Neuroprotective and Antioxidant Potential of *Euphorbia tithymaloides* Ethanolic Leaf Extract Against LPS-Induced Neuroinflammation in Rats

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Abstract

The present study investigated the morphological characteristics, phytochemical composition, acute toxicity, and neuroprotective potential of Euphorbia tithymaloides ethanolic leaf extract (ETE) against lipopolysaccharide (LPS)-induced neuroinflammation in rats. Morphological analysis confirmed characteristic pharmacognostic features, ensuring plant authenticity. Ethanolic extraction yielded 7.72% w/w extract rich in alkaloids, glycosides, flavonoids, tannins, triterpenes, and phenols, as revealed by phytochemical screening, while fractionation separated bioactive components by polarity. Acute oral toxicity studies indicated no adverse effects up to 2000 mg/kg, placing ETE in GHS Category 5. Neuroprotective evaluation showed LPS significantly impaired motor coordination and locomotor activity, increased oxidative stress markers (MDA, nitrite), and caused severe neuronal degeneration. ETE treatment produced dose-dependent improvements in behavioral tests, with the 400 mg/kg dose showing greater recovery than 200 mg/kg, and markedly reduced MDA and nitrite levels. Histopathology confirmed preservation of neuronal architecture and reduced inflammation in treated groups, particularly at higher doses. These findings suggest ETE exerts potent neuroprotective effects through antioxidant and anti-inflammatory mechanisms, supporting its therapeutic potential against neurodegenerative conditions associated with oxidative stress and inflammation.

Keywords: *Euphorbia tithymaloides, neuroprotection, oxidative stress, anti-inflammatory activity, phytochemical screening, lipopolysaccharide-induced neuroinflammation.*

1. Introduction

Neurodegenerative diseases (NDs) are a diverse group of progressive neurological disorders characterized by the gradual deterioration of neuronal structure and function, ultimately leading to significant impairments in motor, cognitive, and emotional abilities (1). The underlying pathogenesis of NDs is heterogeneous, but most share the common hallmark of neuronal loss in the central nervous system (CNS), where neurons serve as the fundamental units responsible for transmitting signals throughout the brain and spinal cord. As these neurons degenerate and die, the CNS undergoes structural and functional decline, manifesting in debilitating clinical symptoms. Parkinson's disease (PD), one of the most prevalent NDs, is primarily associated with the degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to reduced dopamine levels in the striatum and resulting in hallmark motor symptoms such as bradykinesia, rigidity, tremor, and postural instability (2). The pathophysiological hallmarks of PD include dopaminergic neuronal death, largely due to the misfolding and aggregation of α-synuclein into Lewy bodies; mitochondrial dysfunction, which compromises ATP production and elevates oxidative stress; and chronic neuroinflammation, which is increasingly recognized as a key driver of disease progression (3). Mutations in genes

such as PINK1 and Parkin, which are critical for mitophagy, highlight the role of defective mitochondrial quality control in PD pathology (4). Oxidative stress—characterized by an excess of reactive oxygen species (ROS)—contributes to protein oxidation, lipid peroxidation, and DNA damage, further exacerbating neuronal injury. Chronic neuroinflammation, mediated largely by microglia, is a critical pathological process in PD. In response to stimuli such as infections, trauma, toxins, or ischemia, microglia adopt a pro-inflammatory phenotype, releasing cytokines including interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), which disrupt neuronal homeostasis, amplify oxidative stress, and induce apoptosis in dopaminergic neurons (5). Lipopolysaccharide (LPS), a large glycolipid found in the outer membrane of Gram-negative bacteria, is a potent inducer of neuroinflammation and has been widely used in experimental PD models (6). Structurally, LPS comprises lipid A, which anchors it to the bacterial membrane and triggers immune activation; a core oligosaccharide backbone; and an O-antigen side chain, which exhibits variability that aids bacterial evasion of host immunity (7). Mechanistically, LPS activates the Toll-like receptor 4 (TLR4) pathway, initiating a cascade of intracellular signaling events that culminate in the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). This transcription factor drives the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, intensifying oxidative stress, inflammation, and dopaminergic neuronal death—three core pathological features of PD (4). Given the multifactorial nature of PD pathogenesis, therapeutic strategies that target multiple pathways—particularly oxidative stress and inflammation—are of significant interest. In this context, medicinal plants rich in bioactive phytochemicals offer promising neuroprotective potential.

Euphorbia tithymaloides L., commonly known as Devil's Backbone, Slipper Spurge, or Zigzag Plant, is a perennial succulent belonging to the Euphorbiaceae family and has a long history of use in traditional medicine (8). It is native to tropical and subtropical regions, with a global distribution that includes North and South America, Africa, India, Thailand, Malaysia, and the Caribbean Islands. In India, it is found in states such as Gujarat, Maharashtra, Kerala, Karnataka, Tamil Nadu, Assam, and Meghalaya, as well as urban areas (9). Morphologically, *E. tithymaloides* exhibits dorsoventral leaves measuring 5–10 cm in length and 2–4 cm in width, ovate to lanceolate in shape with smooth margins and prominent venation. The leaves are dull green with a glossy sheen, emitting an earthy odor and a bitter taste when fresh. The green cylindrical stems, approximately 50 cm long, develop light brown woody suffusions with age and possess a weak earthy odor and dull bitter taste. Microscopically, the leaf epidermis is covered with a waxy cuticle and has stomata on both surfaces, with mesophyll differentiated into palisade and spongy layers. The stem cortex comprises collenchyma and parenchyma for support and storage, with randomly distributed vascular bundles containing xylem and phloem, and laticiferous cells producing milky latex. The roots possess a well-defined root cap, parenchymatous cortex with starch storage, and an endodermis with Casparian strips regulating nutrient uptake (10).

Phytochemical analyses of *E. tithymaloides* reveal a rich profile of secondary metabolites, including alkaloids (euphorbiaine), triterpenoid terpenes, flavonoids (quercetin, kaempferol), phenolic acids (caffeic acid, ferulic acid), lactones (euphorbiacins), glycosides, and fatty acids (8). These compounds collectively contribute to the plant's broad pharmacological activities. Its anti-inflammatory properties are attributed to the suppression of pro-inflammatory cytokines and enzymes, making it relevant for the management of arthritis and other inflammatory disorders. The plant also exhibits notable analgesic activity, traditionally used for alleviating headaches, muscle pains, and joint aches. Antimicrobial and antifungal activities have been demonstrated, indicating potential applications in infection control and wound healing. Its antioxidant activity is particularly important in the context of neuroprotection, as it can neutralize free

radicals and mitigate oxidative stress—a key driver of neurodegeneration. Moreover, *E. tithymaloides* has been reported to possess hypoglycemic activity, suggesting potential in diabetes management, as well as preliminary antitumor effects by inhibiting cancer cell proliferation in vitro (9). Cardioprotective effects have been observed, potentially reducing the risk of cardiovascular diseases, while emerging evidence suggests neuroprotective activity through the modulation of oxidative and inflammatory pathways in the CNS (11). The flavonoids quercetin and kaempferol, in particular, are known to inhibit microglial activation, reduce oxidative damage, and protect dopaminergic neurons in experimental PD models (8). Given the involvement of oxidative stress and neuroinflammation in PD, the phytochemical composition of *E. tithymaloides* aligns with multiple therapeutic targets, making it a promising candidate for further investigation.

In PD models involving LPS-induced neuroinflammation, plant-based compounds with antioxidant and anti-inflammatory activities have shown the potential to attenuate neurodegeneration, improve neuronal survival, and preserve motor function (5). E. tithymaloides, by virtue of its diverse bioactive constituents, could modulate TLR4/NF-κB signaling, reduce the production of pro-inflammatory cytokines, and enhance endogenous antioxidant defenses, thereby mitigating the dual pathological drivers of PD—oxidative stress and inflammation. This multi-targeted approach is especially valuable given the limitations of current PD treatments, which primarily offer symptomatic relief without halting disease progression. Consequently, the exploration of *E. tithymaloides* in LPS-induced PD models could provide critical insights into plant-based neuroprotective strategies and contribute to the development of novel therapeutic interventions for neurodegenerative diseases.

2. Materials and Methods

2.1 Collection and Authentication of Plant Material

Fresh leaves of *Euphorbia tithymaloides* were collected from forested areas in Bilaspur district, Chhattisgarh, India, during the flowering season (August–October). The plant was identified and authenticated by a certified botanist from the Department of Botany, GGV, Koni, Bilaspur, Chhattisgarh. A herbarium voucher specimen was deposited for reference (Reference No.: Bot./GGV/2025/145). Proper cleaning was performed to remove soil and debris before drying (12, 13, 14).

2.2 Morphological evaluation

Fresh leaves of *Euphorbia tithymaloides* were evaluated for morphological characteristics, including color, odour, taste, shape, size, and texture, following macroscopic identification procedures as per standard pharmacognostical guidelines. Observations were recorded for key organoleptic and structural features (15, 16).

2.3 Extraction of Plant Materials

The dried and coarsely powdered *Euphorbia tithymaloides* leaves (500 g) were subjected to Soxhlet extraction using 95% ethanol for 48 hours to ensure exhaustive extraction of bioactive constituents. The obtained extract was filtered and concentrated under reduced pressure using a rotary evaporator at 40°C to remove the solvent, yielding a semi-solid mass. The ethanolic extract was stored in airtight containers at 4°C until further use. Ethanol was selected as the solvent due to its high efficiency in extracting a wide range of polar and semi-polar phytoconstituents, including flavonoids, alkaloids, phenolics, and glycosides, which are potentially responsible for neuroprotective activity (17, 18, 19).

2.4 Phytochemical Screening of Plant Extract

Preliminary phytochemical screening of the *Euphorbia tithymaloides* leaf extract was performed using standard qualitative tests to identify the major classes of phytoconstituents. These tests followed protocols outlined in Khandelwal (2008) and Harborne (1998) (20, 21, 22).

2.5 Fractionation of Active Ingredients

To obtain the flavonoid-rich fraction, *Euphorbia tithymaloides* leaves were dried, powdered, and extracted using 95% ethanol in a Soxhlet apparatus. The concentrated ethanolic extract was suspended in distilled water and subjected to liquid–liquid partitioning using solvents in increasing polarity: petroleum ether, chloroform, and ethyl acetate. The ethyl acetate layer was separated, concentrated under reduced pressure, and labelled as the flavonoid-rich fraction. This fraction was stored at 4°C for further analysis and biological evaluation.

Fractionate with solvents of increasing polarity:

- Petroleum ether (removes fats)
- Chloroform (removes alkaloids, some steroids)
- Ethyl acetate (concentrates flavonoids and phenolics) (23, 24, 25).

2.6 Approval for Animal Experimentation and Acclimatization

All animal experiments were conducted following ethical guidelines and were approved by the Institutional Animal Ethics Committee (IAEC) under the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Approval No.: SOP/IAEC/2024/11/12). Healthy adult Wistar rats weighing 180–220 g were used for the study. The animals were housed in polypropylene cages under standard laboratory conditions: a 12-hour light/dark cycle, temperature maintained at 25 ± 2°C, and relative humidity of 50–60%. Rats were provided with standard pellet diet and water ad libitum. Prior to experimentation, the animals were acclimatized for a minimum of 7 days (26, 27, 28).

2.7 Acute Oral Toxicity Study

The acute oral toxicity study of *Euphorbia tithymaloides* leaf extract was carried out as per the OECD guideline 423 (Acute Toxic Class Method). Female Wistar albino rats (150–180 g) were divided into five groups (n=3 per group). After overnight fasting, the control group received distilled water, while the test groups were administered single oral doses of the extract at 5, 50, 300, and 2000 mg/kg body weight. Animals were observed individually for signs of toxicity, behavioural changes, and mortality during the first 30 minutes, periodically during the first 24 hours, and then daily for up to 14 days (29, 30, 31).

2.8 Neuroprotective Activity Evaluation

Neuroprotective effects of *Euphorbia tithymaloides* extract (ETE) were assessed in a Parkinson's disease-like rat model induced by intracerebroventricular lipopolysaccharide (LPS, 5 µg/5 µL). Thirty Wistar rats were divided into five groups (n=6): control, LPS, LPS + ETE (200 mg/kg), LPS + ETE (400 mg/kg), and LPS + levodopa (25 mg/kg). Treatments were given orally for 21 days.

Behavioral assessment included the Rotarod test (motor coordination), Actophotometer test (locomotion), and Open Field test (exploration). Biochemical parameters—malondialdehyde (MDA), nitrite, glutathione (GSH), and catalase—were measured in brain homogenates. Histopathology of H&E-stained brain sections was performed to evaluate neuronal degeneration and inflammation. Data were analysed by one-way ANOVA with Tukey's post-hoc test ($p<0.05$) (32 – 50).

3. Results and Discussion

3.1 Morphological Evaluation of Euphorbia tithymaloides

The morphological evaluation of *Euphorbia tithymaloides* leaves revealed light to dark green coloration, ovate to cordate shape, and smooth texture, with sizes ranging from 3–7.2 cm in length and 2–3.5 cm in width. The leaves exhibited a characteristic odour and mucilaginous taste, features commonly associated with members of the Euphorbiaceae family due to latex and mucilage content. These traits correspond with standard pharmacognostic descriptions, supporting accurate identification and authentication of the plant material. Such morphological parameters are essential for ensuring the quality and purity of samples prior to pharmacological and phytochemical investigations.

Fig. no. 3.1 Morphological evaluation of *Euphorbia tithymaloides* leaves

3.2 Preparation of Ethanolic extract of leaves of Euphorbia tithymaloides

The ethanolic (95%) extraction of *Euphorbia tithymaloides* leaves yielded 7.72% w/w brownish-green extract from 500 g of dried plant material, indicating a moderate presence of ethanol-soluble phytoconstituents. Ethanol's intermediate polarity facilitates the extraction of alkaloids, flavonoids, glycosides, saponins, tannins, and phenolics. The extract's colour suggests chlorophyll, tannins, and flavonoid derivatives. A yield above 5% confirms efficient extraction, making ethanol suitable for obtaining bioactive compounds for pharmacological evaluation.

Fig. no. 3.2 (a) Plant Material (*Euphorbia tithymaloides* leaves) Collection, Drying and Grinding

Fig. no. 3.2 (b) Extraction of *Euphorbia tithymaloides* leaves and Extractive Value

3.3 Phytochemical Screening of Plant Extract

Preliminary phytochemical screening of the ethanolic extract of *Euphorbia tithymaloides* revealed the presence of alkaloids, glycosides, flavonoids, carbohydrates, tannins, triterpenes, and phenols, while proteins, diterpenes, and fatty acids were absent. The detected compounds are associated with various pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and neuroprotective effects. The absence of proteins and fatty acids indicates minimal nutritional or lipid content. These results highlight the therapeutic potential of the extract and support its further evaluation in pharmacological studies.

Table no. 3.3 Phytochemical Screening of Plant Extract

S. No.	Chemical Constituent	Test Performed	Inference
1.	Alkaloids	Hager's Test	+
2.	Glycosides	Keller-kiliani Test	+
3.	Flavonoids	Shinoda Test	+
4.	Carbohydrates	Molisch's Test	+
5.	Tannins	Ferric chloride Test	+
6.	Proteins	Biuret Test	-
7.	Diterpenes	Copper acetate Test	-
8.	Triterpenes	Salkowski Test	+
9.	Fatty acids	Sudan IV Test	-
10.	Phenols	Lead Acetate Test	+

Where: (+) = Present and (-) = Absent

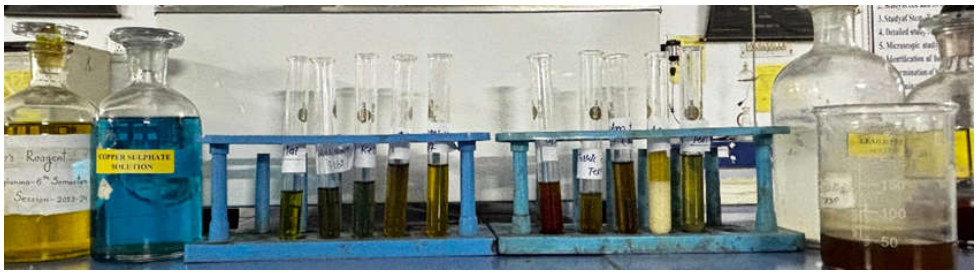


Fig. no. 3.3 Phytochemical Screening of Plant Extract

3.4 Phytochemical Screening of Plant Extract

The ethanolic extract of *Euphorbia tithymaloides* (3.86 g) was fractionated using petroleum ether, chloroform, and ethyl acetate, yielding 54.92% of the total extract. Ethyl acetate gave the highest yield (21.76%, light brown, semi-solid) rich in semi-polar constituents like flavonoids and phenolics. Chloroform yielded 19.69% (greenish-brown, sticky), likely containing alkaloids, terpenoids, and steroids. Petroleum ether produced 13.47% (pale yellow, oily), indicating non-polar compounds such as fatty acids and waxes. Fractionation effectively separated bioactive compounds based on polarity for targeted pharmacological evaluation.

Fig. no. 3.4 Fractionation of Active Ingredients

3.5 Acute Oral Toxicity Study

The acute oral toxicity study of *Euphorbia tithymaloides* extract (ETE) in rats, following OECD guideline 423, showed no mortality or signs of toxicity at doses up to 2000 mg/kg over 14 days. No adverse effects such as tremors, diarrhoea, lethargy, or behavioural changes were observed in any group. These results indicate ETE is safe with a wide margin of safety, placing it in GHS Category 5 (low acute toxicity). The findings support its traditional medicinal use and justify further pharmacological evaluation.

3.6 Neuroprotective Activity Evaluation

3.6.1 Behavioral Analysis

3.6.1.1 Rotarod Test (Motor Coordination)

In the motor-coordination (rotarod) test, LPS significantly reduced performance (62.48 ± 2.10 rotations/sec) compared to control (169.28 ± 1.25, $p < 0.0001$). Treatment with ETE improved motor function dose-dependently: 200 mg/kg (96.38 ± 1.80) and 400 mg/kg (120.4 ± 1.42), with the higher dose showing greater recovery. Levodopa (133.5 ± 1.58) produced the most improvement among treatments. All treatments showed highly significant improvement vs. LPS ($p < 0.0001$).

Table no. 3.6.1.1 Effect of *Euphorbia tithymaloides* extract on motor-coordination test in LPS treated rat

S. No.	Animal Group (n=6)	Group	Rotation per second
1.	Group- I	Control	169.28 ± 1.25
2.	Group- II	LPS	62.48 ± 2.10 a****
3.	Group- III	LPS+ETE (200 mg/kg)	96.38 ± 1.80 b****
4.	Group- IV	LPS+ETE (400 mg/kg)	120.4 ± 1.42 b****
5.	Group- V	LPS+Levodopa (25 mg/kg)	133.5 ± 1.58 b****

Values are expressed as mean ± SEM (n = 6). Statistical significance determined by one-way ANOVA followed by Tukey's post hoc test.

- a = comparison between Control and LPS
- b = comparison between LPS and treatment groups

- **** = $p < 0.0001$

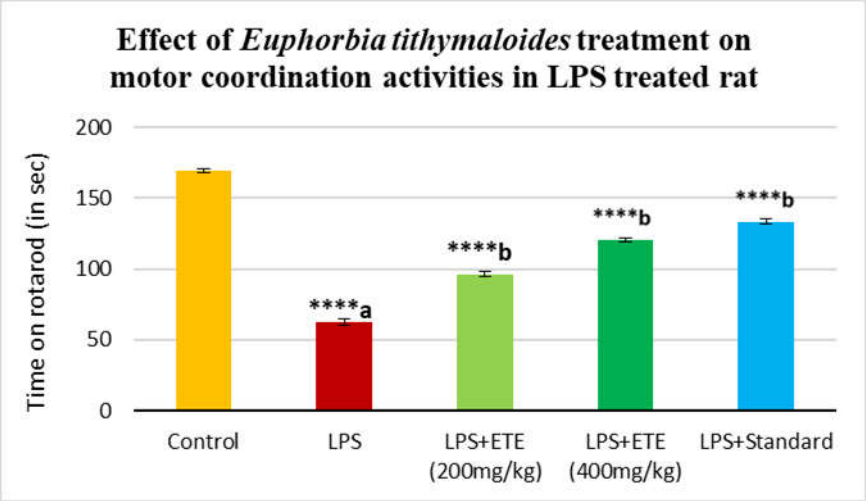


Fig. no. 3.6.1.1 (a) Effect of *Euphorbia tithymaloides* extract on motor-coordination test in LPS treated rat

Fig. no. 3.6.1.1 (b) Rotarod Test (Motor Coordination)

3.6.1.2 Actophotometer Test (Locomotor Activity)

In the locomotor activity test, LPS markedly reduced movement counts (146.41 ± 11.64) compared to control (309.56 ± 6.63, $p < 0.0001$). ETE treatment improved activity in a dose-dependent manner: 200 mg/kg (236.44 ± 6.21) and 400 mg/kg (260.03 ± 7.14). Levodopa (274.3 ± 6.85) produced the highest improvement among treatments. All treatment groups showed highly significant recovery vs. LPS ($p < 0.0001$).

Table No. 3.6.1.2 Effect of *Euphorbia tithymaloides* extract on locomotor activity in LPS treated rat

S. No.	Animal Group (n=6)	Group	Locomotor count/ 5 min
1.	Group- I	Control	309.56 ±6.63
2.	Group- II	LPS	146.41 ±11.64 a****
3.	Group- III	LPS+ETE (200mg/kg)	236.44 ±6.21 b****
4.	Group- IV	LPS+ETE (400mg/kg)	260.03 ±7.14 b****
5.	Group- V	LPS+Levodopa (25mg/kg)	274.3 ±6.85 b****

Values are expressed as mean ± SEM (n = 6). Statistical significance determined by one-way ANOVA followed by Tukey's post hoc test.

- a = comparison between Control and LPS
- b = comparison between LPS and treatment groups

- **** = $p < 0.0001$

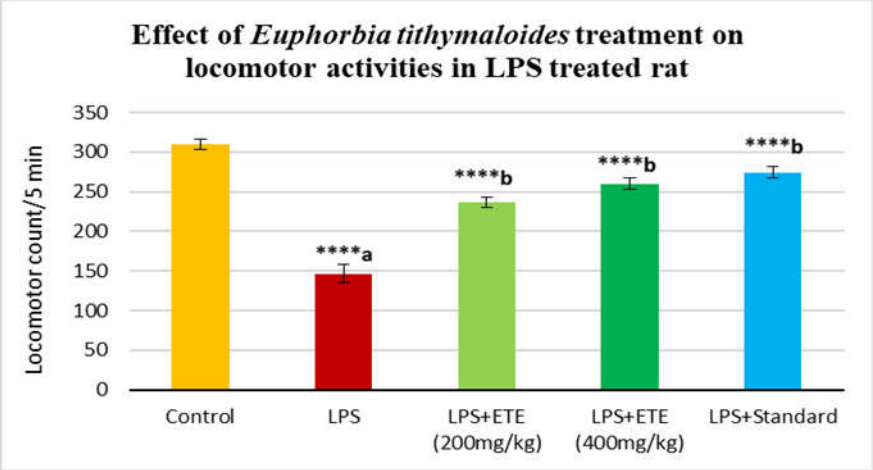


Fig. no. 3.6.1.2 (a) Effect of LPS and Treatments on locomotor activity test of Rat

Fig. no. 3.6.1.2 (b) Actophotometer Test (Locomotor Activity)

3.6.2 Biochemical Analysis

LPS significantly elevated MDA (15.75 ± 0.179) and nitrite (33.75 ± 0.35) levels compared to control (4.093 ± 0.075 and 16.5 ± 0.121; $p < 0.0001$). ETE reduced these oxidative stress markers in a dose-dependent manner: 200 mg/kg (MDA 11.01 ± 0.339; nitrite 23.44 ± 0.241) and 400 mg/kg (MDA 7.7 ± 0.371; nitrite 20.73 ± 0.23). Levodopa showed the greatest reduction (MDA 5.87 ± 0.211; nitrite 18.56 ± 0.195). All treatments significantly reversed LPS-induced increases ($p < 0.0001$).

Table no. 3.6.2 Biochemical Analysis of *Euphorbia tithymaloides* extract

S. No.	Animal Group (n=6)	Group	MDA (nmol/mg/ protein)	Nitrite (µg/mg protein)
1.	Group- I	Control	4.093±0.075	16.5±0.121
2.	Group- II	LPS	15.75±0.179 a****	33.75±0.35 a****
3.	Group- III	LPS+ETE (200mg/kg)	11.01±0.339 b****	23.44±0.241 b****
4.	Group- IV	LPS+ETE (400mg/kg)	7.7±0.371 b****	20.73±0.23 b****
5.	Group- V	LPS+Levodopa (25mg/kg)	5.87±0.211 b****	18.56±0.195 b****

Values are expressed as Mean ± SEM (n = 6). Statistical comparison was done using one-way ANOVA followed by Tukey-Kramer's post hoc test.

- a = Control vs. LPS group
- b = LPS vs. treatment groups (III, IV, V)

- p values: * = $p < 0.05$, ** = $p < 0.01$, **** = $p < 0.0001$

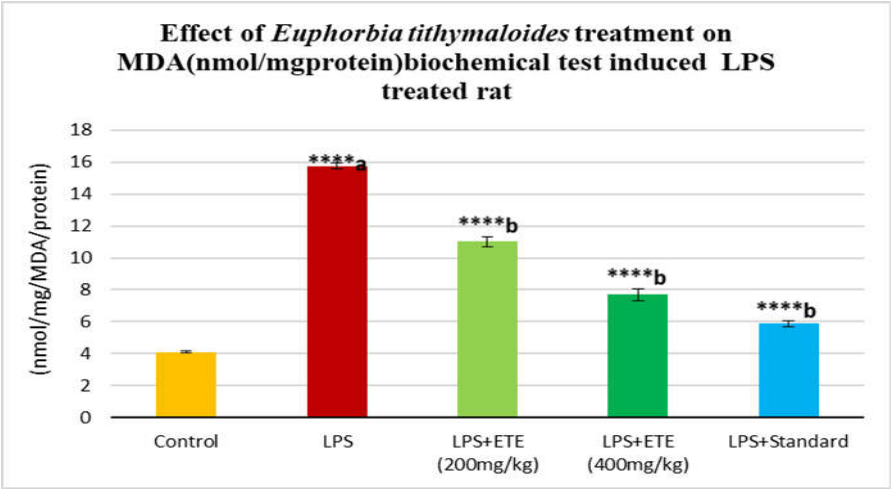


Fig. no. 3.6.2 (a) Malondialdehyde (MDA) Assay – Lipid Peroxidation Estimation

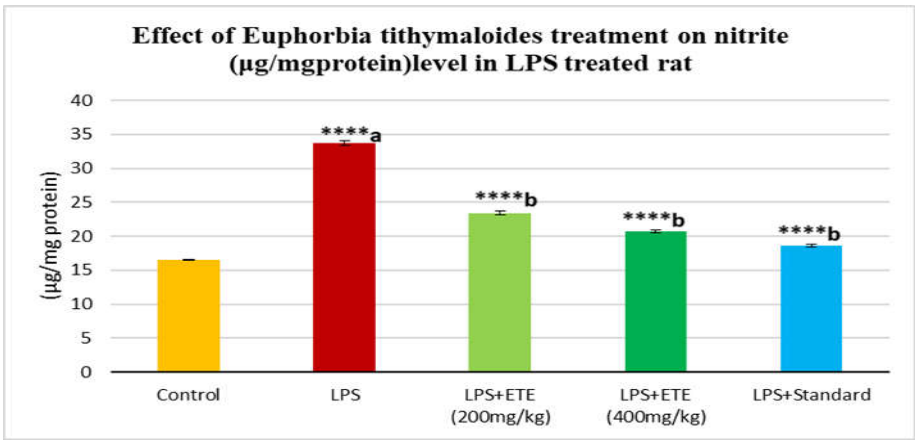
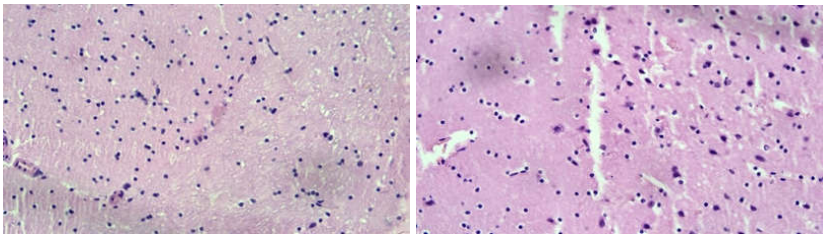


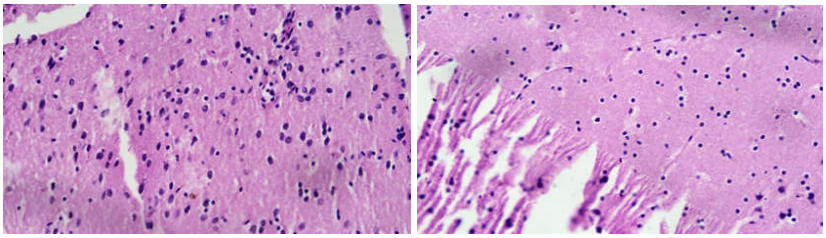
Fig. no. 3.6.2 (b) Nitrite Estimation – Indirect Measure of NO Production

3.6.3 Histopathological Analysis

Histopathological analysis showed that control brains had normal neuronal architecture, while LPS treatment caused severe degeneration with neuronal shrinkage, vacuolization, and pyknotic nuclei. ETE at 200 mg/kg provided moderate protection, reducing inflammation and preserving neuronal structure. At 400 mg/kg, ETE almost fully restored normal architecture, with minimal damage and well-preserved neurons. These results confirm a dose-dependent neuroprotective effect of ETE, with the higher dose showing stronger protection against LPS-induced neuroinflammation.



(i) (ii)



(iii) (iv)

Fig. no. 3.6.3 Microscopic sections illustrating the effects of LPS and ETE on neuronal degeneration in the rat brain, as observed through hematoxylin-eosin staining: (i) Control, (ii) LPS-treated, (iii) LPS + ETE (200 mg/kg), and (iv) LPS + ETE (400 mg/kg)

4. Conclusion

The present study demonstrates that the ethanolic extract of *Euphorbia tithymaloides* (ETE) possesses significant neuroprotective activity against LPS-induced neuroinflammation in rats. Morphological evaluation confirmed proper identification and authentication of plant material, while phytochemical analysis revealed the presence of bioactive constituents such as alkaloids, flavonoids, tannins, triterpenes, and phenols—compounds known for their antioxidant and anti-inflammatory properties. Acute toxicity studies confirmed the safety of ETE up to 2000 mg/kg, indicating a wide safety margin.

Behavioral assessments showed that ETE improved motor coordination and locomotor activity in a dose-dependent manner. Biochemical analysis indicated a marked reduction in oxidative stress markers, including malondialdehyde and nitrite, with the higher dose (400 mg/kg) approaching the efficacy of the standard drug levodopa. Histopathological evaluation supported these findings, revealing substantial preservation of neuronal architecture in ETE-treated groups.

These results suggest that *E. tithymaloides* exerts neuroprotective effects primarily through antioxidant and anti-inflammatory mechanisms. Its safety profile and efficacy support its potential as a therapeutic candidate for neurodegenerative conditions associated with oxidative stress and inflammation. Further studies focusing on molecular mechanisms, active compound isolation, and clinical translation are warranted.

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