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D-Ribose-L-Cysteine Mitigates Bisphenol A-Induced Anxiety and Depressive-Like Behaviour in Experimental Mice

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Authors' contributions

This work was carried out in collaboration among all authors. Author IH designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AEF managed the analysis of the result and literature searches. Authors AAO and ACO performed the statistical analysis. Author EAT wrote the protocol and final draft of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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Abstract

Background: Bisphenol A (BPA) is a widely used industrial chemical with endocrine-disrupting properties, posing significant risks to human health, particularly through its neurotoxic and oxidative stress–inducing effects. Antioxidant-based interventions, such as D-ribose L-cysteine, offer potential in mitigating BPA-induced neurotoxicity by enhancing glutathione-mediated cellular defense mechanisms.

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Aim: This study investigated whether DRLC (D-Ribose-L-Cysteine) alleviates BPA (Bisphenol A)-induced anxiety and depressive-like behaviours in mice.
Study Design: A 14-day experimental study was conducted under standard conditions at the Pharmacology Laboratory, Delta State University, Abraka, Nigeria.
Methodology: Adult male mice were divided into four groups: control (which received the vehicle 10 ml/kg distilled water), BPA-only (10 mg/kg), BPA (10 mg/kg) with DRLC at 50 mg/kg and BPA (10 mg/kg) with DRLC 100 mg/kg. BPA was administered alternately for 14 days to induce neurobehavioural changes, while DRLC was given orally for 14 days. Behavioural tests included the Light/Dark Box, Elevated Plus Maze, Hole Board Test, Tail Suspension Test, Social Interaction Tests, and Forced Swim. Brain biomarkers for inflammation [Myeloperoxidase (MPO)], oxidative/nitrergic stress [Malonaldehyde (MDA)/Nitric Oxide (NO)], anti-oxidative stress (Super oxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), and reduced Glutathione (GSH), and HPA (Hypothalamic Pituitary Adrenal) -axis function were assessed.
Results: BPA exposure led to significant anxiety- and depression-like behaviours and disrupted redox balance, shown by elevated NO, MDA, MPO, and ACTH, and reduced SOD, CAT, GPx, and GSH. DRLC treatment reversed these effects, restoring antioxidant levels and Behavioural function.
Conclusion: DRLC effectively counters BPA-induced neurobehavioural disturbances in mice by restoring redox homeostasis, highlighting its neuroprotective potential.

Keywords: Bisphenol A; D-Ribose L-Cysteine; behavioural changes; antioxidant therapy; neuroprotection.

1. Introduction

Bisphenol A (BPA) is a ubiquitous industrial chemical used in the production of polycarbonate plastics and epoxy resins (Mitta et al., 2025). BPA has become an environmental and public health concern because it poses a significant health risk to exposed individuals (Charkiewicz et al., 2024). Human exposure to BPA could occur via inhalation, dermal, oral, perinatal, or fetal routes (Hahladakis et al., 2023). BPA's widespread application in food and beverage containers, thermal paper receipts, and other consumer products has led to near-universal human exposure (Andonotopo et al., 2025). While the inclusion of BPA in the manufacturing of plastic products has revolutionized numerous industries due to its durable and versatile properties, its endocrine-disrupting potential has raised alarms regarding its potential adverse effects on human health, particularly on neurological function (Feng et al.,2026). The endocrine-disrupting activity of BPA stems from its ability to mimic or interfere with the actions of endogenous hormones, primarily estrogen (Li et al., 2023; Costa & Cairrao, 2024). This interaction can disrupt the delicate hormonal balance within the body, leading to a cascade of effects across multiple organ systems (Ojo et al., 2025). In the context of neurodevelopment, BPA exposure during critical periods, such as prenatal and early postnatal life, has been linked to various neurobehavioral alterations, including impairments in learning and memory (Feng et al.,2026), and social behavior (Zuo et al., 2025). Notably, numerous animal studies have demonstrated that BPA exposure can induce anxiety-like and depressive-like behaviors, suggesting a potential role in the pathophysiology of mood disorders. The mechanisms underlying BPA-induced neurotoxicity are multifaceted and involve oxidative stress and the generation of free radicals. Several pathways, such as estrogen receptor activation, neurotransmitter dysregulation, epigenetic modifications, and inflammation (Abdou et al., 2022), have also been proposed. Given the potential for BPA to induce anxiety-like and depressive-like behaviors, there is a growing need for effective strategies to mitigate its neurotoxic effects. Hence, anti-oxidant based interventions have emerged as a promising approach, with a focus on identifying compounds that can counteract the adverse effects of BPA on the brain. D-ribose L-Cysteine (DRLC) is a prodrug of L-Cysteine that promotes the brain's production of glutathione (Isibor et al.,2022; Isibor et al., 2026). DRLC has emerged as a promising therapeutic agent for enhancing the brain's natural antioxidant defenses. Recent studies by Isibor et al. (2022, 2026) highlight DRLC’s role in stimulating the biosynthesis of glutathione, a master antioxidant essential for maintaining neuronal health. In the presence of environmental toxins particularly with BPA, the brain’s glutathione stores can become depleted, leaving neurons vulnerable to damage. DRLC acts as a metabolic catalyst to replenish these stores, thereby providing a robust shield against neurotoxicity. This property suggests that DRLC could play a pivotal role in preventative strategies against neurodegenerative conditions such as anxiety and depression where oxidative damage is a pivotal hallmark of disease progression.

2. Materials and Methods

2.1 Equipment and Reagents Used

Bisphenol A (Sigma-Aldrich), D-ribose L-Cysteine compound [(4R)-2-[(1R,2R,3R)-1,2,3,4-tetrahy droxybuty1]- 1,3-thiazolidine-4-carboxylic acid] (pharmaceutical-grade formulation), Light/Dark Box, EPM, and Hole Board apparatus, UV-Vis spectrophotometer (for NO, MDA, CAT, GSH, GPx, SOD, MPO assays), Griess reagent (NO detection), TBARS reagent (MDA), DTNB (GSH), o-dianisidine and H_2O_2 (MPO), Centrifuge (10,000 rpm), digital timer, Syringe and needle, gloves, , ELISA kits for ACTH, Dichromate/acetic acid solution (CAT), adrenaline (SOD assay).

2.2 Experimental Animals

Male swiss mice weighing 24.0 -26.0 g used in this study were obtained from the Central Animal House, Faculty of Basic Medical Sciences, Delta State University, Abraka and were housed in plastic cages at room temperature under standard conditions. They were fed with balanced rodent pellet diet and water *ad libithum*. Mice were acclimatized for a period of 10 days before commencement of experiment. The experiment procedure was performed in accordance with the National Institute of Health (NIH) Guideline for the Care and Use of Laboratory Animals.

2.3 Drug Preparation and Treatment Groups

125 mg of DRLC was dissolved in 12.5 ml of distilled water to obtain a stock solution of 10 mg/ml. 0.02 g of BPA was dissolved in 0.2 ml of dimethyl sulfoxide which was further diluted in 20 ml of distilled water. The animals were allotted into four (4) groups (n=6): group 1 received distilled water (10 ml/kg *i.p*), group 2 received BPA (10 mg /kg *p.o*), groups 3 and 4 received BPA (10 mg/kg) with graded doses of DRLC (50 mg/kg and 100 mg/kg) respectively. The doses of DRLC used for this study was based on previous studies (Isibor et al., 2022). Animals were treated for 14 consecutive days. At the end of the experimental protocol, behaviour assessment (Light/Dark Box, Elevated Plus Maze, Hole Board, Forced Swim, Tail Suspension, and Social Interaction Tests) were performed on the animals.

2.4 Behavioural Assessment

2.4.1 Light and Dark Box Test

The Light and Dark Box (LDB) apparatus was used to assess the anxiety-like behavior of mice. The LDB apparatus consists of two compartments, one dark and one lighted (light), connected by a small opening. Each mouse was placed alone in the light compartment with its back to the opening, and they were given five minutes to explore both compartments freely. The total time spent in the light chamber was recorded using a stop watch. Less time in the light zone indicates higher anxiety-like behavior, while more time suggests decreasing anxiety.

2.4.2 Elevated Plus Maze (EPM) Test

The EPM apparatus consists of two open arms (30 × 5 cm) and two closed arms (30 × 5 × 15 cm), all elevated 50 cm above the floor. Each mouse was placed in the center of the maze facing an open arm and allowed to explore for 5 minutes. The number of entries and the amount of time spent in the open arms were recorded using a video-recording system. A decrease in open-arm activity is interpreted as anxiety-like behavior (Eduviere et al., 2022).

2.4.3 Hole Board Test

Exploratory behaviour was evaluated using the Hole Board apparatus consisting of a flat platform (40 × 40 cm) with evenly spaced holes (3 cm in diameter). Mice were placed individually at the centre of the board and observed for 5 minutes. The number of head dips (defined as insertion of the head into a hole) was recorded (using a video recording system) as a measure of exploratory behaviour and anxiety. A lower number of head

dips suggested increased anxiety and depressive-like behaviour, typically associated with reduced exploratory drive and heightened fear.

2.4.4 Tail Suspension Test (TST)

Depressive-like behaviour was assessed using the Tail Suspension Test (Steru et al., 1985). Mice were suspended by the tail using adhesive tape affixed 1 cm from the tip of the tail. Each mouse was suspended for 6 minutes, and the duration of immobility (complete lack of movement) was recorded using a stopwatch, during the last 4 minutes. Increased immobility time is considered an indicator of behavioural despair.

2.4.5 Social Interaction Test

Mice were assessed for social interest and motivation using the social interaction test (Moy et al., 2004). Each test mouse was placed in an open-field arena containing a wire-mesh enclosure housing an unfamiliar mouse (stranger) and an identical empty enclosure on the opposite side. The time spent by the test mouse exploring each enclosure was recorded (using a stopwatch) over 5 minutes. Reduced time interacting with the stranger mouse is indicative of social withdrawal and depressive-like behaviour.

2.4.6 Forced Swim Test (FST)

The FST was conducted using a transparent cylinder (height 25 cm; diameter 20 cm) filled with water (25°C) to a height of 15 cm (Porsolt et al., 1977). Each mouse was placed in the water for 6 minutes, and the immobility time during the last 4 minutes was recorded using a stopwatch. Immobility was defined as the absence of any movement except those necessary to keep the head above water. Increased immobility time reflects depressive-like behaviour.

2.5 Sample Collection

At the end of behavioural assessments, mice were euthanized under deep ether anesthesia. The brains of six animals per group were rapidly excised, and dissected on ice tray at 4°C. Tissues were homogenized in phosphate buffer (0.1 M, pH 7.4) and centrifuged at 10,000 rpm for 10 minutes at 4°C. The resulting supernatants were stored at -20°C for subsequent biochemical analyses.

2.6 Biochemical Assays

2.6.1 Myeloperoxidase (MPO) Activity

MPO activity, a marker of inflammation, was measured using a method described by (Bradley et al., 1982). Brain homogenates were suspended in extraction buffer, freeze-thawed, sonicated, and centrifuged. Supernatants were incubated with o-dianisidine and H_2O_2 in phosphate buffer. The change in absorbance at 450 nm over 3 minutes was recorded, and MPO activity was expressed in U/mg protein.

2.6.2 Determination of Malondialdehyde (MDA) levels

The thiobarbituric acid-reactive substances (TBARS) assay was used to determine MDA concentration ((Ohkawa et al., 1979).). A brain supernatant fraction (0.1 mL) was combined with 0.5 mL of TCA (30%) and 0.5 mL of thiobarbituric acid (0.75%) after being diluted 20 times with 0.15 M Tris-KCl buffer. After that, the reaction mixture was heated for 45 minutes at 80 °C in a water bath. After halting the reaction for ten minutes in an ice-cold water bath, the tubes were centrifuged for five minutes at 4000 rpm. After the supernatant fraction was separated, a UV/Vis spectrophotometer (INESA, 752 N) was used to measure the absorbance at 532 nm. Values were expressed as nmol MDA/mg protein.

2.6.3 Determination of Nitric Oxide (NO)

Griess reagent was used to quantify total nitrite in brain tissue (Green et al., 1982). The supernatant fraction (100 µL) was mixed with 10µL of Griess reagent (1:1 mixture of 1% sulfanilamide in 5% phosphoric acid and

0.1% N-1-naphthyl ethylenediamine dihydrochloride). The nitrite level was determined using a standard curve prepared with sodium nitrite (0-100 μM), and absorbance was measured at 540 nm via a UV-spectrophotometer, and concentrations were expressed as μmol/mg tissue.

2.6.4 Superoxide Dismutase (SOD) Activity

SOD activity was measured based on its ability to inhibit adrenaline oxidation (Misra and Fridovich, 1972). Brain homogenate was added to carbonate buffer, and the reaction was initiated with adrenaline. The change in absorbance at 480 nm was recorded, and SOD activity was calculated as units/min/mg protein.

2.6.5 Catalase (CAT) Activity

CAT activity was determined by adding supernatant to phosphate buffer and hydrogen peroxide. After reaction incubation, dichromate/acetic acid was added and absorbance was read at 570 nm. Results were expressed as μmol H_2O_2 decomposed/min/mg protein (Sinha, 1972).

2.6.6 Glutathione Peroxidase (GPX) Activity

GPX activity was estimated from brain homogenates. The enzyme catalyzed the reduction of hydrogen peroxide using GSH as a substrate. The change in absorbance was measured spectrophotometrically and GPX levels were reported in U/mg protein (Paglia and Valentine, 1967).

2.6.7 Reduced Glutathione (GSH)

GSH levels were determined using the DTNB method. Supernatant was mixed with TCA, centrifuged, and the resulting supernatant was reacted with DTNB. Absorbance was measured at 412 nm, and GSH was expressed in μmol/g tissue (Moron et al., 1979).

2.6.8 Brain Adrenocorticotropic Hormone (ACTH) Concentration

ACTH concentrations in brain extracts were measured using a competitive enzyme-linked immunosorbent assay (ELISA) for mice following manufacturer's instruction (RayBiotech Pharmaceuticals). Samples were diluted appropriately to fall within the linear range of the standard curve (0.1-1000 pg/ml). ACTH levels were normalized to total protein content and expressed as ng per mg protein.

2.7 Statistical Analysis

All experimental results were expressed as mean ± standard error of the mean (SEM). Statistical significance was determined at a threshold of $p < 0.05$. Data obtained from behavioral and biochemical evaluations were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Analyses were performed using GraphPad Prism® version 10.01 (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1 Effect of D-ribose L-Cysteine (DRLC) on anxiety-like behaviour in mice treated with Bisphenol A (BPA) Utilizing the Light and Dark Box

The effect of D-ribose L-Cysteine on anxiety-like behaviour in mice subjected to Bisphenol A on anxiety-like behaviour was evaluated using the LDB on alternate days for a period of 14 days. The result revealed that the amount of time spent in the dark compartment significantly ($p < .05$) [Fig. 1] increased in the Bisphenol A-treated mice when compared with the control group which indicates an increase in anxiety and a marker for anxiety-like behaviour. However, D-ribose L-Cysteine (50mg/kg and 100mg/kg. p.o) significantly ($p < .05$) decreased the amount of time spent in the dark compartment when compared to the Bisphenol A-subjected group.

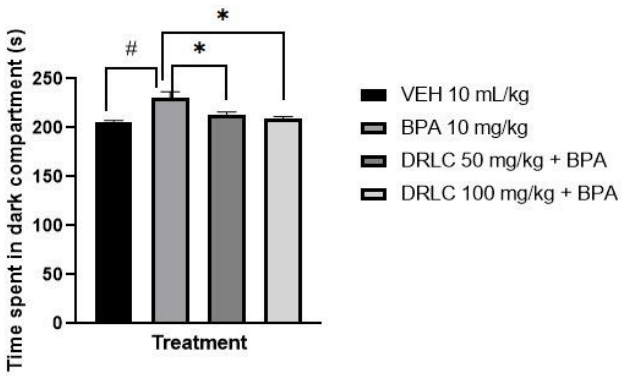

Fig. 1. Effect of D-ribose-L-Cysteine on Bisphenol A-induced anxiety-like behaviour in mice utilizing the Light and Dark Box

*Values represent the mean ± S.E.M of six animals per group; # p< .05 compared to the control group (ANOVA followed by Turkey's post hoc test).; * p< .05 compared to the pathologic group (ANOVA followed by Turkey's post hoc test).*

3.2 Effect of D-ribose L-Cysteine (DRLC) on Anxiety-like Behaviour in Mice Treated with Bisphenol A (BPA) Utilizing the Elevated Plus Maze (EPM)

The effect of D-ribose L-Cysteine on anxiety-like behaviour in mice subjected to Bisphenol A on anxiety-like behaviour was also evaluated using the EPM. The result revealed that the amount of time spent in the closed arm significantly ($p < .05$) [Fig. 2] increased in the Bisphenol A-treated mice when compared with the control group, which indicates an increase in anxiety and a marker for anxiety-like behaviour. However, D-ribose L-Cysteine (50mg/kg and 100mg/kg. p.o) significantly ($p < .05$) decreased the amount of time spent in the closed arm when compared to the Bisphenol A subjected group.

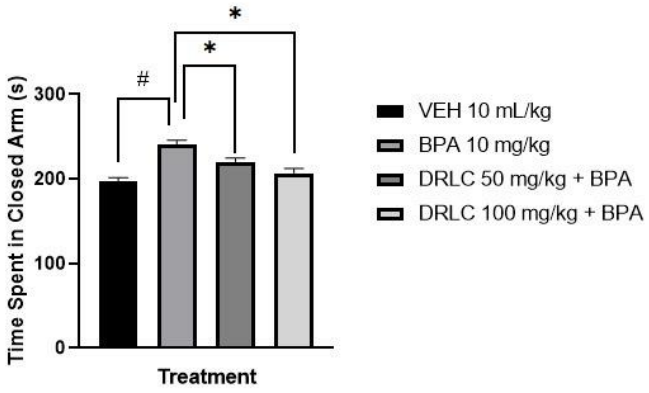

Fig. 2. Effect of D-ribose-L-Cysteine on Bisphenol A-induced anxiety-like behaviour in mice utilizing the Elevated Plus Maze

*Values represent the mean ± S.E.M of six animals per group; # p<0.05 compared to the control group (ANOVA followed by Turkey's post hoc test).; * p<0.05 compared to the pathologic group (ANOVA followed by Turkey's post hoc test)*

3.3 Effect of D-ribose L-Cysteine (DRLC) on Anxiety-like Behaviour in Mice Treated with Bisphenol A (BPA) Utilizing the Hole Board Test (HBT)

The effect of D-ribose L-Cysteine on anxiety-like behaviour in mice subjected to Bisphenol A was also evaluated using HBT. The result revealed that the Frequency of head dips was significantly ($P < .05$) [Fig. 3] reduced in the Bisphenol A-treated mice when compared with the control group, which indicates an increase in anxiety and a marker for anxiety-like behaviour. However, D-ribose L-Cysteine (50mg/kg and 100mg/kg. p.o) significantly ($p < .05$) increased the frequency of head dips when compared to the Bisphenol A subjected group.

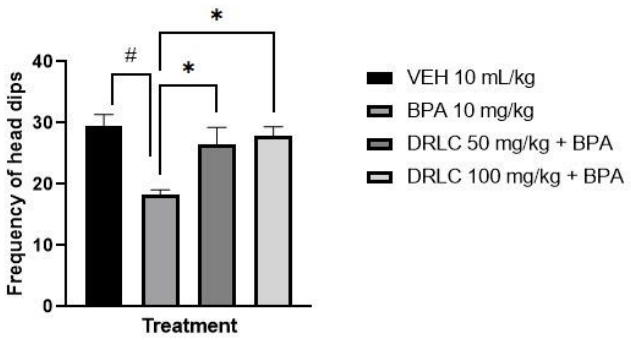

Fig. 3. Effect of D-ribose-L-Cysteine on Bisphenol A-induced anxiety-like behaviour in mice utilizing the Hole Board Test

*Values represent the mean ± S.E.M. of six animals per group; # p < .05 compared to the control group (ANOVA followed by Turkey's post hoc test); * p < .05 compared to the pathologic group (ANOVA followed by Turkey's post hoc test)*

3.4 Effect of D-ribose L-Cysteine (DRLC) on Depressive-like Behaviour in Mice Treated with Bisphenol A (BPA) Utilizing the Tail Suspension Test (TST)

The effect of D-ribose L-Cysteine on depressive-like behaviour in mice subjected to Bisphenol A on alternate days for 14 days was evaluated using the TST. The result revealed that the time of immobility was significantly (P < .05) [Fig. 4] elevated in the Bisphenol A-treated mice when compared with the control group, which indicates an increase in depression and a marker for depressive-like behaviour. However, D-ribose L-Cysteine (50mg/kg and 100mg/kg. p.o) significantly (p < .05) reduced the immobility time when compared to the Bisphenol A subjected group.

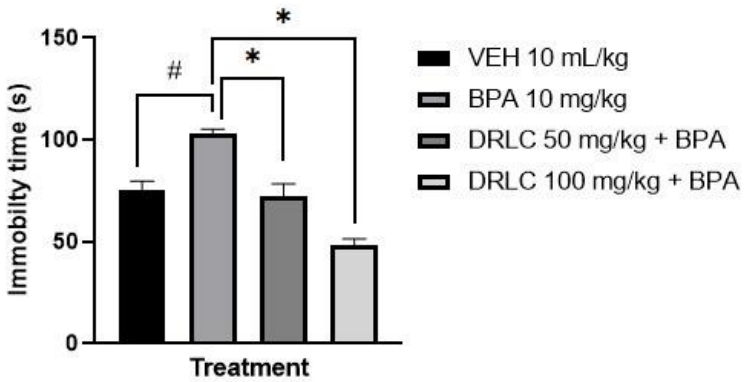

Fig. 4. Effect of D-ribose-L-Cysteine on Bisphenol A-induced depressive-like behaviour in mice utilizing the Tail Suspension Test

*Values represent the mean ± S.E.M of six animals per group; # p < .05 compared to the control group (ANOVA followed by Turkey's post hoc test).; * p <.05 compared to the pathologic group (ANOVA followed by Turkey's post hoc test).*

3.5 Effect of D-ribose L-Cysteine (DRLC) on Depressive-like Behaviour in Mice Treated with Bisphenol A (BPA) Utilizing the Social Interaction Test (SIT)

The effect of D-ribose L-Cysteine on depressive-like behaviour in mice subjected to Bisphenol A was evaluated using SIT. The result revealed that the social preference percentage was significantly (P < .05) [Fig. 5] reduced in the Bisphenol A-treated mice when compared with the control group, which indicates a decrease in social interaction and a marker for depressive-like behaviour. However, D-ribose L-Cysteine (50mg/kg and 100mg/kg. p.o) significantly (p <.05) increased the social preference percentage when compared to the Bisphenol A subjected group.

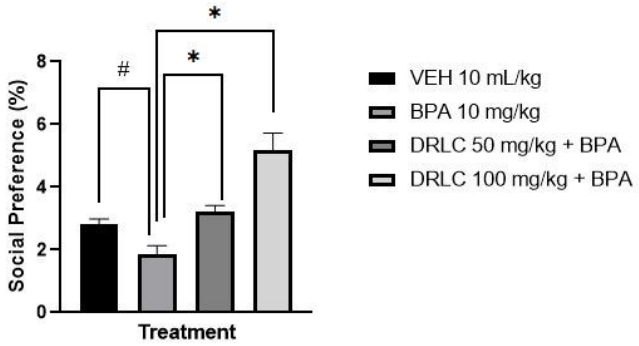

Fig. 5. Effect of D-ribose-L-Cysteine on Bisphenol A induced depressive-like behaviour in mice utilizing the Social Interaction Test

*Values represent the mean ± S.E.M of 6 animals per group; # p<0.05 compared to the control group (ANOVA followed by Turkey's post hoc test); * p<0.05 compared to the pathologic group (ANOVA followed by Turkey's post hoc test)*

3.6 Effect of D-ribose L-Cysteine (DRLC) on Depressive-like Behaviour in Mice Treated with Bisphenol A (BPA) Utilizing the Forced Swim Test (FST)

The effect of D-ribose L-Cysteine on depressive-like behaviour in mice subjected to Bisphenol A on alternate days for 14 days using the FST. The result revealed that the time of immobility was significantly (P <.05) [Fig. 6] elevated in the Bisphenol A-treated mice when compared with the control group, which indicates an increase in depression and a marker for depressive-like behaviour. However, D-ribose L-Cysteine (50mg/kg and 100mg/kg. p.o) significantly (p <.05) reduced the immobility time when compared to the Bisphenol A subjected group.

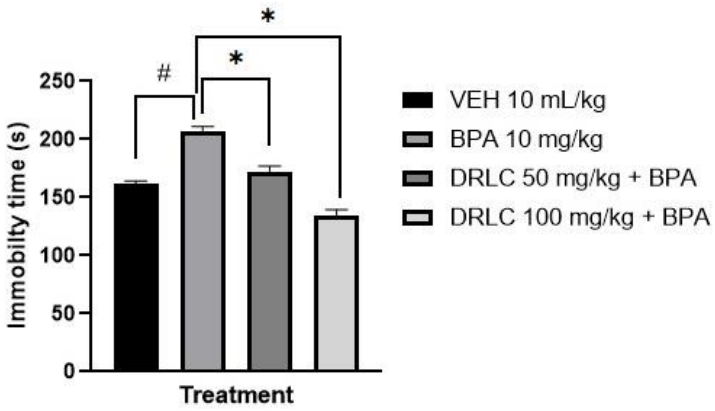

Fig. 6. Effect of D-ribose-L-Cysteine on Bisphenol A induced depressive-like behaviour in mice utilizing the Force Swim Test

*Values represent the mean ± S.E.M of 6 animals per group; # p<0.05 compared to the control group (ANOVA followed by Turkey's post hoc test); * p<0.05 compared to the pathologic group (ANOVA followed by Turkey's post hoc test)*

3.7 Effects of D-ribose L-Cysteine (DRLC) on Brain Myeloperoxidase (MPO) Enzyme Activity in Bisphenol A-Treated Mice

The effect of D-ribose L-Cysteine (DRLC) on brain Myeloperoxidase activity was assayed for in mice subjected to Bisphenol A. The result revealed that the brain MPO activity was significantly ($p < 0.05$) [Fig. 7] elevated in the Bisphenol A (BPA) treated mice when compared with the control group. However, D-ribose L-Cysteine (50mg/kg and 100mg/kg, p.o) significantly ($P < .05$) reduced the activity level of MPO in mice brain serum when compared to the Bisphenol A treated mice.

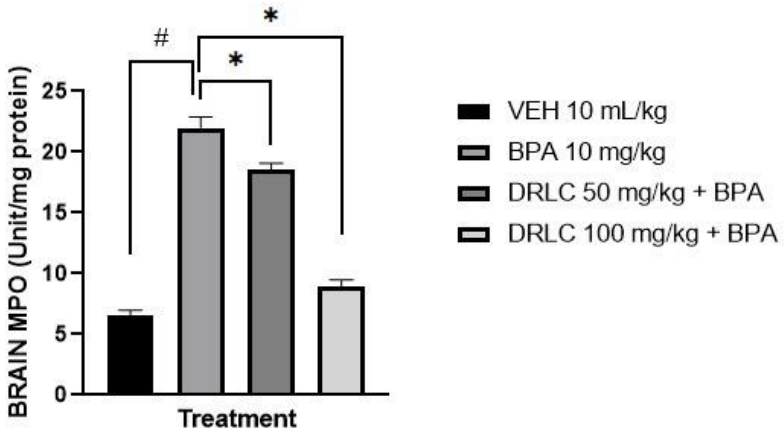

Fig. 7. Effect of D-ribose-L-Cysteine on Bisphenol A-induced anxiety and depressive-like in mice in Myeloperoxidase (MPO) in brain

*Values represent the mean ± S.E.M of six animals per group; # p < .05 compared to the control group (ANOVA followed by Turkey's post hoc test); * p < .05 compared to the pathologic group (ANOVA followed by Turkey's post hoc test)*

3.8 Effects of D-ribose L-Cysteine (DRLC) on Brain Malondialdehyde (MDA) Activity in Bisphenol A-treated Mice

The effect of D-ribose L-Cysteine (DRLC) on brain Malondialdehyde activity in mice subjected to Bisphenol A. The result revealed that the brain MDA activity was significantly ($p < 0.05$) [Fig. 8] elevated in the Bisphenol A (BPA) treated mice when compared with the control group. However, D-ribose L-Cysteine (50mg/kg and 100mg/kg, p.o) significantly ($P < 0.05$) reduced the activity level of MDA in mice brain serum when compared to the Bisphenol A-treated mice.

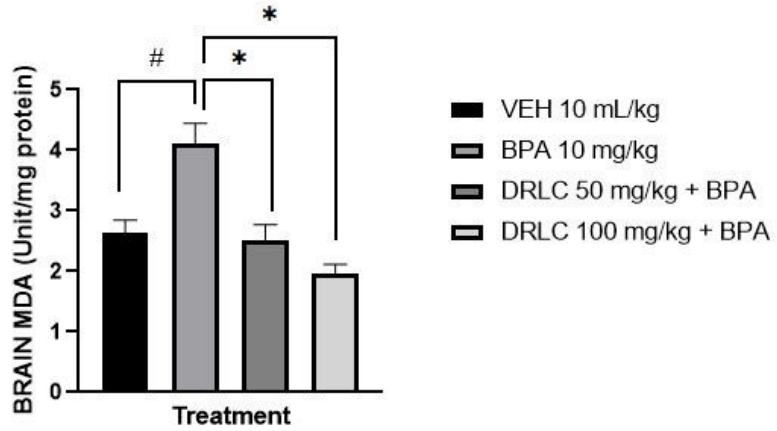

Fig. 8. Effect of D-ribose-L-Cysteine on Bisphenol A induced anxiety and depressive-like behaviour in mice in Malondialdehyde (MDA) in brain

*Values represent the mean ± S.E.M of six animals per group; # p < .05 compared to the control group (ANOVA followed by Turkey's post hoc test); * p < .05 compared to the pathologic group (ANOVA followed by Turkey's post hoc test)*

3.9 Effects of D-ribose L-Cysteine (DRLC) on Brain Nitric Oxide (NO) Activity in Bisphenol A-Treated Mice

The effect of D-ribose L-Cysteine (DRLC) on brain Nitric Oxide activity was assayed for in mice subjected to Bisphenol A. The result revealed that the brain NO activity was significantly ($p < .05$) [Fig. 9] elevated in the

Bisphenol A (BPA) treated mice when compared with the control group. However, D-ribose L-Cysteine (50mg/kg and 100mg/kg, p.o) significantly (P < .05) reduced the activity level of NO in mice brain serum when compared to the Bisphenol A treated mice.

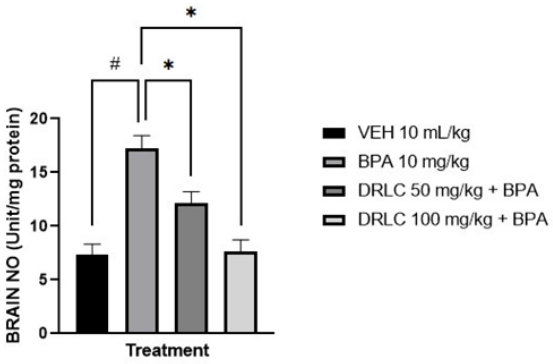

Fig. 9. Effect of D-ribose-L-Cysteine on Bisphenol A-induced anxiety and depressive-like behaviour in mice in Nitric Oxide (NO) in the brain

*Values represent the mean ± S.E.M of six animals per group; # p<0.05 compared to the control group (ANOVA followed by Turkey's post hoc test); * p<0.05 compared to the pathologic group (ANOVA followed by Turkey's post hoc test)*

3.10 Effect of D-ribose L-Cysteine (DRLC) on Brain Superoxide Dismutase (SOD) Level in Mice Treated with Bisphenol A

The effect of D-ribose L-Cysteine (DRLC) on antioxidant status (SOD) was assayed for in mice exposed to Bisphenol A is shown in the [Fig. 10]. Bisphenol A significantly decreased the concentration of SOD in the brain when compared with the control group. One-way ANOVA revealed a significant difference among treatment groups. Post-hoc comparison indicated that treatment with D-ribose L-Cysteine (50 mg/kg and 100 mg/kg, p.o) significantly (p < 0.05) reversed the Bisphenol A induced reduction in SOD concentration.

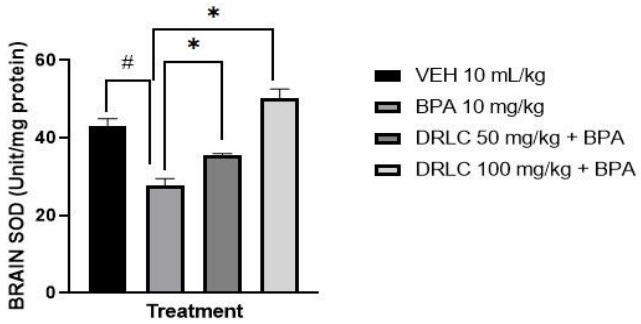

Fig. 10. Effect of D-ribose-L-Cysteine on Bisphenol A induced anxiety and depressive-like behaviour in mice in Superoxide dismutase (SOD) in brain

*Values represent the mean ± S.E.M of six animals per group; # p < .05 compared to the control group (ANOVA followed by Turkey's post hoc test); * p < .05 compared to the pathologic group (ANOVA followed by Turkey's post hoc test).*

3.11 Effects of D-ribose L-Cysteine (DRLC) on Brain Catalase (CAT) Activity in Bisphenol A-Treated Mice

The effect of D-ribose L-Cysteine (DRLC) on brain Catalase (CAT) activity was assayed for in mice subjected to Bisphenol A. The result revealed that the brain CAT activity was significantly (p < 0.05) [Fig. 11] reduced in the Bisphenol A (BPA) treated mice when compared with the control group. However, D-ribose L-Cysteine (50 mg/kg and 100 mg/kg, p.o) significantly (P <0.05) increased the activity level of CAT in mice brain serum when compared to the Bisphenol A-treated mice.

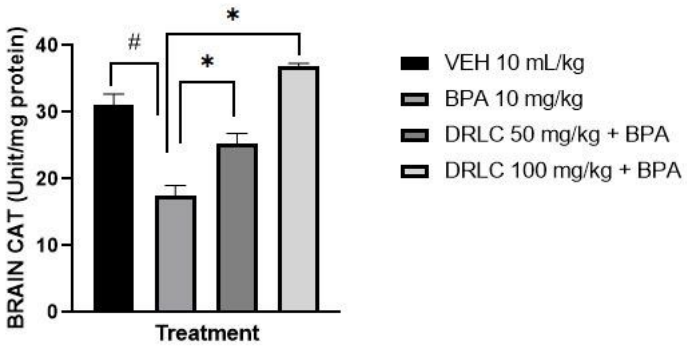

Fig. 11. Effect of D-ribose-L-Cysteine on Bisphenol A induced anxiety and depressive-like behaviour in mice in Catalase (CAT) in brain

*Values represent the mean ± S.E.M for 6 animals per group; # p<0.05 compared to the control group (ANOVA followed by Turkey's post hoc test); * p<0.05 compared to the pathologic group (ANOVA followed by Turkey's post hoc test).*

3.12 Effect of D-ribose L-Cysteine (DRLC) on Brain Glutathione Peroxidase (GPx) Level in Mice Treated with Bisphenol A

The effect of D-ribose L-Cysteine (DRLC) on antioxidant status (GPx) was assayed for in mice exposed to Bisphenol A [Fig. 12]. Bisphenol A significantly decreased the concentration of Glutathione peroxidase (GPx) in the brain when compared with the control group. One-way ANOVA revealed a significant difference among treatment groups. Post-hoc comparison indicated that treatment with D-ribose L-Cysteine (50 mg/kg and 100 mg/kg, p.o) significantly ($p < .05$) reversed the Bisphenol A induced reduction in GPx concentration.

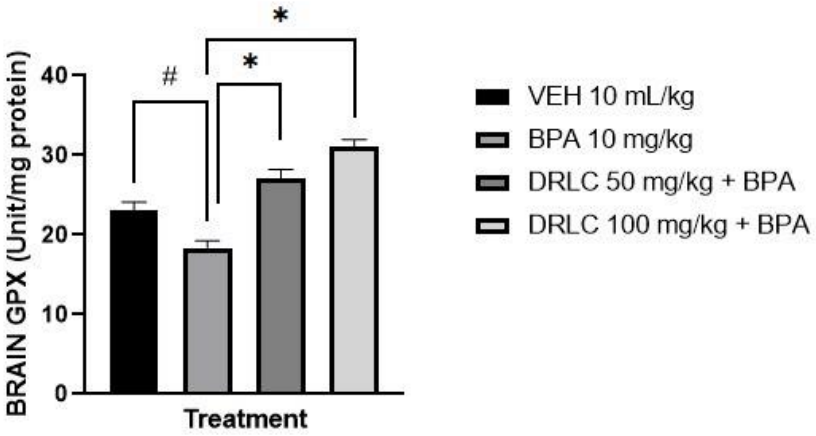

Fig. 12. Effect of D-ribose-L-Cysteine on Bisphenol A-induced anxiety and depressive-like behaviour in mice in Glutathione Peroxidase (GPx) in the brain

*Values represent the mean ± S.E.M for 6 animals per group; # p<0.05 compared to the control group (ANOVA followed by Turkey's post hoc test); * p<0.05 compared to the pathologic group (ANOVA followed by Turkey's post hoc test).*

3.13 Effect of D-ribose L-Cysteine (DRLC) on Brain Reduced Glutathione (GSH) Level in Mice Treated with Bisphenol A

The effect of D-ribose L-Cysteine (DRLC) on antioxidant status (GSH) was assayed for in mice exposed to Bisphenol A is shown [Fig. 13]. Bisphenol A significantly decreased the concentration of reduced Glutathione (GSH) in the brain when compared with the control group. One-way ANOVA revealed a significant difference among treatment groups. Post-hoc comparison indicated that treatment with D-ribose L-Cysteine (50 mg/kg and 100 mg/kg, p.o) significantly ($p < .05$) reversed the Bisphenol A induced reduction in GSH concentration.

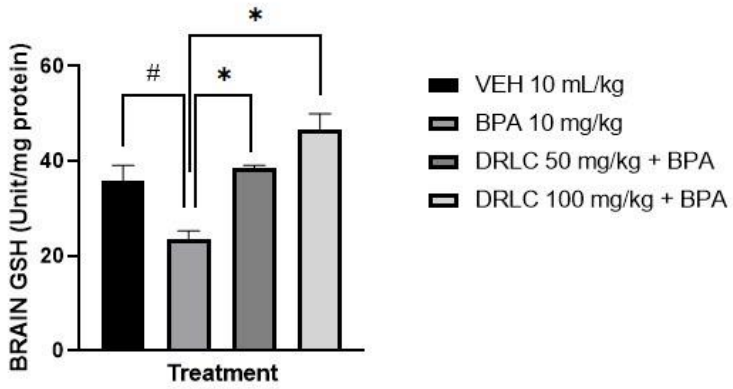

Fig. 13. Effect of D-ribose-L-Cysteine on Bisphenol A-induced anxiety and depressive-like behaviour Glutathione (GSH) level in mice brain

*Values represent the mean ± S.E.M of six animals per group; # p<0.05 compared to the control group (ANOVA followed by Turkey's post hoc test); * p<0.05 compared to the pathologic group (ANOVA followed by Turkey's post hoc test).*

3.14 Effects of D-ribose L-Cysteine (DRLC) on Brain Adrenocorticotropic Hormone Activity in Bisphenol A-Treated Mice

The effect of D-ribose L-Cysteine (DRLC) on brain adrenocorticotropic hormone activity was assayed for in mice exposed to Bisphenol A. The result revealed that the brain adrenocorticotropic activity was significantly ($p < .05$) [Fig. 14] elevated in the Bisphenol A (BPA) treated mice when compared with the control group. However, D-ribose L-Cysteine (50mg/kg and 100mg/kg, p.o) significantly ($P < .05$) reduced the activity level of adrenocorticotropic hormone in mice brain serum when compared to the Bisphenol A treated mice.

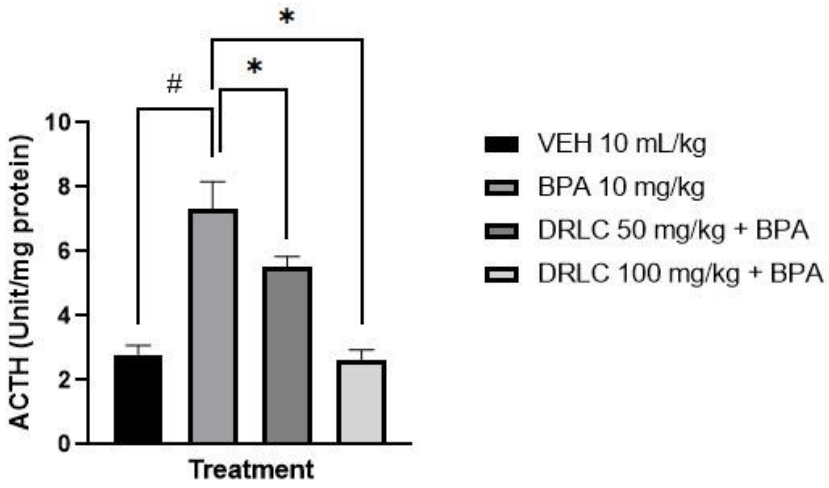

Fig. 14. Effect of D-ribose-L-Cysteine on Bisphenol A induced anxiety and depressive-like behaviour in mice in Adrenocorticotropic (ACTH) hormone in the brain

*Values represent the mean ± S.E.M for 6 animals per group; # p < .05 compared to the control group (ANOVA followed by Turkey's post hoc test); * p < .05 compared to the pathologic group (ANOVA followed by Turkey's post hoc test)*

4. Discussion

The present study evaluated D-Ribose-L-Cysteine Mitigates Bisphenol A-Induced Anxiety and Depressive-Like Behaviours in Experimental Mice. Exposure to bisphenol A on alternate days for 14 days resulted in anxiety-like behaviours characterized by an increase in the amount of time spent in the dark compartment with the LDB (Fig. 1), increase in the amount of time spent in the close arm with the EPM (Fig. 2), reduction in the frequency

of head dips with the HBT (Fig. 3) and depressive-like behaviours characterized by increase in immobility time in the TST (Fig. 4), reduction in social preference percentage using the SIT(Fig. 5), and increase in immobility time with the FST (Fig. 6). These behavioural disturbances were accompanied with remarkable increase in MPO (Fig. 7), MDA (Fig. 8), NO (Fig. 9), ACTH (14) and decrease in SOD (Fig. 10), CAT (Fig. 11), GPx (Fig. 12), GSH (13) in the brain. This findings are currently in line with previously reported studies (Abdou et al., 2022; Li et al.,2024). Neuroinflammation is a key underlying mechanism associated with most neurodegenerative diseases. Bisphenol A administration significantly increased MPO activity which is a well-documented marker of neutrophil infiltration and inflammatory response. Increased activity in MPO is indicative of inflammatory pathway activation within the brain. This can lead to impairment in neuronal intergrity and synaptic function, which are processes implicated in the pathophysiology of mood disorder. Bisphenol A is known to impair synaptic plasticity and emotional regulation thus contributing to anxiety and depressive-like behaviour (JI & YU, 2025). Besides being an endocrine disruptor (Costa & Cairrao, 2024), BPA enhance the production of reactive oxygen specie which is evident in figures 7and Fig. 8. Bisphenol A is also seen to cause increase in ACTH level. This could be as a result of hyperactivation of the HPA- axis, resulting in sustained glucocorticoid release and impaired stress feedback mechanism. The resulting endocrine disruption results in anxiety and depressive-like behaviours in established models. Particularly, treatment with DRLC (50 mg/kg and 100 mg/kg) significantly mitigated these behavioural and biochemical derangement, implicative of its therapeutic effect. These findings are similar to previously reported studies (Okoh et al., 2020; Isibor et al.,2022; Isibor et al., 2025).

5. Conclusion

D-Ribose L-Cysteine significantly attenuates anxiety-like and depressive-like behaviours induced by Bisphenol A in mice. Its action involves the restoration of antioxidant defenses, reduction of oxidative/nitrosative stress, and normalization of stress hormone levels.

6. Limitation

Despite these interesting findings from this study, key neurotransmitters such as serotonin and dopamine levels were not assessed.

Consent

It is not applicable.

Ethical Approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the “ethics committee of Faculty of Allied Health Sciences, Delta State University, Abraka, with approval number RBC/FBMC/DELSU/24/645.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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