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ORIGINAL RESEARCH

Assessment of the Impact of *Bidens pilosa* on Behavioral, Oxidative Stress and Cerebellar Cortical Histoarchitectural Alterations During Bisphenol A Exposure in Mice

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Purpose: The use of medicine plants in the management of various human ailments have gained lots of attention in recent time; hence, the present study aimed to investigate the impact of *Bidens pilosa* on the behavioral, oxidative stress and cerebellar cortical histoarchitectural alterations during bisphenol A exposure in mice.

Methods: Thirty (30) adult male mice were divided into six groups. Group 1 received distilled water (2 mls/kg b.w). and group 2 was treated with (bisphenol A) BPA (100 mg/kg body weight). Groups 3–5 were co-treated with varying doses of *Bidens pilosa* (250 mg/kg, 500 mg/kg, and 1000 mg/kg b.w). and group 6 received vitamin C (60 mg/kg b.w). along with BPA. The animals underwent neurobehavioral tests (beam walking and wire hang). Other parameters evaluated included body weight, oxidative stress biomarkers, and cerebellar histology.

Results: Animals treated with high doses of *Bidens pilosa* spent less time crossing the beam during the beam walking test and more time in the wire hang test than those treated with BPA alone. Lowered malondialdehyde level and higher catalase and superoxide dismutase activity was observed in *Bidens pilosa* treated groups than in the BPA-only group. Histological examination revealed a significant improvement in the cerebellar tissue structure in *Bidens pilosa* treated groups, particularly at higher doses.

Conclusion: *Bidens pilosa* demonstrated potential protective effects against BPA-induced oxidative stress and negative histological changes in the cerebellar cortex, suggesting its therapeutic potential for mitigating BPA neurotoxicity. Further research is needed to explore the therapeutic applicability.

Keywords: cerebellar cortex, *Bidens pilosa*, bisphenol A, oxidative stress, neuroprotection

Introduction

Bisphenol A (BPA) is a high-production volume industrial chemical widely used in the manufacture of epoxy resins and polycarbonate plastics.[1,2] BPA is an integral component in the production of various consumer goods, including food and beverage containers, water bottles, baby feeding bottles, inner linings of canned foods, pharmaceutical delivery systems, and dental sealants. Due to its wide usage, BPA is continuously released into the environment, with large quantities finding their way into aquatic ecosystems.[3] Environmental exposure to BPA poses a global threat to aquatic organisms, livestock, and humans, primarily through bioaccumulation and food chain transfer. The most common route for BPA exposure are through the consumption of contaminated food and drink, skin contact, or inhalation. It then builds up in biological tissues and may have long-term detrimental effects.[4,5]

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BPA is an endocrine disruptor (EDC) that interacts with hormonal signalling pathways and mimic estrogen. Researches have linked exposure to BPA to several negative health problems, such as immune system related disorder, metabolic disorders, neurodevelopmental abnormalities, and reproductive dysfunction.[1,6–9] The growing neurological system's susceptibility to BPA's neurotoxic effects, particularly in fetuses and young children, is a cause for great concern. According to scientific research, BPA can cause inflammation, neuronal degeneration, and oxidative stress in critical brain areas, including the cerebellar cortex, which is essential for motor coordination.[10] Recent data suggests that BPA has significant neurotoxic effects, mostly via triggering apoptotic pathways and oxidative stress in brain regions. By producing more reactive oxygen species (ROS), compromising mitochondrial function, and upsets of neural homeostasis.[1,11] Long-term BPA exposure has also been linked to neurodegenerative alterations, including astrogliosis, Purkinje cell loss, and neuronal shrinkage, which can affect cognitive and motor function.[12] Given the global health concerns associated with BPA exposure, there is a growing interest in identifying safer, more effective protective interventions. Plant-based treatments have become more popular for complementary and alternative therapies in recent years, especially those with a traditional therapeutic background.[13–21] Among these, *Bidens pilosa*, commonly known as blackjack, has attracted scientific attention due to its rich pharmacological profile. A member of the Asteraceae family, *Bidens pilosa* is widely distributed across tropical and subtropical regions and is renowned for its adaptability and resilience.[22–24] Traditionally, *Biden pilosa* plant has been used to treat a variety of ailments, including gastrointestinal disturbances, respiratory disorders, and inflammatory conditions.[25,26] Phytochemical studies of *Bidens pilosa* have revealed several bioactive compounds, including diterpenes, flavonoids, alkaloids, saponins, and polyacetylenes.[25] The phytochemical compounds present in *Bidens pilosa* exhibit neuroprotective, anti-inflammatory, and antioxidant qualities, indicating that *Bidens pilosa* may be a viable natural remedy for BPA-induced neurotoxicity.

Despite widespread evidence of BPA's neurotoxicity in most brain regions, particularly in the cerebellum, there is a lack of effective, accessible, and affordable therapeutic agents to mitigate its adverse effects. Moreover, the neuroprotective potential of *Bidens pilosa* in the context of BPA exposure has not been adequately explored. This study builds on existing knowledge of *Bidens pilosa*'s pharmacological potential by investigating its protective effects on the cerebellar cortex during BPA exposure in mice. Hence, the aim of the present study was to examine the impact of *Bidens pilosa* on behavioral, oxidative stress and cerebellar cortical histoarchitectural alterations during BPA exposure in mice. Exploring such natural products and plants may provide safer alternatives for mitigating the neurotoxic risks posed by environmental pollutants like BPA.

Materials and Methods

Chemical and Drug Preparation

Bisphenol A (CAS Number: 80–05-7 ADR/PG) was obtained from LOBA CHEMIE PVT Ltd. Vitamin C (1000 mg) was purchased from a reputable drug store in Ishaka Bushenyi.

Acquisition of Plant Material and Extraction

Fresh leaves of *Bidens pilosa* were collected from around Ishaka and verified in the Herbarium unit of the Biology Department of Mbarara University of Science and Technology. The plant was assigned the authentication number AAI-2024-001 by Dr Olet Eunice. The leaf extract of *Bidens pilosa* was prepared as described by Li et al[27] with minor modifications. Fresh leaves were shade-dried and grounded into fine powder using an electric blender (WARING MX-1100XT). Powdered plant leaves (500 g each) were soaked in 1500 mL of distilled water for 48 h with intermittent shaking every 12 h. The mixture was drained and filtered using cloth gauze and Whatman filter paper 1.[28] The filtrate was then concentrated using a rotary evaporator at a temperature of 45°C. The extract was then stored in the refrigerator at −20°C and protected from light to prevent possible photodegradation using amber-colored glass container from where the extract was retrieved for use during each administration.

Qualitative phytochemical screening of the methanol fraction of *Bidens pilosa* leaves was performed using standard methods to determine the presence of chemical constituents.[29] For quantitative screening, the total polyphenol content of the extract was determined as described by Geremu[30] and the total flavonoid content was estimated as described by

Shraim & Hijji.[31] Both qualitative and quantitative screening were repeated in each cases to improve the reliability of the obtained results.

Acute Toxicity Study

The up-and-down (OECD 425) method was employed to assess the acute oral toxicity of the methanol extract of *Bidens pilosa*, following the approach described by Santello & Volterra.[32] Following the OECD 425 guidelines, the upper limit was 5000 mg/kg because the toxicity of this plant sourced in Western Uganda and its methanol extract has not been previously reported. This technique effectively determines the toxicological profile of the extract while minimizing the number of animals used. By the end of the procedure, six female mice were utilized for the acute toxicity study.

Experimental Animals

Thirty (30) adult male mice were obtained from the animal house of Kampala International University, Western Campus, Uganda. The animals were kept in a room with regulated temperature, 12-hour light and 12-hour dark cycles, and 20–22 °C ambient temperature. The experimental animals were allowed free access to food and water, without restrictions. Experimental animals were cared for and maintained in accordance with the guidelines for the care and use of laboratory animals.

Dosage Determination

A dose of 100 mg/kg bw was adopted for BPA administration (orally) as reported by Gurmeet et al.[33] The adopted dose of Vitamin C was 200 mg/kg, as previously described by Greggi et al.[34] The dosages of *Bidens pilosa* were 200 mg/kg (4% of the LD50), 400 mg/kg (8% of the LD50), and 600 mg/kg (12% of the LD50) as low, medium, and high doses, respectively since the oral LD_{50} of the methanolic extract of *Bidens pilosa* was greater than 5000 mg/kg in mice. Our choice of the different doses of *Bidens pilosa* was also supported by Ezeonwumelu et al.[35]

The stock solution for BPA was prepared by dissolving 100 mg of BPA in 10 mL of olive oil, *Bidens pilosa* extract was prepared by dissolving 100 mg of extract in 5 mL of distilled water, vitamin C was prepared by dissolving 100 mg of extract in 10 mL of distilled water. The volume of BPA, *Bidens pilosa* and vitamin C to be administered to the experimental animals were subsequently calculated based on the body weight of each of the experimental animal and dosage (BPA – 100 mg/kg; *Bidens pilosa* – 200, 400, and 600 mg/kg; vitamin C – 200 mg/kg).

Experimental Design

The experimental animals were randomly assigned to six groups (n = 5) according to the ARRIVE guidelines. Group 1 administered 2 mL/kg of distilled water (Normal Control), Group 2 was administered 100 mg/kg bw BPA (negative control), Group 3 was administered 100 mg/kg bw BPA + 200 mg/kg bw B.P, Group 4 was administered 100 mg/kg bw BPA + 400 mg/kg bw B. P, Group 5 was administered 100 mg/kg bw BPA + 600 mg/kg bw B.P, and Group 6 was administered 100 mg/kg bw BPA + 200 mg/kg bw vitamin C.

Administration was performed via gastric intubation for 4 weeks. The experimental animals were subjected to beam walking and wire hang tests as outlined by Ayuba[13] The weights of the animals were measured weekly until the end of the experimental period using a digital weighing balance (Kern EMB 1000–2 Precision Balance Weighing Scale). At the end of the experiment, the animals were euthanized using 5 mg/kg of thiopental sodium (intraperitoneal injection) as described by Usman et al.[17] This was followed by a mid-sagittal incision through the skull to expose the cerebellum for excision. The removed cerebellum was weighed and prepared for histological processing and oxidative stress analysis. The tissue for the histological study was fixed in 10% neutral buffered formalin, whereas samples for the oxidative stress study were homogenized in phosphate buffer.

Behavioral Study

Beam Walking Test

This test evaluates balance and fine motor coordination. The beam walking setup included two poles holding one-meter beams, which had a 12 mm wide flat surface and were elevated 50 cm above the table This apparatus effectively detects subtle motor skills and balance impairments that other tests, such as rotarods, might not capture. The dark box represents

the beam endpoint. Approximately ten minutes before training, mice were introduced into a room containing the beam apparatus. The mouse was then carefully positioned at the center of the beam, facing one of its ends. The mice were permitted to move towards the end of the dark box, and the time it took to reach the dark box was monitored; each mouse underwent three trials.[13]

Wire Hang Test

A custom-built wire hang test apparatus was used to assess the grip strength of the mice. A circular wire was hung to allow mice to grasp the underside. The optimal duration for the mouse to remain on the wire was set at 180 seconds. Suspension time (time until the mouse fell) was recorded as described by Ayuba et al.[13]

Oxidative Stress Assessment

The activities of key oxidative stress biomarkers and enzymes, such as malondialdehyde, superoxide dismutase, and catalase, were evaluated in cerebellar tissue homogenates from mice across the experimental groups. The cerebellar tissue was homogenized in cold 0.1M Tris-HCl buffer (pH 7.4; 1:5 w/v) and centrifuged for 10 min at 3000 × g to produce a pellet, which was discarded, and the supernatant was used for the quantification of malondialdehyde, superoxide dismutase, and catalase. The tissue levels of malondialdehyde were determined as described by Rizzo.[36] The tissue SOD level of superoxide dismutase was assessed as described by Gueroui & Kechrid.[37] Catalase levels in the cerebellum were determined as described by Afolabi et al.[38]

Tissues Processing and Staining

The fixed tissues were grossed and processed as described by Ayuba et al.[13] The tissue processing stages included dehydration in graded alcohol, clearing with xylene, infiltration with molten paraffin wax, and embedding in paraffin wax. Paraffin provides tissue stability to aid in sectioning during microtomy. The ribbons produced during microtomy were placed on clean slides, dewaxed, cleared with xylene, and rehydrated using graded alcohol in a descending order. The slide preparations were then stained using hematoxylin and eosin, as described by Feldman and Wolfe,[39] and visualized using a light microscope fitted with a camera.

Statistical Analysis

Data were analyzed using GraphPad Prism® version 5.01 (San Diego, CA, USA). One-way analysis of variance (ANOVA) was used to compare the means of quantitative variables in the present study, followed by Tukey's post-hoc test, where necessary; p-value was set to be or less than 0.05.

Result

Phytochemical Profile of the Methanol Extract of Bidens Pilosa Leaf

Qualitative Phytochemical Analysis

Phytochemical studies revealed the presence of various compounds in the extract, including polyphenols, anthocyanosides, anthracenosides, flavonoids, coumarins, glycosides, saponins, tannins, and reducing compounds (Table 1).

Quantitative Phytochemical Analysis

Quantitative phytochemical screening revealed that the methanol leaf extract of *Bidens pilosa* (BP) contained 16.7 ± 0.4 mg/g of total polyphenols and 10.8 ± 0.2 mg/g of total flavonoids as shown below in Figure 1.

Acute Toxicity Study

The result of the acute toxicity study revealed absence of visible signs of toxicity at 175, 550, 1750 and 5000 mg/kg dose in the first, second, and third phase of the test. No mortality was observed at 175, 550, 1750, and 5000 mg/kg dose in the first, second, third, and fourth phase of the test (Table 2).

Table 1 Phytochemical Profile of *Tamarindus Indica*

Phytochemical	Qualitative Test Result
Saponins	–
Reducing compounds	+
Tannin	+
Polyphenol	+
Alkaloid salts	–
Anthocyanosides	+
Anthracenosides	+
Flavonoid	++
Coumarins	+
Steroid glycosides	+

Notes: + denotes presence; – denotes absence.

Neurobehavioral Result

Beam Walking Test Result

The results of the beam walking test revealed that the animals in the 2 mL/kg of distilled water (normal control) and 100 mg/kg BPA + 1000 mg/kg BP (high dose) treated groups took less time to cross the beam when compared to the 100 mg/kg BPA treated group. However, no significant difference was observed in the time required to cross the beam after running the ANOVA test (Figure 2).

Wire Hang Test

The ANOVA results of the wire hang test revealed that the animals in the 2 mL/kg distilled water (normal control) and 100 mg/kg BPA + 1000 mg/kg BP (high dose) treated groups had a higher hold time than those in the other treatment groups. However, there was no significant difference in the reported hold times between treatment groups after running the ANOVA test (Figure 3).

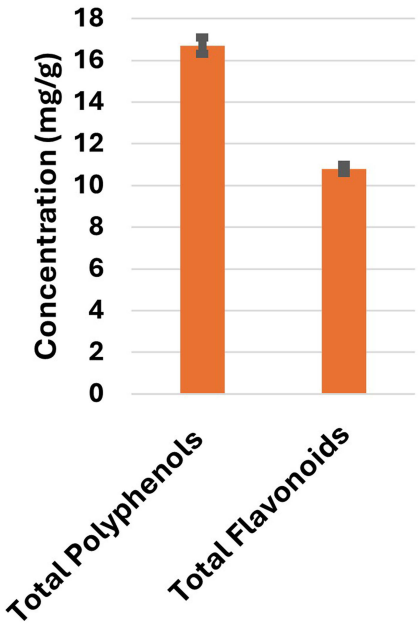

Figure 1 The concentration of the total polyphenols and total flavonoids in the aqueous leaf extract of *Tamarindus indica*. Results are presented as Mean ± SEM.

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Table 2 Acute Toxicity Using the Up and Down Method

Phase	Dose of *Bidens Pilosa*	n	Observation	Mortality After 24 Hours
One	175 mg/kg	1	No visible sign of toxicity	NA
Two	550 mg/kg	1	No visible sign of toxicity	NA
Three	1750 mg/kg	1	No visible sign of toxicity	NA
Four	5000 mg/kg	1	No visible sign of toxicity	NA
	5000 mg/kg	1	Reduced activity which resolved with 2 hours	NA
	5000 mg/kg	1	No visible sign of toxicity	NA

Abbreviations: n, Number of animals used; NA, Not application (no mortality).

Morphometric Study Result

The result of the ANOVA test showed there was no significant difference in the mean body weight change in the experimental animals at weeks 1, 2, 3, and 4 across the different groups. However, the weight decreased in the 100 mg/kg BPA and 100 mg/kg BPA + 250 mg/kg treated groups throughout the administration period (Table 3). There was also no significant difference in the organ body weight ratio of the cerebellum between groups (Figure 4).

Oxidative Stress Result

The result of the ANOVA test revealed significantly lower levels of catalase in the cerebellar tissue of the 100 mg/kg BPA, 100 mg/kg BPA + 250 mg/kg BP, and 100 mg/kg BPA + 1000 mg/kg BP-treated groups than in the 2 mL/kg of distilled water. The 100 mg/kg BPA + 1000 mg/kg BP and 100 mg/kg BPA + 60 mg/kg Vit C treated group had significantly higher levels of catalase in cerebellar cortical tissue than the 100 mg/kg BPA treated group (Figure 5).

Significantly higher levels of malondialdehyde were observed in the cerebellar tissue of the 100 mg/kg BPA-treated group than in the 2 mL/kg distilled water treated group. The 100 mg/kg BPA + 250 mg/kg BP, 100 mg/kg BPA + 500 mg/kg BP, 100 mg/kg BPA + 1000 mg/kg BP, and 100 mg/kg BPA + 60 mg/kg Vitamin C treated groups had significantly lower levels of malondialdehyde in the cerebellar cortical tissue than in the 100 mg/kg BPA treated group (Figure 6).

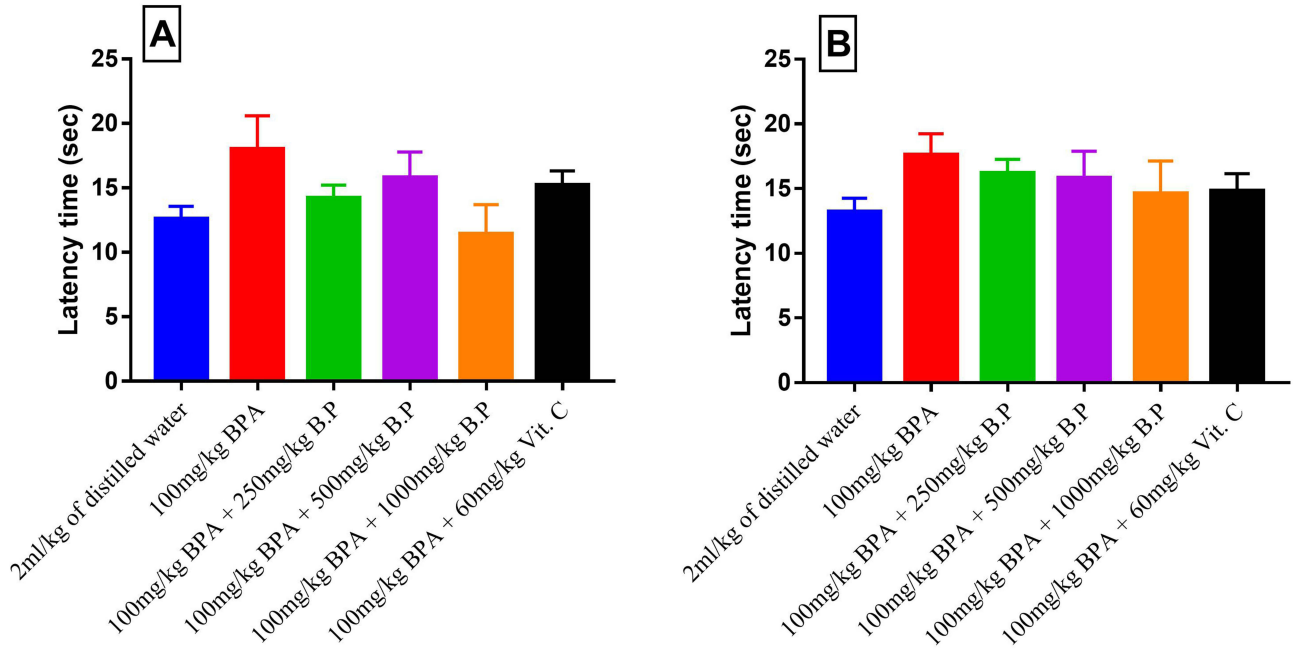

Figure 2 The result of the ANOVA for beam walking Test Result for the different test groups. (**A**) Time taken to cross the beam on week 2 and (**B**) Time taken to cross the beam on week 4. The differences were considered significant at $p<0.05$ (n = 5).
Abbreviations: BPA, Bisphenol A; BP, *Biden pilosa.*

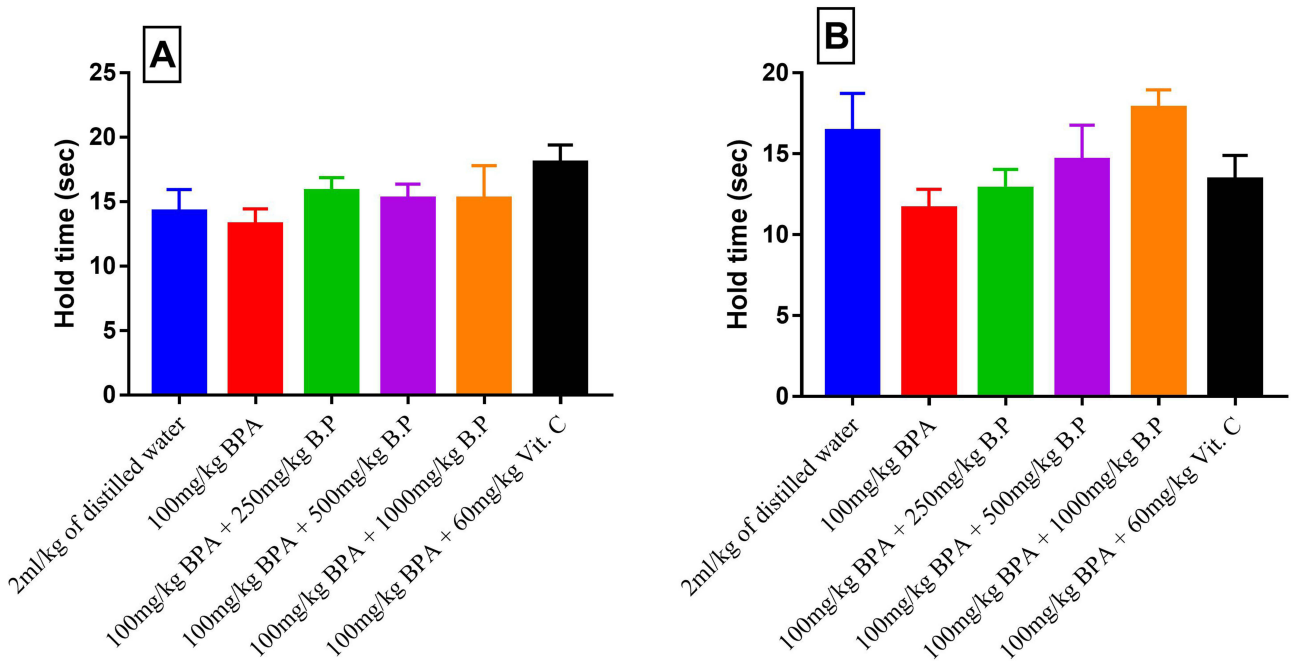

Figure 3 The result of the ANOVA for wire hang test; (**A**) Hang time on the 2nd week and (**B**) Hang time on the 4th week. The differences were considered significant at $p<0.05$ (n = 5).
Abbreviations: BPA, Bisphenol A; BP, *Biden pilosa.*

Significantly low levels of superoxide dismutase were observed in cerebellar tissue in the 100 mg/kg BPA, 100 mg/kg BPA + 250 mg/kg BP, 100 BPA + 500 mg/kg BP, and 100 mg/kg BPA + 60 mg/kg Vit. C treated groups when compared with the distilled water-treated group. Higher levels of cerebellar tissue superoxide dismutase concentration were observed in the 100 mg/kg BPA + 1000 mg/kg BP treatment group than in the 100 mg/kg BPA-treated group (Figure 7).

Histology Study

Histological examination of the cerebellar cortex showed normal histoarchitecture in the 2 mL/kg distilled water group (Figure 8A). A marked alteration was observed in the cerebellar cortex following the administration of 100 mg/kg BPA, as evidenced by the presence of pyknotic cells and reduced linear distribution of Purkinje cells (Figure 8B–D). The administration of 1000 mg/kg BP and 60 mg/kg vitamin C significantly improved the histoarchitecture of the cerebellar cortex (Figure 8E and F).

Table 3 The Result of the ANOVA for Body Weight Change from the Different Treatment Groups

Treatment	Week 1	Week 2	Week 3	Week 4
2 mL/kg of distilled water	37.12±0.34	37.36±0.27	37.90±0.43	37.00±0.61
100 mg/kg BPA	38.44±1.47	36.98±1.66	37.55±1.19	36.86±0.85
100 mg/kg BPA + 250 mg/kg BP	37.18±1.72	35.37±1.53	37.14±1.70	36.46±1.55
100 mg/kg BPA + 500 mg/kg BP	38.56±1.53	37.76±1.45	38.15±1.32	38.42±1.59
100 mg/kg BPA + 1000 mg/kg BP	37.58±1.21	37.25±1.12	36.81±1.37	36.27±1.32
100 mg/kg BPA + 60 mg/kg Vit. C	36.3±1.78	34.29±1.50	34.73±1.95	35.75±1.87

Notes: The differences were considered significant at $p<0.05$ (n = 5).
Abbreviations: BPA, Bisphenol A; BP, *Biden pilosa.*

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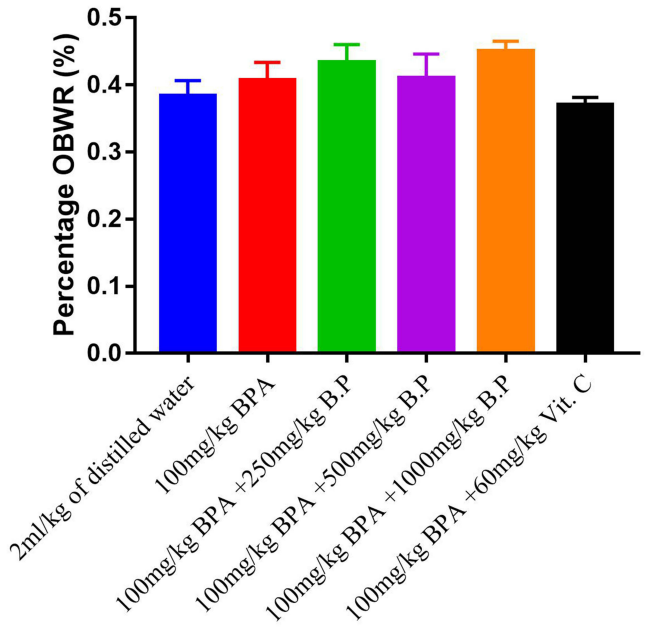

Figure 4 The result of the ANOVA for the percentage organ body weight ratio (OBWR) of the cerebellum. The differences were considered significant at $p<0.05$ (n = 5).
Abbreviations: BPA, Bisphenol A; BP, *Biden pilosa*.

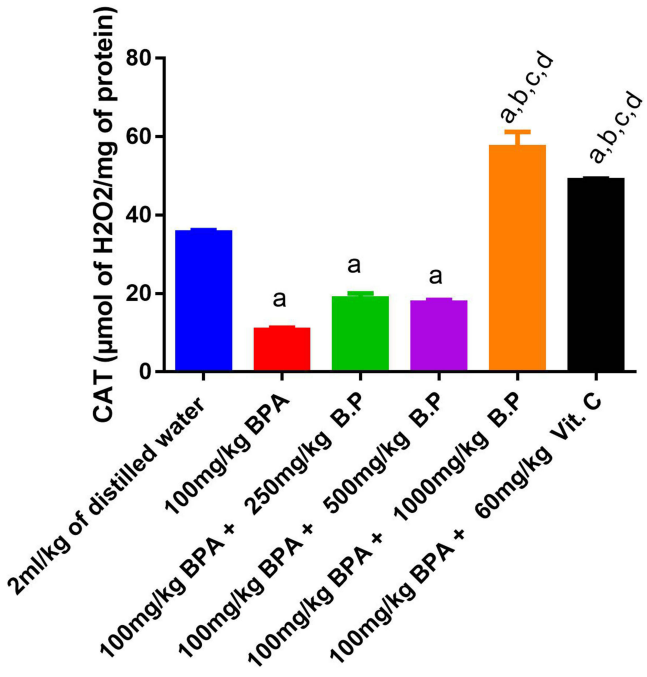

Figure 5 The result of the ANOVA test for the level of tissue catalase in the cerebellum from all the treatment groups. [a–d]Indicate significant differences when compared to 2mL/kg of distilled water, 100 mg/kg BPA, 100 mg/kg BPA + 250 mg/kg BP, and 100 mg/kg BPA + 500 mg/kg BP respectively at $p<0.05$.
Abbreviations: BPA, Bisphenol A; BP, *Biden pilosa*; CAT, Catalase.

Discussion

The qualitative phytochemical analysis of *Bidens pilosa* extract in the present study revealed the presence of bioactive compounds, including polyphenols, flavonoids, tannins, anthocyanosides, anthracenosides, coumarins, and steroid glycosides. On the other hand, the quantitative assay revealed the presence of high levels of polyphenols and flavonoids. The identified compounds are known to play very important role in mitigating oxidative stress-induced neuronal damage.[40]

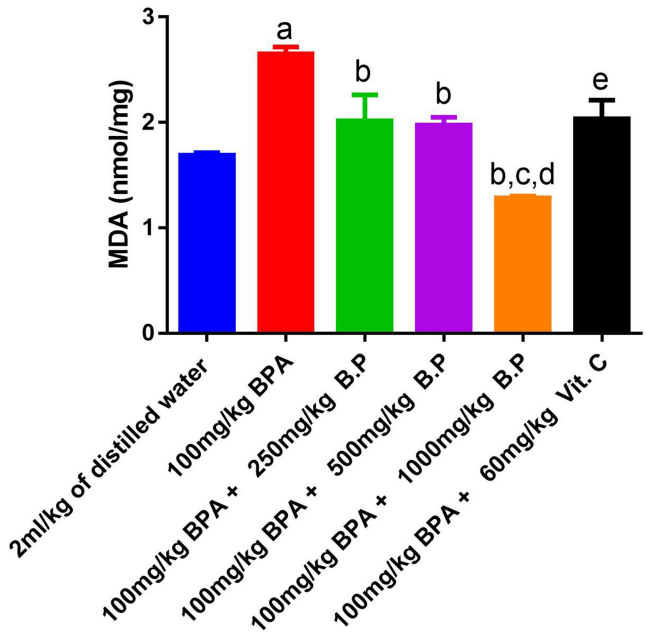

Figure 6 The result of the ANOVA for the levels of tissue malondialdehyde in the cerebellum from all the treatment groups. $^{a-e}$ indicate significant differences when compared to 2mL/kg of distilled water, 100 mg/kg BPA, 100 mg/kg BPA + 250 mg/kg BP, 100 mg/kg BPA + 500 mg/kg BP, and 100 mg/kg BPA + 1000 mg/kg BP respectively at $p<0.05$.
Abbreviations: BPA, Bisphenol A; BP, *Biden pilosa*; MDA, Malondialdehyde.

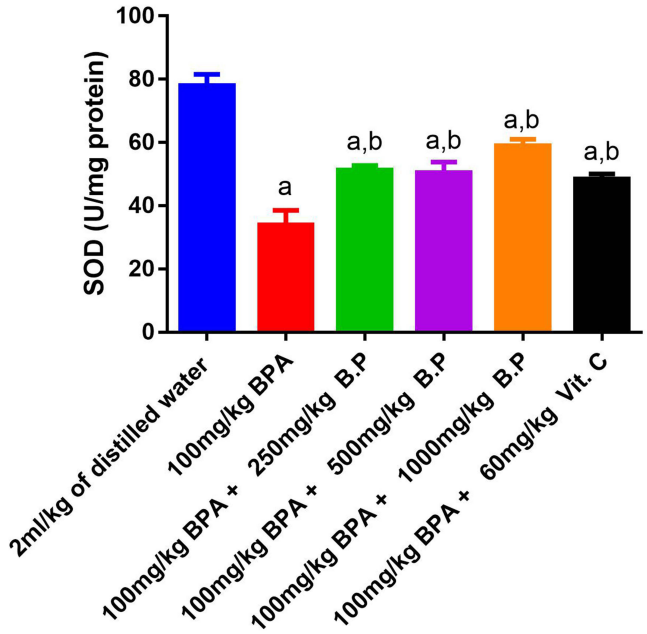

Figure 7 The result of the ANOVA test for the level of tissue super oxide dismutase in the cerebellum from all the treatment groups. ^{a} and ^{b} indicate significant difference when compared to 2 mL/kg of distilled water and 100 mg/kg BPA respectively at $p<0.05$.
Abbreviations: BPA, Bisphenol A; BP, *Biden pilosa*; SOD, Super oxide dismutase.

The result of the oral acute toxicity study using female mice in the present study revealed that the LD50 of *Bidens pilosa* was greater than 5000 mg/kg bw. Plant extract with LD50 greater than 5000 mg/kg are often considered to be relatively safe;[41] hence, highlighting key fact about possible safety of *Bidens pilosa.*

Neurobehavioral assessments have shed light on the functional impact of *Bidens pilosa* in counteracting Bisphenol A (BPA)-induced motor deficits. Although the beam walking test did not show statistically significant differences across the treatment

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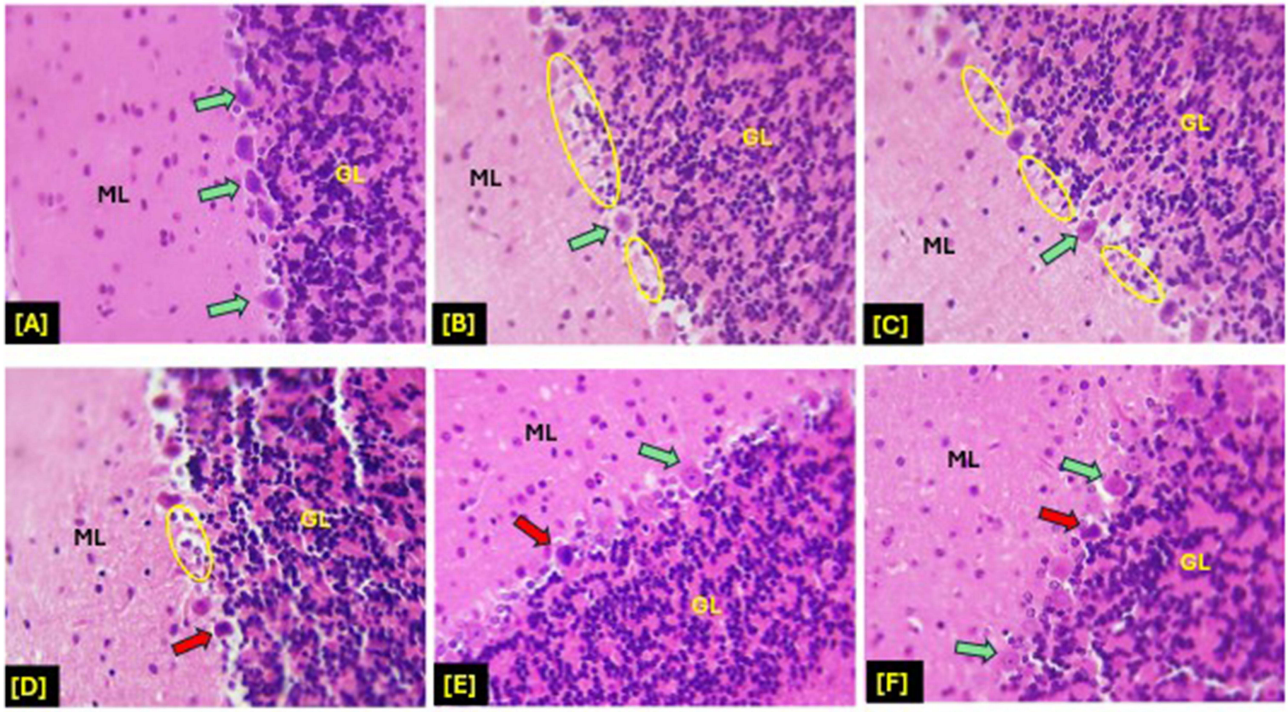

Figure 8 The Photomicrograph of the cerebellar cortex from the treatment groups. (**A**) 2 mL/kg of distilled water; (**B**) 100 mg/kg BPA; (**C**) 100 mg/kg BPA + 250 mg/kg BP; (**D**) 100 mg/kg BPA + 500 mg/kg BP; (**E**) 100 mg/kg BPA + 1000 mg/kg BP; (**F**) 100 mg/kg BPA + 60 mg/kg Vit. C; Purkinje cell (Green arrow); Degenerating Purkinje cell (Red arrow); Region with reduced linear distribution of Purkinje cells (Yellow circles) (H&E; ×400).
Abbreviations: ML, Molecular layer; GL, Granular layer.

groups, the high-dose *Bidens pilosa* group (100 mg/kg BPA + 1000 mg/kg *Bidens pilosa*) showed improved motor coordination, as evidenced by a reduced crossing time. This finding suggests a potential neuroprotective effect that may be attributed to the antioxidant properties of very important phytochemical present within *Bidens pilosa* extract. These findings suggest that *Bidens pilosa* like other plants rich in antioxidant phytochemicals, may offer protective benefits against BPA-induced neurotoxicity, which is consistent with previous studies that demonstrated the neuroprotective potential of antioxidant-rich plants on motor function.[42] However, the lack of statistical significance underscores the need for other behavioral studies, larger sample sizes and longer treatment durations to confirm these findings.

The wire hang test showed that animals treated with a high dose (1000 mg/kg) of *Bidens pilosa* had longer hang times, indicating enhanced neuromuscular strength. This suggests that *Bidens pilosa* may protect against BPA-induced neuromuscular impairments. BPA exposure has been shown to compromise neuromuscular integrity and locomotor activity via oxidative stress, which leads to impaired motor function.[6] Notably, the improvements observed with *Bidens pilosa* were comparable to the neuroprotective effects of vitamin C, an FDA-approved water-soluble antioxidant known to enhance motor coordination and neuromuscular strength through similar mechanisms.[43] These findings underscore the potential of *Bidens pilosa* as an possible alternative or complementary treatment to vitamin C, supporting its use in mitigating BPA-induced neuromuscular deficits.

Gross morphological analysis indicated that BPA exposure did not significantly alter the body weight, although a decreasing trend was observed in the 100 mg/kg BPA and 100 mg/kg BPA + 250 mg/kg *Bidens pilosa* groups. This suggests that BPA induces metabolic disturbances, possibly through endocrine disruption, as documented by Maniradhan and Calivarathan[2] and Hong et al.[44] The absence of significant weight loss in the high-dose *Bidens pilosa* group suggests that the plant extract may offer systemic protection beyond neuroprotection, potentially mitigating the BPA-induced metabolic dysregulation. The organ-body weight ratio of the cerebellum remained unchanged, indicating that BPA and *Bidens pilosa* administration did not result in remarkable gross anatomical changes. However, this does not preclude the possibility of histological or molecular alterations within this brain region, necessitating further investigation using advanced imaging and molecular techniques.

In our study, oxidative stress biomarkers and antioxidant enzyme assay provided compelling evidence for the neuroprotective role of *Bidens pilosa*. Bisphenol A exposure significantly reduced cerebellar catalase (CAT) activity, while the high-dose *Bidens pilosa* group exhibited significantly higher catalase levels than the BPA-only group. CAT is an important antioxidant enzyme that neutralizes hydrogen peroxide and protects against oxidative damage.[45] This finding is consistent with studies showing that polyphenol-rich plant extracts boost endogenous antioxidant defenses against neurotoxicity.[19] The restoration of superoxide dismutase (SOD) activity in the high-dose *Bidens pilosa* group reinforced its antioxidant-linked neuroprotective effects. BPA-induced oxidative stress suppresses SOD, a critical enzyme that neutralizes superoxide radicals and protects against oxidative neurodegeneration.[46,47] The high-dose *Bidens pilosa* group showed significantly higher SOD activity than the BPA-only group, suggesting that *Bidens pilosa* enhances antioxidant defenses. This result is consistent with those of previous studies indicating that flavonoids and polyphenols stimulate endogenous antioxidant enzyme expression,[48] thereby counteracting neurotoxic insults. Similarly, vitamin C restores SOD activity, supporting its role in combating oxidative stress and neurodegeneration.[49] The comparable effects of *Bidens pilosa* and vitamin C highlight the potential of *Bidens pilosa* as a viable alternative or adjunct to the conventional antioxidants.

Additionally, lipid peroxidation, as measured by malondialdehyde (MDA) levels, was significantly elevated in BPA-treated animals, confirming the oxidative stress-inducing properties of BPA. The reduction in MDA levels in the high-dose *Bidens pilosa* group further supports its neuroprotective role by preventing oxidative damage to the neuronal membranes. These findings align with previous research demonstrating that plants rich in antioxidants, such as *Bidens pilosa* reduce oxidative stress markers and lipid peroxidation, thereby offering protection against neuronal damage.[50] The effect of *Bidens pilosa* in oxidative stress by Bidens pilosa was comparable to that by vitamin C, which is known to decrease MDA levels and reduce oxidative damage in neuronal tissues.[51]

Histological findings confirmed the protective effects of *Bidens pilosa* against BPA-induced neurotoxicity. The BPA-treated group showed cerebellar tissue alterations, including pyknotic neurons and disrupted Purkinje cell distribution. These alterations have been reported as early signs of neurodegeneration Bi et al.[52] These findings align with previous studies indicating that BPA induces negative histological changes in the cerebellum, neuronal apoptosis and synaptic dysfunction through oxidative stress.[53] The high-dose *Bidens pilosa* group exhibited remarkable preservation of cerebellar histoarchitecture, with well-preserved Purkinje cells and fewer signs of neurodegeneration. These findings underscore the neuroprotective role of *Bidens pilosa*, which is likely attributable to its antioxidant properties. The neuroprotective effects observed in this group were consistent with those of vitamin C, which has been shown to preserve neuronal integrity and protect against oxidative stress.[54]

Despite these promising findings, this study had several limitations. The lack of statistically significant differences in some behavioral tests suggests that other behavioral studies such as rotarod, larger sample sizes or longer treatment durations may be necessary to fully capture the neuroprotective effects of *Bidens pilosa*. The present submission did not use isolated active compound for administration which could have helped in pinpointing the active compound responsible for the protective potential of *Bidens pilosa* in the present study. Nonetheless, the findings from this study contribute to the growing body of evidence supporting plant-based interventions for neuroprotection and highlight the need for further translational studies to explore their clinical potential.

Conclusion

This study identified *Bidens pilosa* as a plant with neuroprotective potential, particularly through its antioxidant and cerebellar tissue-protective effects. High doses of *Bidens pilosa* enhanced catalase and SOD activity, reduced oxidative stress, and preserved cerebellar histomorphology. These findings suggest *Bidens pilosa* is a promising plant-based neuroprotective agent, comparable to established natural antioxidants such as vitamin C. Given the widespread exposure to neurotoxins, such as BPA, *Bidens pilosa* could serve as a valuable adjunct in mitigating neurotoxicity. Future studies should explore oxidative stress markers and histological analyses to provide compelling evidence of neuroprotection, and explore additional molecular pathways, including inflammatory and apoptotic signaling cascades, to better understand the underlying mechanisms. Research on the bioavailability and pharmacokinetics of *Bidens pilosa* phytochemicals is warranted to optimize dosing regimens for clinical applications. Elucidating its therapeutic potential can pave the way for translational applications in the clinical setting. These findings contribute to the growing body of evidence supporting the use of plant-based interventions for neuroprotection and emphasize the need for further research to unlock their health benefits.

Data Sharing Statement

All datasets used in this article can be accessed from the corresponding author upon reasonable request.

Ethical Consideration

Ethical approval was obtained from the Kampala International University Research and Ethics Committee (reference number: KIU-2024-697).

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Disclosure

The authors report there are no conflicts of interest in this work.

References

1. Costa HE, Cairrao E. Effect of bisphenol A on the neurological system: a review update. *Arch Toxicol*. 2023;98(1):1–73. doi:10.1007/S00204-023-03614-0
2. Maniradhan M, Calivarathan L. Bisphenol A-induced endocrine dysfunction and its associated metabolic disorders. *Endocrine, Metab Immune Disord*. 2022;23(4):515–529. doi:10.2174/1871530322666220928144043
3. Wang Y, Gai T, Zhang L, Chen L, Wang S. Ecotoxicology and environmental safety neurotoxicity of bisphenol A exposure on Caenorhabditis elegans induced by disturbance of neurotransmitter and oxidative damage. *Ecotoxicol Environ Saf*. 2023;252(November 2022):114617. doi:10.1016/j.ecoenv.2023.114617
4. Elston DM, Ukić Š. Bisphenol A – an environmental and human threat. *J Am Academy Dermatol*. 2021;86(4):295–304. doi:10.1016/j.jaad.2021.01.086
5. Mishra A, Goel D, Shankar S. Environmental concerns, and microbial remediation bisphenol A contamination in aquatic environments: a review of sources, environmental concerns, and microbial remediation. *Environ Monit Assess*. 2023;195(11):1352. doi:10.1007/s10661-023-11977-1
6. Htae AE, Al-Shami AS. Chronic exposure to bisphenol A induces behavioural, neurochemical, histological, and ultrastructural alterations in the ganglia tissue of the date mussels Lithophaga lithophaga. *Environ Sci Pollut Res*. 2023;30(50):109041–109062. doi:10.1007/S11356-023-29853-3
7. Malits J, Attina TM, Karthikraj R, et al. Renal function and exposure to bisphenol A and phthalates in children with chronic kidney disease. *Environ Res*. 2018;167:575–582. doi:10.1016/j.envres.2018.08.006
8. Ma Y, Liu H, Wu J, et al. The adverse health effects of bisphenol A and related toxicity mechanisms. *Environ Res*. 2019;176(June):108575. doi:10.1016/j.envres.2019.108575
9. Chidinma E, Oviosun A, Johnson N, Anyanwu EG. Phytomedicine plus hesperidin protects against spermatological damages and testicular apoptosis induced by bisphenol-A in adult male Wistar rats. *Phytomedicine Plus*. 2025;5(2):100797. doi:10.1016/j.phyplu.2025.100797
10. Santoro A, Chianese R, Troisi J, et al. Neuro-toxic and reproductive effects of BPA. *Curr Neuropharmacol*. 2019;17(12):1109–1132. doi:10.2174/1570159X17666190726112101
11. Helli B, Parisa S, Seyed N, et al. The protective effects of syringic acid on bisphenol A – induced neurotoxicity possibly through AMPK / PGC - 1α / Fndc5 and CREB / BDNF signaling pathways. *Mol Neurobiol*. 2024;61(10):7767–7784. doi:10.1007/s12035-024-04048-0
12. Rodríguez Mesa XM, Contreras Bolaños LA, Modesti Costa G, Mejia AL, Santander González SP. A bidens pilosa L. non-polar extract modulates the polarization of human macrophages and dendritic cells into an anti-inflammatory phenotype. *Mol*. 2023;28(20):7094. doi:10.3390/MOLECULES28207094
13. Ayuba JT, Okesina AA, Usman IM, et al. Galinsoga parviflora restored associated motor coordination through increased linear distribution of Purkinje Cells in mercury chloride-induced toxicity of mices cerebellum. *African J Cell Pathol*. 2022;14(1):1–8. doi:10.5897/AJCPATH2022.0035
14. Oviosun A, Godson Emeka A, Chidinma OE, Nebeuwa Emmanuel O, Santos Ehizokhale E, Anthony EI. Therapeutic properties of ficus exasperata extract on gentamicin induced kidney damage. *Eur J Pharm Med Res*. 2022;10(10):50–55.
15. Ninsiima HI, Eze ED, Ssekatawa K, et al. Green tea silver nanoparticles improve physiological motor and cognitive function in BALB/c mice during inflammation. *Heliyon*. 2023;9(3):e13922. doi:10.1016/J.HELIYON.2023.E13922
16. Onohuean H, Alagbonsi AI, Usman IM, et al. Annona muricata Linn and Khaya grandifoliola C.DC. reduce oxidative stress in vitro and ameliorate plasmodium berghei-induced parasitemia and cytokines in BALB/c mice. *J Evidence-Based Integr Med*. 2021:26. doi:10.1177/2515690X211036669/ASSET/IMAGES/LARGE/10.1177_2515690X211036669-FIG2.JPEG
17. Usman IM, Agbon AN, Ivang AE, et al. Ethyl acetate fraction of Tamarindus indica leaf ameliorates aluminium chloride induced neural damage in neonatal Wistar rats. *J Trace Elem Miner*. 2023;100047. doi:10.1016/J.JTEMIN.2023.100047
18. Yusuf HR, Musa SA, Agbon AN, et al. Hepatoprotective potential of Tamarindus Indica following prenatal aluminum exposure in Wistar rat pups. *Toxicol Reports*. 2023;10:376–381. doi:10.1016/J.TOXREP.2023.03.002
19. Usman IM, Etukudo EM, Owembabazi E, et al. Medicinal plants with neuroprotective potential in East African countries: a systematic review. *Phytomedicine Plus*. 2025;100744. doi:10.1016/J.PHYPLU.2025.100744
20. Usman I, Adebisi S, Musa S, Iliya I. Glial fibrillary acid protein expression and behavioral changes in hippocampus following prenatal co-administration of ethyl acetate leaf fraction of Tamarindus Indica and aluminum chloride in Wistar rats. *Niger J Exp Clin Biosci*. 2022;10(1):1. doi:10.4103/njecp.njecp_34_21
21. Usman I, Buraimoh A, Ibegbu A. Histological and biochemical studies of Tamarindus indica pulp extract on the cerebral cortex in prenatal ethanol exposure in Wistar rats. *J Exp Clin Anat*. 2016;15(2):96. doi:10.4103/1596-2393.200919

22. Barugahare B. Effect of the leaf extract of bidens pilosa on haemostasis. *J Adv Biol Biotechnol*. 2020;23(5):18–22. doi:10.9734/JABB/2020/v23i530155
23. Mtenga DV, Ripanda AS. A review on the potential of underutilized Blackjack (Biden Pilosa) naturally occurring in sub-Saharan Africa. *Heliyon*. 2022;8(6):. doi:10.1016/j.heliyon.2022.e09586
24. Mtenga DV, Ripanda AS. Heliyon review article A review on the potential of underutilized Blackjack (Biden Pilosa) naturally occurring in sub-Saharan Africa. *Heliyon*. 2022;8(May):e09586. doi:10.1016/j.heliyon.2022.e09586
25. Idris OA, Kerebba N, Horn S, Maboeta MS, Pieters R. Phytochemical-based evidence of the health benefits of bidens pilosa extracts and cytotoxicity. *Chem Africa*. 2023;6(4):1767–1788. doi:10.1007/s42250-023-00626-2
26. Ajanaku CO, Echeme JO, Mordi RC, Ajani OO, Okere DU, Kayode AA. Antimicrobial and GC-MS analyses of stem and root extracts of bidens pilosa Linn. *Oriental J Chem*. 2019;35(2):839–848. doi:10.13005/ojc/350247
27. Li XK, Motwani M, Tong W, Bornmann W, Schwartz GK. Huanglian, A Chinese herbal extract, inhibits cell growth by suppressing the expression of cyclin B1 and inhibiting CDC2 kinase activity in human cancer cells. *Mol Pharmacol*. 2000;58(6):1287–1293. doi:10.1124/mol.58.6.1287
28. Michael EJ, Lissu JP, Mvungi E, Mpinda C, Stephano F, Kidima W. Acute toxicity assessment of centella asiatica and bidens pilosa aqueous crude leaf extracts in pregnant rat model. *Tanzania J Sci*. 2024;50(2):204–223.
29. Trease GE, Evans WC. *Pharmacognosy*. 13th. Evans WC, ed.. Bailliere Tindall; 1989.
30. Geremu M, Tola YB, Sualeh A. Extraction and determination of total polyphenols and antioxidant capacity of red coffee (Coffea arabica L.) pulp of wet processing plants. *Chem Biol Technol Agric*. 2016;3(1):25. doi:10.1186/s40538-016-0077-1
31. Shraim AM, Ahmed TA, Rahman MM, Hijji YM. Determination of total flavonoid content by aluminum chloride assay: a critical evaluation. *LWT*. 2021;150:111932. doi:10.1016/j.lwt.2021.111932
32. Santello M, Toni N, Volterra A. Astrocyte function from information processing to cognition and cognitive impairment. *Nat Neurosci*. 2019;22(2):154–166. doi:10.1038/s41593-018-0325-8
33. Gurmeet K, Rosnah I, Normadiah MK, Das S, Mustafa AM. Detrimental effects of bisphenol A on development and functions of the male reproductive system in experimental rats. *Excli J*. 2014;13:151–160.
34. Greggi Antunes LM, Darin JD, Bianchi MD. Protective effects of vitamin C against cisplatin-induced nephrotoxicity and lipid peroxidation in adult rats: a dose-dependent study. *Pharmacol Res*. 2000;41(4):405–411. doi:10.1006/phrs.1999.0600
35. Ezeonwumelu JOC, Ntale M, Ogbonnia SO, et al. Analgesic appraisal of bidens pilosa (Asteraceae) leaf extracts used in management of oral lesion pain in HIV/AIDS patients in rodents. *Pharmacol Pharm*. 2018;9(6):175–192. doi:10.4236/PP.2018.96014
36. Rizzo M. Measurement of malondialdehyde as a biomarker of lipid oxidation in fish. *Am J Anal Chem*. 2024;15(09):303–332. doi:10.4236/ajac.2024.159020
37. Gueroui M, Kechrid Z. Evaluation of some biochemical parameters and brain oxidative stress in experimental rats exposed chronically to silver nitrate and the protective role of vitamin E and selenium. *Toxicol Res*. 2016;32(4):301. doi:10.5487/TR.2016.32.4.301
38. Afolabi OB, Oloyede OI, Olayide II, et al. Antioxidant enhancing ability of different solvents extractable components of talinum triangulare in some selected Tissue homogenates of albino rats -in vitro. *J Appl Pharm Sci*. 2015;5(9):056–061. doi:10.7324/JAPS.2015.50911
39. Feldman AT, Wolfe D. Tissue processing and hematoxylin and eosin staining. *Methods Mol Biol*. 2014;1180:31–43. doi:10.1007/978-1-4939-1050-2_3
40. ul HSS, Samanta S, Dash R, et al. The neuroprotective effects of fisetin, a natural flavonoid in neurodegenerative diseases: focus on the role of oxidative stress. *Front Pharmacol*. 2022;13:1015835. doi:10.3389/FPHAR.2022.1015835/BIBTEX
41. Erhirhie EO, Ihekwereme CP, Ilodigwe EE. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. *Interdiscip Toxicol*. 2018;11(1):5. doi:10.2478/INTOX-2018-0001
42. Katiyar D, Saxena R, Kumar A, et al. A comprehensive review of the protective effects of herbals against toxicity of Bisphenol-A. *Toxin Rev*. 2024;43(3):329–357. doi:10.1080/15569543.2024.2329907
43. Zhang W, Xiao D, Mao Q, Xia H. Role of neuroinflammation in neurodegeneration development. *Signal Transduct Target Ther*. 2023;8(1):1–32. doi:10.1038/s41392-023-01486-5
44. Hong X, Zhou Y, Zhu Z, et al. Environmental endocrine disruptor Bisphenol A induces metabolic derailment and obesity via upregulating IL-17A in adipocytes. *Environ Int*. 2023;172:107759. doi:10.1016/J.ENVINT.2023.107759
45. Jomova K, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Arch Toxicol*. 2024;98(5):1323–1367. doi:10.1007/S00204-024-03696-4
46. Houldsworth A. Role of oxidative stress in neurodegenerative disorders: a review of reactive oxygen species and prevention by antioxidants. *Brain Commun*. 2023;6(1). doi:10.1093/BRAINCOMMS/FCAD356
47. Chidambaram SB, Anand N, Varma SR, et al. Superoxide dismutase and neurological disorders. *IBRO Neurosci Rep*. 2024;16:373–394. doi:10.1016/J.IBNEUR.2023.11.007
48. Rao MJ, Zheng B. The role of polyphenols in abiotic stress tolerance and their antioxidant properties to scavenge reactive oxygen species and free radicals. *Antioxidants*. 2025;14(1):74. doi:10.3390/ANTIOX14010074
49. Ghasemi SG, Khoshrou A, Kakhki S, et al. Ascorbic acid supplementation improves adolescent stress-induced cognitive impairment through restoration of behavioral, biochemical and electrophysiological alterations in male rats. *Neuroscience*. 2024;549:55–64. doi:10.1016/J.NEUROSCIENCE.2024.04.012
50. Ashok A, Andrabi SS, Mansoor S, Kuang Y, Kwon BK, Labhasetwar V. Antioxidant therapy in oxidative stress-induced neurodegenerative diseases: role of nanoparticle-based drug delivery systems in clinical translation. *Antioxidants*. 2022;11(2):408. doi:10.3390/ANTIOX11020408
51. Semwal BC, Singh B, Murti Y, Singh S. Therapeutic potential of ascorbic acid in the management of alzheimer's disease: an update. *Curr Pharm Biotechnol*. 2023;25(2):196–212. doi:10.2174/1389201024666230804102617/CITE/REFWORKS
52. Bi SS, Talukder M, Sun XT, et al. Cerebellar injury induced by cadmium via disrupting the heat-shock response. *Environ Sci Pollut Res*. 2022;30(9):22550–22559. doi:10.1007/S11356-022-23771-6
53. Shen Y, Li X, Wang H, et al. Bisphenol A induced neuronal apoptosis and enhanced autophagy in vitro through Nrf2/HO-1 and Akt/mTOR pathways. *Toxicology*. 2023;500:153678. doi:10.1016/J.TOX.2023.153678
54. Kumar RR, Singh L, Thakur A, Singh S, Kumar B. Role of vitamins in neurodegenerative diseases: a review. *CNS Neurol Disord*. 2021;21(9):766–773. doi:10.2174/1871527320666211119122150