/mL)
Control	40.73 $\pm$ 0.57	7.18 $\pm$ 2.25	4.57 $\pm$ 0.50
Propofol 0.5	19.82 $\pm$ 0.21*	7.91 $\pm$ 2.65	4.90 $\pm$ 0.19
Propofol 1.0	26.03 $\pm$ 0.88#	1.10 $\pm$ 0.11*	4.98 $\pm$ 0.54
Propofol 1.5	31.04 $\pm$ 4.25#	1.21 $\pm$ 0.22*	5.04 $\pm$ 0.02

Data are presented as mean $\pm$ SEM (n = 10 rats per group). *p < 0.05 versus the control group.

3.8 Histomorphological Alterations in the Dentate Gyrus and Prefrontal Cortex

3.8.1 Haematoxylin and Eosin Histology

Representative H&E-stained photomicrographs of the dentate gyrus and prefrontal cortex are presented in **Figure 8 a and 8 b**. Histological examination of the dentate gyrus (Figure 8a) revealed preserved cytoarchitecture and neuronal density in the control group. Animals administered 0.5 mg/kg propofol showed mild neuronal distortion and a slight reduction in granular-cell density. At 1.0 mg/kg, moderate neuronal morphological alterations, including pyknotic nuclei and disruption of the granular cell layer, were observed. The 1.5 mg/kg group exhibited more pronounced neuronal

morphological alterations, reduced neuronal density, and distortion of the hippocampal architecture. The prefrontal cortex (Figure 8b) of control animals showed preserved neuronal organisation and cytoarchitecture. The 0.5 mg/kg propofol group exhibited mild neuronal shrinkage and cellular distortion. At 1.0 mg/kg, moderate neuronal morphological alterations, sparse neuronal profiles, and mild vacuolation were observed. The 1.5 mg/kg group demonstrated more pronounced neuronal morphological alterations, reduced neuronal profiles, and distortion of the neuropil.

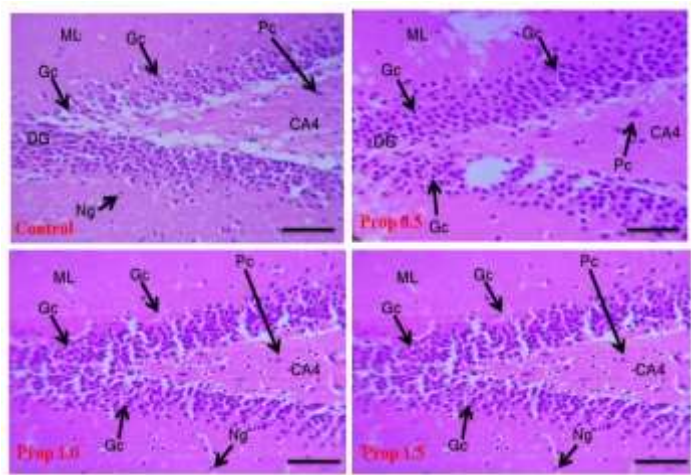


Figure 8a. Representative photomicrographs of the dentate gyrus following repeated propofol administration (H&E, $\times 400$). Control group showing preserved granular cell layer and normal hippocampal cytoarchitecture. Propofol 0.5 group showing mild neuronal distortion and early morphological alterations. Propofol 1.0 group showing moderate neuronal morphological alterations, pyknotic nuclei, and vacuolation. Propofol 1.5 group showing marked neuronal morphological alterations. DG: Dentate gyrus, GC: Granule Cell, ML: Molecular layer, PC: Pyramidal Cell, Ng: Neuroglia, CA4: Cornus ammonis 4 region.

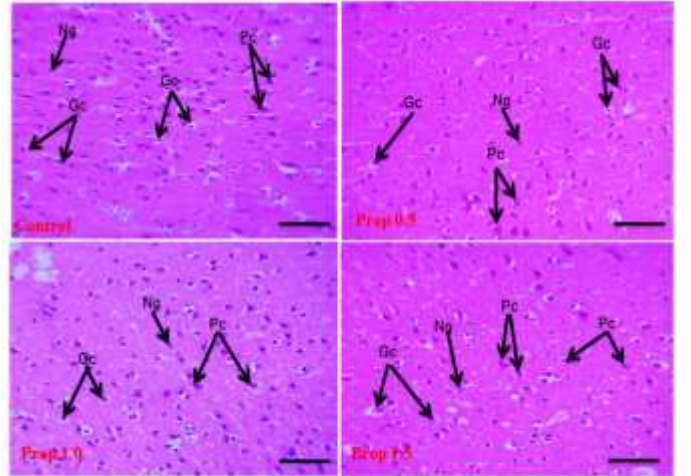


Figure 8b. Representative photomicrographs of the Prefrontal cortex following repeated propofol administration (H&E, $\times 400$). Control group showing preserved cortical organisation and neuronal morphology. Propofol (PROP) 0.5 group showing mild neuronal shrinkage and distortion. Propofol (PROP) 1.0 group showing moderate cortical morphological alterations and vacuolation. Propofol (PROP) 1.5 group showing marked neuronal morphological alterations, distorted neuropil, and reduced neuronal profiles. GC: Granule Cell, PC: Pyramidal Cell, Ng: Neuroglia

3.8.2 Cresyl Violet Histology

Representative Cresyl Violet-stained sections are presented in **Figure 9a and 9b**. Cresyl Violet staining demonstrated intense Nissl staining and preserved neuronal morphology in the dentate gyrus of control animals. The 0.5 mg/kg propofol group showed mild chromatolytic changes and reduced staining intensity. At 1.0 mg/kg, moderate chromatolysis and reduced neuronal staining were observed, whereas the 1.5 mg/kg group exhibited more pronounced chromatolysis and reduced neuronal profiles. In the prefrontal cortex, control animals demonstrated intense Nissl staining with preserved neuronal morphology. The 0.5 mg/kg group showed mild chromatolysis and reduced staining intensity, while the 1.0 mg/kg group demonstrated moderate chromatolysis and neuronal morphological alterations. The 1.5 mg/kg group showed marked reduction in Nissl staining and pronounced chromatolytic changes.

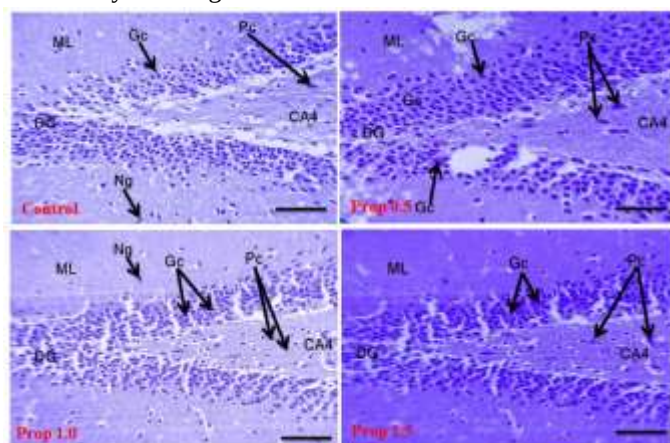


Figure 9a. Representative photomicrographs of the dentate gyrus following repeated propofol administration (Cresyl Violet, ×400).

Control group showing intense Nissl staining and preserved neuronal morphology. Propofol 0.5 group showing mild chromatolysis and reduced staining intensity. Propofol 1.0 group showing moderate chromatolysis and reduced neuronal staining. Propofol 1.5 group showing pronounced chromatolysis and reduced neuronal profiles. DG: Dentate gyrus, GC: Granule Cell, ML: Molecular layer, PC: Pyramidal Cell, Ng: Neuroglia, CA4: Cornu ammonis 4 region.

Discussion

The present study demonstrates that repeated propofol administration was associated with alterations across several behavioural, circulating biochemical, and brain histomorphological endpoints. However, the effects were not uniform across all measures, indicating a complex and non-linear response rather than a consistent dose-dependent adverse effect.

Body-weight change did not differ significantly among the experimental groups despite a significant alteration in feed intake. This suggests that, under the present exposure conditions, repeated propofol administration did not produce a statistically demonstrable overall effect on body-weight change during the

study period. Feed intake, however, showed a non-linear pattern, with increased intake at 0.5 mg/kg and reduced intake at 1.0 and 1.5 mg/kg. The reduction in feed intake at the higher doses occurred alongside numerically lower body-weight gain, although the absence of a significant between-group difference in body-weight change prevents attribution of the latter specifically to altered food consumption or nutrient utilisation. Although there is a dearth of scientific information on the effects of repeated propofol on body weight and feed intake indices this study demonstrated that repeated administration of subanaesthetic doses of propofol can alter feed intake and by extension body weight.

Locomotor and exploratory behaviours showed a similarly non-linear response. The 0.5 mg/kg group exhibited higher line-crossing and rearing frequencies, whereas these measures were reduced at the higher doses. The administration of anaesthetic doses of propofol has been associated with the potentiation of gamma amino butyric acid-A (GABA_A) receptor-mediated inhibitory neurotransmission and with a resultant central nervous system depression [1, 2], this study shows that repeated administration of subanaesthetic doses of propofol is associated with central excitatory effects at the lowest dose and central depressant effects at the two higher doses [1]. The reduction in spontaneous activity observed at the higher doses may therefore reflect greater pharmacological suppression of motor and exploratory activity following repeated exposure. Conversely, the increased activity observed at 0.5 mg/kg may represent a behavioural response to a subanaesthetic exposure rather than evidence of neurotoxicity. Information from extant literature shows the development of paradoxical excitation phenomenon with low or subanaesthetic concentrations of propofol. Temporary motor activation, anxiety relief, or reward-seeking behaviours are by propofol at these doses [1, 23, 24].

The elevated-plus-maze findings demonstrated increased open-arm exploration and reduced closed-arm occupancy following propofol exposure. Subhypnotic doses of propofol have previously been associated with anxiolytic effects [1], and the present findings are therefore compatible with a decrease in anxiety-related [1, 23, 24] behavioural response. Spatial working-memory performance, assessed using spontaneous alternation in the Y-maze, was significantly altered, with the lowest performance observed at the highest propofol dose. Spatial working memory depends on coordinated hippocampal and prefrontal cortical function, making this finding relevant to the histomorphological alterations observed in these regions. Experimental studies have similarly reported cognitive and hippocampal consequences following prolonged propofol exposure, particularly under conditions of increased neuronal vulnerability [23, 24]. The present findings extend these observations by demonstrating an association between repeated

propofol exposure and altered spatial working-memory performance in adult Wistar rats.

The neurochemical findings revealed distinct responses among dopamine, acetylcholine and serotonin. Circulating dopamine showed the most pronounced and progressive alteration, decreasing from 71.19 ± 0.47 pg/mL in controls to 43.49 ± 0.37 pg/mL at the highest propofol dose. Dopamine contributes to locomotor activity, motivation, attention and cognitive processing [18]; consequently, the reduction in circulating dopamine occurred alongside reduced locomotor and exploratory activity at the higher doses. The direction of the present dopamine finding differs from some observations following acute propofol exposure. For example, microdialysis studies have reported increased cortical dopamine and serotonin activity during propofol infusion [25, 26]. This apparent discrepancy may reflect important differences in experimental design, including acute versus repeated exposure and regional brain measurements versus whole brain concentrations. Acetylcholine concentrations increased at the medium and high propofol doses, with the highest mean concentration observed at 1.0 mg/kg. Acetylcholine has important roles in attention, learning and memory, and alterations in cholinergic signalling have been implicated in cognitive responses associated with anaesthesia. Experimental evidence indicates that propofol can interact with cholinergic mechanisms, although the direction of these effects varies according to the experimental model and exposure conditions [2, 27]. The non-linear increase in acetylcholine observed in the present study therefore warrants further investigation, particularly using direct measurements of cholinergic markers within specific brain regions. Serotonin also exhibited a non-linear response, with the highest mean concentration occurring at 1.0 mg/kg and a reduction at 1.5 mg/kg. Serotonin is involved in the regulation of anxiety, arousal, mood and behavioural responses [14]. Experimental research has also implicated serotonergic signalling in propofol-associated neuronal effects [24, 25].

The oxidative-stress findings provide additional evidence of altered systemic redox status following repeated propofol exposure. MDA concentration increased significantly, with the highest mean concentration observed at 1.5 mg/kg, suggesting increased lipid peroxidation at the highest exposure level. Interestingly, TAC also increased markedly at 1.5 mg/kg. The concurrent increase in MDA and TAC should not be interpreted as evidence of simple antioxidant depletion. Rather, the increase in TAC may represent a compensatory or adaptive response occurring in the presence of increased oxidative challenge. This interpretation is consistent with the recognised antioxidant properties of propofol. Previous studies have demonstrated free-radical-scavenging activity and attenuation of lipid peroxidation by propofol in experimental models [3-5]. More recent work has also implicated cellular redox pathways in the antioxidant

effects of propofol [5]. Thus, the increased TAC observed at the highest dose may reflect activation or recruitment of endogenous antioxidant defences in response to oxidative stress. Nevertheless, because only MDA and TAC were assessed, the specific redox mechanisms underlying these changes cannot be determined from the present study.

The inflammatory findings were similarly selective. Serum TNF- α concentration was significantly reduced following propofol administration, whereas IL-1 β did not differ significantly among groups. IL-10 was also significantly reduced at the 1.0 and 1.5 mg/kg doses. Previous experimental studies have demonstrated that propofol can inhibit inflammatory signalling and microglial activation [3, 28]. The reduction in circulating TNF- α observed in the present study is therefore consistent with previously reported anti-inflammatory effects of propofol. However, the concurrent reduction in IL-10 indicates that the inflammatory response cannot be characterised simply as an anti-inflammatory effect. IL-10 is an important regulatory cytokine involved in limiting inflammatory responses, and its reduction may reflect altered immune regulation rather than straightforward suppression of inflammation. Furthermore, because the cytokines were measured in serum rather than brain tissue, the present findings should be interpreted as alterations in circulating inflammatory markers rather than direct evidence of altered neuroinflammation. Recent experimental studies have demonstrated that propofol-associated neuronal alterations may involve inflammatory and intracellular signalling pathways. In developing rats, prolonged propofol exposure has been associated with activation of NLRP3/caspase-1-related pathways, hippocampal injury and cognitive abnormalities [7, 29]. Other experimental work has implicated calpain-TRPC6 signalling in propofol-associated neuronal injury and behavioural abnormalities [30]. These observations provide potential mechanistic contexts for the present findings, although such pathways were not directly investigated in this study and therefore cannot be inferred as mechanisms underlying the observed changes.

The histological findings demonstrated progressive morphological alterations in both the dentate gyrus and prefrontal cortex with increasing propofol dose. The dentate gyrus showed preserved cytoarchitecture in control animals, followed by mild neuronal distortion at 0.5 mg/kg, more pronounced morphological alterations and pyknotic nuclei at 1.0 mg/kg, and marked structural alterations with reduced neuronal profiles at 1.5 mg/kg. A comparable pattern was observed in the prefrontal cortex, where mild neuronal shrinkage and distortion at 0.5 mg/kg progressed to more pronounced morphological alterations, vacuolation, reduced neuronal profiles and neuropil distortion at the higher doses. These observations are broadly consistent with experimental evidence suggesting that prolonged propofol exposure may affect hippocampal neuronal integrity

and neurogenesis [31, 32]. The parallel changes observed in the dentate gyrus and prefrontal cortex are also of potential functional relevance given the involvement of these regions in spatial memory, executive processing and behavioural regulation. However, the present histological assessment was primarily morphological. H&E and Cresyl Violet staining can demonstrate alterations in cellular organisation, neuronal morphology, chromatolysis, pyknosis and vacuolation, but they do not independently establish apoptosis, definitive neuronal death or a specific molecular pathway of neurodegeneration.

Taken together, the behavioural, biochemical and histological findings indicate that repeated propofol exposure was associated with alterations across multiple biological domains. Reduced locomotor and spatial working-memory performance at higher doses occurred alongside alterations in circulating dopamine, acetylcholine and serotonin, increased MDA, altered TAC and changes in selected inflammatory cytokines. These changes were accompanied by progressive histomorphological alterations in the dentate gyrus and prefrontal cortex. Although the temporal and dose-related concurrence of these findings suggests that they may be biologically related, the present experimental design does not establish causal relationships between individual biochemical changes and the observed behavioural or histological outcomes.

The study provides evidence that repeated propofol exposure is associated with alterations in behavioural performance, circulating neurochemical and biochemical parameters, and brain histomorphology in Wistar rats. The non-linear responses observed across several endpoints further indicate that the biological effects of repeated propofol administration cannot be adequately characterised as uniformly dose-dependent. Future studies should incorporate sex-stratified analyses, direct assessment of brain neurotransmitter systems, molecular markers of oxidative stress and neuroinflammation, and specific markers of neuronal integrity and cell death to clarify the mechanisms underlying these observations.

Conclusion

Repeated propofol administration was associated with significant alterations in behavioural performance, circulating neurochemical measures, oxidative balance, inflammatory markers, and brain histomorphology in Wistar rats. These effects included alterations in locomotor and exploratory behaviour, changes in anxiety-related exploration and Y-maze performance, a marked reduction in circulating dopamine, increased acetylcholine at the medium and high doses, non-linear changes in serotonin, increased MDA and TAC, reduced TNF- α and IL-10, and progressive histomorphological abnormalities in the dentate gyrus and prefrontal cortex. Overall, the findings indicate that repeated propofol exposure produces complex, parameter-dependent effects that are not uniformly dose-dependent. The observed behavioural, circulating biochemical,

and histomorphological changes should not be interpreted as definitive evidence of clinical neurotoxicity, particularly because the study did not directly assess neuronal cell death or region-specific brain neurochemistry. Nevertheless, the findings provide experimental evidence supporting further investigation of the long-term consequences, reversibility, and molecular mechanisms underlying repeated propofol exposure.

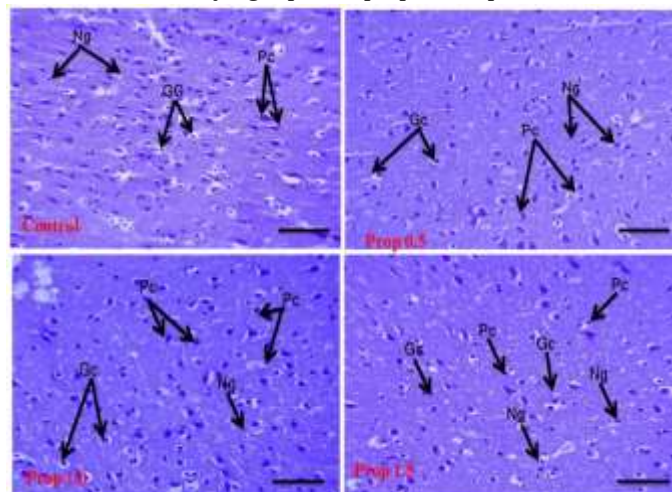


Figure 9b. Representative photomicrographs of the prefrontal cortex following repeated propofol administration (Cresyl Violet, $\times 400$). Control group showing intense Nissl staining and preserved neuronal morphology. Propofol 0.5 group showing mild chromatolysis and reduced staining intensity. Propofol 1.0 group showing moderate chromatolysis and neuronal morphological alterations. Propofol 1.5 group showing marked reduction in Nissl staining and pronounced chromatolytic changes. GC: Granule Cell, PC: Pyramidal Cell, Ng: Neuroglia

Limitations

Several limitations should be acknowledged. First, the biochemical sample size was smaller than the behavioural sample size, which reduces the statistical power and generalisability of the biochemical findings. Second, histological evaluation was primarily descriptive and was not accompanied by quantitative neuronal counting or immunohistochemical assessment of apoptosis, microglial activation, or astrogliosis. Third, the peripheral biochemical measurements may not directly reflect neurotransmitter concentrations or neurochemical activity within specific brain regions. Fourth, the study did not assess whether the observed behavioural, biochemical, or histomorphological changes were reversible following cessation of propofol administration. In addition, the inclusion of both male and female animals without sex-stratified statistical analysis limits interpretation of potential sex-related differences in response to repeated propofol exposure. Future studies should incorporate quantitative stereological assessment and complementary markers such as NeuN, GFAP, Iba-1, cleaved caspase-3, and TUNEL, together with more comprehensive measures of oxidative stress, antioxidant enzymes, and mitochondrial function. Direct assessment of neurotransmitters within relevant brain regions

would also strengthen interpretation of the neurochemical findings. Longitudinal behavioural and biochemical assessments following cessation of propofol administration would further help establish the persistence or reversibility of the observed effects

Acknowledgement

The authors acknowledge the technical staff and laboratory personnel of the Department of Pharmacology and Therapeutics who assisted with animal care, behavioural testing, biochemical analyses and histological processing.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The Authors in this paper declare that there is no conflict of interest related to the content of this manuscript.

Data Availability

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

Ethical Approval

All experimental procedures involving animals were conducted in accordance with institutional guidelines for the care and use of laboratory animals. Ethical approval for the research was granted by the Ethical Committee of College of Health Sciences, LAUTECH with approval number **LCHS/REC-00020/26**.

Received: 25th August, 2026, Revised 1st September, 2026, Accepted: 11th September, 2026, **Published online:25/9/26**

References

- Jeong S, Lee HG, Kim WM, Jeong CW, Lee SH, Yoon MH, Choi JI. Increase of paradoxical excitement response during propofol-induced sedation in hazardous and harmful alcohol drinkers. *Br J Anaesth*. 2011 Dec;107(6):930-3. doi: 10.1093/bja/aer275.
- Chen ZW, Chintala SM, Bracamontes J, Sugawara Y, Pierce SR, Varga BR, Smith EH, Edge CJ, Franks NP, Cheng WWL, Akk G, Evers AS. Three classes of propofol binding sites on GABA_A receptors. *J Biol Chem*. 2024 Oct;300(10):107778. doi: 10.1016/j.jbc.2024.107778.
- Guan S, Sun L, Wang X, Huang X, Luo T. Propofol inhibits neuroinflammation and metabolic reprogramming in microglia *in vitro* and *in vivo*. *Front Pharmacol*. 2023 Jun 13;14:1161810. doi: 10.3389/fphar.2023.1161810.
- Li L, Liu X, DU J, Yang W, Fu R, Li Y, Zhao W, Wang H. Propofol mitigates brain injury and oxidative stress, and enhances GABAA receptor $\alpha 1$ subunit expression in a rat model of lithium chloride-pilocarpine induced status epilepticus. *Turk J Med Sci*. 2023 May 25;53(5):1058-1066. doi: 10.55730/1300-0144.5670.
- Fan W, Zhu X, Wu L, Wu Z, Li D, Huang F, He H. Propofol: an anesthetic possessing neuroprotective effects. *Eur Rev Med Pharmacol Sci*. 2015 Apr;19(8):1520-9.
- Kotani Y, Shimazawa M, Yoshimura S, Iwama T, Hara H. The experimental and clinical pharmacology of propofol, an anesthetic agent with neuroprotective properties. *CNS Neurosci Ther*. 2008 Summer;14(2):95-106. doi: 10.1111/j.1527-3458.2008.00043.x
- Zheng H, Xiao X, Han Y, Wang P, Zang L, Wang L, Zhao Y, Shi P, Yang P, Guo C, Xue J, Zhao X. Research progress of propofol in alleviating cerebral ischemia/reperfusion injury. *Pharmacol Rep*. 2024 Oct;76(5):962-980. doi: 10.1007/s43440-024-00620-6.
- Zhang J, Li Y. Propofol-Induced Developmental Neurotoxicity: From Mechanisms to Therapeutic Strategies. *ACS Chem Neurosci*. 2023 Mar 15;14(6):1017-1032. doi: 10.1021/acschemneuro.2c00755.
- Bosnjak ZJ, Logan S, Liu Y, Bai X. Recent Insights Into Molecular Mechanisms of Propofol-Induced Developmental Neurotoxicity: Implications for the Protective Strategies. *Anesth Analg*. 2016 Nov;123(5):1286-1296. doi: 10.1213/ANE.0000000000001544.
- Xiong M, Zhang L, Li J, Eloy J, Ye JH, Bekker A. Propofol-Induced Neurotoxicity in the Fetal Animal Brain and Developments in Modifying These Effects—An Updated Review of Propofol Fetal Exposure in Laboratory Animal Studies. *Brain Sciences*. 2016; 6(2):11. <https://doi.org/10.3390/brainsci6020011>
- Sigurdsson T, Duvarci S. Hippocampal-Prefrontal Interactions in Cognition, Behavior and Psychiatric Disease. *Front Syst Neurosci*. 2016 Jan 26;9:190. doi: 10.3389/fnsys.2015.00190.
- Sheffler ZM, Niculescu AG, Roza E, Vladăncescu O, Grumezescu AM, Teleanu DM. Neurotransmitters-Key Factors in Neurological and Neurodegenerative Disorders of the Central Nervous System. *Int J Mol Sci*. 2022 May 25;23(11):5954. doi: 10.3390/ijms23115954.
- Ravishankar, Gokare A., and Akula Ramakrishna, eds. *Serotonin and melatonin: their functional role in plants, food, phytomedicine, and human health*. CRC press, 2017.
- Gonzales EL, Yang SM, Choi CS, Mabunga DF, Kim HJ, Cheong JH, Ryu JH, Koo BN, Shin CY. Repeated neonatal propofol administration induces sex-dependent long-term impairments on spatial and recognition memory in rats. *Biomol Ther (Seoul)*. 2015 May;23(3):251-60. doi: 10.4062/biomolther.2014.120. Epub 2015 May 1.
- Onaolapo, A. Y., O. J. Onaolapo, and P. U. Nwoha. "Methyl aspartylphenylalanine, the pons and cerebellum in mice: An evaluation of motor, morphological, biochemical, immunohistochemical and apoptotic effects." *Journal of chemical neuroanatomy* 86 (2017): 67-77.
- Olofinnade AT, Onaolapo OJ, Onaolapo AY Clomipheneletrozole administration can modulate neurobehavioural and neurochemical indices in hyperandrogenic female rats. *Acta Bioscientia* 2025;1(3):111-122. <https://doi.org/10.71181/actabioscientia12260>
- Falade, Joshua, Adejoke Y. Onaolapo, and Olakunle J. Onaolapo. "Evaluation of the behavioural, antioxidative and histomorphological effects of folic acid-supplemented diet in dexamethasone-induced depression in mice." *Central Nervous System Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Central Nervous System Agents)* 21.1 (2021): 73-81.
- Onaolapo, Olakunle James, and Adejoke Yetunde Onaolapo. "Acute low dose monosodium glutamate retards novelty induced behaviours in male Swiss albino mice." *Neurosc Behav Health* 3, no. 4 (2011): 1-56.
- Onaolapo, Olakunle J., Adejoke Y. Onaolapo, Tope A. Omololu, Adedunke T. Oludimu, Toluwale Segun-Busari, and Taofeeq Omoleke. "Exogenous testosterone, aging, and changes in behavioral response of gonadally intact male mice." *Journal of Experimental Neuroscience* 10 (2016): JEN-S39042.
- Onaolapo, Olakunle J., Adejoke Y. Onaolapo, Moses A. Akanmu, and Gbola Olayiwola. "Caffeine/sleep-deprivation interaction in mice produces complex memory effects." *Annals of Neurosciences* 22, no. 3 (2015): 139.Onaolapo OJ, Odeniyi AO, Jonathan SO, Samuel MO, Amadiogwu D, Olawale A, Tiarniyi AO, Ojo FO, Yahaya HA, Ayeni OJ, Onaolapo AY. An Investigation of the Anti-Parkinsonism Potential of Co-enzyme Q₁₀ and Co-enzyme Q₁₀ /Levodopa-carbidopa Combination in Mice. *Curr Aging Sci*. 2021;14(1):62-75. doi: 10.2174/1874609812666191023153724.
- Onaolapo AY, Akinrinde ES, Wahab KA, Olofinnade AT, Onaolapo OJ, Histopathological and Computational Histomorphometric Evaluation of the Protective Effects of Quercetin in High-Fat High-Sugar Diet-Induced Metabolic Syndrome in Wistar Rats *Acta Bioscientia* 2026;2(4):458-472. <https://doi.org/10.71181/actabioscientia12610>
- Abe T, Kurabe M, Nakamura Y, Sasaki M, Seino Y, Furutani K, Ueno M, Baba H. Propofol regulates arousal by enhancing inhibitory synaptic transmission of noradrenergic neurons in the locus coeruleus of adult male mice. *Sci Rep*. 2025 Oct 28;15(1):37725. doi: 10.1038/s41598-025-22005-2.

24. Huang J, Hu Y, Jing S, Bai F, Long Z, Wu Z, Fang L, Cao L, Deng Y, Bao X, Li H. Propofol Promotes Anesthesia Through the Activation of Centrally-Projecting Edinger-Westphal Nucleus Urocortin 1-Positive Neurons. *Neurosci Bull.* 2025 Jun;41(6):1109-1114. doi: 10.1007/s12264-025-01401-8.
25. Shyr MH, Tsai TH, Yang CH, Chen HM, Ng HF, Tan PP. Propofol anesthesia increases dopamine and serotonin activities at the somatosensory cortex in rats: a microdialysis study. *Anesth Analg.* 1997 Jun;84(6):1344-8. doi: 10.1097/0000539-199706000-00031.
26. Wang Y, Yu T, Yuan C, Yuan J, Luo Z, Pan Y, Zhang Y, Zhang Y, Yu B. Effects of propofol on the dopamine, metabolites and GABAA receptors in media prefrontal cortex in freely moving rats. *Am J Transl Res.* 2016 May 15;8(5):2301-8.
27. Chen L, Yang ZL, Cheng J, Zhang PP, Zhang LS, Liu XS, Wang LC. Propofol decreases the excitability of cholinergic neurons in mouse basal forebrain via GABA_A receptors. *Acta Pharmacol Sin.* 2019 Jun;40(6):755-761. doi: 10.1038/s41401-018-0168-6.
28. Abdolmohammadi P, Bietar B, Zhou J, Lehmann C. Mechanisms of Action of Propofol in Modulating Microglial Activation in Ischemic Stroke. *Molecules.* 2025; 30(13):2795. <https://doi.org/10.3390/molecules30132795>
29. Zhang Z, Chen H, Han L, Liu K, Du S, Gao R. Inhibition of the NLRP3/caspase-1 cascade related pyroptosis relieved propofol-induced neuroinflammation and cognitive impairment in developing rats. *Free Radic Biol Med.* 2024 Nov 20;225:87-97. doi: 10.1016/j.freeradbiomed.2024.09.038.
30. She YJ, Xu HP, Gao Y, Wang Q, Zheng J, Ruan X. Calpain-TRPC6 signaling pathway contributes to propofol-induced developmental neurotoxicity in rats. *Neurotoxicology.* 2023 Mar;95:56-65. doi: 10.1016/j.neuro.2023.01.004.
31. Qiao H, Li Y, Xu Z, Li W, Fu Z, Wang Y, King A, Wei H. Propofol Affects Neurodegeneration and Neurogenesis by Regulation of Autophagy via Effects on Intracellular Calcium Homeostasis. *Anesthesiology.* 2017 Sep;127(3):490-501. doi: 10.1097/ALN.0000000000001730.
32. Zhou H, Zhou H, Tan X, Jin Y, Choi TY, Dong C. High-Dose Propofol Induces Cytotoxicity by Elevating Intracellular Ca²⁺ via GABA_A Receptor and IP₃R in HT22 Cells. *Drug Des Devel Ther.* 2026 Mar 10;20:566272. doi: 10.2147/DDDT.S566272.

Cite as: Ajadi EO, Ajao JA, Oladipupo BM, Ajadi MO, Eyitayo FO, Onaolapo AY, Onaolapo OJ. Behavioural, Neurochemical, and Brain Histomorphological Effects of Repeated Propofol Administration in Wistar Rats. *Acta Bioscientia* 2026:2(5);632-644. <https://doi.org/10.71181/actabioscientia12701>