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Research Article

Dose-Dependent Immunohistochemical Modulation of Astrocyte Activation by *Phyllanthus amarus* in Paraquat-Induced Hippocampal Neurotoxicity

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Abstract

Background: Neurotoxicity, characterized by adverse effects on the structure or function of the central and peripheral nervous systems as a result of exposure to biological, chemical, or physical agents, remains a significant challenge in medical science. Available drugs often provide only symptomatic relief and are associated with various side effects, necessitating the exploration of novel protective agents. The present study aimed to evaluate the role of *Phyllanthus amarus*, a medicinal plant known for its antioxidant and anti-inflammatory properties, in paraquat-induced neurotoxicity in Wistar rats.

Results: The results of the study showed that *Phyllanthus amarus* attenuated paraquat-induced neurotoxicity in a dose-dependent manner. While the low (200 mg/kg) and medium (300 mg/kg) doses produced mild to moderate reductions in GFAP immunoreactivity, the high dose (400 mg/kg) exhibited a more pronounced protective effect, indicating enhanced neuroprotection at higher doses.

Conclusion: *Phyllanthus amarus* demonstrates dose-dependent neuroprotective properties in paraquat-induced neurotoxicity, and these findings emphasize the importance of dosage optimization for therapeutic application. These results support further investigation into the therapeutic application of *Phyllanthus amarus* in neurodegenerative conditions involving oxidative stress and glial activation.

Keywords: *Neurotoxicity, Dose-Dependent, Phyllanthus amarus, Neuroprotection.*

Introduction

The metabolic need of the brain is very high, as the brain consumes about 20% of the entire body's oxygen demand while only making up 2% of the body mass [1]. In addition to its structure, the brain is further divided into the forebrain, midbrain, and hindbrain, each with distinct functions. For example, the forebrain is considered to be the most developed part of the brain because it contains very important features such as the thalamus, hypothalamus, cerebral cortex, etc., which are responsible for functions such as memory, reasoning, and learning [2].

Furthermore, there are two distinct cell components of the brain which are the neurons and glial cells. The neurons are electrically excitable cells that transmit and process neural information through electrochemical signalling, while the glial cells are non-neuronal supportive cells that are greatly involved in maintaining neural homeostasis, regulating the blood-brain barrier (BBB), providing metabolic support to neurons, and mediating CNS immune responses [3].

A major bilateral structure of immense importance in the limbic system of the brain is the hippocampus. It is located within the medial temporal lobe with its core anatomical structures, such as CA1, CA2, CA3, and CA4, and the dentate gyrus, each with distinct cellular structures contributing to the overall hippocampal function [4]. The hippocampal circuit is formed by the hippocampal areas such as CA1–CA3, which is fundamental for memory storage and retrieval, whereas the dentate gyrus is involved in pattern separation and adult hippocampal neurogenesis [5].

A major characteristic of the hippocampus is that it is highly vulnerable to reactive oxygen species (ROS), which makes it prone to oxidative stress. This happens because of its high metabolic demand due to its high content of polyunsaturated fatty acids (PUFAs) prone to lipid peroxidation, high iron content facilitating hydroxyl radical generation via the Fenton reaction, and relatively low expression of endogenous antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT) [6]. These characteristics collectively distinguish the hippocampus as the primary site of injury in experimental models of environmental neurotoxicant exposure.

The hippocampus is an extension of the temporal part of the cerebral cortex. It can be distinguished externally as a layer of densely packed neurons, which curls into an S-shaped structure on the edge of the temporal lobe. Therefore, it is known as a part of the limbic lobe (limbic means border). Though it lies sub-cortically, it is not totally considered to be a sub-cortical structure. It is a part of hippocampal formation and has many limbs [2]. Furthermore, since alterations in learning and memory are a common consequence of toxicant exposure, it is possible that the hippocampus is an important target site for neurotoxicity. It is the earliest and most severely affected structure in several neurological disorders such as Alzheimer's disease and epilepsy [7].

In the central nervous system, astrocytes are the most abundant glial cells participating in different homeostatic functions, including neurotransmitter recycling and maintenance of metabolic support of neurons. Under normal conditions, astrocytes exhibit a resting, ramified morphology with slender processes [8]. In response to neuronal injury, oxidative stress, or neuroinflammation, astrocytes respond by undergoing reactive astrogliosis, which is a process characterised by cellular hypertrophy, process elongation, increased proliferative activity, and marked upregulation of their principal cytoskeletal protein, Glial Fibrillary Acidic Protein (GFAP) [9].

GFAP is a type III intermediate filament protein constituting the primary structural component of the astrocyte cytoskeleton. It is universally recognised as the most specific and reliable immunohistochemical marker for astrocyte identification and the semi-quantitative assessment of reactive astrogliosis in the CNS [10]. Elevated GFAP immunoreactivity in the hippocampus reliably reflects ongoing neuroinflammation or toxic insult and has been consistently documented in experimental models of neurotoxicant exposure. Immunohistochemistry (IHC) enables the precise spatial localization of GFAP expression across distinct hippocampal subfields, rendering it an indispensable tool in neuropathological investigations and toxicological research [11].

Medicinal plants are the major sources of numerous valuable chemicals and/or drugs. Over 1300 medicinal plants are used in European countries, and out of them, 90% are from wild sources. According to the International Union for Conservation of Nature and the World Wildlife Fund, about 50,000–80,000 flowering plants are used because of their medicinal values [12]. Medicinal plants have been used by all cultures throughout history as remedies for human diseases. A large majority of rural and urban dwellers in Nigeria still rely on traditional medicines to meet their primary health care needs [13]. Traditional herbal medicines still remain the basic health care means for a large majority of rural and urban dwellers in Nigeria.

Phyllanthus amarus is an herbal plant belonging to the Euphorbiaceae family. It has approximately 800 species which are found in tropical and subtropical countries of the world. The plant has been found in the Philippines, Cuba, Nigeria, and India among others. Extract of the plant has been reported to possess pharmacological effects such as antibacterial, antiviral, anticancer, antiamnesic, antioxidative, antimicrobial, antileptospiral, anticonvulsant, and anti-inflammatory activities. *Phyllanthus amarus* has been used as a chemoprotective, antimutagenic, nephroprotective, cardioprotective, hepatoprotective, and hypoglycemic agent [14].

Paraquat (PQ) is a well-known neurotoxicant and poses a greater risk for human and animal health. As a type of pesticide in agriculture, paraquat is a commonly used herbicide that has been recognized as a neurotoxicant and is connected to an elevated risk of Parkinson's disease and neuropathologies similar to Parkinson's disease [15]. Paraquat acts by generation of reactive oxygen species, which destroy cells by peroxidation of the lipid cell membrane. The organs most affected are the kidney, liver, heart, and lungs. The herbicide is known to concentrate in lung tissue and cause progressive and irreversible damage (pneumonitis and fibrosis), with death occurring anywhere between 5 to 31 days after lung injury [16]. It is generally believed that the pathogenesis of paraquat poisoning mainly involves the generation of superoxide radicals, followed by the activation of inflammatory cells, apoptosis, and other processes [16].

Although the protective nature of *Phyllanthus amarus* against oxidative stress has been identified in recent studies, there is no study on its effect on paraquat-induced neuronal damage, specifically in relation to hippocampal GFAP expression. Given that paraquat induces neurotoxicity through oxidative stress mechanisms and that the hippocampus is highly vulnerable to such damage, investigating the modulatory effect of *Phyllanthus amarus* on GFAP expression is necessary to better understand its potential neuroprotective role.

This study aimed at the effect of doses of aqueous leaf extract of *Phyllanthus amarus* on paraquat-induced neurotoxicity in the hippocampus of adult Wistar rats. The mechanism of action of *Phyllanthus amarus* is poorly understood. This study was focused on elaborating the neuroprotective effect of *Phyllanthus amarus* on paraquat-induced neurotoxicity in Wistar rats. This study provides insight into the potential neuroprotective role of *Phyllanthus amarus* in paraquat-induced neurotoxicity and its modulation of hippocampal GFAP expression

Methods

Study Aim

This study aimed at the effect of aqueous leaf extract of *Phyllanthus amarus* on paraquat induced neurotoxicity in the hippocampus of adult Wistar rats.

Study Design and Experimental Setting

Neurobehavioral assessment was conducted on experimental day 8 and day 15 using Y-maze. All experimental procedures were conducted in a quiet room in the Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Medical Sciences, Ondo City, Ondo State, Nigeria.

Experimental Animals

Forty-two (42) apparently healthy albino Wistar rats, weighing between 100-150 g, were obtained from the Animal House at the University of Medical Sciences, Ondo City, Ondo State, Nigeria. The animals were housed in well-ventilated plastics with saw dust or shavings as bedding, fed on standard rodent feed, and allowed free access to tap water ad libitum under standard laboratory conditions. The rats underwent maximum acclimatization (28 days) before the actual commencement of the experiment.

Plant Collection and Authentication

Fresh leaves of *Phyllanthus amarus* were collected in bulk in the swampy or riverside areas of Federal Housing Estate Farm, Igba, Ondo City, Ondo State, and were immediately taken for identification at the Plant Biology Department, Adeyemi College of Education, Ondo City, Ondo State, Nigeria, and the Batch No: ACE/BIO/22/010 was provided. Also, it was authenticated at the Biological Sciences Department, University of Medical Sciences, Ondo City, Ondo State, and the authentication number: UNIMEDP.B.T.H013 was given.

Drugs and Chemicals

Paraquat manufactured by Hubei Xianlong Chemical Industry Co., Int. No. 36 Yanjiang East Road, Xiantao, Hubei, China, was purchased from Uche Care Pharmaceutical Store in Aba, Abia State, Nigeria.

Levodopa (Sinemet) tablet with batch number 6637200-1 manufactured by Mylan Pharmaceuticals, Inc., Morgantown, WV 26505, USA, was purchased from Uche Care Pharmaceutical Store in Aba, Abia State, Nigeria.

Materials

Other materials used in the course of the study included measuring cylinders, digital weighing balance, cages, water bottles, gloves and nose masks, vital feed, saw dust for bedding, distilled water, digital water bath, microscope, glass slides, cover slips, stains, fixative and bottles for fixing tissues, dissecting set, conical flask, oral gavage needle, beakers and syringes, Y-maze test set-up, etc.

Preparation of Plant Extract

The harvested fresh leaves were shade dried for 7 days with irregular sun drying for better grinding. The dried leaves were then ground into coarse powder using a grinding machine and kept in an airtight condition for extraction. Six hundred grams (600 g) of the powder was soaked in 6 liters of cold distilled water in a conical flask. After 48 hours, the solution (mixture of *Phyllanthus amarus* leaves powder and distilled water) was filtered using a filtered rag and funnel. The filtrate was allowed to settle for a while, followed by decantation of the supernatant. The supernatant was heated (steamed) to dryness in a beaker using a digital water bath (Model DK-420) at 90 °C.

Preparation of Drug Solutions

15.23 g of the extract was measured and dissolved in 150 mL of distilled water. This made up the working solution. From a freshly prepared working solution, a portion was measured out for animal administration. Different doses of extract were administered to experimental animals by oral gavage for the experimental period.

3.5 mL of paraquat was measured and added to 245 mL of distilled water to form a solution to aid administration to the experimental animals by oral gavage.

0.14 g of levodopa was dissolved in 50 mL of distilled water to form a solution to aid administration of the drug to the experimental animals by oral gavage.

Experimental Design and Treatment Groups

The animals were divided into six (6) groups of seven (7) animals each. Group 1 served as the control group and was administered distilled water (0.5 mL/kg), while groups 2, 3, 4, 5, and 6 served as experimental groups. Group 2 was administered paraquat (10 mg/kg) + L-dopa (10 mg/kg) as reference drug, group 3 was administered paraquat (10 mg/kg), while groups 4, 5, and 6 were administered paraquat + aqueous leaves extract of *Phyllanthus amarus* at doses of 200 mg/kg, 300 mg/kg, and 400 mg/kg, respectively. The experiment lasted for a period of 15 days (N = 7). The route of administration was oral. [18].

Neurobehavioral Assessment (Y-Maze Test)

Y-maze consists of a three-dimensional arm mounted in the shape of Y. Y-maze spontaneous alternation is a behavioral test for measuring the willingness of rodents to explore new environments. It is also used to assess behavioral tasks in preclinical research for studying spatial learning and memory of rodents [18].

Animal Sacrifice and Tissue Collection

Animals were anesthetized with diethyl ether and sacrificed by cervical dislocation on the 16th day after a 24-hour window following the last administration. The skulls were opened using bone forceps, and the brains were excised by sagittal incision and fixed in 10% neutral buffered formalin for processing. The recommended procedure of Dury and Wallington (1980) was adopted.

Tissue Processing

All tissues were dehydrated by immersion through ascending grades of ethanol. The dehydrated tissues were cleared in xylene, infiltrated in molten paraffin wax, embedded, sectioned using a rotary microtome, floated in a water bath, mounted on slides, and dried.

Immunohistochemistry (GFAP)

The sections were deparaffinized in xylene and taken to water with descending grades of alcohol. Antigen retrieval was performed. The slides were washed in phosphate buffered saline (PBS). Endogenous peroxidase blocking was carried out using 0.3% hydrogen peroxide in PBS.

The sections were blocked in 2.5% normal animal serum and incubated in primary antibody (anti-GFAP) at 1:7500 for 3 hours at room temperature. Sections were incubated in ImmPRESS Polymer Anti-Rabbit IgG reagent, and colour was developed with DAB peroxidase substrate kit. Sections were counter-stained in haematoxylin, dehydrated, cleared, and mounted [17]

Photomicrography and Image Analysis

The processed tissues were viewed under a digital light microscope, and digital photomicrographs were taken by an attached camera at ×400, ×100, and ×40 magnifications using OMAX software. NIH-sponsored Image software was used for digital analysis of photomicrographs using the cell counter plugin [19].

One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to analyze data from body weight and GFAP immune reactivity. GraphPad Prism 8 was used for statistical analysis. Data were expressed as mean ± SEM, and statistical significance was set at $p < 0.05$.

Results

1. Y-Maze (Spontaneous Alternation)

Spontaneous alternation behavior in the Y-maze test is presented in Fig. 1A–B. Paraquat administration resulted in a significant reduction in spontaneous alternation compared with the control group on both Day 8 and Day 15 ($p < 0.05$), indicating impaired spatial working memory. Treatment with *Phyllanthus amarus* produced an improvement in alternation performance. The low (200 mg/kg) and medium (300 mg/kg) doses showed significant improvement relative to the paraquat group, while the high dose (400 mg/kg) showed mild increase in spontaneous alternation on Day 8 and significant increase on Day 15 compared with the paraquat-treated group ($p < 0.05$), suggesting improved cognitive function. The L-Dopa-treated group also showed significant improvement compared with the paraquat group.

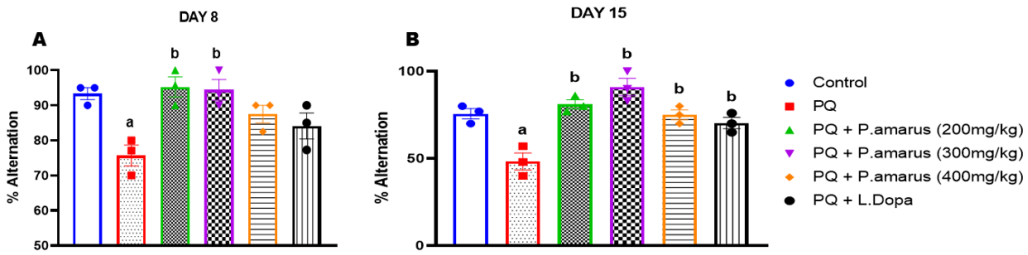

Figure 1 A-B: Effects of *Phyllanthus amarus* extract on spontaneous alternation behavior in the Y-maze test in paraquat-induced neurotoxicity in adult Wistar rats on Day 8 and Day 15. Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. a indicates a significant difference compared with the control group ($p < 0.05$), while b indicates a significant difference compared with the paraquat (PQ) group ($p < 0.05$). PQ = paraquat; P. amarus = *Phyllanthus amarus*; L-Dopa = levodopa.

2. Y-Maze (Arm Entries)

The number of arm entries is shown in Fig. 2A–B. Paraquat administration caused a reduction in exploratory activity compared with the control group, as indicated by a decrease in total arm entries. Treatment with *Phyllanthus amarus* resulted in a slight increase in arm entries across all doses on Day 8, although the changes were not statistically significant. On Day 15, treatment with *Phyllanthus amarus* resulted in significant increase in arm entries across the low and medium dose. This suggests improved exploratory behavior compared with the paraquat group, suggesting partial restoration of locomotor activity.

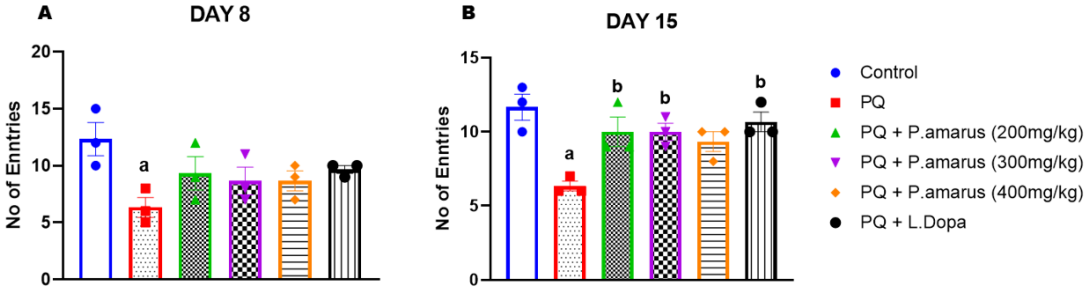

Figure 2 A-B: Effects of *Phyllanthus amarus* extract on number of arm entries in the Y-maze test in paraquat-induced neurotoxicity in adult Wistar rats on Day 8 and Day 15. Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. **a** indicates a significant difference compared with the control group ($p < 0.05$), while **b** indicates a significant difference compared with the paraquat (PQ) group ($p < 0.05$). **PQ** = paraquat; **P. amarus** = *Phyllanthus amarus*; **L-Dopa** = levodopa.

3. Histology – Hippocampal CA3

Histological assessment of the hippocampal CA3 region is presented in Fig. 3.0. The control group exhibited normal cytoarchitecture with well-arranged pyramidal neurons. In contrast, the paraquat-treated group showed disrupted neuronal organization, with evidence of neuronal degeneration and increased glial cell presence. Treatment with *Phyllanthus amarus* resulted in varying degrees of structural preservation. The low and medium doses showed mild improvement, while the high dose (400 mg/kg) demonstrated marked preservation of pyramidal neurons and overall cytoarchitecture comparable to the control group. The L-Dopa group also showed notable structural recovery.

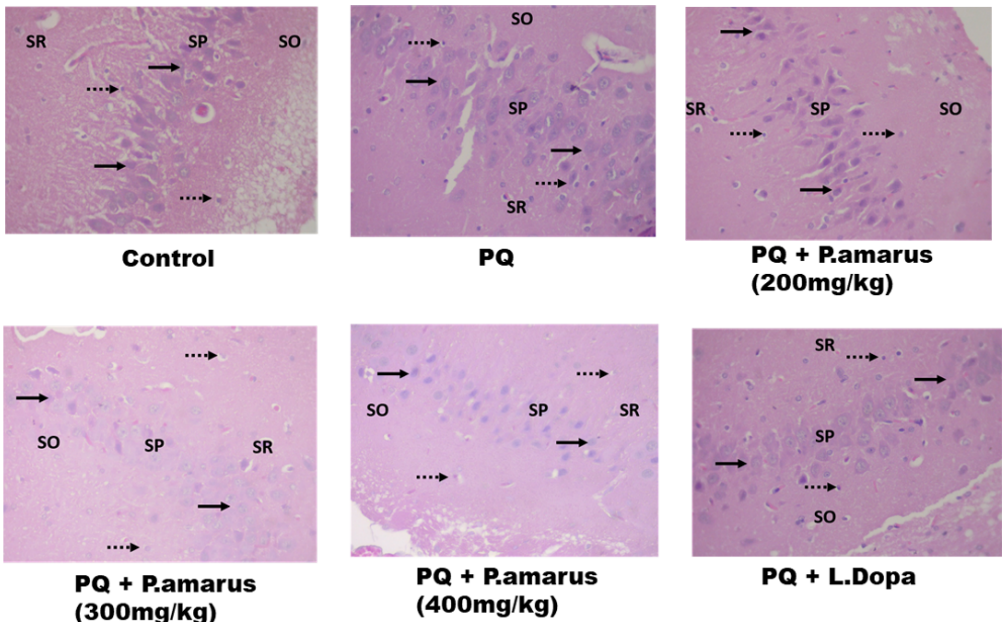

Figure 3. Histological assessment of the hippocampal CA3 region following treatment with *Phyllanthus amarus* extract in paraquat-induced neurotoxicity in Wistar rats. Photomicrographs show the hippocampal CA3 architecture in the control, paraquat-treated, paraquat + *Phyllanthus amarus* extract (200, 300, and 400 mg/kg), and paraquat + L-dopa groups. Sections were stained with hematoxylin and eosin (H&E) and viewed at ×400 magnification. SR – stratum radiatum; SP – stratum pyramidalis; SO – stratum oriens; arrows – pyramidal neurons; dashed arrows – glial cells.

4. Histology – Hippocampal Dentate Gyrus

Histological findings in the dentate gyrus are shown in Fig. 4.0. The control group displayed normal granular layer organization with densely packed neurons. The paraquat-treated group exhibited disruption of the granular layer and increased glial cell infiltration. Treatment with *Phyllanthus amarus* improved cellular organization in a dose-dependent manner. The highest dose (400 mg/kg) showed the most pronounced protective effect, with improved neuronal arrangement and reduced glial presence. Similar improvements were observed in the L-Dopa-treated group.

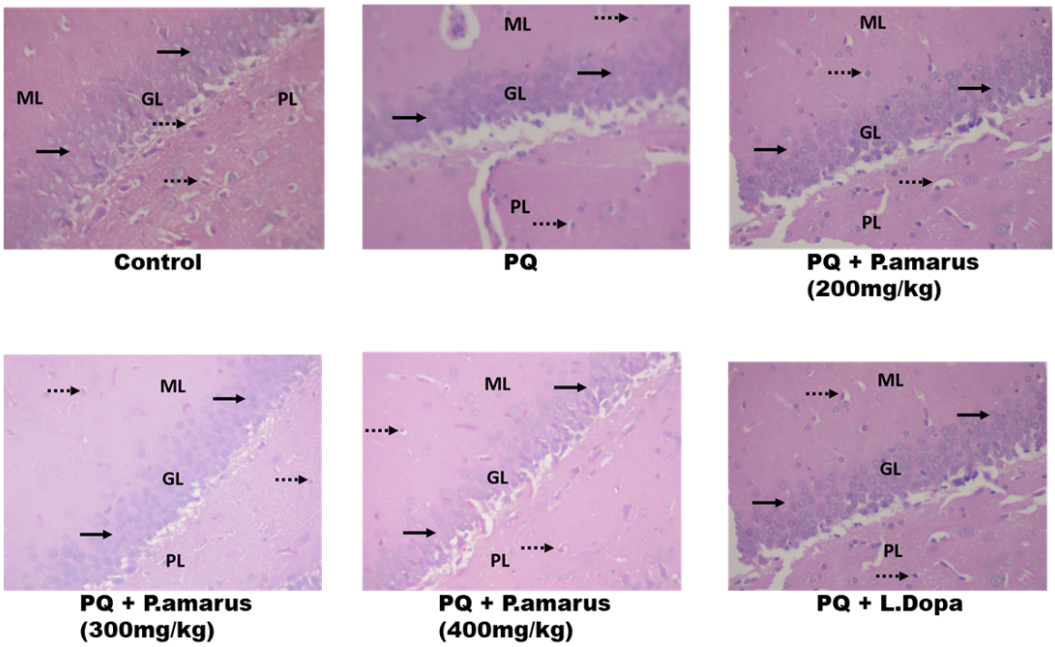

Figure 4. Histological assessment of the hippocampal dentate gyrus (DG) following treatment with *Phyllanthus amarus* extract in paraquat-induced neurotoxicity in Wistar rats. Photomicrographs show the dentate gyrus architecture in the control, paraquat-treated, paraquat + *Phyllanthus amarus* extract (200, 300, and 400 mg/kg), and paraquat + L-dopa groups. Sections were stained with hematoxylin and eosin (H&E) and observed at ×400 magnification. mL – molecular layer; GL – granular layer; PL – polymorphic layer; arrows – granular neurons; dashed arrows – glial cells.

5. GFAP – Hippocampal CA3

GFAP immunoreactivity in the CA3 region is shown in Fig. 5.0. The control group exhibited minimal GFAP expression, indicating normal astrocytic activity. In contrast, paraquat administration significantly increased GFAP immunoreactivity, which indicates astrocyte activation and neuroinflammation. Treatment with *Phyllanthus amarus* reduced GFAP expression in a dose-dependent manner. The high dose (400 mg/kg) showed a marked reduction in GFAP-positive astrocytes compared with the paraquat group ($p < 0.05$), while the low and medium doses showed only mild reductions. The L-Dopa group also demonstrated decreased GFAP expression.

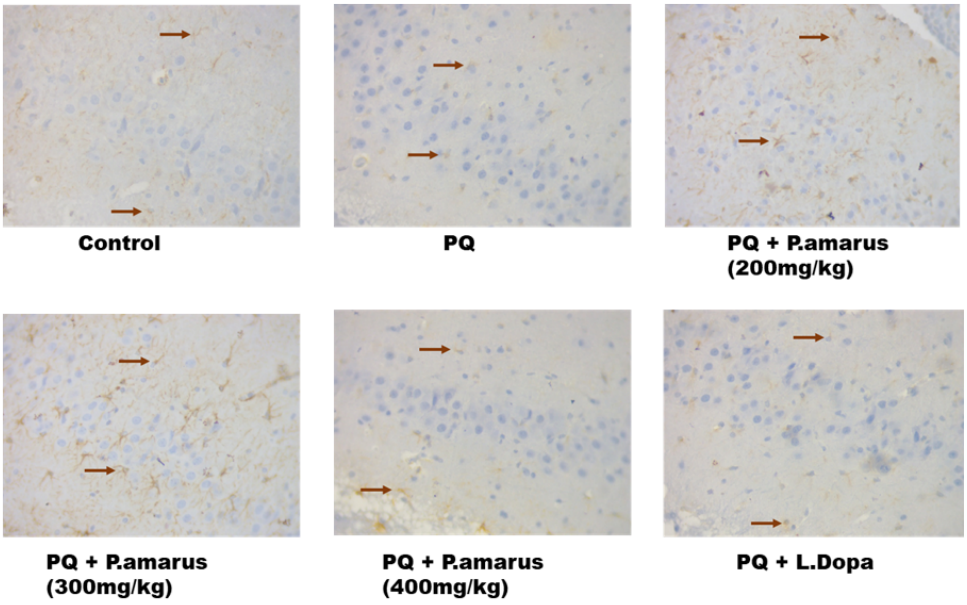

Figure 5. Immunohistochemical assessment of GFAP expression in the hippocampal CA3 region following treatment with *Phyllanthus amarus* extract in paraquat-induced neurotoxicity in Wistar rats. Photomicrographs show GFAP immunoreactivity in the hippocampal CA3 of the control, paraquat-treated, paraquat + *Phyllanthus amarus* extract (200, 300, and 400 mg/kg), and paraquat + L-dopa groups. GFAP staining was used to visualize astrocytes and sections were observed at ×400 magnification. Arrows indicate GFAP-expressing astrocytes.

6. GFAP – Hippocampal Dentate Gyrus

GFAP expression in the dentate gyrus is presented in Fig. 6.0. Similar to the CA3 region, paraquat exposure significantly increased GFAP immunoreactivity compared with the control group. Treatment with *Phyllanthus amarus* reduced GFAP expression across all doses, with the most pronounced reduction observed at 400 mg/kg. The L-Dopa group also showed decreased GFAP levels compared with the paraquat group, indicating attenuation of astrocytic activation.

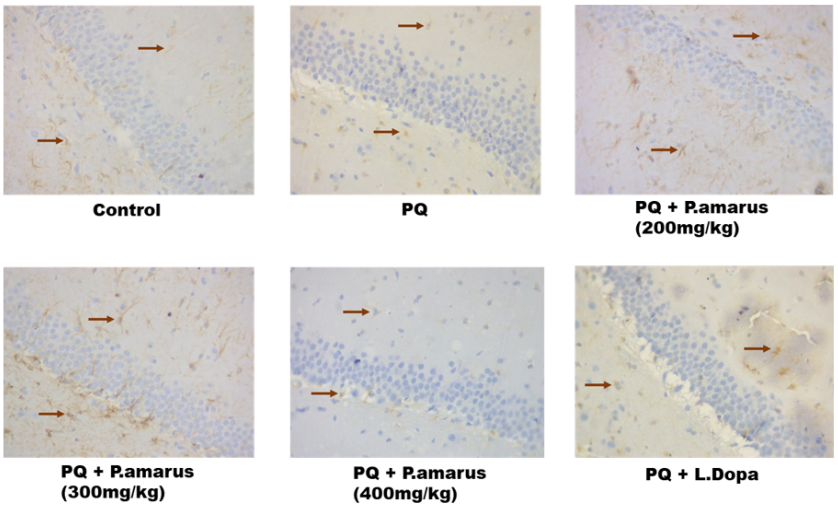

Figure 6. Immunohistochemical assessment of GFAP expression in the hippocampal dentate gyrus (DG) following treatment with *Phyllanthus amarus* extract in paraquat-induced neurotoxicity in Wistar rats. Photomicrographs show GFAP immunoreactivity in the dentate gyrus of the control, paraquat-treated, paraquat + *Phyllanthus amarus* extract (200, 300, and 400 mg/kg), and paraquat + L-dopa groups. GFAP staining was used to visualize astrocytes and sections were observed at ×400 magnification. Arrows indicate GFAP-expressing astrocytes.

7. GFAP Quantification

Quantitative analysis of GFAP-positive cells is shown in Fig. 7.0 The graph shows the number of GFAP-positive astrocytes in the CA3 and DG regions of the control and treated groups. In the CA3 region, the control group showed the lowest number of GFAP-positive cells, whereas the paraquat-treated group showed the highest number with a significant increase compared with the control group. Treatment with *Phyllanthus amarus* extract at 400 mg/kg and L-Dopa significantly reduced the number of GFAP-positive cells compared with the paraquat group. In the DG region, the control group also showed the lowest GFAP expression, while the paraquat group showed the highest level with a significant difference compared with the control group. All P. amarus-treated groups (200, 300, and 400 mg/kg) and the L-Dopa group showed a significant reduction in GFAP-positive cells compared with the paraquat group.

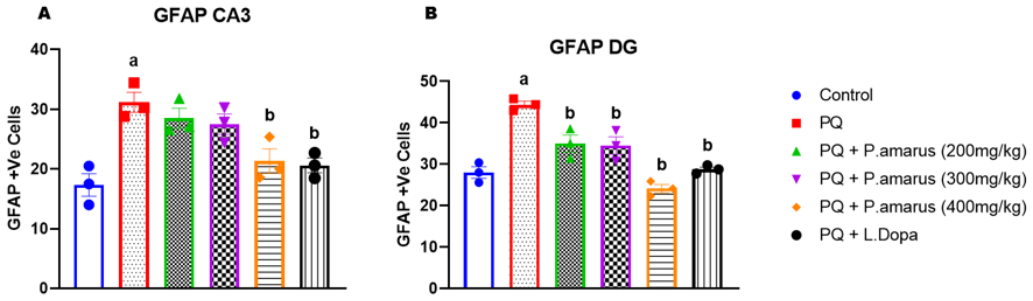

Figure 7. Quantification of GFAP-positive cells in the hippocampal CA3 and dentate gyrus (DG) regions following treatment with *Phyllanthus amarus* extract in paraquat-induced neurotoxicity in Wistar rats. Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. **a** indicates a significant difference compared with the control group ($p < 0.05$), while **b** indicates a significant difference compared with the paraquat (PQ) group ($p < 0.05$). PQ = paraquat; P. amarus = *Phyllanthus amarus*; L-Dopa = levodopa.

Discussion

The present study investigated the effect of aqueous leaf extract of *Phyllanthus amarus* on paraquat-induced neurotoxicity in the hippocampus of adult Wistar rats, with emphasis on astrocytic activation as assessed by GFAP immunoreactivity. The findings demonstrate that paraquat exposure produced clear behavioral, histological, and astrocytic alterations in the hippocampus, while treatment with *Phyllanthus amarus* attenuated many of these changes, particularly at higher doses.

Behavioral assessment using the Y-maze test revealed that paraquat administration impaired spontaneous alternation behavior, which is commonly used as an indicator of spatial working memory. The observed reduction in alternation performance suggests disruption of hippocampal-dependent cognitive processing following paraquat exposure as shown in Fig. 1.0. This observation is consistent with previous reports indicating that paraquat-induced oxidative stress disrupts neuronal signaling within hippocampal circuits responsible for memory and learning [20]. Treatment with *Phyllanthus amarus* improved spontaneous alternation behavior relative to the paraquat-treated group as shown in Fig 1, which suggests a partial restoration of hippocampal function. The improvement in behavioral performance may show preservation of neuronal integrity and synaptic communication within hippocampal networks.

Histological analyses further supported the behavioral findings. In the hippocampal CA3 region (Fig. 3.0) and dentate gyrus (Fig. 4.0), paraquat exposure resulted in alterations in neuronal organization accompanied by an increased presence of glial cells. Such structural disturbances are commonly associated with neurotoxic injury and are indicative of cellular stress within hippocampal tissue [21,22]. The pyramidal neurons of the CA3 region appeared less organized in the paraquat-treated animals compared with the control group as shown in Fig. 3.0, while the dentate gyrus exhibited changes in the arrangement of granular neurons as shown in Fig. 4.0. These morphological disturbances are consistent with the vulnerability of the hippocampus to oxidative damage due to its high metabolic demand and lipid content [21].

Administration of *Phyllanthus amarus* produced varying degrees of preservation of hippocampal cytoarchitecture. Animals treated with the plant extract showed improved neuronal organization relative to the paraquat group, particularly at the higher dose. The protective effect observed in the CA3 and dentate gyrus regions suggests that the extract may mitigate structural damage associated with oxidative neurotoxicity. The presence of relatively preserved pyramidal neurons and granular cells in the treated groups indicates that the extract may support neuronal survival under toxic conditions.

A major focus of the present study was the evaluation of astrocytic responses to paraquat exposure. Astrocytes play a critical role in maintaining neuronal homeostasis and responding to central nervous system injury [23]. Increased GFAP expression is widely recognized as a hallmark of reactive astrogliosis and is commonly observed following neurotoxic insults [24]. In the present study, paraquat administration markedly increased GFAP immunoreactivity in both the CA3 (Fig. 5.0) and dentate gyrus (Fig. 6.0) regions of the hippocampus. The elevated number of GFAP-positive astrocytes observed in the paraquat group reflects astrocyte activation in response to neuronal stress or injury. Reactive astrocytosis following paraquat exposure is likely related to the generation of reactive oxygen species and subsequent inflammatory signaling within the brain [25]. Paraquat undergoes redox cycling that produces superoxide radicals, leading to oxidative stress and cellular damage. Astrocytes respond to such insults by undergoing structural and biochemical changes, including increased expression of GFAP [24,26]. The elevated GFAP immunoreactivity observed in the present study therefore indicates that paraquat induced a strong neuroinflammatory response within the hippocampus as shown in Fig. 5.0 and Fig. 6.0. Treatment with *Phyllanthus amarus* resulted in a reduction in GFAP immunoreactivity compared with the paraquat group. The decrease in the number of GFAP-positive astrocytes suggests that the extract attenuated astrocytic activation associated with paraquat-induced neurotoxicity. Notably, the highest dose of the extract demonstrated the most pronounced reduction in GFAP expression, indicating a dose-dependent response. This observation suggests that the protective effect of the plant extract may become more evident as the administered dose increases. The reduction in astrocytic activation observed in the treated groups may be related to the antioxidant and anti-inflammatory properties previously attributed to *Phyllanthus amarus*. The plant contains several bioactive compounds, including flavonoids, lignans, and polyphenolic constituents, which have been reported to possess free-radical scavenging activity. By reducing oxidative stress, these compounds may limit the cellular damage that triggers astrocytic activation. Consequently, decreased GFAP expression in the treated animals may reflect a lower degree of neuronal injury within the hippocampus.

The quantitative analysis of GFAP-positive cells further supported the immunohistochemical observations. The paraquat-treated group exhibited the highest number of GFAP-positive astrocytes in both hippocampal regions examined, whereas treatment with *Phyllanthus amarus* significantly reduced these values as shown in Fig. 7.0. The effect was particularly evident at the highest dose, which produced GFAP levels closer to those observed in the control group. These findings show that the extract may exert a protective influence against astrocyte-mediated neuroinflammatory responses induced by paraquat.

The use of levodopa as a reference treatment in this study also provided a comparative perspective. Levodopa administration reduced GFAP expression relative to the paraquat group, indicating that pharmacological intervention can attenuate astrocytic activation under neurotoxic conditions. The similar trend observed in the *Phyllanthus amarus*-treated groups suggests that the plant extract may exert effects comparable to established neuroprotective interventions, although the mechanisms involved may differ.

Conclusion

In conclusion, the findings of this study suggest that paraquat exposure disrupts hippocampal structure and function, leading to behavioral deficits, neuronal alterations, and astrocytic activation. Treatment with *Phyllanthus amarus* attenuated several of these changes, particularly at higher doses, indicating a potential protective effect against paraquat-induced neurotoxicity. The reduction in GFAP expression observed in the treated animals suggests that the extract may limit reactive astrogliosis and associated neuroinflammatory responses.

These findings are relevant as they highlight the potential of *Phyllanthus amarus* as a neuroprotective agent against oxidative stress–induced neurotoxicity, a key mechanism underlying several neurodegenerative disorders such as Parkinson's disease. This study therefore contributes to the growing body of evidence supporting the therapeutic relevance of plant-derived antioxidants in neuroprotection and underscores the importance of dose optimization in achieving maximal efficacy.

Further studies are needed to better understand how *Phyllanthus amarus* produces its neuroprotective effects. Future research focusing on oxidative stress, inflammation, and neuronal survival pathways could help explain how the plant extract protects brain cells from damage caused by toxic agents. However, the findings from this study already suggest that *Phyllanthus amarus* may help protect the hippocampus against paraquat-induced damage.

List of Abbreviations

GFAP — Glial Fibrillary Acidic Protein

CNS — Central Nervous System

BBB — Blood-Brain Barrier

ROS — Reactive Oxygen Species

PUFAs — Polyunsaturated Fatty Acids

SOD — Superoxide Dismutase

CAT — Catalase

IHC — Immunohistochemistry

PQ — Paraquat

DG — Dentate Gyrus

CA1, CA2, CA3, CA4 — Cornu Ammonis regions 1, 2, 3, and 4 of the hippocampus

PBS — Phosphate Buffered Saline

DAB — Diaminobenzidine

IgG — Immunoglobulin G

H&E — Hematoxylin and Eosin

NIH — National Institutes of Health

SEM — Standard Error of the Mean

ANOVA — Analysis of Variance

L-Dopa — Levodopa (L-3,4-dihydroxyphenylalanine)

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Consent for Publication

Not applicable

Further studies are needed to better understand how *Phyllanthus amarus* produces its neuroprotective effects. Future research focusing on oxidative stress, inflammation, and neuronal survival pathways could help explain how the plant extract protects brain cells from damage caused by toxic agents. However, the findings from this study already suggest that *Phyllanthus amarus* may help protect the hippocampus against paraquat-induced damage.

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Competing Interest

The authors declare that they have no competing interest.

Ethical Approval

The experimental protocol and sample size were in accordance with standard procedures and complied with the guidelines of the National Institutes of Health (NIH) for the care and use of laboratory animals. Ethical approval for this study was granted by the University of Medical Sciences Animal Research Ethics Committee with approval number UNIMED-AREC/Apv/2022/106.

Authors Contribution

Iretiogo Esther Faturoti: Conceptualization, study design, experimental procedures, behavioral assessments, data curation, statistical analysis, manuscript drafting, and manuscript revision.

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Khadijat Opeyemi Olaoye: Experimental procedures, sample preparation, laboratory analyses, data interpretation, literature review, manuscript writing, and manuscript revision.

Titilayo Ayomipo Akinlose: Study supervision, project administration, experimental procedures, data interpretation, critical review of the manuscript, and final approval of the manuscript.

All authors contributed to the conduct of the experiments, participated in the preparation and revision of the manuscript, and approved the final version of the manuscript.

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