Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 7 Issue 9 September 2025

Research Article

Neuroprotective Potential of *Tamarindus indica* Fruit Pulp Extract Against Acrylonitrile-Induced Neuroinflammation in Male Albino Rats

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Received: July 30, 2025

Published: August 30, 2025

Abstract

Neurodegenerative diseases, characterized by neuroinflammation, pose significant global health challenges, exacerbated by environmental toxins like acrylonitrile (AN). This study investigates the anti-inflammatory potential of *Tamarindus indica* fruit pulp ethanolic extract in an AN-induced neuroinflammation model in male Wistar rats. Seventy rats were divided into seven groups, receiving varying doses of *T. indica* extract (200 or 400 mg/kg body weight) either preventively or therapeutically alongside AN exposure, with normal and positive controls. Brain homogenates were analyzed for pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) using ELISA techniques. Results revealed significant reductions in cytokine levels in both preventive and treatment groups compared to the negative control, with the lowest IL-6 levels in the high-dose treatment group (4.899 ± 0.023 pg/mL) and the lowest IL-1β and TNF-α levels in the low-dose preventive group. These findings suggest that *T. indica* extract effectively mitigates AN-induced neuroinflammation, likely through modulation of inflammatory pathways, highlighting its potential as a cost-effective, natural therapeutic for neurodegenerative diseases.

Keywords: Neuroinflammation; *Tamarindus indica*; Acrylonitrile; Pro-Inflammatory Cytokines; Neurodegenerative Diseases

Introduction

Neurodegenerative diseases, affecting millions globally, pose a growing challenge as populations age, with conditions like Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis driving significant morbidity [1]. These disorders involve neuroinflammation, protein aggregation, mitochondrial dysfunction, and oxidative stress, exacerbated by environmental toxins like acrylonitrile (AN) [2,3]. AN's neurotoxicity, linked to glutathione depletion and cyanide production, disrupts cellular homeostasis, promotes inflammation, and targets astrocytes, increasing glioma risk. It also induces endoplasmic reticulum stress, triggering the unfolded protein response, though excessive stress may lead to harmful autophagy. Emerging evidence suggests that hydrogen sulfide and Nrf2 signaling may mitigate AN-induced damage, yet their therapeutic potential remains underexplored [4].

Natural compounds, particularly flavonoids and phenolics, offer promising neuroprotective strategies due to their anti-inflammatory properties [3]. In West Africa, plants from the Fabaceae family, such as *Tamarindus indica*, are valued for their phytochemical richness [5]. *T. indica* fruit pulp, abundant in flavonoids like epicatechin and apigenin, shows potent anti-inflammatory activity, but its anti-inflammatory efficacy against AN-induced neurodegeneration is unknown. This study evaluates the anti-inflammatory capacity of *T. indica* fruit pulp ethanolic extract in an acrylonitrile-induced rat model, aiming to establish its potential as a cost-effective, natural therapeutic for neuroinflammation in neurodegenerative diseases.

Literature Review

Cognitive decline, neuronal loss, inflammation, and cell death through necroptosis or apoptosis are characteristics of neurodegenerative diseases. Autism spectrum disorder (ASD) is an instance of a neurological ailment that is linked to social and learning difficulties and inflammation in the central nervous system [3]. Drug discovery has advanced significantly because of the centuries-long usage of herbal remedies to treat a variety of illnesses worldwide. Natural products have been shown to have neuroprotective qualities; they work with various receptors in a variety of ways to help control brain chemicals, and these substances have well-established neuroprotective properties. Africa's traditional medicine (ATM), one of the oldest medical systems, is practiced throughout West Africa, a region renowned for its abundant plant biodiversity. Boasting over 45,000 native plant species, representing a quarter of global flora, the region holds immense potential for therapeutic applications. Studies demonstrate that the seed extract of *T. indica* exhibits significant anti-inflammatory, anti-nociceptive, and anti-arthritic properties in animal models. ATM's significance as a source of novel biopharmaceutical chemicals and sustainable healthcare solutions is highlighted by its affordability, safety, and efficacy in treating neurological and chronic illnesses [5]. Several therapeutic plants reduce inflammation by blocking the enzyme cyclooxygenase-1 (COX-1), which is linked to amyloid plaques in microglia. Gliomas, especially astrocyte-related tumors, are the most common brain tumors linked to acrylonitrile (AN) exposure. Astrocytes are a target of AN-induced neurotoxicity because they accumulate more AN than microglia, while being plentiful in the brain and essential for neuronal function [4].

Methodology

Plant material and extraction

T. indica fruit pods were collected from Kwara State University, Malete, Nigeria, authenticated at the University of Ilorin Herbarium (voucher: UILH/001/1155), and processed as described previously by Ajani and Usman (2020) [6]. Briefly, 200 g of air-dried fruit pulp was macerated in 2 L 70% ethanol for 72 h, blended, filtered, and concentrated to dryness at 60°C. The crude extract was stored at 4°C.

Experimental animals and experimental design

Seventy (70) male Wistar rats with an average weight of 90 ± 5 g were purchased from Umaraahmarkeen Nigeria Global Ventures, Kwara State, Nigeria. The animals were kept in metabolic cages at the animal holding unit of the Department of Biochemistry, Kwara State University, Malete, with optimum environmental conditions (temperature: 23 ± 1°C, photoperiod: 12 h light/dark cycle, relative humidity: 45-50%). The animals were allowed free access to food (TOPFEED Grower Mash) and freshwater ad libitum. All animals were acclimated to laboratory conditions for seven days prior to the start of the experiment. The study was conducted after receiving approval from the Ethical Committee for the Use of Laboratory Animals at the Department of Biochemistry, Kwara State University, Malete, Nigeria.

The animals were allocated into seven groups, each containing 10 rats, grouped according to their body weight (g). Throughout the 28-day experiment, all rats were allowed access to clean drinking water and dried pelletized animal feed ad libitum. The experimental rats received treatments as outlined below:

- Group 1 (Normal control group) – Daily oral administration of 1 mg/kg body weight (b.w) normal saline for 28 days.
- Group 2 (Preventive group 1) – Daily oral administration of 200 mg/kg b.w *T. indica* fruit pulp extract for the first 14 days, followed by the concomitant daily oral administration of 1 mg/kg b.w of acrylonitrile and 200 mg/kg b.w *T. indica* fruit pulp extract for the subsequent 14 days.
- Group 3 (Preventive group 2) – Daily oral administration of 400 mg/kg b.w *T. indica* fruit pulp extract for the first 14 days followed by the concomitant daily oral administration of 1 mg/kg b.w of acrylonitrile and 400 mg/kg b.w *T. indica* fruit pulp extract for the subsequent 14 days.
- Group 4 (Treatment group 1) – Daily oral administration of 1 mg/kg b.w of acrylonitrile for the first 14 days followed by the daily oral administration of 200 mg/kg b.w *T. indica* fruit pulp extract for the subsequent 14 days.
- Group 5 (Treatment group 2) – Daily oral administration of 1 mg/kg b.w of acrylonitrile for the first 14 days, followed by the daily oral administration of 400 mg/kg b.w *T. indica* fruit pulp extract for the subsequent 14 days.

- Group 6 (Positive control) – Daily oral administration of 1 mg/kg b.w of acrylonitrile for the first 14 days, followed by the daily oral administration of 0.5 mg/kg b.w donezepil hydrochloride for the subsequent 14 days.
- Group 7 (Negative control) – Daily oral administration of 1 mg/kg b.w of acrylonitrile for the first 14 days and were left untreated for the subsequent 14 days.

Neurodegeneration was established by administering acrylonitrile (1 mg/kg) for 14 days in the albino rats**,** leading to neuroinflammation, evident by elevated interleukin 1β and 6 levels.

Animal sacrifice and preparation of brain homogenates

Upon completion of the experiments, rats in each group were euthanized using chloroform anesthesia. The brain tissue was carefully excised immediately after sacrifice and homogenized in ice-cold phosphate-buffered saline (PBS) in a 1:10 (w/v) tissue-to-buffer ratio. The resulting homogenate was centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant was collected via careful siphoning with a clear syringe and kept in sample bottles for neuroinflammation analyses.

Evaluation of neuroinflammation status

ELISA Assay for Interleukin-6, Interleukin-1β, and TNF- α

The concentrations of interleukin-1β, interleukin-6, and TNF-α were determined in the prepared plasma using enzyme-linked immunosorbent assay (ELISA) kits, employing a modified sandwich ELISA principle as described by the manufacturer (Cayman Chemical) and [7].

Description of immunometric ELISA

This immunometric assay employs a double-antibody sandwich method. Each well of the provided microwell plate is pre-coated with a rat monoclonal antibody specific to rat IL-6, IL-1β, or TNF-α, respectively. This antibody will bind any IL-6, IL-1β, or TNF-α introduced into the well. Standards or biological test samples are incubated on the antibody-coated plate, and the plate is then rinsed before the addition of a second, non-overlapping biotin-conjugated rat monoclonal antibody specific for rat IL-6, IL-1β, or TNF-α, utilized to identify the bound IL-6, IL-1β, or TNF-α. Horseradish peroxidase (HRP)-conjugated streptavidin is used to recognize the sandwiches. The levels of IL-6, IL-1β, or TNF-α are quantified by assessing the enzymatic activity of HRP with the chromogenic substrate TMB (3,3',5,5'-tetramethylbenzidine). After a sufficient period, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured between 420 nm - 450 nm. The intensity of this color, determined spectrophotometrically, is directly proportional to the amount of bound HRP-streptavidin conjugate, which in turn is proportional to the concentration of IL-6, IL-1β, or TNF-α.

Principle

In this assay, each well on the microtiter plate had been pre-coated with a specific antibody. Standards or samples were introduced into the wells, enabling the target antigen to bind to the antibody. Unbound substances were subsequently washed away. A biotin-conjugated detection antibody, specific to the captured antigen, was then introduced. Following the removal of unbound detection antibody through washing, an avidin-horseradish peroxidase (HRP) conjugate was introduced, binding to the biotin. A tetramethylbenzidine (TMB) substrate was introduced, leading to color development through its reaction with the HRP enzyme. The reaction was halted by the addition of a sulfuric acid stop solution, which caused a color change from blue to yellow. The optical density (OD) of the wells was measured at a wavelength of 450 nm. The optical density (OD) values were compared to a standard curve created using known antigen concentrations to quantify the antigen levels in the samples.

Interleukin-6 assay

- Exactly 100 µL of standard, blank, or sample was added to each well, and the plate was incubated for 1 hour at room temperature on an orbital shaker.
- The wells were washed five times with 350 µL of wash buffer.
- Thereafter, 100 µL of Anti-IL-6 (rat) Biotin Conjugate was added, and the plate was incubated for 1 hour at room temperature on an orbital shaker, followed by five washes as described above.
- Streptavidin-HRP working solution (100 µL) was added, and the plate was incubated for 30 minutes at room temperature on an orbital shaker, followed by five washes as described above.

- TMB substrate solution (100 µL) was then added, and the plate was incubated for 30 minutes at room temperature, protected from light.
- Exactly 100 µL of HRP stop solution was added, and the OD was measured at 450 nm.

Interleukin-1 β assay

- Exactly 100 µL of standard, blank, or sample was added to each well, and the plate was incubated for 2 hours at 37°C.
- The liquid was aspirated without washing, and 100 µL of Detection Reagent A working solution was added, followed by incubation for 1 hour at 37°C.
- The wells were washed three times with 350 µL of wash buffer.
- Thereafter, 100 µL of Detection Reagent B working solution was added, and the plate was incubated for 30-60 minutes at 37°C, followed by five washes.
- TMB substrate solution (90 µL) was then added, and the plate was incubated for 15-30 minutes at 37°C, protected from light.
- Exactly 50 µL of stop solution was added, and the OD was measured at 450 nm.

TNF- α assay

- Exactly 100 µL of standard or sample was added to each well, and the plate was incubated for 90 minutes at 37°C.
- The liquid was removed, and 100 µL of biotinylated detection antibody working solution was added, followed by incubation for 1 hour at 37°C.
- The wells were washed three times with 350 µL of wash buffer.
- HRP conjugate working solution (100 µL) was added, and the plate was incubated for 30 minutes at 37°C, followed by five washes.
- TMB substrate solution (90 µL) was added, and the plate was incubated for 15 minutes at 37°C, protected from light.
- Exactly 50 µL of stop solution was added, and the OD was measured at 450 nm.

Statistical analysis

All data were expressed as the mean of six replicates ± standard error of the mean (S.E.M). Statistical evaluation of data was performed using Graphpad version 10 using one-way analysis of variance (ANOVA). Followed by Tukey's posthoc test for multiple comparisons. Results were deemed statistically significant at $p \leq 0.05$, corresponding to a 95% confidence level.

Results

Figures 1, 2, and 3 show bar charts illustrating significant reductions in IL-6, IL-1β, and TNF-α levels in the preventive and treatment groups compared to the negative and normal control groups. The negative control group exhibited the highest levels of IL-6, IL-1β, and TNF-α at 10.623 ± 0.285 pg/mL, 74.889 ± 1.889 pg/mL, and 287.143 ± 6.016 pg/mL, respectively. Conversely, the lowest levels of IL-1β and TNF-α were recorded in preventive group 1, showing statistically significant differences compared to the other groups. In contrast, the result of IL-6 shows a different trend, with the lowest value observed in the treatment group 2 at 4.899 ± 0.023 pg/mL, with significant statistical differences observed across all the experimental groups.

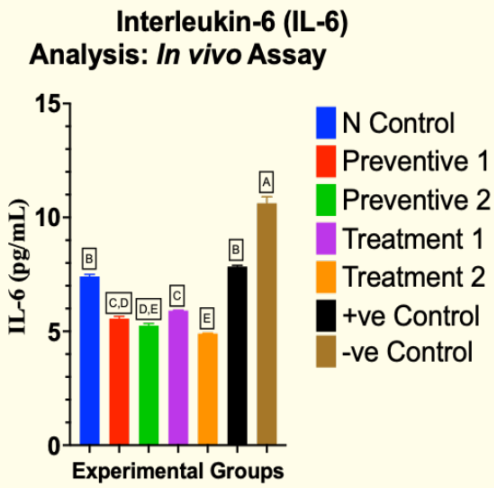

Figure 1: Effect of Tamarindus indica fruit pulp extract on brain homogenate Interleukin-6 (IL-6) levels in male Wistar rats across the experimental groups.
Results are Mean ± S.E.M, (n = 6). Bars with different letters are significantly different ($p < 0.05$).

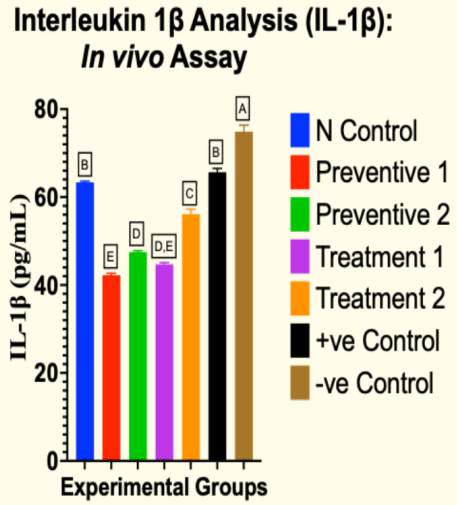

Figure 2: Effect of Tamarindus indica fruit pulp extract on brain homogenate Interleukin-1β (IL-1β) levels in male Wistar rats across the experimental groups.
Results are Mean ± S.E.M, (n = 6). Bars with different letters are significantly different ($p < 0.05$).

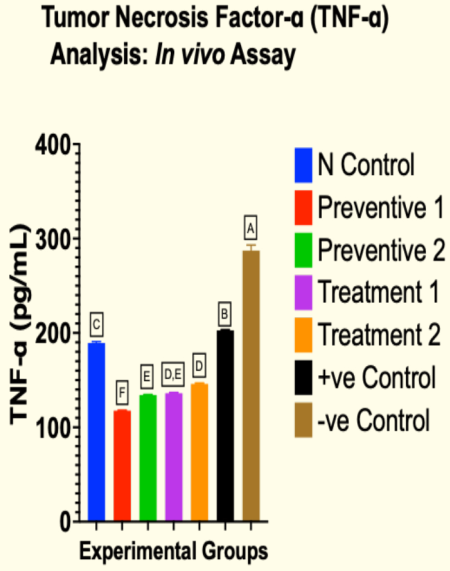

Figure 3: Effect of Tamarindus indica fruit pulp extract on brain homogenate Tumor Necrosis Factor-α (TNF-α) levels in male Wistar rats across the experimental groups.
Results are Mean ± S.E.M, (n = 6). Bars with different letters are significantly different ($p < 0.05$).

Discussion

IL-6, IL-1β, and TNF-α evaluation in experimental animal brain homogenate

Neuroinflammation, mediated by pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF-α), plays a central role in acrylonitrile-induced neurodegeneration. This study demonstrated the ability of *T. indica* fruit pulp extract to significantly reduce the levels of these cytokines in brain homogenates. IL-6 is a versatile cytokine associated with neuroinflammation and the activation of microglia. Elevated IL-6 levels exacerbate neuronal damage by amplifying inflammatory cascades and disrupting synaptic function. In this study, the extract significantly reduced IL-6 levels, indicating its ability to suppress inflammatory signaling pathways and protect neuronal tissues.

IL-1β is a potent pro-inflammatory cytokine involved in activating microglia and astrocytes, leading to chronic inflammation in the central nervous system (CNS). The observed suppression of IL-1β levels by *T. indica* extract highlights its potential to modulate neuroinflammation and prevent cytokine-induced neurotoxicity.

TNF-α contributes to blood-brain barrier disruption, synaptic dysfunction, and neuronal apoptosis. The significant reduction in TNF-α levels suggests that the extract effectively mitigates inflammatory responses, further supporting its neuroprotective role.

These findings align with the work of Merecz-Sadowska., *et al.* (2020) [8], who demonstrated that polyphenolic compounds in *T. indica* modulate inflammatory signaling pathways by inhibiting nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs). The extract significantly reduced pro-inflammatory cytokines, including IL-6, IL-1β, and TNF-α, in the brain homogenate of treated rats compared to controls. These cytokines are critical mediators of neuroinflammation, and their suppression by *T. indica* aligns with its anti-inflammatory properties observed computationally. Statistically significant differences across experimental groups highlight the extract's potent anti-inflammatory effects.

In the context of neuroinflammation, the study's findings on cytokine levels are particularly noteworthy. The significant reduction in brain homogenate levels of pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α after treatment with *T. indica* pulp suggests a modulatory effect on neuroinflammatory pathways. These results are consistent with prior research indicating that dietary antioxidants can attenuate neuroinflammation, which is a critical factor in the progression of neurodegenerative diseases [9]. The neuroprotective effects of *T. indica* fruit pulp may, therefore, be attributed to both its antioxidant properties and its ability to modulate inflammatory responses in the brain.

Conclusion

This study demonstrates that *Tamarindus indica* fruit pulp ethanolic extract possesses potent anti-inflammatory properties, significantly reducing pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) in an acrylonitrile-induced neuroinflammation model in Wistar rats. The extract's efficacy in both preventive and therapeutic applications underscores its potential to modulate neuroinflammatory pathways, as supported by significant reductions in cytokine levels compared to controls. These findings highlight *T. indica* as a promising, cost-effective natural therapeutic for mitigating neuroinflammation associated with neurodegenerative diseases. Further studies are warranted to elucidate the precise molecular mechanisms and to explore clinical applications in human populations, potentially offering a sustainable approach to managing the growing burden of neurodegenerative disorders.

Bibliography

1. Wang W., *et al*. "Resveratrol: Multi-Targets Mechanism on Neurodegenerative Diseases Based on Network Pharmacology". *Frontiers in Pharmacology* 11 (2020).

2. Agrawal MY., *et al*. "Research Trend and Detailed Insights into the Molecular Mechanisms of Food Bioactive Compounds against Cancer: A Comprehensive Review with Special Emphasis on Probiotics". *Cancers (Basel)* 14.22 (2022).

3. Rasool M., *et al*. "Recent updates in the treatment of neurodegenerative disorders using natural compounds". *Evidence-based Complementary and Alternative Medicine* 2014 (2014).

4. Yang B., *et al*. "Activation of autophagic flux and the Nrf2/ARE signaling pathway by hydrogen sulfide protects against acrylonitrile-induced neurotoxicity in primary rat astrocytes". *Archives of Toxicology* 92.6 (2018): 2093-2108.

5. Ayeni EA., *et al*. "Medicinal Plants for Anti-neurodegenerative diseases in West Africa". *Journal of Ethnopharmacology* 285 (2022): 114468.

6. Ajani EO and Usman LA. "Tamarindus indica fruit pulp restores reproductive function in sodium fluoride administered rats". *The FASEB Journal* 34.S1 (2020): 1-1.

7. Ye Z., *et al*. "Analytical and clinical evaluation of a novel assay for measurement of interleukin 6 in human whole blood samples". *Journal of Clinical Laboratory Analysis* 35.11 (2021): 1-6.

8. Merecz-Sadowska A., *et al*. "Anti-inflammatory activity of extracts and pure compounds derived from plants via modulation of signaling pathways, especially pi3k/akt in macrophages". *International Journal of Molecular Sciences* 21.24 (2020): 1-32.

9. Vasconcelos AR., *et al*. "Nrf2/ARE pathway modulation by dietary energy regulation in neurological disorders". *Frontiers in Pharmacology* 10 (2019): 1-18.