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# Ameliorative Potential of *Euphorbia hirta* (Linn.) Extract against Lipopolysaccharide Induced-neuroinflammation and Oxidative Damage in Rats

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## Abstract

**Objective:** To find out the antioxidant and anti-neuroinflammatory capabilities of *Euphorbia hirta* Linn. methanol extract in LPS-induced to oxidative stress animal model.

**Methods:** Lipopolysaccharide (LPS) was used to induce oxidative stress in rats. Oxidative stress bio-markers (TBARS, GSH, CAT and SOD) were evaluated. Enzyme-linked immunosorbent assay (ELISA) and Griess assay were used analysis of cytokines (TNF-α, IL-6 and IL-1β) and nitric oxide (NO) level in brain tissues respectively. An anxiety was calculated by elevated plus maze test.

**Result:** LPS treated rats showed markedly depleted brain reduced glutathione (GSH) level (56.25%), superoxide dismutase (SOD) level (43.93%) and catalase (CAT) level (58.55%) in brain tissue, whereas a significant ($p<0.01$) raise in malondialdehyde (MDA) level (106.67%), nitric oxide (NO) level (149.0%) and also depicts a significant increase in levels of cytokines mainly TNF-α (60 %), IL-6 (50.28%) and IL-1β (106.77%) in the brain tissue. After 21 days, rats were supplementated orally with methanol extract of *Euphorbia hirta* (MEEH) in two different doses 100 mg/kg and 200 mg/kg, resulted in normalization of GSH, CAT and SOD levels in dose dependent manner, while a significant ($p<0.01$) decreased in the MDA, cytokines and nitric oxide levels in the brain tissue, compared with LPS group. The behavioral study of time spent in open arms using maze model showed a significant results.

**Conclusion:** The pro-inflammatory mediators TNF-α, IL-6, IL-1β and oxidative stress bio-markers (TBARS, GSH, CAT and SOD) are inhibited by MEEH. Present study showed that the MEEH exhibited strong anti-oxidant properties.

**Keywords:** Cytokine; *Euphorbia hirta;* Lipopolysaccharide; Oxidative stress

## Introduction

Microglial activation play a critical role in neuro-inflammation and also involved in various neuropathological conditions which are seen in several neurodegenrative diseases such as Spinocerebellar ataxia, Multiple sclerosis, Parkinson's disease and Alzheimer's disease [1,2]. Stimulation of microglia marks in release of different pro-inflammatory intermediaries and free radicals [3]. It is acclaimed that decrease of pro-inflammatory mediators such as nitric oxide (NO), tumor necrosis factor-alpha (TNF-α) and other inflammatory cytokines in activated microglia could reduce the acerbity of these disorders [4].

*Euphorbia hirta* (family: euphorbiaceae), is a perennial herb widely found in India and other countries. It has enormous medicinal importance in the treatment of verities of gastrointestinal disorders), bronchial, respiratory diseases, antidiabetic, immunomodulatory and nephroprotective activity [5-7].

In this study, we undertake to determine whether *Euphorbia hirta* Linn. extract possesses anti-neuroinflammatory activity in LPS-stimulated oxidative stress. In addition, its anti-oxidant potential was assessed to confirm its anti-neuroinflammatory effects and compare with quercetin a known antioxidant.

## Materials and Methods

### Chemicals and drugs

Thiobarbituric acid (TBA); 2,6,-di-tert-butyl-4-hydroxy-toluene (BHT); trichloroacetic acid (TCA); Hydrogen peroxide ($H_2O_2$); EDTA; Tris buffer; Potassium dihydrogenortho phosphate; Disodium hydrogen ortho phosphate were obtained from CDH, Mumbai, NADPH; DTNB were obtained from Hi Media, Mumbai, cytokine ELISA kits obtain from reputed international company Cayman Chemical USA, Lipopolysaccharide Serotype *E. coli* 0111:B4 were obtained from Sigma Chemicals, USA.

### Animals

Rats (8 weeks), Sprague Dawley weighing 150-160 g were solicited from Laboratory Animal Division, Central Drug Research Institute (CDRI), Lucknow, India. In group wise seven rats were kept in cage and maintained standard housing condition (room temperature 25 ± 1°C and humidity 60–65%) with 12 h light and dark cycle. The food and water were available adlibitum. Ethical approval was obtained from the Ethical Committee, Integral University, Lucknow, India (Approval No-IU/Pharm/Ph.D/CPCSEA/12/03).

### Preparation of the *Euphorbia hirta* Linn. extract and authentication

Whole plant of *Euphorbia hirta* was obtained in month of October and November 2011 from National Botanical Research Institute (NBRI)

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Herbal Garden at NBRI Campus, Lucknow and was authenticated by Dr. D.V. Amla, voucher of specimen (no. NBRI/CIF/255/2011).

The plant *Euphorbia hirta* was air-dried and pulverized. The powdered material (500 g) were packed in muslin cloth and kept in soxhlet extractor for extraction (50°C) with methanol for 72 h. The methanolic extract was filtered through Whatman paper no. 42 and filtrates were concentrated under reduced pressure and vacuum dried (yield: 12.2%).

### Phytochemical screening test

Analysis of MEEH for its active natural principles was conducted using the methods, chemicals and reagents earlier described [8]. Killer-Killani, borntrager's and legal test for glycosides; ferric chloride, lead acetate and gelatin for phenolic compounds; ammonia and shinoda. The flavonoids and steroids test were performed as well.

### Acute toxicity study

The acute toxicity of the extract of Root of *Euphorbia hirta* Linn. was evaluated in mice using the up and down procedure (OECD, 2001). Mice received methanolic extract at various doses (500-2000 mg/kg) orally by gavage. The animals were observed for toxic symptoms continuously for the first 4 h after dosing and survivors was noted after 24 h. Remaining rats were treated daily for 14 days [9].

### Lipopolysaccharide-induced oxidative stress in rats

Animals were divided into seven groups randomly: Group I (Control group) 1% Carboxy methyl cellulose (CMC) 5 ml/kg p.o. once a day, and single dose of normal saline at day 21. Group II (LPS group) 1% CMC 5 ml/kg p.o. once a day, and single dose of LPS Serotype E. coli 0111:B4 at day 21. Groups III (standard groups) Quercetin100 mg/kg p.o. daily, and one dose of LPS at day 21 Groups IV and V (Drugs treated groups) 100 mg/kg and 200 mg/kg p.o. daily, and one dose of LPS at day 21 Groups VI 200 mg/kg of MEEH and 100 mg/kg standard drug p.o., and one dose of LPS at day 21.VII (perse group) 200 mg/kg p.o. daily for 21 days. Anxiety was assessed in the elevated plus mazes 4 h after the LPS or saline administration on 21 day. Rats were transcardially perfused with cold saline followed by 4% formalin in phosphate buffer-saline (o.1 M; PH 7.4). The brains were removed and store -80°C and later use for biochemical estimation.

### Elevated plus maze test

The elevated plus maze consisted of two opposite open arms (50 cm long, 10 cm wide) and two opposite closed arms of the same size, enclosed by 40 cm high walls. The apparatus was elevated 50 cm above the floor and illuminated from the top. The time spent in the open and closed arms of individual animals were observed for 5 min. The number of entries and the time spent in the open and closed arms were reported. Rats enter more frequently in the closed arms. The maze was cleaned after each trial to remove any residue or odour of the animals [10].

### Determination of antioxidant status of the extract

**Assay of brain MDA content:** 1 ml of 10% tissue homogenate, 0.5 ml of 30% TCA and 0.5 ml of 0.8% TBA reagent were taken in test tube kept in shaking water bath for 30 minutes at 80°C and then kept in ice-cold water for 30 minutes. These were centrifuged at 3000 rpm for 15 minutes and the absorbance of the supernatant was read at 540 nm against appropriate blank. The specific activity of MDA was expressed in nmoles MDA/mg protein [11].

**Assay of brain glutathione:** Brain tissue homogenate (5-8 ml of 0.02 M EDTA), 4.0 ml of cold distilled water and 1 ml of 50% trichloroacetic acid (TCA) was mixed thoroughly by vortex mixer for 10 minutes and centrifuged at 6000 rpm for 15 minutes. 2 ml of the supernatant was mixed with 4.0 ml of 0.4 M Tris buffer (pH 8.9). The whole solution was mixed well and 0.1 ml of 0.01M DTNB was added and absorbance was read at 412 nm against a reagent within 5 minutes. The specific activity of GSH was expressed in µg/mg protein [12].

**Assay of brain catalase:** Brain tissue homogenate (50 mM/L potassium phosphate buffer) was centrifuged at 10,000 rpm at 4°C in a cooling centrifuge for 20 minutes. Supernatant (50 µl) was added to cuvette containing 2.95 ml of 19 mM/L solution of $H_2O_2$ prepared in potassium phosphate buffer. The absorbance was taken at 240 nm for 3 times in 1 minute interval. The specific activity of CAT was expressed in nmol $H_2O_2$/mg protein [13].

**Assay of Superoxide dismutase:** 100 µl of cytosolic supernatant (Tris HCl buffer - pH 8.5, 25 µl of pyrogallol was kept in test tube and absorbance at 420 nm are recorded at 1 minute interval for 3 minutes.. The specific activity of SOD was expressed in Units/mg protein [14].

### Measurement of cytokines level

TNF-α, IL-1β and IL-6, in brain were determined by using cytokine ELISA kits (eBioscience and Cayman Chemical USA). Tissue cytokine concentrations were expressed as picograms of antigen per milligram of protein.

### Measurement of nitric oxide (NO): Griess reaction

Animals were sacrificed and tissues were extracted with PBS (pH 7.4) and placed on ice cold water. 50 µl sample was added with 100 µl of Griess reagent and was incubate for about 10 minutes at room temperature and absorbance was measured at 540. Calculations were done after generating a standard curve from sodium nitrite in the same buffer as used for preparation of homogenate [15].

### Statistical analysis

Statistical analysis was executed by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. P values < 0.05 were considered as significant.

## Results

MEEH has shown the presence of alkaloids, triterpenes, tannins, phenol, flavonoids and sterols.

### Acute toxicity of extract

MEEH up to a dose of 2000.0 mg/kg was administered orally to mice and no toxicity results found during the evaluated period. One-tenth and one-twenty of the maximum tolerated dose of the extract tested (2000 mg/kg) for acute toxicity did not indicate mortality and were selected for evaluation of the effect of *Euphorbia hirta* Linn i.e. 100 and 200 mg/kg.

### Assessment of elevated Plus maze test

LPS induced an anxiogenic effect was reduced by *Euphorbia hirta* Linn. extract and significantly ($P<0.001$) increased the time spent in open arm in plus maze (Table 1).

### Malondialdehyde (MDA) content in brain

MDA in rat brain, the obtained result (Table 2) explain that LPS administration into rats markedly increased the level of brain MDA compared to the control animals. However, animals administered

both MEEH (100 mg/kg and 200 mg/kg p.o daily) in 21 consecutive days and a single dose of LPS at the 21 day showed marked reduction (p<.001) in brain MDA .

### Reduced glutathione (GSH) content in brain

A significant depletion of brain GSH was noticed in LPS treated rats. Administration of 100 mg/ kg and 200 mg/kg MEEH as an oral daily for 21 days as well as LPS significantly increased (p<.001) brain GSH content as it reached normal levels (Table 2).

### Catalase (CAT) content in brain

LPS administration decreases the level of SOD in to rats. Animal administered MEEH 100 mg/kg and 200 mg/kg p.o. daily for 21 days marked increase(p<.001) in SOD (Table 2).

### Superoxide dismutase (SOD) content in brain

Animal administered MEEH 100 mg/kg and 200 mg/kg p.o. daily for 21 days and single dose of LPS marked increase(p<.001) in SOD in comparison with only LPS-administered animals (Table 2), whereas LPS administration decreases the level of SOD.

### Cytokines content in brain

Cytokines level were significantly reduced (p<.001) as compared to control (Figure 1). Result shows the effect of the significant and dose dependent recovery on the LPS induced elevation of the cytokines levels in animals (Figure 1).

### Nitric oxide content in brain

Levels of NO were significantly reduced (p<.001) in MEEH (100 mg/kg and 200 mg/kg) treated rats as compared to control (Figure 2).

| Groups | Drug treatments | Time duration in open arm in sec | No of entries in open arm |
|---|---|---|---|
| I | Control | 204.6 ± 5.115 | 6.6 ± 0.509 |
| II | LPS | 99.6 ± 3.558$^{***}$ | 2.00 ± 0.136$^{***}$ |
| III | QT | 180.8 ± 3.338$^{###}$ | 5.2 ± 0.374$^{###}$ |
| IV | $EH_1$ | 143.4 ± 3.187$^{###}$ | 3.2 ± 0.372$^{ns}$ |
| V | $EH_2$ | 181.4 ± 2.943$^{###}$ | 4.2 ± 0.374$^{##}$ |
| VI | $EH_2$+QT | 198.8 ± 3.513$^{###}$ | 6 ± 0.447$^{###}$ |
| VII | $EH_2$ perse | 200.44 ± 3.172 | 6.2 ± 0.200 |

**Table 1:** Assessment of Elevated Plus maze test. (LPS-Lipopolysaccharide, $EH_1$-Lower dose of *Euphorbia hirta* extract, $EH_2$- Higher dose of *Euphorbia hirta* extract extract, QT-Quercetin. Results are expressed as mean ± SEM. The results were analyzed by Analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. n=6 (Number of animal per group) $^{###}$= p<0.001, $^{##}$= p<.01, $^{#}$ = p<0.05, $^{***}$ = p<0.001, $^{#}$ *Vs.* Group II, $^{*}$ *Vs.* Group I).

| Drug treatments | TBARS(nmole MDA/mg protein) | GSH(µg/mg protein) | CAT(nmol$H_2O_2$/ mg protein) | SOD(Units/mg protein) |
|---|---|---|---|---|
| Control | 3.658 ± 0.076 | 3.452 ± 0.080 | 22.2 ± 0.860 | 2.604 ± 0.106 |
| LPS | 7.56 ± 0.096$^{***}$ | 1.51 ± 0.050$^{***}$ | 9.0 ± 0.707$^{***}$ | 1.46 ± 0.1281$^{***}$ |
| QT | 3.79 ± 0.051$^{###}$ | 3.13 ± 0.070$^{###}$ | 19.4 ± 0.500$^{###}$ | 2.42 ± 0.128$^{#}$ |
| $EH_1$ | 5.48 ± 0.066$^{###}$ | 1.99 ± 0.101$^{##}$ | 13.4 ± 0.927$^{#}$ | 1.92 ± 0.106$^{#}$ |
| $EH_2$ | 4.744 ± 0.068$^{###}$ | 2.20 ± 0.034$^{##}$ | 14.4 ± 0.812$^{##}$ | 2.16 ± 0.0400$^{###}$ |
| $EH_2$+QT | 3.646 ± 0.033$^{###}$ | 3.45 ± 0.127$^{###}$ | 21.2 ± 0.860$^{###}$ | 2.54 ± 0.107$^{###}$ |
| $EH_2$ *perse* | 3.562 ± 0.082 | 3.578 ± 0.0754 | 22.4 ± 1.07 | 2.56 ± 0.103 |

**Table 2:** Assessment of oxidative stress markers. [LPS-Lipopolysaccharide, $EH_1$-Lower dose of *Euphorbia hirta* extract, $EH_2$- Higher dose of *Euphorbia hirta* extract extract, QT-Quercetin. Results are expressed as mean ± SEM. The results were analyzed by Analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. n=6 (Number of animal per group) $^{###}$= p<0.001, $^{##}$= p<.01, $^{#}$ = p<0.05, $^{***}$ = p<0.001, $^{#}$ *Vs.* Group II (LPS), $^{*}$ *Vs.* Group I (Control)].

## Discussion

Bioactive components of medicinal plant are recognized for their powerful biological activities for good health and remedies from disease [16]. Stimulation of microglia and the successive release of inflammatory cytokines and noxious free radicals are the symbols of neuro inflammation detected in numerous neurodegenerative diseases. Thus, inhibition of cytokine production or function serves as a key mechanism in the control of inflammatory responses in neuro-degeneration [17].

In the present study, administration of LPS resulted oxidative stress due to anxiogenic response and damage in brain tissue in rats. This effect was corroborated with an increase in the concentration of lipid peroxidation, cytokines, nitric oxide and decrease GSH, SOD and Catalase.

LPS is well known fact that oxidative stress agents are generated by ROS and production of proinflammatory cytokines [18]. Tissues injury is one of the most common disorders due to peroxidation effects of lipids [19]. Brain tissues are abundant in polyunsaturated fatty acids and are known for its high oxygen uptake. Therefore, it is more susceptible to oxidative stress than other tissues [20].

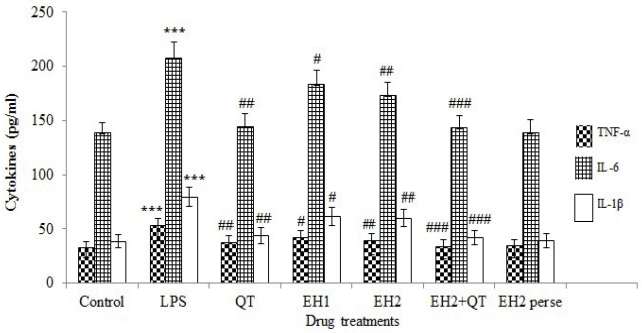


**Figure 1:** Effect of methanolic extract of *Euphorbia hirta* Linn. on LPS- activated pro-inflammatory cytokines production. [LPS-Lipopolysaccharide, $EH_1$- Lower dose of *Euphorbia hirta* extract, $EH_2$- Higher dose of *Euphorbia hirta* extract extract, QT-Quercetin. Results are expressed as mean ± SEM. The results were analyzed by Analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. n=6 (Number of animal per group) $^{###}$= p<0.001, $^{##}$= p<.01, $^{#}$ = p<0.05, $^{***}$ = p<0.001, $^{#}$ *Vs.* Group II (LPS), $^{*}$ *Vs.* Group I (Control)].

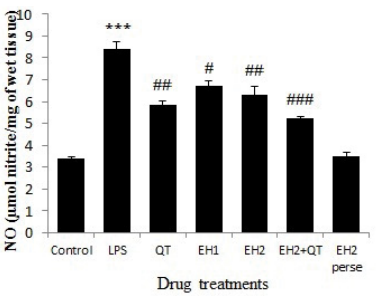


**Figure 2:** Effect of MEEH extract on NO Production in LPS-stimulated rats brain. [LPS-Lipopolysaccharide, $EH_1$- Lower dose of *Euphorbia hirta* extract, $EH_2$- Higher dose of *Euphorbia hirta* extract extract, QT-Quercetin. Results are expressed as mean ± SEM. The results were analyzed by Analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. n=6 (Number of animal per group) $^{###}$= p<0.001, $^{##}$= p<.01, $^{#}$ = p<0.05, $^{***}$ = p<0.001, $^{#}$ *Vs.* Group II (LPS), $^{*}$*Vs.* Group I (Control)].

Methanolic extract of *Euphorbia hirta* Linn. and quercetin exhibited antioxidant effect by increasing anti stress enzymes level(GSH, CAT and SOD), and decreasing MDA levels. Normal level of CAT, SOD, cytokines and NO in brain tissues, may be the presence of polyphenols/ alkaloids, in the extract.

## Conclusion

In conclusion, Oral administration of methanolic extract of *Euphorbia hirta* Linn. protected rats from LPS induced neuroinflammation. The MEEH also exhibited powerful antioxidant effects. These observations suggest that MEEH may be clinically viable protection against variety of conditions where cellular damage is a consequence of oxidative stress. The present study gives a special note that methanolic extract of *Euphorbia hirta* (MEEH) as a neuroprotective agent.

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