BMC Neuroscience

RESEARCH

Open Access

Diethyl nitrosamine-induces neurobehavioral deficit, oxido-nitrosative stress in rats' brain: a neuroprotective role of diphenyl diselenide

Solomon Owumi[1*], Joseph Chimezie[2], Praise Dyap Emmanuel[1], Anthony Chukwuma Okeibuno[1] and Olatunde Owoeye[3]

Abstract

Diethylnitrosamine (DEN), a common dietary carcinogen, is associated with neurotoxicity in humans and animals. This study investigated the neuroprotective effects of diphenyl diselenide (DPDS) against DEN-induced neurotoxicity in male Albino Wistar rats (n = 40). Rats were randomly distributed into cohorts and treated as follows: vehicle control (corn oil 2 mL/kg; gavage), DPDS-only (5 mg/kg; gavage) and DEN-only (200 mg/kg; single dose *i.p.*). Also, two other rat cohorts were pre-treated with DPDS (3 or 5 mg/kg) for 15 days (day: 0–15), subsequently administered with DEN (200 mg/kg) and continuously treated with DPDS for another 7 days, (days:15–21). Behavioural tests (OFT- using the open field test; NORT- novel object recognition test; FST- forced swimming test and Y-maze) were conducted from days 19–21, followed by biochemical analysis of the hippocampus and prefrontal cortex for oxidative stress, inflammation, neurotransmitter metabolic enzyme, and histopathology. DEN-treated rats exhibited decreased locomotor activity, spatial memory function and antioxidant activity, increased oxidative and nitration stress, anxiety, and depressive-like behaviour, causing histoarchitectural damage in prefrontal and hippocampal cortices. DPDS treatment (pre- and post-DEN exposure) significantly alleviated these neurotoxic, oxidative, and nitration effects, reversed DEN-induced histopathological alterations, and improved locomotive and cognitive functions. In conclusion, DPDS demonstrates potent neuroprotective effects against DEN-induced toxicity, likely through enhanced endogenous antioxidant capacity that mitigates oxido-nitrative damage. These findings suggest that the organo-selenium -DPDS- is a promising chemotherapeutic agent potent in alleviating DEN-mediated neurotoxicity and maintaining brain health.

Keywords Diethylnitrosamine, Diphenyl diselenide, Behavioural deficit, Oxido-nitrosative stress

*Correspondence:
Solomon Owumi
zicri@hotmail.com
Full list of author information is available at the end of the article

Introduction

Diphenyl diselenide (DPDS) is an organo-selenium compound known for its antioxidant [1, 2], anti-inflammatory [2, 3], chemoprotective [4, 5], antidepressant, and memory-enhancing properties [6, 7]. DPDS increases endogenous glutathione peroxidase-related activity by mediating antioxidant signalling mechanisms involved in the activation of nuclear factor erythroid 2-related factor 2 (Nrf2) nuclear translocation, thereby promoting the expression of heme oxygenase 1 (HO-1) and the thioredoxin antioxidant defence system [3, 8]. DPDS is known to suppress the I kappa B kinase/Nuclear Factor-kappa B (IκB/NF-κB) pathway and activate the nucleotide-binding oligomerisation domain containing leucine-rich repeats (NOD- LRR)- and pyrin domain-containing protein 3 (NLRP3) inflammasome in amyotrophic lateral sclerosis (ALS) model [9], leading to the deactivation of microglia and contributes to neuroprotective activity. Previous studies have shown that DPDS exhibits neurobehavioral benefits and enhances cholinergic transmission [10, 11]. The role of DPDS in suppressing Amyloid-β1 (Aβ1)-42 and heat shock protein 16.2 (hsp-16.2) expression in the *Caenorhabditis elegans* model has also been reported [12]. DPDS has garnered attention for its ability to combat oxidative stress, reduce neuronal damage and demonstrate promising results in various experimental models of neurodegenerative disorders [3, 9, 12, 13]. Moreover, DPDS presents favourable pharmacokinetic profiles and low toxicity, thus enhancing DPDS's appeal as a candidate for therapeutic intervention. DPDS offers potential therapeutic strategies to preserve neuronal function and mitigate the adverse effects of environmental and food-based carcinogenic exposure on neurological health. Despite the above-mentioned advantages, the exploration of DPDS as a neuroprotective agent against Diethyl nitrosamine (DEN)-induced neurotoxicity remains unknown.

DEN is a neurotoxin commonly found in various substances, such as pharmaceutical agents, cigarette smoke, industrial waste, agricultural chemicals, cosmetics, and fried and cured foods [14, 15]. Regrettably, it is challenging to avoid inadvertent exposure to DEN owing to its ubiquity. Daily exposure to DEN at these doses of 0.04 and 2.2 μg/kg/day ultimately results in mutation and the onset of cancer, respectively [16–18]. DEN-induced neurodegeneration has been linked to either direct neurotoxic injury, neural injury or hepatotoxicity [15], incumbent on DEN's metabolic biotransformation by the cytochrome P_{450} (CYP_{450}) enzymes, producing a highly toxic reactive ethyl radicals ($CH_3CH_2^+$) metabolite [15]. The generated ethyl radicals exacerbate reactive oxygen species (ROS) and xanthine oxidase system while covalently interacting with cellular macromolecules, leading to deoxyribonucleic acid (DNA) damage, lipid peroxidation (LPO), protein damage, and downregulating antioxidant signalling pathways [15, 19]. Particularly, DEN-caused AD-type neurodegeneration may be linked to mutagenicity and carcinogenesis via the alkylation mechanism, which increases oxide-nitrative and inflammatory stressors, furthering DNA damage [19, 20]. The preceding biochemical derangement promotes a permissive microenvironment implicated in neurobehavioral deficits [19, 21–24], cognitive challenges [25] and disruption of normal neuronal integrity and function [26]. However, based on documented literature, DEN-induced deficits in spatial working memory in animal models are scanty, suggesting that the effect of DEN on cognition and its mechanism is yet to be well explored.

Nevertheless, no study has shown the role of DPDS on DEN-induced neurotoxicity in rats despite extensive literature reports on oxidative stress- a hallmark of DEN-induced neurotoxicity. We, therefore, hypothesised and investigated a likely role for DPDS in abating DEN-induced neuronal toxicity in albino Wistar rats. Subsequently, we assessed rats' neuro-behaviour, and the brain's prefrontal and hippocampal cortices by histopathology. We also use biochemical toxicology approaches to evaluate the experimental animals' oxidative and inflammatory status and acetylcholinesterase activity. Finally, as we report here, this study deciphers the underlying mechanisms of DPDS as a likely neuroprotective-enhancing agent.

Materials and methods

Chemicals

Diphenyl diselenide ($C_6H_5SeSeC_6H_5$; 1666–13-3, ≥ 98.0%), Diethyl nitrosamine ($(C_2H_5)_2NNO$, 55–18-5) were obtained from Sigma-Aldrich, (St. Louis, MO, Co. USA). All other chemicals used in this study were of analytical grade.

Experimental animals

Experimental male albino Wistar rats (170 ± 10 g; n = 40, age of 10 weeks) were used for this study. The rats were procured from the University of Ibadan Central Animal Facility and housed in polycarbonate cages in the Department of Biochemistry Experimental Animal House. They were subjected to a 12 h light/dark photoperiod and standard room temperature to acclimatise. During this period, the rats were fed a standard rodent diet (Breedwell ™ Feeds, Ibadan, Nigeria) and free access to clean tap water. All the experimental protocols followed the Guide for the Care and Use of Laboratory Animals, United States National Institute of Health" and were approved by the University of Ibadan Experimental Animal Use Ethical Committee (UI-ACUREC/068-0524/06).

Experimental design and treatments

After acclimatising the experimental animals for one week, the rats were randomly assigned to five (5) groups of eight rats each and treated as depicted below. Group 1 received the vehicle (corn oil only: 2 mL/kg/); Group 2 received a single intraperitoneal (*i.p.*) dose of DEN (200 mg/kg bw) dispersed in the vehicle corn oil on day 15, and Group 3 received DPDS 5 mg/kg bw orally. Groups 4–5 received diphenyl diselenide (DPDS) orally for 21 consecutive days and a single *i.p.* dose of DEN (200 mg/kg) on day 15. This experiment's experimental dose for DEN [27] and DPDS [7, 10, 28] follows previously published doses. While corn oil served as the vehicle for both DEN and DPDS, as depicted in Fig. 1 and outlined below:

Group 1: Untreated Control (Corn Oil (vehicle) only 2 mL/kg; *p.o*).

Group 2: DEN only (200 mg/kg; single dose *i.p;* on day 15).

Group 3: DPDS only (5 mg /kg; *p.o;* daily for 21 days).

Group 4: DPDS (3 mg /kg; *p.o* daily for 21 days) + DEN (200 mg/kg; single dose *i.p;* on day 15).

Group 5: DPDS (5 mg /kg; *p.o* daily for 21 days) + DEN (200 mg/kg; single dose *i.p;* on day 15).

All treatments were carried out daily between 9:00 am and 10:30 am for 21 days.

Behavioural test

Behavioural tests were conducted using the Y-maze test, elevated plus maze (EPM), open field test (OFT), NORT, and FST on days 19, 20, and 21 h. after corn oil and DPDS administration (Fig. 1).

Y-maze test

The Y-maze apparatus was used to evaluate the effects of DPDS on spatial learning memory in rats [29, 30]. The apparatus consisted of three similarly spaced arms labelled A, B, or C (41 long × 10 wide × 10 high cm) at 120° and elevated 50 cm above the ground. On day 19, each rat was placed in one of the arm compartments and allowed to move freely for 5 min using a video recorder. An entry into all three arms consecutively defined the sequence of arm entries and correct alternation (ABC, ACB, BCA, CAB, CBA, and BAC). For accuracy, the total number of arm entries, alternate entries, track plots, and heat maps were measured using the ANY-maze version 7.4 tracking software. The frequency of alternate arm entry (AAE) was recorded, and the percentage of spontaneous alternation (PSA) was calculated as the ratio of correct alternations to the total number of arms entered minus two, multiplied by one hundred. The apparatus was cleaned with 70% alcohol and allowed to dry between the sessions.

Elevated plus maze (EPM)

The elevated plus maze test was conducted to evaluate the anxiety-like activity of DPDS in rats [31]. The EPM apparatus consisted of two open arms (50 × 10 × 40 cm) and two closed arms (50 × 10 cm) connected perpendicularly with a centre zone (10 × 10 cm) elevated 50 cm above ground level. Briefly, on day nineteen, each animal was

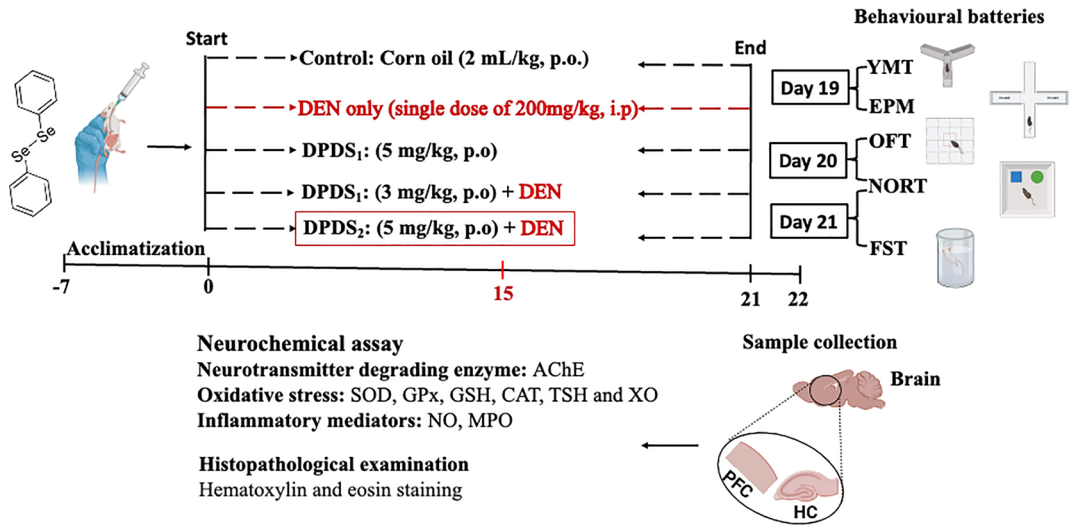

Fig. 1 Effect of DPDS pre-treatments on DEN intoxicated rats experimental protocol. *DEN* Diethyl nitrosamine; *DPDS* Diphenyl diselenide; *YMT* Y-maze test; *EPM* elevated plus maze, *OFT*, open field test, *NORT* novel object recognition test, *FST* forced swiming test, *AChE* acetylcholinesterase, *SOD* superoxide dismutase, *CAT* catalase, *GPx* glutathione peroxidase, *GSH* reduced glutathione, *CAT* catalase total sulfhydral, *TSH* xanthine oxidase, *XO* nitric oxide, *MPO* myeloperoxidase

placed in the centre zone and allowed to explore all arms for approximately 5 min, and the video was recorded. Two trained observers blinded to the experimental treatment recorded the time spent in the open and closed arms and were located away from the animal view. The olfactory cues left by the previously tested animals were cleaned using 70% alcohol after each investigation.

Open field test (OFT)

The open field test (OFT) assessed locomotive and exploratory behaviours using a wooden open field area (100 × 100 × 60 cm). On day twenty, each experimental animal was placed in the centre of the OFT area and allowed to explore freely for approximately 10 min using a video recorder. Pre-recorded videos were analysed using ANY-maze software version 7.4, and the distance travelled, mean speed, number of line crossings, path efficiency, tract plot, and heat map were obtained. The olfactory cues left by the previously tested animals were cleaned using 70% alcohol after each investigation.

Novel recognition test (NORT)

The novel object recognition test was used to assess recognition memory function, as previously described by [32]. NORT was conducted briefly in a wooden open field area (100 cm × 100 cm × 60 cm) for 4 h. Following the habituation of the animal in the open field for 10 min without the objects, each rat was trained and placed singly in the OFT apparatus with two identical objects (object A) for 10 min for object familiarisation. Meanwhile, on day twenty-one, after object familiarisation, the animals were exposed to two identical objects (object A and object B) for a test period of 5 min using a video recorder. Pre-recorded videos were analysed using ANY-maze software version 7.4, and the time spent exploring the novel object (B), the familiar object, the tract plot, and the heatmap were obtained. The object recognition memory function was calculated mathematically as an explorative index: explorative index (EI) = (time spent in novel object)/(total object exploration time) and discrimination index (DI) as time exploring novel object (B) minus time exploring familiar object (A) to total object exploration time. The olfactory cues left by the previously tested animals were cleaned using 70% alcohol after each investigation.

Force swimming test (FST)

The FST was employed to indicate the anti-depressive-like activity of DPDS, as described by [33]. On day 21, after 4 hours, the NORT, FST was performed using clear polyvinyl chloride (PVC) cylinders (50 cm tall × 20 cm diameter) containing ¾-filled water and a standard temperature of 23–25 °C. Each rat was singly immersed in water and allowed to swim freely for 6 min. Immobility time was recorded for the last 4 min by two trained researchers blinded to the treatment protocol when the rat showed no further activity other than that required to keep its head above water. The water was systematically renewed at the end of each experiment.

Biochemical assay

Rats were sacrificed at the end of the experimental timeline and behavioural tests, following 12–13 h fasting by decapitation under carbon dioxide asphyxiation. The brain tissue of each experimental animal was removed, rinsed in ice-cold potassium chloride (KCl) solution and sectioned into the prefrontal cortex and hippocampus. The brain sections were homogenised in 0.1 M Phosphate buffer (1:9 w/v, pH 7.4) and centrifuged at 10,000 rpm for 15 min at 4 C to obtain supernatants for biochemical assay, including acetylcholinesterase activity, oxidative and nitrosative stress biomarkers.

Measurement of brain acetylcholinesterase (AChE)

The supernatant of the prefrontal and hippocampal cortices was used to evaluate cholinergic function by measuring AChE activity in the brain regions, as previously reported by [34]. Briefly, 0.4 mL of brain supernatant was combined with 2.6 mL of sodium phosphate buffer (0.1 M, pH 7.4), 0.1 mL of DTNB (5′,5′-dithiobis-2-nitrobenzoic acid), and 0.1 mL of acetylthiocholine iodide. The colour change indicates AChE activity resulting from the reaction between thiocholine and DTNB. The absorbance of the reaction mixture at 412 nm was measured using a UV spectrophotometer (752P UV–VIS).

Determination of brain antioxidant biomarkers

Following the sacrifice of the experimental animal under light ether anaesthesia and CO_2 asphyxiation [35–37], the brain was carefully excised and, under ice-cold conditions, homogenised in 50 mM Tris–HCl buffer at a pH of 7.4. The supernatant was obtained upon cold centrifugation at 4 C and 12,000 g for 15 min and afterwards used for biochemical analysis. Total protein concentration from the prefrontal and hippocampal was evaluated according to Bradford's previously reported method [38, 39]. Briefly, 50 µl of the prefrontal and hippocampal cortex supernatant, 1.95 mL of distilled water, and 3 mL of Bradford reagent were added to the test tube to obtain a mixture incubated at room temperature for 30 min and read at 540 nm and protein concentration was determined using the standard curve.

Reduced glutathione (GSH) estimated in 5, 5′-dithiobis-2-nitrobenzoic acid (DTNB; Ellman's reagent) as previously reported by Jollow et al. [40] by adding 80 µL of sample was added into 80 µL of precipitate solution

(sulfosalicylic acid, 4%) incubated at 4 °C for 10 min and centrifuged at 4000 rpm, 4 °C for 5 min. Then, 50 µL supernatant mixed with 150 µL of DTNB (0.1 M, pH 8.5) incubated for 5 min were read at 412 nm absorbance against blank (GSH stock solution; 40 mg of GSH; 0.1 M phosphate buffer) after 10 min.

The glutathione peroxidase (GPX) activity was measured according to the procedure described by Rotruck et al. [41]. The mixture consists of 10 mM sodium azide, 4 mM GSH, 10% trichloroacetic acid, 2,5 mM hydrogen peroxide, 0.1 M pH 7.4 phosphate buffer and 50 µL of sample respectively, incubated for 3 min at 37 C and centrifuge for 3000 rpm for 5 min to obtain a supernatant mixture. 50 µL of supernatant mixture and distilled water was added to 100 µL Dipotassium hydrogen orthophosphate (0.3 mM), and 50 µL of Ellman's DTNB and absorbance was read against blank at 412 nm. GPx activity was expressed as µmoles of residual GSH/mg protein.

Catalase (CAT) activity was determined according to the method of Claiborne [42] following the addition of 2.95 mL phosphate buffered hydrogen peroxide and 50 µL of sample or standard in a cuvette, mixed gently by inversion and read at 240 nm absorbance for 5 min.

Using Misra and Fridovich's method [43], Superoxide dismutase (SOD) activity was determined by adding 2.5 mL of carbonate buffer (0.05 M, pH 10.2) in a cuvette with 50 µL of sample or standard and 0.3 mL of epinephrine was added. Meanwhile, the change in absorbance was recorded at 480 nm every 30 s for 2 min.

Glutathione-S-transferase (GST) activity was according to the Habig et al. method [44] by adding a mixture of 10 µL of 1-chloro-2,4-dinitrobenzene (CDNB), 170 µL of the reaction mixture and 20 µL of samples or standard in a 96 healthy plate and read at 340 nm absorbance for 3 min every 30 s.

Total Sulfhydryl group levels were determined using dithionetrobenzoic acid (DTNB) in the prefrontal and hippocampal cortices supernatant [45, 46]. Briefly, 130 µL of reaction mixture consisting of sample, phosphate buffer and distilled water in a ratio of (3:2:5) was mixed with 15 µL of Ellman's reagent incubated at room temperature for 2 min and absorbance read at 412 nm.

Determination of neural oxido-inflammatory responses

The brain oxidant biomarker, such as xanthine oxidase (XO), was assessed using the Bergmeyer et al. method [47] briefly, 80 µL of xanthine solution (0.15 m M, pH 7.5) was pipetted into 8 µL of sample into a microplate and absorbance measured for 3 min at 290 nm, xanthine oxidase activity expressed as µmole/min/mg protein.

Nitric Oxide (NO) levels were estimated using Griess reagent, and sample supernatant aliquots (1:1) were mixed and incubated for 15 min, and absorbance read at 540 nm [48]. The total nitrite level was calculated from the absorbance obtained from a standard solution, and the results were expressed in units per mg protein.

The level of myeloperoxidase (MPO) activity was evaluated in *O*-dianisidine dihydrochloride as previously described [49, 50], using the prefrontal and hippocampal tissue homogenate. Briefly, 200 µL of O-dianisidine dihydrochloride was mixed with7 µL of sample in a microplate reader, and absorbance was measured at 30 s intervals for 4 min at 460 nm. MPO activity was expressed in µmol/mg protein.

Histological protocol

Following sacrifice, the brain tissue was dissected, submerged in 10% neutral buffered formalin, and processed for paraffin embedding. Brain sections were cut using a microtome and stained with hematoxylin and eosin (H&E) [51]. Light microscopy was used to determine the histoarchitecture of the prefrontal and hippocampal cortices at 400X magnification. The number of prefrontal and hippocampal cortices (CA3) pyramidal dark stained neurons were counted (n = 3/group of 5 photomicrographs average) using ImageJ software.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9.0. One-way analysis of variance (ANOVA) was used to determine the difference between the five groups, and means were compared using the Tukey multiple comparison test. All data obtained from this study (biochemical and histomorphometry) were expressed as mean ± SEM. The results were considered statistically significant at *$P < 0.05$ vs control or DEN untreated cohort.

Results

DPDS treatment prevented DEN-induced locomotive deficit

The impact of DPDS on rat's locomotive and explorative behaviour is displayed in Fig. 2 (a & b). Compared to the control, animals treated with a neurogenic dose of DEN (200 mg/kg) resulted in a significant increase ($p < 0.05$) in path efficiency [$F(4, 20) = 70.10$, $P < 0.0001$] and a decrease in distance travelled [$F(4, 20) = 46.76$, $P < 0.0001$] and mean speed $F(4, 20) = 29.11$, $P < 0.0001$]. The DEN-treated group also observed deterioration in the track plot and heat map. However, DPDS treatment attenuated DEN-induced reduction in locomotive and explorative activities by significantly decreasing the path efficiency and increasing the distance travelled and mean speed.

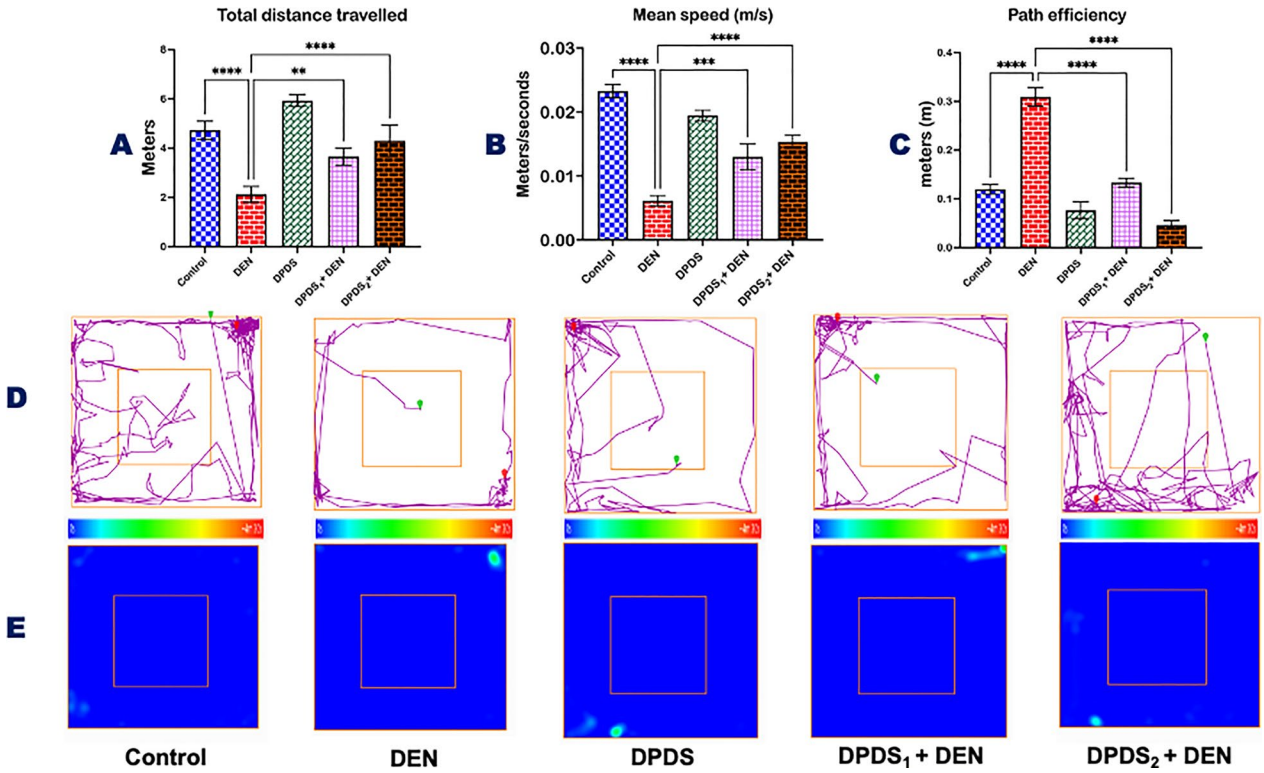

Fig. 2 Effect of DPDS pre-treatments on locomotive function of DEN intoxicated rats. **A**–**C** Distance travelled, mean speed, path efficiency. **D** Track plot. **E** Heat map. Values are expressed as mean ± SEM (n = 5). **P < 0.01, ***P < 0.001, ****P < 0.0001. compared to the control and DEN-only groups. control, DEN Diethyl nitrosamine, or DPDS Diphenyl diselenide

DPDS prevented DEN-induced anxio-depressive-like behavior

As shown in Fig. 3. DEN-only treated rats observed a significant increase in time spent in the closed arm [F (4, 20) = 16.58, P < 0.0001) and a significant decrease ($p < 0.05$) in the open arm [F (4, 20) = 191.0, P < 0.0001] of the elevated plus maze compared with the control. DEN-only treated cohort observed a significant

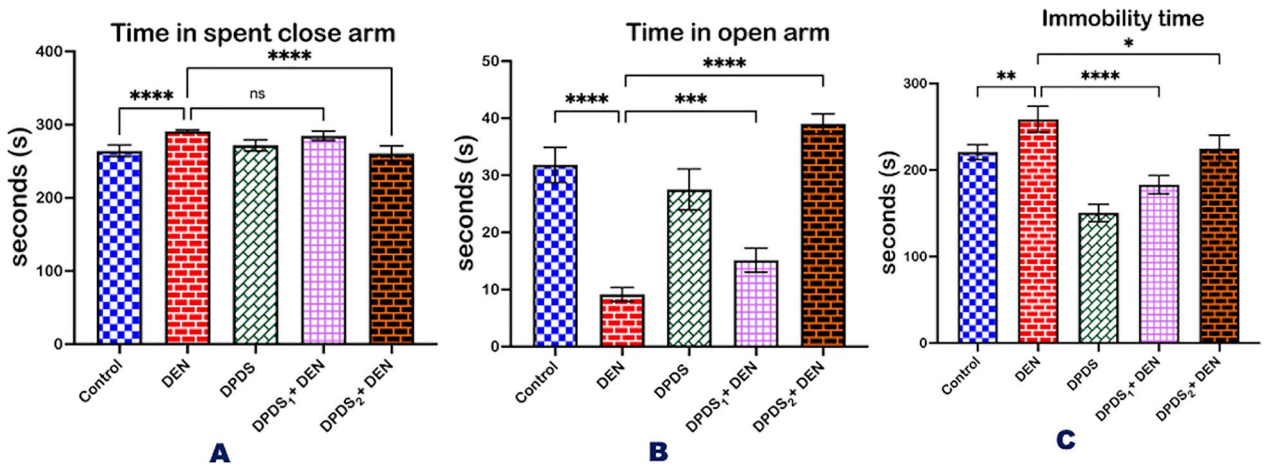

Fig. 3 Effect of DPDS pre-treatments on anxious-depressive behaviour of DEN intoxicated rats. **A** Time spent in closed arm, **B** in open arm, **C** Immobility time. Values are expressed as mean ± SEM (n = 5). **P < 0.01, ***P < 0.001, ****P < 0.0001 compared to control and DEN-only group. control, *DEN* Diethyl nitrosamine, *DPDS* Diphenyl diselenide

increase in immobility time on the tail suspension test relative to the control. DPDS treatment annulled the anxiogenic and depression caused by DEN by increasing the time spent in the open arm and reducing immobility time.

DPDS prevented cognitive impairment in DEN-treated experimental rats

As depicted in Figs. 4, 5. Cognitive impairment was observed in DEN-only treated rats as indicated by a significant reduction in the frequency of alternate arm entry [F (4, 20) = 6.438, P = 0.0017] and percentage spontaneous alternation [F (4, 20) = 46.39, P < 0.0001] on y-maze test **(**Fig. 4**),** reduction in the explorative index on the novel object [F (4, 20) = 36.05, P < 0.0001], time spent with a novel object [F (4, 20) = 17.33, P < 0.0001] and an increase in the explorative index on the familiar object [F (4, 20) = 9.768, P = 0.0002] (Fig. 5) compared to control. The representative of the track plot and heat map confirmed DEN-induced cognitive impairment. However, DPDS + DEN treatment reversed DEN cognitive impairment by increasing spontaneous alternation, explorative index on the novel object, time spent with a novel object and decreased explorative index on the familiar object.

DPDS inhibited hippocampal and prefrontal cortex acetylcholinesterase (AChE) activity

The impact of co-exposure to DEN and DPDS on AChE activity in rats' hippocampus and prefrontal cortex is shown in Fig. 6. Compared with the control, animals treated with DEN alone observed a significant increase ($p < 0.05$) in hippocampal and prefrontal cortex AChE activities [F (4, 15) = 12.22, P = 0.0001; F (4, 15) = 11.54, P = 0.0002]. Conversely, the group treated with different doses of DPDS (3 and 5 mg/kg) + DEN showed a significant decrease in AChE activity in both the hippocampal and prefrontal cortices relative to the DEN cohort.

DPDS improved antioxidant enzymes activities in the prefrontal cortex and hippocampus of DEN-treated rats

Hippocampal and prefrontal cortex sample oxidative stress biomarker activities in experimental groups are shown in Figs. 7, 8, respectively. DEN-only treated experimental rats exhibited a decrease ($p < 0.05$) in the SOD [F (4, 15) = 4.635, P = 0.0123; F (4, 15) = 9.562, P = 0.0005], CAT [F (4, 15) = 115.6, P < 0.0001; F (4, 15) = 71.04, P < 0.0001], GSH [F (4, 15) = 8.859, P = 0.0007; F (4, 15) = 32.61, P < 0.0001] and GPx [F (4, 15) = 110.8, P < 0.0001; F (4, 15) = 20.06, P < 0.0001] activity in the hippocampus and prefrontal cortex with only

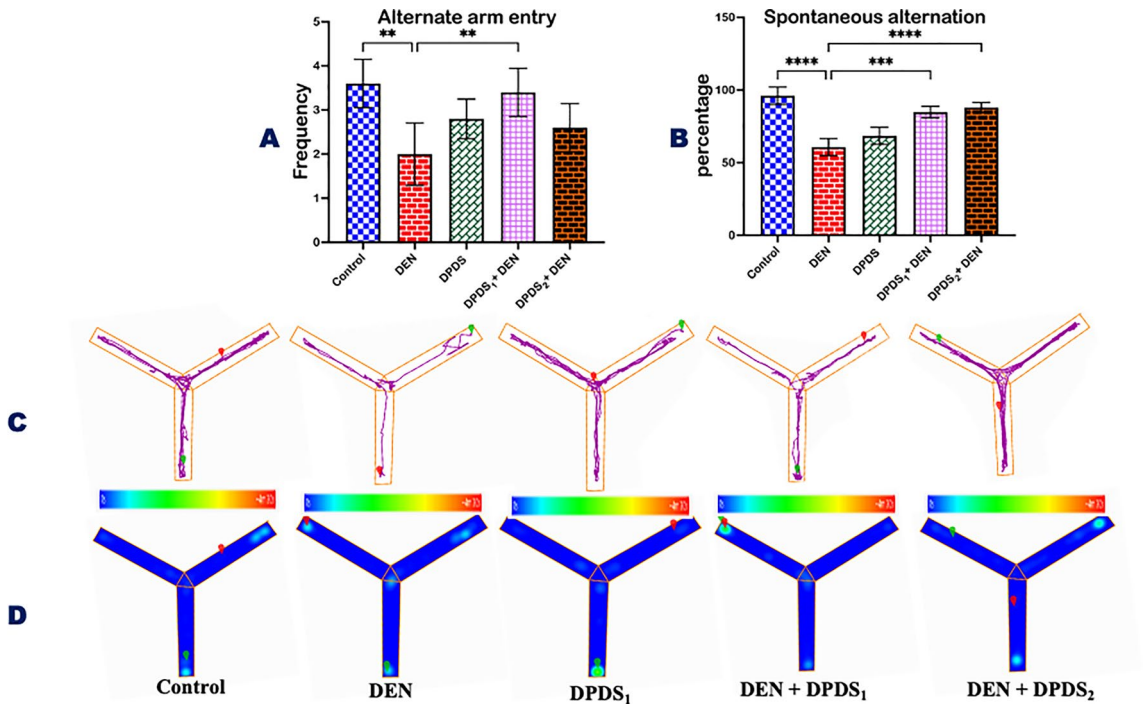

Fig. 4 Effect of DPDS pre-treatments on cognitive behaviours of DEN intoxicated rats on y-maze test. **A** alternate arm entry, **B** spontaneous alternation, **C** track plot, and **D** heat map. Values are expressed as mean ± SEM (n = 5). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 compared to control and DEN-only group. control, DEN Diethyl nitrosamine, or DPDS Diphenyl diselenide

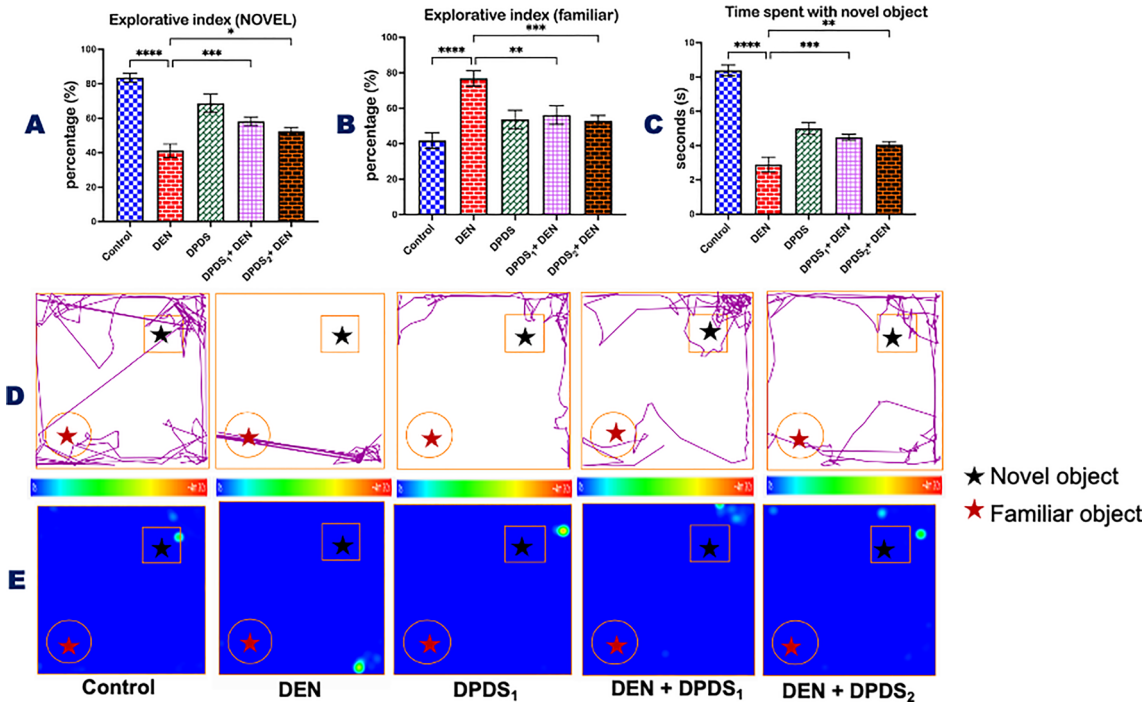

Fig. 5 Effect of DPDS pre-treatments on cognitive behaviours of DEN intoxicated rats on NORT test. **A** explorative index on the novel object, **B** explorative index on the familiar object, **C** time spent with a novel object, **D** track plot, and **E** heat map. Values are expressed as mean ± SEM (n = 5). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 compared to control and DEN-only group. control, DEN Diethyl nitrosamine, or DPDS Diphenyl diselenide. A red star is a familiar object, and a blue star is a novel object

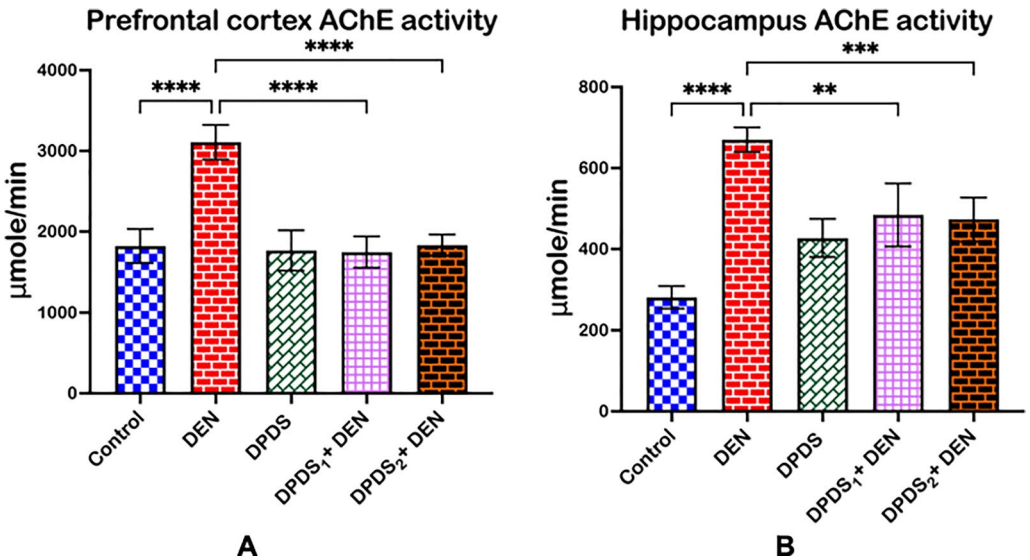

Fig. 6 Effect of DPDS pre-treatments on the prefrontal cortex and hippocampal AChE activities of DEN intoxicated rats. Values are expressed as mean ± SEM (n = 5). **P < 0.01, ***P < 0.001, ****P < 0.0001 compared to control and DEN-only group. *AChE* acetylcholinesterase, control, *DEN* Diethyl nitrosamine, *DPDS* Diphenyl diselenide

a decrease in TSH [F (4, 15) = 3.198, P = 0.0436; F (4, 15) = 19.41, P < 0.0001] activity in the prefrontal cortex relative to control. On the other hand, different doses of DPDS + DEN treated rats lessened the effect of DEN by increasing the activity of SOD, CAT and GPx activity in the prefrontal cortex and hippocampus, with no change

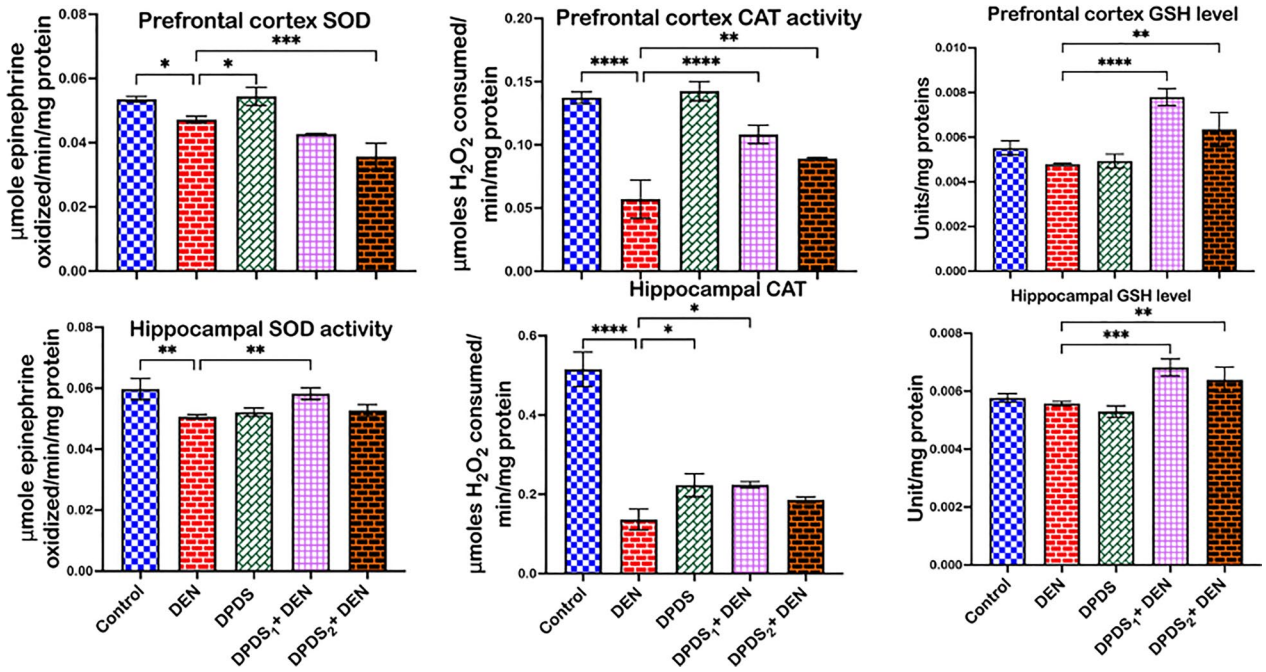

Fig. 7 Effect of DPDS pre-treatments on the prefrontal cortex and hippocampal GPx, CAT, and SOD activities of DEN intoxicated rats. Values are expressed as mean ± SEM (n = 5). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.000 compared to control and DEN-only group. SOD superoxide dismutase CAT catalase, GSH reduced glutathione, DEN Diethyl nitrosamine, DPDS Diphenyl diselenide

in the prefrontal cortex and hippocampal TSH compared to those in rats co-exposed to DEN.

DPDS mitigated oxido-nitrosative biomarkers in the Prefrontal Cortex and Hippocampus of DEN-treated rats

As shown in Fig. 9, compared to the control, rats exposed to DEN-only treated rats observed an increase ($p < 0.05$) in the hippocampal and prefrontal cortex XO [F (4, 15) = 38.73, P < 0.0001; F (4, 15) = 17.77, P < 0.0001], NO [F (4, 15) = 19.26, PP < 0.0001; F (4, 15) = 31.33, P < 0.0001] and MPO [F (4, 15) = 30.23, P < 0.0001; F (4, 15) = 21.50, P < 0.0001] levels. Conversely, rats co-treated with different doses of DPDS (3 and 5 mg/kg) + DEN revealed a significant decrease in the prefrontal cortex and hippocampal XO, NO and MPO compared to DEN.

DPDS reversed prefrontal cortex and hippocampus histological architecture

The control group displayed typical neurons of the prefrontal cortex pyramidal cell. At the same time, DEN-only treated rats showed pyknotic, necrotic and degenerated neurons in the prefrontal cortical pyramidal layer with elevated dark stained neuronal count [F (4, 10) = 12.24, P = 0.0007] relative to the control (Fig. 10). However, different doses of DPDS cotreated with DEN showed normal neurons with mild degeneration in the prefrontal cortex pyramidal layer. Similarly, the hippocampal section CA3 pyramidal layer in the control rat was typical. In contrast, the DEN-only treated rat showed shrinkage of CA3 pyramidal neurons and multiple vacuolations with marked dark stained neuronal count [F (4, 10) = 19.16, P = 0.0001] compared to the control (Fig. 11). DPDS reversed CA3 pyramidal neuronal shrinkage and vacuolations in DEN treatment.

Discussion

Diethylnitrosamine (DEN) is a carcinogen associated with cognitive decline and neurodegeneration [19, 22, 23, 25]. Studies have shown that DEN at low doses can impair skilled motor performance, learning, and memory while increasing biomarkers of neuro-inflammatory by upregulating amyloid-β, phospho-tau, ubiquitin immunoreactivities, tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) in the hippocampus [19, 25, 52]. Clinical studies have also reported cognitive impairments, persistent anxiety, depression, and reduced quality of life among cancer patients and survivors [53–55], particularly those with brain tumors [22, 56, 57].

The neuroprotective role of diphenyl diselenide (DPDS) against DEN-mediated neuronal oxido-nitrosative stress in rats is discussed based on our experimental findings corroborating previous literature. The brain is a complex organ with intricate neuronal connections whose

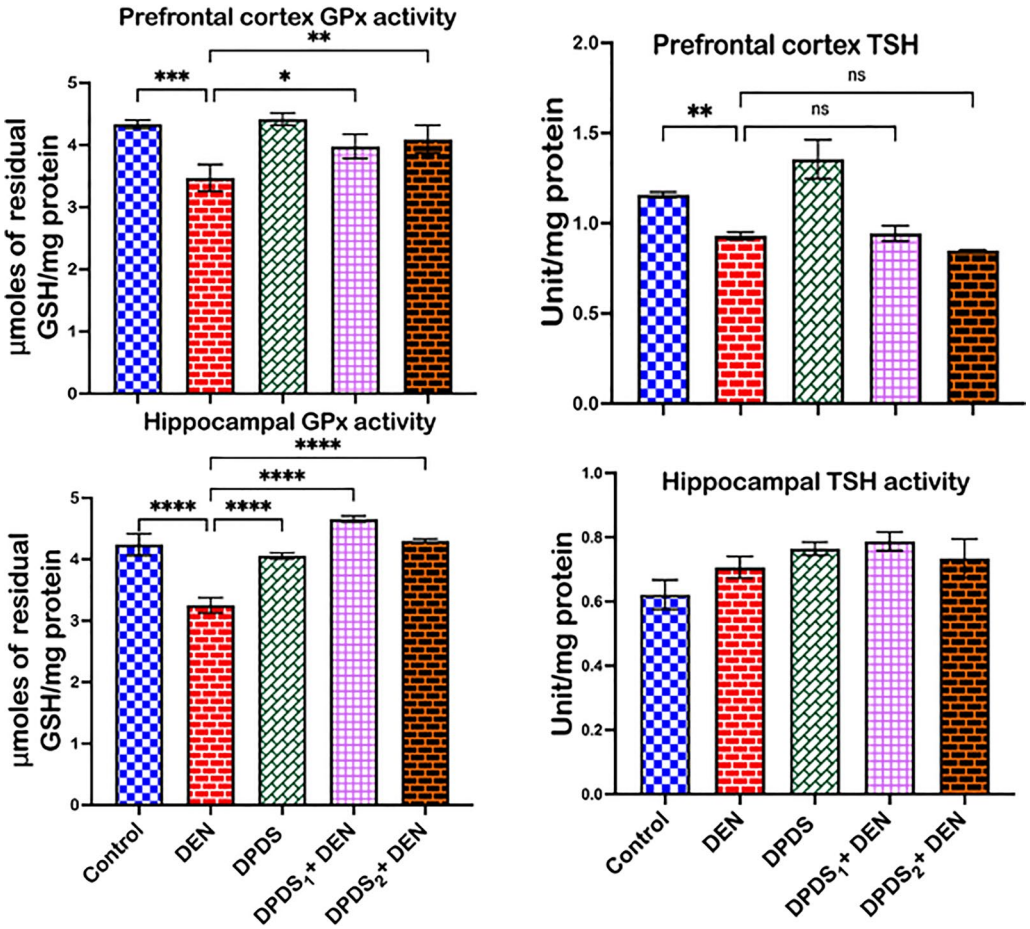

Fig. 8 Effect of DPDS pre-treatments on the prefrontal cortex and hippocampal GPx and TSH activities of DEN intoxicated rats. Values are expressed as mean ± SEM (n = 5). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.000 compared to control and DEN-only group. GPx; glutathione peroxidase, total sulfhydryl; TSH, DEN; Diethyl nitrosamine, DPDS Diphenyl diselenide

functions are influenced by environmental stressors [58], including neurotoxins and carcinogens [59]. Of note are the roles played by the nervous system in carcinogenesis [60–62], modulating tumour tissue immune cells, nerve responses, and neurotransmission, which may influence carcinogenesis and cancer progression [63]. We demonstrated that neurogenic doses of DEN led to hypo-locomotion, anxiogenic-depression-related behaviour and altered spatial working memory in experimental rats. The OFT, EPM, FST, YMT and NORT used in this study are well-known behavioural batteries that provide valuable information on neurobehavioral phenotypes. Rats treated with DEN alone exhibited impairment in their learning and memory, as previously reported [32, 64, 65], locomotive and explorative dysfunction, and anxio-depressive-like symptoms in rodent models [66–68]. Another analogue of DEN, N-Nitrosodiethylamine (NDMA), has been reported to cause a remarkable decrease in neuromuscular strength and cognition in treated mice [23, 25, 26]; on the other hand, DPDS reduced locomotive deficit, cognitive impairment and anxiety-depressive-like behaviour in our experimental model of DEN-induced neuronal toxicity; similarly, DPDS has been reported to alleviate toxicity in different rodent models [69–71]. Relative to the untreated control, groups of rats treated with DPDS alone exhibited increases in total locomotive activity, lessened anxio-depressive-like behaviour and improved spatial memory and learning, suggesting an improvement beyond baseline motor and cognitive functions, aligning with the previous report of Stangherlin et al., [72].

Similarly, DEN-induced hepatocarcinoma has been associated with psychological stress and alteration in brain neurotransmitters, including noradrenaline and dopamine [73]. Similarly, NDMA has also been reported to downregulate neurotransmitters, including dopamine, serotonin and acetylcholinesterase [26]. To underscore the mechanism involved in the neurotoxic effect of DEN

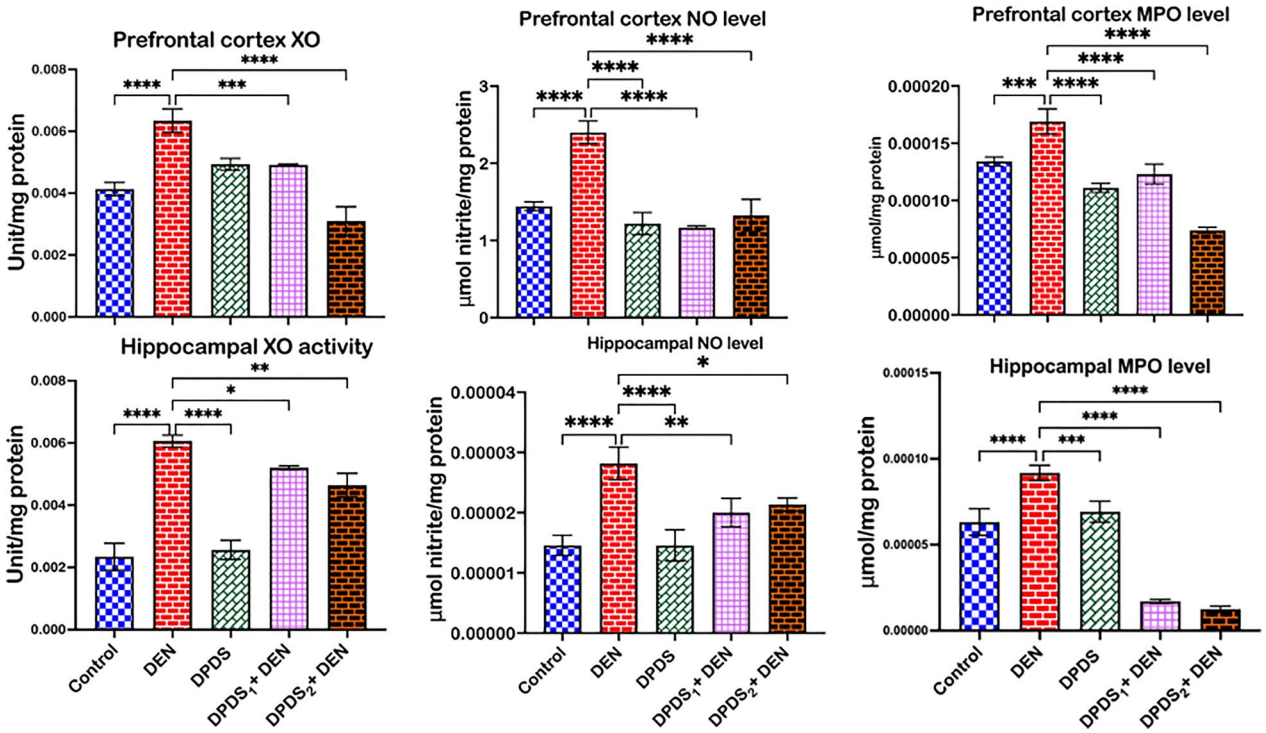

Fig. 9 Effect of DPDS pre-treatments on the prefrontal cortex and hippocampal XO, NO and MPO level of DEN intoxicated rats. Values are expressed as mean ± SEM (n = 5). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.000 compared to control and DEN-only group. xanthine oxidase XO, NO nitric oxide, MPO myeloperoxidase, DEN Diethyl nitrosamine, DPDS Diphenyl diselenide

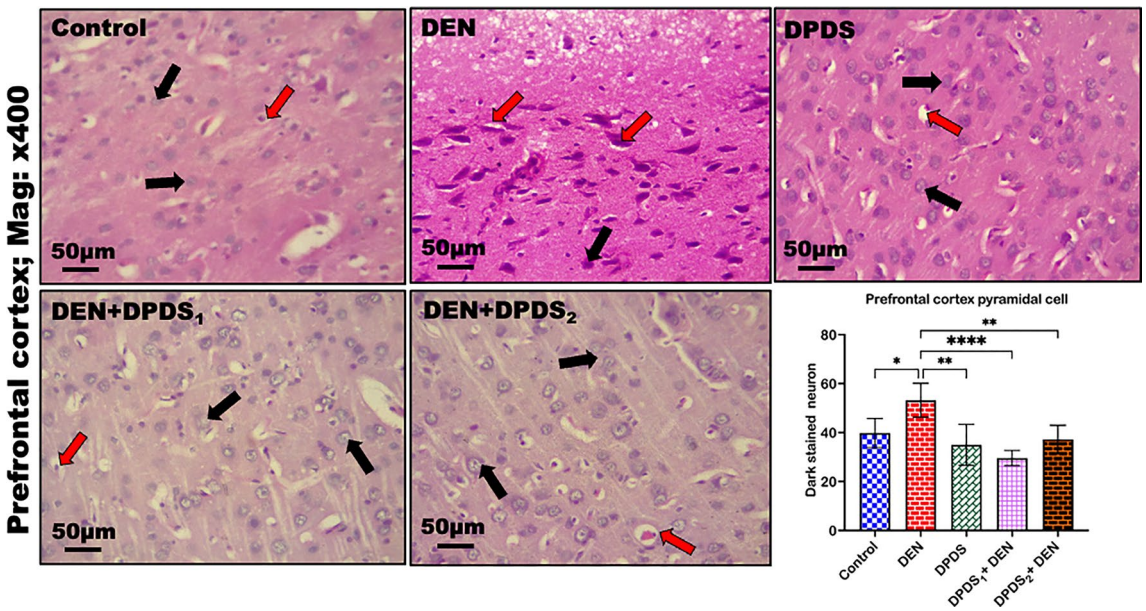

Fig. 10 Histological and histomorphometry evaluation of the prefrontal cortex section pyramidal neuron using H&E, 400X. DEN: Diethylnitrosamine, DPDS: Diphenyl diselenide. The prefrontal cortex section shows normal neurons (black arrows), and the prefrontal cortex has pyknotic, necrotic and shrinkage neurons (red arrows). Values are expressed as mean ± SEM (n = 5). *P < 0.05, **P < 0.01, ****P < 0.000 1 compared to control and DEN-only group

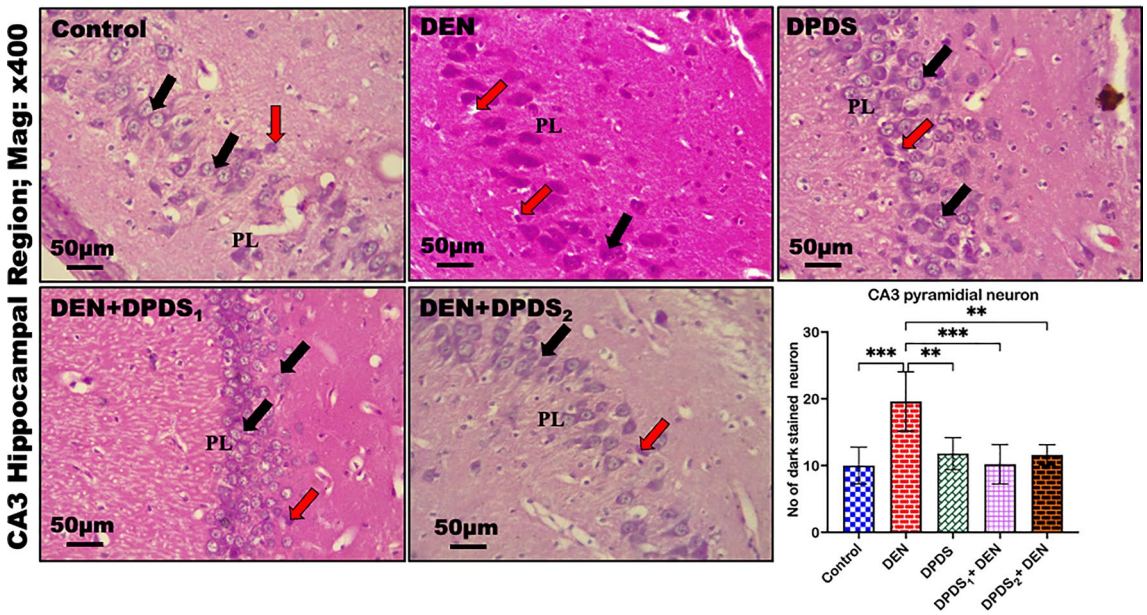

Fig. 11 Histological and histomorphometry evaluation of the hippocampal section (CA3) pyramidal neuron using H&E, 400X. *DEN* Diethylnitrosamine, *DPDS* Diphenyl diselenide, *PL* pyramidal layer. The hippocampal section shows normal neurons (black arrows) and hippocampal pyknotic, necrotic and degenerated neurons (red arrows). Values are expressed as mean ± SEM (n = 5). *P < 0.05, **P < 0.01, ***P < 0.001 compared to control and DEN-only group

exposure, we investigated its role in the cholinergic system, focusing on acetylcholinesterase activity (AChE), an essential enzyme necessary to maintain cholinergic neuronal transmission balance, by inhibiting acetylcholine (ACh) activity and blocking ACh postsynaptic signal transmission [74]. Our finding demonstrated that rats exposed to DEN-only caused a significant increase in the activity of the cholinergic neurotransmitter-degrading enzyme, indicated by an increase in prefrontal and hippocampal cortices AChE activity. Our findings of DEN-induced ACh hypofunction in cortical and hippocampal cortices agree with a previous study by Picciotto et al. [75]. The cortical and hippocampal cortices brain regions are well-known for motor function, memory consolidation, and retrieval [76, 77]. Disruption in the cholinergic function of the hippocampal-prefrontal network remains the central underlying pathology in Alzheimer's disease [78]. Altered AChE activity has been linked with motor dysfunction, anxio-depressive behaviour [79] and impaired learning and memory [80], as observed in the current study. Noteworthily, neurotoxicity is linked to cholinergic dysfunction typified by low acetylcholine activity, resulting in memory and learning dysfunctions [74]. Experimental rats pre-treated with DPDS showed reduced AChE activity in the prefrontal cortex and hippocampus relative to the DEN-only treated group, suggesting the restoration of the cholinergic function and improved cognitive and motor function. Low AChE may indicate high acetylcholine activity and activation of anti-apoptotic pathways, as reported in the literature [81]. DPDS-supplementation has been reported to enhance exploratory behaviour and reduce cerebrum and cerebellar AChE activity in rats [7] and *Ctenopharyngodon idella* [69] exposed to doxorubicin and methyl mercury, respectively.

Besides DEN-induced dysfunctions in neurotransmission, DEN has also been implicated in oxidative stress, chronic neuroinflammation, apoptosis and neurotoxic injury [55]. Hence, we demonstrate the protective role of DPDS in DEN-induced oxidative and nitrosative stress. Experimental rat cohorts treated with DEN-only showed a marked increase in oxido-nitrosative stress exemplified by decreased activity of endogenous enzymatic antioxidants and glutathione in rats' hippocampus and prefrontal cortex. The preceding findings are in agreement with previous reports that dimethyl nitrosamine (DMN), a nitrosamine compound as DEN, caused oxido-nitrosative stress in both rat brains [82], rat and rat kidneys [27, 83], rats' liver, stomach, colon [14] and the testis of both rat and mice [83, 84]. In post-cultured-mitotic rat CNS neurons treated with DEN [19, 52, 54], studies have reported upregulated phosphorylated tau protein, amyloid-beta protein, 8-hydroxy-2'-deoxyguanosine, 4-hydroxy-2-nonenal, and mitochondrial dysfunction, which are linked to neuronal cell death and neurotoxicity. These effects may be attributed to DEN-mediated metabolic pathways and

transformation processes, which produce reactive oxygen species (ROS), inflammation, DNA damage, and apoptosis in neuronal cells [19, 21, 23, 85].

DPDS exhibit antioxidants, cholinesterase, and inflammation-inhibitory activities [7, 9, 86, 87]. DPDS pretreatment has been reported to inhibit LPS-induced activation of the IκB/NF-κB pathway and reduce NO and ROS levels [9]. These reported beneficial biological effects of DPDS may make it a promising therapeutic agent against neuronal damage in cancer, cancer treatment, and predisposition to carcinogens. Hence, we postulate that DPDS may alleviate DEN-induced neurotoxicity. DPDS-pretreated rats demonstrated antioxidant activities and mitigated the oxido-nitrosative stress in the hippocampus and prefrontal cortex by increasing endogenous antioxidants and glutathione and reducing nitrosative biomarkers. These results suggested that DPDS can effectively abate DEN-induced oxidative stress and nitrosative processes in the brain, which underscores its potential to prevent brain-related pathology, including chemotherapy-induced neurotoxicity, traumatic brain injury, Stroke, Alzheimer's and Parkinson's diseases.

Our finding demonstrated neurotoxic injury in single intraperitoneal administration of DEN in rats, resulting in prefrontal and hippocampus (corneum ammonis areas three: CA3) pyramidal neuron pyknosis, chromatolysis, vacuolation and degeneration in line with earlier studies [23, 88]. DPDS pretreated groups prevented DEN-induced pyramidal neuronal injury in the prefrontal cortex and hippocampal CA3 region, which play an important role in episodic memory. The CA3 region is especially well known for rapidly storing and retrieving associative memories [89, 90]. It consists of unique projection to the DG via mossy fibre and CA1 via Schaffer collateral while receiving input through the perforant path directly from the entorhinal cortex [90, 91]. CA3 region has also been documented in the literature, as most CA regions with large populations of pyramidal cells are linked to hippocampal trisynaptic connection, ensuing spatial memory retrieval and learning [92, 93]. Noteworthily, damage to CA3 pyramidal cells has been linked to neurodegeneration as it is documented to be highly susceptible to neurological disorders [89]. Similarly, the prefrontal cortex is critical in executive functions such as working memory, thought, attention, planning, value-based decision-making and motor planning [76, 94].

Our histomorphometry study showed CA3 pyramidal cell shrinkage, vacuolations, and increased darkly stained neurons in a singly intraperitoneal dose of DEN-intoxicated rats, suggesting severe CA3 pyramidal cell damage. DEN-intoxicated rats also demonstrated prefrontal cortex and pyramidal cell damage, confirming possible injury in the prefrontal-cortical-hippocampal connection in the DEN-only group. Of note, damage in the prefrontal-cortical-hippocampal connection is linked with impaired behaviour, including anxiety, depression, impaired cognition and motor learning, a condition associated with neurologic and neuropsychiatric disorders [76, 95, 96]. DPDS pretreatment prevented DEN-induced prefrontal cortex and hippocampal damage, providing prefrontal cortex and hippocampal structural recovery and suggesting possible neuro-preventive or protective benefits of DPDS in neurologic and neuropsychiatric disorders.

In conclusion, DPDS pre-treatment protected against toxicities in experimental rats intoxicated with DEN by improving the neurochemical function, behavioural and histomorphometry alterations through modulation of acetylcholinesterase activity and brain oxido-nitrosative stress. Meanwhile, additional pathways, such as anti-inflammatory and anti-apoptotic pathways, were not explored. However, further studies are required to fully unravel the entire mechanism, including the anti-inflammatory and anti-apoptotic pathways by which DPDS confers its neuroprotective effect since the present study was focused on oxido-nitrosative effects, neurotransmitters degrading enzymes and histomorphometrical parameters. Besides the efforts of others, we intend to investigate the roles of pro- and anti-inflammatory cytokines, apoptotic neurogenesis biomarker, astrocyte and astrogliosis in the mechanism of DPDS pre-treatment to abate DEN-induced neuronal toxicity.

Acknowledgements
Not applicable.

Declaration of generative AI and AI-assisted technologies in the writing process
During the preparation of this work, the corresponding author (Solomon Owumi) used Claude AI to abridge the Abstract of this manuscript alone within the permissive word count of this Journal from the initial 350 abstract word count. After using Claude AI, the corresponding author (Solomon Owumi) reviewed and edited the content as needed and took full responsibility for the publication's content.

Author contributions
SO, and OO: Conceptualization, Supervision. JC, PDE, ACO, OO and PTO: Project administration, Methodology, Investigation. SO, JC, PDE, ACO, OO and PTO: Data curation, Formal analysis, Writing – original draft, Writing – review & editing.

Funding
This study was carried out without a specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data availability
The generated data from the current study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

The University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC) approved this study (Approval Number: UI-ACUREC/068-0524/06. All experiments were performed according to relevant guidelines and regulations and adhered to the ARRIVE guidelines (https://www.arriveguidelines.org) to report animal experiments.

Consent for publication

Not applicable.

Competing interest

The authors declare no competing interests.

Author details

[1]Cancer Research and Molecular Biology Laboratories, Department of Biochemistry, Faculty of Basic Medical Sciences, University of Ibadan, Change-Lab-Changing Lives; Rm NB 302, Ibadan, Oyo State 200005, Nigeria. [2]Department of Physiology, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Oyo State, Nigeria. [3]Department of Anatomy, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Oyo State, Nigeria.

Received: 2 September 2024 Accepted: 20 December 2024
Published online: 25 December 2024

References

1. Wang X, et al. Diphenyl diselenide alleviates Tert-Butyl hydrogen peroxide-induced oxidative stress and lipopolysaccharide-induced inflammation in rat glomerular mesangial cells. Int J Mol Sci. 2022;23(19):11215.
2. Doleski PH, et al. Diphenyl diselenide modulates nucleotidases, reducing inflammatory responses in the liver of Toxoplasma gondii-infected mice. Purinergic Signal. 2017;13(4):489–96.
3. Quispe RL, et al. Diphenyl diselenide protects neuronal cells against oxidative stress and mitochondrial dysfunction: Involvement of the glutathione-dependent antioxidant system. Redox Biol. 2019;20:118–29.
4. Fulco BCW, et al. Diphenyl diselenide is as effective as Ebselen in a juvenile rat model of cisplatin-induced nephrotoxicity. J Trace Elem Med Biol. 2020;60: 126482.
5. Alvarez-Perez M, et al. Selenides and diselenides: a review of their anticancer and chemopreventive activity. Molecules. 2018;23(3):628.
6. Adedara IA, et al. Diphenyl diselenide protects against mortality, locomotor deficits and oxidative stress in drosophila melanogaster model of manganese-induced neurotoxicity. Neurochem Res. 2016;41(6):1430–8.
7. Da-Silva OF, et al. Diphenyl diselenide through reduction of inflammation, oxidative injury and caspase-3 activation abates doxorubicin-induced neurotoxicity in rats. Neurochem Res. 2024;49(4):1076–92.
8. Mancini G, et al. Diphenyl diselenide improves the antioxidant response via activation of the Nrf-2 pathway in macrophage cells. Free Radic Biol Med. 2014;75(Suppl 1):S40.
9. Zhang C, et al. Diphenyl diselenide protects motor neurons through inhibition of microglia-mediated inflammatory injury in amyotrophic lateral sclerosis. Pharmacol Res. 2021;165: 105457.
10. Adedara IA, et al. Diphenyl diselenide abrogates brain oxidative injury and neurobehavioural deficits associated with pesticide chlorpyrifos exposure in rats. Chem Biol Interact. 2018;296:105–16.
11. Baldissera MD, et al. Diphenyl diselenide dietary supplementation alleviates behavior impairment and brain damage in grass carp (*Ctenopharyngodon idella*) exposed to methylmercury chloride. Comparative Biochem Physiol Part C: Toxicol Pharmacol. 2020;2020(229):210.
12. Zamberlan DC, et al. Diphenyl-diselenide suppresses amyloid-beta peptide in Caenorhabditis elegans model of Alzheimer's disease. Neuroscience. 2014;278:40–50.
13. de Freitas AS, et al. Diphenyl diselenide, a simple organoselenium compound, decreases methylmercury-induced cerebral, hepatic and renal oxidative stress and mercury deposition in adult mice. Brain Res Bull. 2009;79(1):77–84.
14. Mansour DF, et al. The carcinogenic agent diethylNitrosamine induces early oxidative stress, inflammation and proliferation in rat liver, stomach and colon: protective effect of ginger extract. Asian Pac J Cancer Prev. 2019;20(8):2551–61.
15. Shanab O, et al. Modulatory mechanisms of copper(II)-albumin complex toward N-nitrosodiethylamine-induced neurotoxicity in mice via regulating oxidative damage, inflammatory, and apoptotic signaling pathways. Ecotoxicol Environ Saf. 2024;270: 115841.
16. Li Y, Hecht SS. Metabolic activation and DNA interactions of carcinogenic N-Nitrosamines to which humans are commonly exposed. Int J Mol Sci. 2022;23(9):4559.
17 Janmeda P, et al. A systematic review on multipotent carcinogenic agent, N-nitrosodiethylamine (NDEA), its major risk assessment, and precautions. J Appl Toxicol. 2024. https://doi.org/10.1002/jat.4574.
18. Johnson GE, et al. Permitted daily exposure limits for noteworthy N-nitrosamines. Environ Mol Mutagen. 2021;62(5):293–305.
19. de la Monte SM, Tong M. Mechanisms of nitrosamine-mediated neurodegeneration: potential relevance to sporadic Alzheimer's disease. J Alzheimers Dis. 2009;17(4):817–25.
20. de la Monte SM, Wands JR. Alzheimer's disease is type 3 diabetes-evidence reviewed. J Diabetes Sci Technol. 2008;2(6):1101–13.
21. Elguindy NM, Yacout GA, El Azab EF. Amelioration of DENA-induced oxidative stress in rat kidney and brain by the essential oil of Elettaria cardamomum. Beni-Suef Univ J Basic Appl Sci. 2018;7(3):299–305.
22. Pendergrass JC, Targum SD, Harrison JE. Cognitive impairment associated with cancer: a brief review. Innov Clin Neurosci. 2018;15(1–2):36–44.
23. Cicek B, et al. Artichoke (Cynara Scolymus) methanolic leaf extract alleviates diethylNitrosamine-induced toxicity in BALB/c mouse brain involvement of oxidative stress and apoptotically related Klotho/PPAR-gamma signaling. J Pers Med. 2012;12(12):2012.
24. Hamdi BA. Remedy efficiency through paclitaxel on rat (Rattus norvegicus) brain injury and risk premium of N-diethyl nitrosamine (DEN) Injection Ibn AL-Haitham. J Pure Appl Sci. 2023;36(2):43–54.
25. Ponce-Lopez T, et al. Metformin prevents NDEA-induced memory impairments associated with attenuating beta-amyloid, tumor necrosis factor-alpha, and interleukin-6 levels in the hippocampus of rats. Biomolecules. 2023;13(9):1239.
26. Rajawat NK. Neurotoxicity of N-Nitrosodimethylamine (NDMA) in Swiss albino mice and neuroprotection by nanocurcumin. Mater Today Proc. 2022;69:A30–41.
27. Singh D, et al. Amelioration of diethylNitrosamine (DEN) induced renal oxidative stress and inflammation by Carissa carandas embedded silver nanoparticles in rodents. Toxicol Rep. 2021;8:636–45.
28. Wang X, et al. Diphenyl diselenide alleviates diabetic peripheral neuropathy in rats with streptozotocin-induced diabetes by modulating oxidative stress. Biochem Pharmacol. 2020;182: 114221.
29. Foda AM, et al. Pterostilbene ameliorates cognitive impairment in polycystic ovary syndrome rat model through improving insulin resistance via the IRS-1/PI3K/Akt/GSK-3beta Pathway: a comparative study with metformin. ACS Chem Neurosci. 2024;15(16):3064–77.
30. Jung DH, et al. Therapeutic effects of anodal transcranial direct current stimulation in a rat model of ADHD. Elife. 2020. https://doi.org/10.7554/eLife.56359.
31. Pellow S, et al. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods. 1985;14(3):149–67.
32 Lueptow LM. Novel object recognition test for the investigation of learning and memory in mice. J Vis Exp. 2017. https://doi.org/10.3791/55718-v.
33. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature. 1977;266(5604):730–2.
34. Ellman GL, et al. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88–95.
35 Hawkins P, et al. A Good Death? Report of the second newcastle meeting on laboratory animal Euthanasia. Animals. 2016;6(9):50.
36. Association. Report of the AVMA Panel on Euthanasia. J Am Vet Med Assoc. 2000;218(5):669–96.
37. Owumi S, et al. Prepubertal repeated berberine supplementation enhances cerebrocerebellar functions by modulating neurochemical and behavioural changes in wistar rats. J Mol Neurosci. 2024;74(3):72.

38. Owumi SE, et al. An in vivo and in silico probing of the protective potential of betaine against sodium fluoride-induced neurotoxicity. BMC Pharmacol Toxicol. 2024;25(1):87.
39. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248–54.
40. Jollow DJ, et al. Bromobenzene-induced liver necrosis protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology. 1974;11(3):151–69.
41. Rotruck JT, et al. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973;179(4073):588–90.
42 Claiborne A. Catalase activity Handbook of methods for oxygen radical research. Cambridge: CRC Press; 1985.
43. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247(10):3170–5.
44 Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J Biol Chem. 1974;249(22):7130–9.
45. Hu ML. Measurement of protein thiol groups and glutathione in plasma. Methods Enzymol. 1994;233:380–5.
46. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70–7.
47 Bergmeyer HI, Grassl GK. Methods of enzymatic analysis. New York: Academic Press Inc; 1974.
48. Green LC, et al. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem. 1982;126(1):131–8.
49. Granell S, et al. Heparin mobilizes xanthine oxidase and induces lung inflammation in acute pancreatitis. Crit Care Med. 2003;31(2):525–30.
50. Adedara IA, Owumi SE. Neurobehavioral and biochemical responses to artemisinin-based drug and aflatoxin B(1) co-exposure in rats. Mycotoxin Res. 2023;39(1):67–80.
51 Bancroft JD. Theory and practise of histological techniques Churchill Livingstone. Philadelphia: Elsevier; 2008.
52. Tong M, Longato L, de la Monte SM. Early limited nitrosamine exposures exacerbate high fat diet-mediated type 2 diabetes and neurodegeneration. BMC Endocr Disord. 2010;10:4.
53. Pitman A, et al. Depression and anxiety in patients with cancer. BMJ. 2018;361: k1415.
54. Naser AY, et al. Depression and anxiety in patients with cancer: a cross-sectional study. Front Psychol. 2021;12: 585534.
55. Orszaghova Z, Mego M, Chovanec M. Long-term cognitive dysfunction in cancer survivors. Front Mol Biosci. 2021;8: 770413.
56. Giovagnoli AR. Investigation of cognitive impairments in people with brain tumors. J Neurooncol. 2012;108(2):277–83.
57. Owumi SE, Adebisi G. Epirubicin treatment induces neurobehavioral, oxido-inflammatory and neurohistology alterations in rats: protective effect of the endogenous metabolite of tryptophan—3-indolepropionic acid. Neurochem Res. 2023;48(9):2767–83.
58. Bassett DS, Gazzaniga MS. Understanding complexity in the human brain. Trends Cogn Sci. 2011;15(5):200–9.
59. Cardenas-Iniguez C, Burnor E, Herting MM. Neurotoxicants, the developing brain, and mental health. Biol Psychiatry Glob Open Sci. 2022;2(3):223–32.
60. Mravec B. Neurobiology of cancer: definition, historical overview, and clinical implications. Cancer Med. 2022;11(4):903–21.
61. Tibensky M, Mravec B. Role of the parasympathetic nervous system in cancer initiation and progression. Clin Transl Oncol. 2021;23(4):669–81.
62. Kumaria A. Parasympathetic influences in cancer pathogenesis: further insights. Clin Transl Oncol. 2021;23(7):1491–3.
63. Kuol N, et al. Role of the nervous system in cancer metastasis. J Exp Clin Cancer Res. 2018;37(1):5.
64. Bak J, et al. Effect of rotation preference on spontaneous alternation behavior on Y maze and introduction of a new analytical method, entropy of spontaneous alternation. Behav Brain Res. 2017;320:219–24.
65. Kim J, et al. A quantitative analysis of spontaneous alternation behaviors on a Y-maze reveals adverse effects of acute social isolation on spatial working memory. Sci Rep. 2023;13(1):14722.
66 Ronquillo J, et al. Nature and nurture: comparing mouse behavior in classic versus revised anxiety-like and social behavioral assays in genetically or environmentally defined groups. bioRxiv. 2023;10:210.
67 Naiho AO, et al. Treatment with N-acetylcysteine and/or zinc sulfate restores neurobehavioral functions through modulation of neurochemical activities in mice exposed to bonny light crude oil. Nutrire. 2024. https://doi.org/10.1186/s41110-024-00262-x.
68. Wolf A, et al. A comprehensive behavioral test battery to assess learning and memory in 129S6/Tg2576 mice. PLoS ONE. 2016;11(1): e0147733.
69. Baldissera MD, et al. Diphenyl diselenide dietary supplementation alleviates behavior impairment and brain damage in grass carp (Ctenopharyngodon idella) exposed to methylmercury chloride. Comp Biochem Physiol C Toxicol Pharmacol. 2020;229: 108674.
70. Glaser V, et al. Protective effects of diphenyl diselenide in a mouse model of brain toxicity. Chem Biol Interact. 2013;206(1):18–26.
71. Trevisan da Rocha J, et al. Effects of diphenyl diselenide on depressive-like behavior in ovariectomized mice submitted to subchronic stress: involvement of the serotonergic system. Psychopharmacology. 2012;222(4):709–19.
72. Stangherlin EC, et al. Sub-chronical exposure to diphenyl diselenide enhances acquisition and retention of spatial memory in rats. Brain Res. 2008;1201:106–13.
73. Ghosh AS, et al. Effect of DENA induced hepatocarcinogenesis on neuroendocrine levels in male rats. Indian J Exp Biol. 2008;46(7):498–504.
74 Chen ZR, et al. Role of cholinergic signaling in Alzheimer's Disease. Molecules. 2022;27(6):110.
75. Picciotto MR, Higley MJ, Mineur YS. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. Neuron. 2012;76(1):116–29.
76. Sigurdsson T, Duvarci S. Hippocampal-prefrontal interactions in cognition, behavior and psychiatric disease. Front Syst Neurosci. 2015;9:190.
77. Preston AR, Eichenbaum H. Interplay of hippocampus and prefrontal cortex in memory. Curr Biol. 2013;23(17):R764–73.
78 Kim S, et al. Alteration of Neural Pathways and Its Implications in Alzheimer's disease. Biomedicines. 2022. https://doi.org/10.3390/biomedicines10040845.
79. Naiho AO, et al. Correction: Treatment with N-acetylcysteine and/or zinc sulfate restores neurobehavioral functions through modulation of neurochemical activities in mice exposed to bonny light crude oil. Nutrire. 2024. https://doi.org/10.1186/s41110-024-00262-x.
80. Bali YA, et al. Learning and memory impairments associated to acetylcholinesterase inhibition and oxidative stress following glyphosate based-herbicide exposure in mice. Toxicology. 2019;415:18–25.
81 Pérez-Aguilar B, et al. Changes in the acetylcholinesterase enzymatic activity in tumor development and progression. Cancers. 2023;15(18):4629.
82. Imosemi IO, Owumi SE. Diethylnitrosamine induced oxidative stress and morphological changes in the cerebellum of wistar rat. Arch Bas App Med. 2008;8:55–60.
83. Somade OT, et al. Cnidoscolus aconitifolius leaf extract and ascorbate confer amelioration and protection against dimethyl nitrosamine-induced renal toxicity and testicular abnormalities in rats. Toxicol Rep. 2021;8:1098–108.
84. Habiba ES, et al. Sericin and melatonin mitigate diethylnitrosamine-instigated testicular impairment in mice: Implications of oxidative stress, spermatogenesis, steroidogenesis, and modulation of Nrf2/WT1/SF-1 signaling pathways. Life Sci. 2023;334: 122220.
85. Ghufran H, et al. Standardization of diethylnitrosamine-induced hepatocellular carcinoma rat model with time based molecular assessment. Exp Mol Pathol. 2021;123: 104715.
86. Refaay DA, et al. Evaluation of novel multifunctional organoselenium compounds as potential cholinesterase inhibitors against Alzheimer's disease. Med Chem Res. 2022;31(6):894–904.
87. Kumawat A, et al. Organoselenium compounds as acetylcholinesterase inhibitors: evidence and mechanism of mixed inhibition. J Phys Chem B. 2021;125(6):1531–41.
88. E, I.I.O.a.O.S*Diethylnitrosamine Induced Oxidative Stress and Morphological Changes in the Cerebellum of Wistar Rat.* Arch. Bas. App. Med, 2019. **8** (2020): 55–60.
89. Cherubini E, Miles R. The CA3 region of the hippocampus: how is it? What is it for? How does it do it? Front Cell Neurosci. 2015;9:19.
90. Rebola N, Carta M, Mulle C. Operation and plasticity of hippocampal CA3 circuits: implications for memory encoding. Nat Rev Neurosci. 2017;18(4):208–20.

91. Bettio LEB, et al. Interplay between hormones and exercise on hippocampal plasticity across the lifespan. Biochim Biophys Acta Mol Basis Dis. 2020;1866(8): 165821.
92 Rao YL, et al. Hippocampus and its involvement in Alzheimer's disease: a review. 3 Biotech. 2022. https://doi.org/10.1007/s13205-022-03123-4.
93. Lin X, et al. Noncanonical projections to the hippocampal CA3 regulate spatial learning and memory by modulating the feedforward hippocampal trisynaptic pathway. PLoS Biol. 2021;19(12): e3001127.
94. Chafee MV, Heilbronner SR. Prefrontal cortex. Curr Biol. 2022;32(8):R346–51.
95. Chen Y, et al. Prefrontal cortical circuits in anxiety and fear: an overview. Front Med. 2022;16(4):518–39.
96. Larsen NY, et al. Layer III pyramidal cells in the prefrontal cortex reveal morphological changes in subjects with depression, schizophrenia, and suicide. Transl Psychiatry. 2022;12(1):363.

Publisher's Note