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ORIGINAL ARTICLE

Neuroprotective Role of *Phyllanthus acidus* Against Nicotine-Induced Neuroinflammation and Memory Impairment in Experimental Rats

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ABSTRACT

The neuroprotective effect of Phyllanthus acidus extract against nicotine-induced neuroinflammation and cognitive impairment in experimental rats was examined in this work. Four groups of adult rats were created: control, nicotine-exposed (1 mg/kg, i.p.), Phyllanthus acidus-treated (100 mg/kg) and nicotine + Phyllanthus acidus-treated groups. Significant memory impairments increased oxidative stress in brain regions, especially in the hippocampus and cerebral cortex, and heightened neuroinflammatory markers were all brought on by nicotine treatment. In rats given nicotine, histopathological analysis showed altered cellular architecture, inflammatory cell infiltration, and neuronal degeneration. On the other hand, Phyllanthus acidus extract therapy significantly increased antioxidant enzyme activity, decreased pro-inflammatory cytokines, and enhanced behavioral performance. Additionally, it enhanced histoarchitecture and maintained neuronal integrity in the impacted brain areas. According to the results, Phyllanthus acidus significantly reduces oxidative stress and neuroinflammation. As a result, it might be a useful medication to stop the neurodegenerative alterations and cognitive deficits brought on by nicotine.

KEYWORDS: Nicotine, Phyllanthus acidus, oxidative stress, neuroinflammation, memory loss, histopathology and neuroprotection

INTRODUCTION

A significant global health concern, neurotoxicity and neurodegenerative diseases are becoming more common as a result of exposure to chemicals, the environment, and lifestyle choices. Nicotine, an alkaloid found in tobacco, is one of many neurotoxic substances that have been linked to oxidative stress, neuroinflammation, and cognitive impairment (Islam, *et al.,* 2021). Chronic exposure to nicotine has been demonstrated to have detrimental effects on neuronal integrity and brain function, despite the fact that nicotine is frequently linked to brief stimulatory effects on the central nervous system (Benowitz, 2010; Mishra *et al.,* 2015). A growing body of research suggests that nicotine upsets redox equilibrium by producing too many reactive oxygen species (ROS), which causes lipid peroxidation, mitochondrial malfunction, and neuronal death (Uddin *et al.,* 2020; Kaur, *et al.,* 2022).

Neuroinflammation is becoming more widely acknowledged as a crucial factor in nicotine-induced

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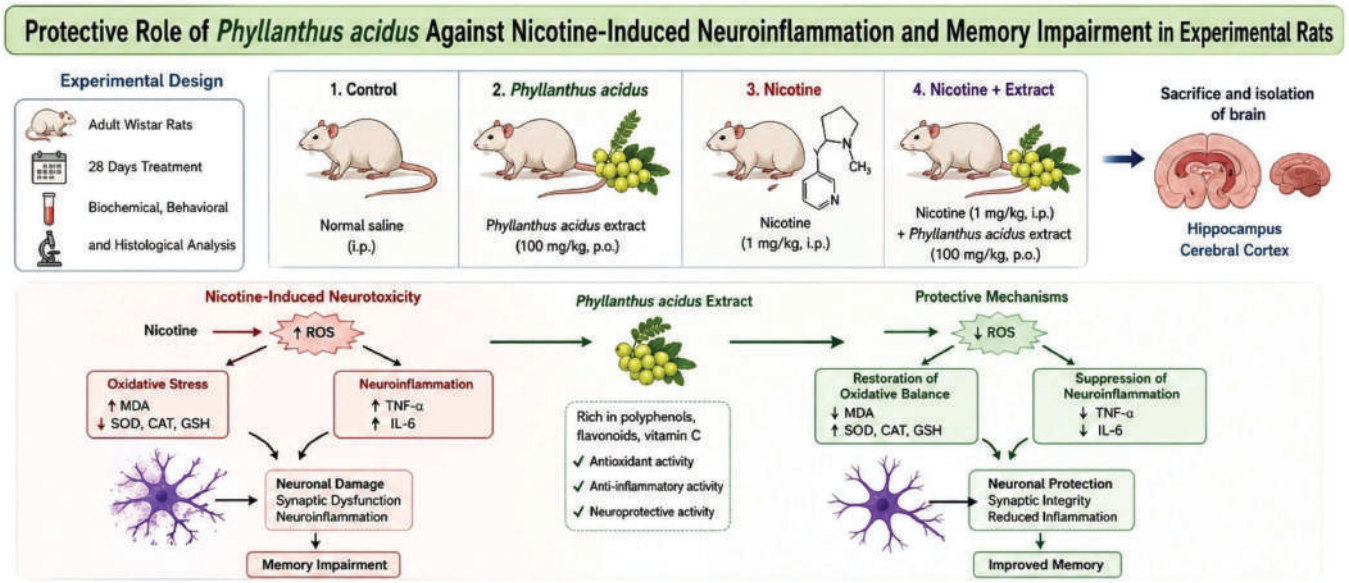

Figure 1: Graphical Representation.

neurotoxicity, along with oxidative stress. The brain's inflammatory reactions are largely mediated by pro-inflammatory cytokines like interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). According to Glass *et al.* (2010), long-term stimulation of these cytokines causes neuronal degeneration, synaptic dysfunction, and microglial activation. Targeting both pathways is crucial for successful neuroprotection, since recent research has shown how oxidative stress and inflammation interact to drive neurodegenerative processes (Singh *et al.,* 2022; Tran *et al.,* 2023). Additionally, the hippocampus—a portion of the brain essential for memory and learning—is especially susceptible to inflammatory and oxidative assaults, which could account for the cognitive deficits linked to nicotine use (Li, *et al.,* 2021).

Nicotine poisoning also affects neurotransmitter systems, especially the cholinergic system, which is crucial for memory and learning. Acetylcholine transmission, synaptic plasticity, and neuronal communication in the hippocampus have all been found to be disrupted by long-term nicotine use (Uddin *et al.,* 2020). The behavioral deficiencies shown in spatial learning and memory tasks like the Morris water maze may be caused by these changes. Furthermore, by reducing ATP generation and encouraging apoptotic pathways, nicotine-induced mitochondrial dysfunction worsens neuronal damage.

The role of natural antioxidants in reducing neurotoxicity has received more attention in recent years. Because they can target several pathways at once, such as oxidative stress, inflammation and apoptosis, chemicals originating from plants have demonstrated remarkable therapeutic potential. Particularly, polyphenols and flavonoids are known to improve endogenous antioxidant defenses, suppress inflammatory cascades, and modify important signaling pathways (Prananda *et al.,* 2023). Phytochemicals can successfully cross the blood–brain barrier and have neuroprotective benefits by controlling redox-sensitive pathways and lowering neuroinflammation, according to studies (Tran *et al.,* 2023; Kumar, *et al.,* 2020).

Phyllanthus acidus is an excellent option for neuroprotective studies in this regard. Phyllanthus acidus may have anti-apoptotic and neurodegenerative effects in addition to its antioxidant qualities, according to new research. According to experimental research, plant extracts can prevent neuronal cell death by regulating mitochondrial activity and preventing caspase activation. Additionally, Phyllanthus acidus's capacity to scavenge free radicals and shield neuronal membranes from lipid peroxidation may be attributed to the availability of prana ascorbic acid and other micronutrients (Rahman *et al.,* 2015).

By methodically examining biochemical, inflammatory, behavioral, and histological markers in the hippocampus and cerebral cortex after nicotine exposure and Phyllanthus acidus treatment, the current work fills this gap. The need for safe and efficient substitutes is highlighted by the rising prevalence of neurodegenerative diseases and the shortcomings of existing treatment strategies. Phyllanthus acidus and other natural compounds present a possible path for the creation of new neuroprotective drugs. The goal of the current investigation was to assess Phyllanthus acidus extract's neuroprotective potential against oxidative stress, neuroinflammation, and cognitive impairment caused by nicotine in experimental rats.

MATERIALS AND METHODS

Experimental Animals

An accredited animal facility provided adult male Wistar albino rats weighing 180–220 g, which were kept in conventional laboratory settings (22 ± 2°C, 12 h light/dark cycle, 50–60% humidity). The animals had unlimited access to water and a typical pellet diet. In compliance with the regulations set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, 2003), Government of India, all experimental Ethics Committee (IAEC). The Institutional Animal Ethics Committee approved the protocol.

Preparation of *Phyllanthus acidus* Extract

The Fresh Phyllanthus acidus fruits and leaves were gathered, verified, shade-dried, and ground into a powder. Ethanol was used in a Soxhlet system to remove the powdered particles. A rotary evaporator was used to concentrate the extract under low pressure, and it was then kept at 4°C until needed. Based on initial research and reports from the literature, the extract dose was set at 100 mg/kg body weight.

Chemicals and Reagents

All additional analytical-grade compounds, including nicotine hydrogen tartrate salt, were purchased from reputable vendors. Oxidative stress marker biochemical assay kits were purchased from reputable commercial vendors.

Experimental Design

48 rats in all were split up into four groups at random (n = 12 per group). Group II was given Phyllanthus acidus extract (100 mg/kg, orally), while Group I (control) was given regular saline. Group IV received a combination of nicotine (1 mg/kg, i.p.) and Phyllanthus acidus extract (100 mg/kg, orally), while Group III received nicotine (1 mg/kg body weight, intraperitoneally). For 28-days in a row, all therapies were administered.

Behavioral Assessment

Standard behavioral paradigms, such as the Morris Water Maze (MWM) for spatial learning and memory, the Y-maze test for spontaneous alternation behavior, and the Novel Object Recognition Test (NORT) for recognition memory, were used to assess cognitive function and memory. To assess cognitive ability, important metrics such escape delay, percentage alternation, and discriminating index were noted.

Biochemical Analysis

After undergoing a 28-days treatment, the animals were euthanized by cervical dislocation. The liver tissue was then extracted at a temperature of 4°C. Subsequently, the tissue was rinsed with ice-cold saline solution. It was then submerged in liquid nitrogen and promptly stored in a deep freezer at a temperature of -80°C in order to facilitate subsequent biochemical analysis. The activity of superoxide dismutase (SOD) was measured in tissue homogenates using the method of Misra and Fridovich (1972) on a Hitachi U-2000 spectrophotometer. The absorbance was measured at 480 nm for 4 minutes. The activity of SOD was expressed as the amount of enzyme needed to inhibit the oxidation of epinephrine by 50%, which is equal to 1 unit (U) per milligram of protein. The activity of catalase (CAT) was determined at room temperature using the method of Aebi (1984). The concentration of reduced glutathione (GSH) in liver homogenates was measured using the method described by Aker boom and Sies (1981). The MDA levels measured by Ohkawa *et al.* (1979). All enzyme activities were expressed as per milligram of protein, and the tissue protein concentration was estimated using the method of Lowry, Rosebrough, Farr and Randall (1951) with bovine serum albumin (BSA) as a standard. To evaluate inflammatory responses, neuroinflammatory markers like TNF-α and IL-6 were also examined.

Histopathological Examination

Brain tissues were treated, embedded in paraffin, and preserved in 10% formalin. Hematoxylin and eosin (H&E)-stained sections (5 μm) were inspected under a light microscope for structural changes, inflammation, and neuronal integrity.

Statistical Analysis

The standard error of the mean (SEM) was used to express the data. Using GraphPad Prism, a one-way ANOVA and Tukey's post hoc test were used for statistical analysis. Statistical significance was defined as $p < 0.05$.

RESULTS

Effect on Behavioral Parameters

In comparison to control animals, nicotine-treated rats showed significant cognitive impairment, as shown by lower discriminating index in the novel object recognition test ($p < 0.05$), decreased spontaneous alternation in the Y-maze, and increased escape latency in the Morris water maze. In rats exposed to nicotine, treatment with Phyllanthus acidus extract dramatically enhanced behavioral performance, suggesting that learning and memory functions had been restored.

Control of animals showed a steady decrease in escape latency during training days, as shown in Table 1, indicating typical learning and memory acquisition. Rats given Phyllanthus acidus alone also displayed a similar decrease in

escape latency, suggesting that the extract had no negative effects on cognitive function. On the other hand, rats given with nicotine showed significantly longer escape latency on all days, and by Day 4, the effect was more noticeable (40 ± 2 s; $p < 0.05$ vs. control), suggesting that their spatial learning and memory were compromised. A deficiency in the task's acquisition and retention phases is suggested by the consistently greater latency across sessions.

Notably, escape latency was lower in the Phyllanthus acidus extract group than in the nicotine group (20 ± 2 on Day 4; $p < 0.05$), indicating a significant improvement in performance. The significant improvement suggests a partial restoration of cognitive function, even though the values did not entirely return to control levels. Overall, these results show that whereas Phyllanthus acidus has a protective impact by improving learning and memory function, probably through its antioxidant and anti-inflammatory pathways, nicotine causes considerable cognitive damage.

Table 1: Behavioral Assessment (Morris Water Maze – Escape Latency).

Group	Day 1	Day 2	Day 3	Day 4
Control	45 ± 3	32 ± 2	20 ± 2	15 ± 1
Phyllanthus acidus	44 ± 3	30 ± 2	19 ± 2	14 ± 1
Nicotine	60 ± 4	55 ± 3	48 ± 3	40 ± 2 ***
Nicotine + Extract	50 ± 3	40 ± 3	28 ± 2	20 ± 2 ###

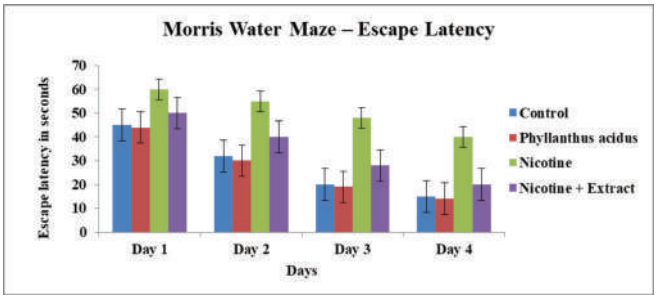

Figure 2: Escape Latency Test (MWM).

Effect on Oxidative Stress Markers

When nicotine was administered to brain tissues, lipid peroxidation (MDA levels) significantly increased while antioxidant enzyme activities (SOD, CAT, and GSH) significantly decreased ($p < 0.05$). By lowering MDA levels and bringing antioxidant enzyme activity back to normal, co-administration of Phyllanthus acidus extract considerably reduced oxidative stress.

According to Table 2, nicotine administration significantly increased MDA levels in the cerebral cortex and hippocampus when compared to the control group ($p < 0.05$), indicating increased oxidative stress and lipid peroxidation.

Table 2: Effect of *Phyllanthus acidus* on MDA (nmol/mg protein) Levels in Hippocampus and Cerebral Cortex.

Group	Hippocampus	Cerebral Cortex
Control	2.6 ± 0.2	2.4 ± 0.2
Phyllanthus acidus (100 mg/kg)	2.4 ± 0.3	2.2 ± 0.3
Nicotine (1 mg/kg, i.p.)	6.2 ± 0.4 ***	5.5 ± 0.3 ***
Nicotine + Extract	3.3 ± 0.3 ###	3.0 ± 0.2 ###

The hippocampus (6.2 ± 0.4 nmol/mg protein) showed a larger increase than the cerebral cortex (5.5 ± 0.3 nmol/mg protein), indicating that the hippocampal area is more vulnerable to oxidative damage caused by nicotine.

The non-toxic and safe profile of Phyllanthus acidus extract was confirmed by the fact that treatment with it alone did not significantly alter MDA levels when compared to the control. In contrast to the nicotine-treated group, co-administration of Phyllanthus acidus with nicotine significantly decreased MDA levels in the hippocampus (3.3 ± 0.3) and cerebral cortex (3.0 ± 0.2) ($p < 0.05$). The significant decrease suggests a significant abatement of lipid peroxidation, even though the levels did not entirely return to control values.

Table 3: SOD Activity (U/mg protein) in Hippocampus and Cerebral Cortex.

Group	Hippocampus	Cerebral Cortex
Control	12.8 ± 0.7	12.2 ± 0.6
Phyllanthus acidus	13.5 ± 0.8	12.9 ± 0.7
Nicotine	6.1 ± 0.5 ***	6.7 ± 0.4 ***
Nicotine + Extract	11.0 ± 0.6 ###	10.5 ± 0.5 ###

Superoxide dismutase (SOD) activity was significantly lower in the hippocampus and cerebral cortex following nicotine administration than in the control group ($p < 0.05$), as indicated in Table 3. This suggests compromised antioxidant defense and increases oxidative stress. The hippocampus (6.1 ± 0.5 U/mg protein) showed a somewhat higher drop than the cerebral cortex (6.7 ± 0.4 U/mg protein), indicating that the hippocampal area is more susceptible to nicotine-induced oxidative damage. When compared to the control, treatment with Phyllanthus acidus alone did not significantly change SOD activity; on the contrary, it slightly increased, suggesting a potential improvement in endogenous antioxidant capability. Significantly, compared to the nicotine-treated group ($p < 0.05$), co-administration of Phyllanthus acidus and nicotine significantly increased SOD activity in the hippocampus (11.0 ± 0.6) and cerebral cortex (10.5 ± 0.5). The significant recovery indicates a protective impact against oxidative stress even though the enzyme levels did not fully return to control values.

Table 4: CAT (µmol/min/mg protein) Activity in Hippocampus and Cerebral Cortex.

Group	Hippocampus	Cerebral Cortex
Control	56.5 ± 2.2	54.0 ± 2.0
Phyllanthus acidus	58.1 ± 2.3	56.8 ± 2.2
Nicotine	26.8 ± 1.8 ***	29.5 ± 1.9 ***
Nicotine + Extract	47.2 ± 2.1 ###	45.8 ± 2.0 ###

As shown in Table 4, nicotine administration significantly decreased catalase (CAT) activity in the cerebral cortex and hippocampus when compared to the control group ($p < 0.05$), suggesting increased oxidative stress and reduced enzymatic detoxification of hydrogen peroxide. The hippocampus (26.8 ± 1.8 µmol/min/mg protein) showed a more significant drop than the cerebral cortex (29.5 ± 1.9 µmol/min/mg protein), indicating that the hippocampal area is more vulnerable to oxidative damage caused by nicotine.

In comparison to the control, treatment with Phyllanthus acidus alone did not result in any appreciable negative effects and showed a modest increase in CAT activity, suggesting its supportive function in preserving antioxidant status. Crucially, when compared to the nicotine-treated group, co-administration of Phyllanthus acidus and nicotine markedly increased CAT activity in the hippocampus (47.2 ± 2.1) and cerebral cortex (45.8 ± 2.0) ($p < 0.05$). The significant improvement indicates the successful restoration of antioxidant defense mechanisms, even though the values did not completely return to control levels. Overall, these results show that while Phyllanthus acidus has a protective impact by increasing enzymatic antioxidant capacity and lowering oxidative stress, nicotine significantly reduces catalase activity in brain tissues.

Table 5: GSH (µg/mg protein) Levels in Hippocampus and Cerebral Cortex.

Group	Hippocampus	Cerebral Cortex
Control	8.8 ± 0.5	8.4 ± 0.4
Phyllanthus acidus	9.3 ± 0.6	8.9 ± 0.5
Nicotine	4.0 ± 0.3 ***	4.5 ± 0.3 ***
Nicotine + Extract	7.5 ± 0.4 ###	7.1 ± 0.4 ###

In comparison to the control group, nicotine administration significantly decreased reduced glutathione (GSH) levels in the hippocampus and cerebral cortex ($p < 0.05$), as Table 5 illustrates. This suggests a higher oxidative burden and weakened cellular antioxidant defense. The hippocampus (4.0 ± 0.3 µg/mg protein) showed a somewhat higher decline than the cerebral cortex (4.5 ± 0.3 µg/mg protein), indicating that the hippocampal area is more vulnerable to nicotine-induced oxidative stress.

Phyllanthus acidus treatment alone did not significantly lower GSH levels; rather, it slightly raised them in comparison to the control, suggesting that it may improve endogenous antioxidant status. Notably, when compared to the nicotine-treated group, co-administration of Phyllanthus acidus and nicotine significantly restored GSH levels in the cerebral cortex (7.1 ± 0.4) and hippocampus (7.5 ± 0.4) ($p < 0.05$). The significant recovery indicates successful reduction of oxidative stress, even though the results did not fully return to control levels. Overall, these results show that nicotine upsets intracellular redox balance by reducing GSH, whereas Phyllanthus acidus counteracts this effect by raising glutathione levels, which helps explain its neuroprotective effects.

Effect on Neuroinflammatory Markers

Pro-inflammatory cytokines (TNF-α and IL-6) were significantly higher in nicotine-treated rats than in controls ($p < 0.05$). Nevertheless, Phyllanthus acidus extract administration significantly decreased these cytokine levels, indicating its strong anti-inflammatory properties.

Table 6: TNF-á (pg/mg protein) Levels in Hippocampus and Cerebral Cortex.

Group	Hippocampus	Cerebral Cortex
Control	26 ± 3	24 ± 2
Phyllanthus acidus	24 ± 2	22 ± 2
Nicotine	72 ± 5 ***	64 ± 4 ***
Nicotine + Extract	40 ± 4 ###	36 ± 3 ###

In comparison to the control group, nicotine administration significantly increased TNF-α levels in the hippocampus and cerebral cortex ($p < 0.05$), as Table 6 illustrates, suggesting the production of a severe neuroinflammatory response. The hippocampus showed a greater increase (72 ± 5 pg/mg protein) than the cerebral cortex (64 ± 4 pg/mg protein), indicating that the hippocampus is more vulnerable to inflammatory damage caused by nicotine.

Phyllanthus acidus's non-inflammatory and safe profile was confirmed by the fact that treatment with it alone did not significantly alter TNF-α levels when compared to control. In contrast to the nicotine-treated group, co-administration of Phyllanthus acidus with nicotine significantly decreased TNF-α levels in the hippocampus (40 ± 4) and cerebral cortex (36 ± 3) ($p < 0.05$). The significant decrease shows successful abatement of inflammation, even though the cytokine levels did not fully revert to baseline levels. Overall, these results show that nicotine causes significant neuroinflammation in brain tissues, especially in the hippocampus, whereas Phyllanthus acidus has a protective impact by reducing the production of pro-inflammatory cytokines, which adds to its neuroprotective potential.

Table 7: IL-6 (pg/mg protein) Levels in Hippocampus and Cerebral Cortex.

Group	Hippocampus	Cerebral Cortex
Control	19 ± 2	17 ± 2
Phyllanthus acidus	18 ± 2	16 ± 2
Nicotine	55 ± 4 ***	49 ± 3 ***
Nicotine + Extract	31 ± 3 ###	27 ± 3 ###

Note: ***p < 0.05 vs Control; ###p < 0.05 vs Nicotine group

Nicotine administration significantly raised IL-6 levels in the hippocampus and cerebral cortex when compared to the control group ($p < 0.05$), as shown in Table 7, suggesting activation of neuroinflammatory pathways. The hippocampal region appears to be more sensitive to nicotine-induced inflammatory stress, as seen by the rise in the hippocampus (55 ± 4 pg/mg protein) compared to the cerebral cortex (49 ± 3 pg/mg protein). The non-inflammatory nature of Phyllanthus acidus was confirmed by the fact that treatment with it alone did not significantly change IL-6 levels when compared to control. Crucially, when Phyllanthus acidus and nicotine were administered together, IL-6 levels in the hippocampus (31 ± 3) and cerebral cortex (27 ± 3) were considerably lower than those in the nicotine-treated group ($p < 0.05$). The significant drop suggests that inflammatory reactions have been effectively suppressed, even though the levels did not completely return to control values. Overall, these results show that nicotine causes significant neuroinflammation, especially in the hippocampus, while Phyllanthus acidus reduces this effect by suppressing pro-inflammatory cytokines like IL-6, suggesting its neuroprotective function.

Histopathological Findings

Both the control and Phyllanthus acidus-treated groups' neural architecture were normal, according to histological investigation, suggesting no harmful effects. On the other hand, nicotine exposure led to severe neurodegenerative alterations in the cerebral cortex and hippocampus, with the hippocampus being more affected. These changes included pyknotic nuclei, vacuolation, neuronal degeneration, disturbed layer organization, and decreased neuronal density. Although slight changes remained, co-treatment with Phyllanthus acidus markedly improved histological characteristics in both locations, demonstrating less neuronal injury, enhanced cellular structure, and preservation of neuronal integrity. Because of its antioxidant and

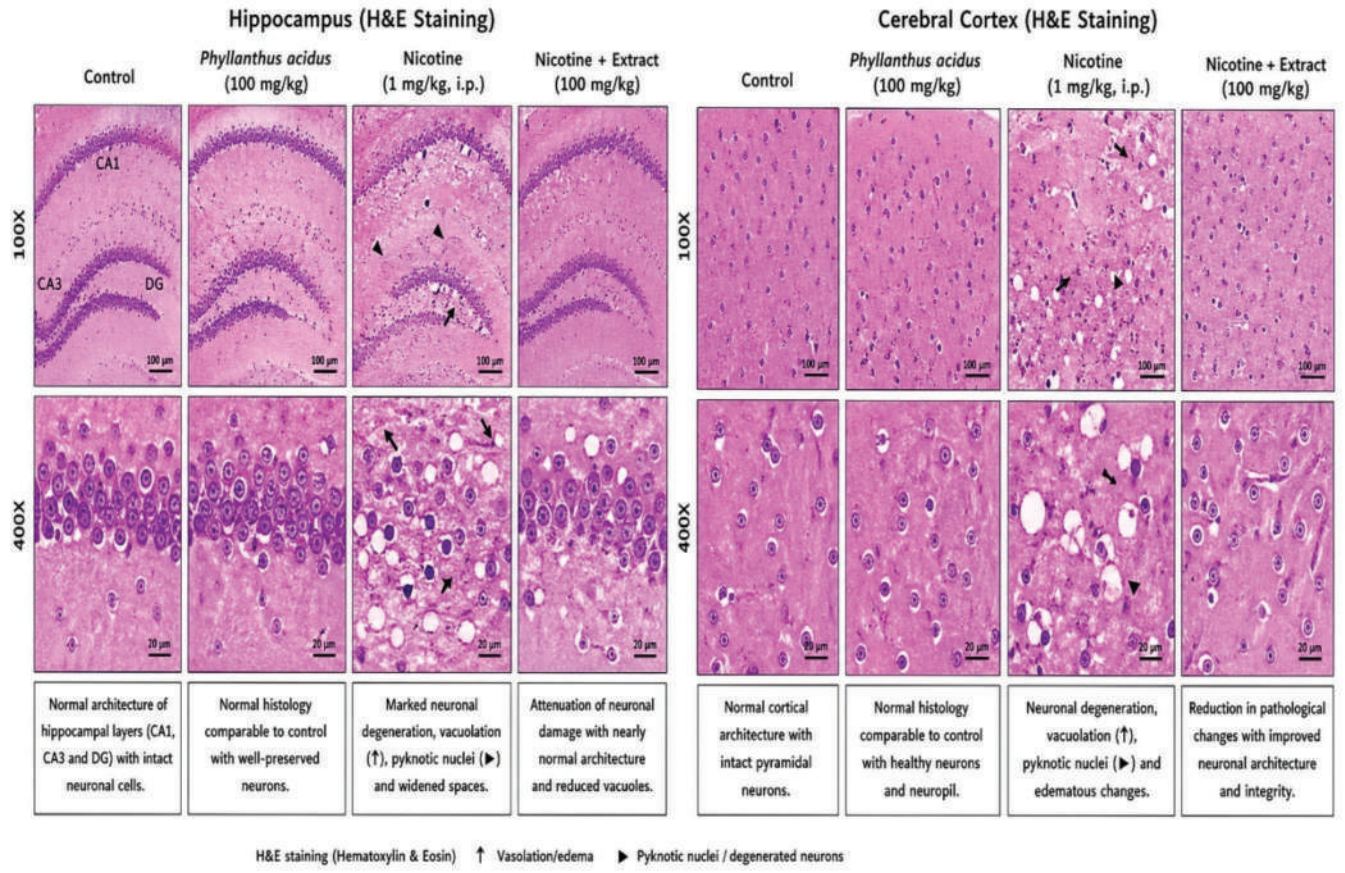

Figure 3: Histopatological Analysis.

anti-inflammatory qualities, Phyllanthus acidus generally exhibits substantial neuroprotective effects against nicotine-induced brain damage.

DISCUSSION

The current work shows that exposure to nicotine causes considerable oxidative stress, neuroinflammation, and cognitive impairment in experimental rats; the effects are more noticeable in the hippocampus than in the cerebral cortex. These results are in line with research showing that nicotine and other neurotoxic substances increase reactive oxygen species (ROS), which causes lipid peroxidation, antioxidant depletion, and damage to neurons. The study's findings of elevated MDA levels and decreased antioxidant enzymes (SOD, CAT, and GSH) support the idea that oxidative stress causes brain damage.

Our findings are consistent with other research demonstrating that high ROS production causes neurodegeneration and disturbs cellular homeostasis (Uddin *et al.,* 2016; Sinha, *et al.,* 2022). According to research on oxidative stress mechanisms, an excess of free radicals damages DNA, proteins, and lipids, which eventually leads to neurodegenerative diseases (Dar, *et al.,* 2021). Significantly, the hippocampus's high metabolic activity and comparatively low antioxidant defense capacity have been shown to make it more susceptible to oxidative damage, which validates our findings of more significant biochemical and histological changes in this area. Nicotine-treated rats showed a substantial increase in pro-inflammatory cytokines (TNF-α and IL-6), which further supports the role of neuroinflammation in nicotine-induced neurotoxicity. These results are consistent with recent studies showing that inflammation and oxidative stress are closely related processes that lead to neuronal dysfunction and cognitive impairment. According to Resent research, inflammatory mediators including TNF-α and IL-6, which activate microglial cells and encourage neuronal death, are critical in the development of neurodegenerative disorders (Tran *et al.,* 2023; Gbdamosi & Akinmoladun (2023)). It's interesting to note that Phyllanthus acidus extract administration considerably decreased inflammatory cytokines, restored antioxidant enzyme levels, and suppressed oxidative stress markers. These results imply that Phyllanthus acidus's neuroprotective benefits are mediated by both anti-inflammatory and antioxidant mechanisms (Bhattarai, *et al.,* 2021). High concentrations of phenolic and flavonoid chemicals, which are known to scavenge free radicals and boost endogenous antioxidant defenses, have been found in Phyllanthus acidus, according to earlier research (Diniz, *et al.,* 2020).

Recent research on the Phyllanthus species, which shows that plant extracts greatly enhance cognitive function and lower oxidative stress by increasing antioxidant enzyme activity and lowering lipid peroxidation, further supports our findings (Prananda *et al.,* 2023). Additionally, research has shown that Phyllanthus species can enhance memory function and alter cholinergic activity, which is consistent with the behavioral changes seen in our Morris water maze results (Ali, *et al.,* 2020; Elufioye, *et al.,*2022). Phyllanthus acidus's neuroprotective role is further reinforced by the observed improvement in cognitive function in the group treated with it (Pohl, *et al.,* 2022). Improved spatial learning and memory are suggested by the decrease in escape latency in behavioral tests, most likely as a result of hippocampal neuronal integrity being preserved. This is in line with previous studies showing that by preserving synapse function and safeguarding brain structures, natural antioxidants can correct memory impairments (Chen, *et al.,*2021; Wang, & Wang, 2023).

The current study's histopathological results corroborate the biochemical findings. Phyllanthus acidus therapy significantly decreased nicotine-induced pyknotic nuclei, vacuolation, and neuronal degeneration. Studies where antioxidant-rich plant extracts maintained neuronal architecture and stopped neurodegeneration under oxidative stress conditions have shown similar neuroprotective benefits. All things considered, the current study offers compelling proof that Phyllanthus acidus significantly protects against nicotine-induced neurotoxicity. Reduction of oxidative stress, inhibition of inflammatory reactions, and maintenance of neuronal structure and function seem to be the protective processes. These results demonstrate Phyllanthus acidus's medicinal promise as a natural remedy for oxidative stress and inflammation-related neurodegenerative diseases.

CONCLUSION

The findings unequivocally show that whereas nicotine causes severe oxidative stress, neuroinflammation, and cognitive impairment, the antioxidant and anti-inflammatory qualities of Phyllanthus acidus extract successfully counteract these effects.

Conflict of interest: None

REFERENCES

Benowitz, N. L. (2010). Nicotine addiction. *New England Journal of Medicine, 362*(24), 2295–2303. https://doi.org/10.1056/NEJMra0809890

Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C., & Gage, F. H. (2010). Mechanisms underlying inflammation in neurodegeneration. *Cell, 140*(6), 918–934. https://doi.org/10.1016/j.cell.2010.02.016

Mishra, A., Chaturvedi, P., Datta, S., Sinukumar, S., Joshi, P., & Garg, A. (2015). Harmful effects of nicotine. *Indian Journal of Medical and Paediatric Oncology, 36*(1), 24–31. https://doi.org/10.4103/0971-5851.151771

Prananda, A. T., Dalimunthe, A., Harahap, U., Simanjuntak, Y., Peronika, E., Karosekali, N. E., Hasibuan, P. A. Z., Syahputra, R. A., Situmorang, P. C., & Nurkolis, F. (2023). Phytochemical

composition and pharmacological properties of *Phyllanthus* species: A review. *Frontiers in Pharmacology, 14*, 1288618. https://doi.org/10.3389/fphar.2023.1288618

Rahman, M. M., Islam, M. B., Biswas, M., & Alam, A. H. M. K. (2015). In vitro antioxidant and cholinesterase inhibitory activities of *Phyllanthus acidus*. *BMC Complementary Medicine and Therapies, 15*, 403. https://doi.org/10.1186/s12906-015-0930-y

Singh, A., Kukreti, R., Saso, L., & Kukreti, S. (2022). Oxidative stress: A key modulator in neurodegenerative diseases. *Antioxidants, 11*(5), 1023. https://doi.org/10.3390/antiox11051023

Tran, N. K. S., Trinh, T. A., Pyo, J., Kim, C. G., Park, J. G., & Kang, K. S. (2023). Neuroprotective effects of natural compounds against oxidative stress-induced neuronal damage. *Antioxidants, 12*(8), 1651. https://doi.org/10.3390/antiox12081651

Uddin, M. S., Mamun, A. A., Hossain, M. S., Ashaduzzaman, M., Noor, M. A. A., Hossain, M. S., Uddin, M. J., Sarker, J., & Asaduzzaman, M. (2016). Neuroprotective effects of *Phyllanthus acidus* on learning and memory impairment. *Advances in Alzheimer's Disease, 5*(2), 53–72. https://doi.org/10.4236/aad.2016.52005

Uddin, M. S., Tewari, D., Mamun, A. A., Kabir, M. T., Niaz, K., Wahed, M. I. I., Barreto, G. E., & Ashraf, G. M. (2020). Emerging promise of antioxidants in the treatment of neurodegenerative diseases. *Biomedicine & Pharmacotherapy, 121*, 109594. https://doi.org/10.1016/j.biopha.2019.109594

Ali, T., Badshah, H., Kim, T. H., & Kim, M. O. (2020). Melatonin attenuates nicotine-induced oxidative stress and neuroinflammation in the brain. *Antioxidants*, 9(8), 724. https://doi.org/10.3390/antiox9080724

Bhattarai, G., Poudel, S. B., Kook, S. H., & Lee, J. C. (2021). Anti-inflammatory and neuroprotective effects of plant-derived compounds. *Molecules*, 26(19), 5936. https://doi.org/10.3390/molecules26195936

Chen, X., Drew, K. L., Berney, W., & Lei, G. (2021). Neuroprotective natural products for Alzheimer's disease. *Cells*, 10(6), 1309. https://doi.org/10.3390/cells10061309

Dar, N. J., & Hamid, A. (2021). Neurodegeneration and oxidative stress: Therapeutic approaches. *Springer Nature Reviews Neuroscience*, 22(3), 167–182. https://doi.org/10.1007/s12035-021-02345-2

Diniz, B. S., Reynolds, C. F., & Butters, M. A. (2020). Neuroinflammation and cognitive impairment: A systematic review. *Molecular Neurobiology*, 57(1), 122–135. https://doi.org/10.1007/s12035-019-01745-0

Elufioye, T. O., Habtemariam, S., & McGaw, L. J. (2022). Phytochemical and pharmacological properties of medicinal plants with neuroprotective potential. *Pharmaceuticals*, 15(3), 287. https://doi.org/10.3390/ph15030287

Gbdamosi, I. T., & Akinmoladun, A. C. (2023). Natural products as modulators of oxidative stress in neurodegenerative diseases. *Antioxidants*, 12(4), 812. https://doi.org/10.3390/antiox12040812

Islam, M. T., Quispe, C., El-Kersh, D. M., & Shill, M. C. (2021). Neuropharmacological effects of medicinal plants: A review. *Frontiers in Pharmacology*, 12, 704921. https://doi.org/10.3389/fphar.2021.704921

Kaur, R., Mehan, S., & Khanna, D. (2022). Role of oxidative stress and inflammation in nicotine-induced neurotoxicity. *Molecular Neurobiology*, 59(5), 2994–3010. https://doi.org/10.1007/s12035-022-02789-3

Khan, H., Ullah, H., Aschner, M., Cheang, W. S., Akkol, E. K., & Sobarzo-Sánchez, E. (2020). Neuroprotective effects of phytochemicals. *Biomolecules*, 10(3), 297. https://doi.org/10.3390/biom10030297

Kumar, A., Singh, A., & Ekavali. (2020). A review on Alzheimer's disease pathophysiology and its management. *Pharmacological Reports*, 72(4), 1097–1111. https://doi.org/10.1007/s43440-020-00121-8

Li, S., Pu, X. P., & Zhang, W. (2021). Neuroprotective effects of natural antioxidants. *Oxidative Medicine and Cellular Longevity*, 2021, 6621234. https://doi.org/10.1155/2021/6621234

Martins, N., Barros, L., & Ferreira, I. C. F. R. (2020). In vivo antioxidant activity of phenolic compounds. *Molecules*, 25(14), 3291. https://doi.org/10.3390/molecules25143291

Pohl, F., Lin, P. K. T., & Lokeshwar, B. L. (2022). Role of phytochemicals in neuroprotection. *Nutrients*, 14(5), 1012. https://doi.org/10.3390/nu14051012

Sinha, K., Das, J., Pal, P. B., & Sil, P. C. (2020). Oxidative stress and neurodegeneration: The therapeutic potential of antioxidants. *Springer Neurochemical Research*, 45(7), 1533–1549. https://doi.org/10.1007/s11064-020-03030-2

Wang, J., & Wang, F. (2023). Role of inflammation and oxidative stress in neurodegenerative diseases. *Cells*, 12(2), 345. https://doi.org/10.3390/cells12020345