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ORIGINAL ARTICLE

Erigeron linifolius Attenuates Lipopolysachharide-Induced Depressive-Like Behavior in Mice by Impeding Neuroinflammation, Oxido-Nitrosative Stress, and Upregulation of Tropomyosin Receptor Kinase B-Derived Neurotrophic Factor and Monoaminergic Pathway in the Hippocampus

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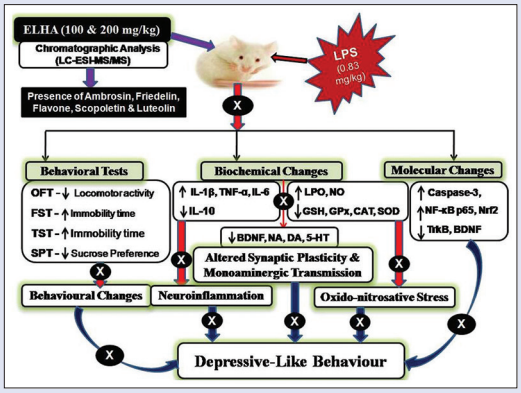
ABSTRACT

Background: Immuno-inflammatory, oxido-nitrosative stress, brain-derived neurotrophic factor-tropomyosin receptor kinase (BDNF-TrkB), and monoaminergic pathways involve in the pathophysiology of depression. *Erigeron linifolius* (*EL*) possesses antioxidant, anticonvulsant, antitumor, and hypoglycemic activities. **Objective:** To investigate the effect of *EL* hydroalcoholic extract (ELHA) against lipopolysachharide (LPS)-induced depressive-like behavior and neurochemical alterations in mice. **Materials and Methods:** Mice were pretreated with vehicle, imipramine (10 mg/kg, intraperitoneally [i. p.]), and ELHA (100 and 200 mg/kg, per oral) for 14 days, and depressive-like behavior was induced by LPS (0.83 mg/kg, i. p.). Depressive-like behavior in mice was evaluated by open-field test, forced swimming test (FST), and tail suspension test (TST). Cytokines (tumor necrosis factor-alpha [TNF-α], interleukin-1β [IL-1β], IL-6, and IL-10), BDNF, monoamines (noradrenaline [NA], dopamine, 5-hydroxy), and oxido-nitrosative stress parameters (lipid peroxidation [LPO], glutathione [GSH], GSH peroxidase [GPx], catalase [CAT], superoxide dismutase [SOD], nitric oxide) were measured in the hippocampus (HC). Hippocampal caspase-3, nuclear factor-kappa B phosphor 65 (NF-κB p65), nuclear-related factor 2 (Nrf2), and TrkB and BDNF levels were measured by reverse transcriptase-polymerase chain reaction (RT-PCR) and immunoblotting. **Results:** Liquid chromatography-electrospray ionization-mass spectroscopy (MS)/MS analysis revealed the presence of ambrosin, friedelin, flavone, scopoletin, and luteolin in ELHA. ELHA showed significant antidepressant-like effect by decreasing the immobility time in FST and TST in LPS-challenged mice. Moreover, ELHA significantly attenuated TNF-α, IL-1β, IL-6, and LPO levels in LPS-challenged mice, whereas LPS-induced decrease in hippocampal IL-10, BDNF, GSH, GPx, CAT, SOD, and monoamine levels was also restored significantly by ELHA. RT-PCR and immunobloting results showed that ELHA significantly attenuated the upregulation of Caspase-3, NF-κB, p65, and Nrf2 and downregulation of TrkB and BDNF levels in the HC of LPS-challenged mice. **Conclusion:** The findings demonstrated antidepressant-like action of ELHA which could be due to the inhibition of neuroinflammation, oxido-nitrosative stress, and upregulation of TrkB-BDNF and monoaminergic pathways in the HC.

Key words: Brain-derived neurotrophic factor, depression, *Erigeron linifolius*, lipopolysachharide, monoamines, neuroinflammation

SUMMARY

- Lipopolysachharide (LPS) induced depressive-like behavior in mice by increasing immobility time in forced swimming test (FST) and tail suspension test (TST)
- Chronic pretreatment of *Erigeron linifolius* hydroalcoholic extract (ELHA) abrogated LPS-induced increase in immobility time in FST and TST
- LPS-induced oxidative stress was also prevented by ELHA pretreatment
- ELHA pretreatment attenuated neuroinflammation by decreasing hippocampal interleukin-1β and tumor necrosis factor-alpha and increasing interleukin-10 level in LPS-challenged mice
- ELHA also increased the BDNF and tropomyosin receptor kinase B level in the hippocampus of LPS-subjected mice
- Reduced level of monoamines such as dopamine, norepinephrine, and 5-hydroxy was also restored in LPS-challenged mice after ELHA pretreatment
- ELHA might be useful in the treatment of psychiatric disorders such as depression associated with neuroinflammation and oxido-nitrosative stress and monoamine imbalance.



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Abbreviations used: LPS: Lipopolysaccharide; TrkB: Tropomyosin receptor kinase B; BDNF: Brain-derived neurotrophic factor; ELHA: *Erigeron linifolius* hydroalcoholic extract; BW: Body weight; I. P.: Intraperitoneally; OFT: Open-field test; FST: Forced swimming test; TST: Tail suspension test; TNF-α: Tumor necrosis factor-alpha; IL-1β: Interleukin-1β; IL-6: Interleukin-6; NA; Noradrenaline; DA: Dopamine; 5-HT: 5-Hydroxy tryptamine; LPO: Lipid peroxidation; GSH: Glutathione; CAT: Catalase; SOD: Superoxide dismutase; NO: Nitric oxide; NF-κB p65: Nuclear factor-kappa B phosphor 65; Nrf2: Nuclear-related factor 2; WHO: World Health Organization.

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INTRODUCTION

Depression is a commonly occurring and debilitating affective disorder characterized by persistent sadness and loss of interest in activities affecting daily routine of life.[1] A WHO report revealed that 322 million people accounting 4.4% of the world's populations are living with depression, and now it has become the leading cause of illness and disability across the globe.[2] It accounts for loss of about 800,000 lives every year, and is a major contributor for the burden of suicide and ischemic heart disease.[2,3] Nearly half of the people with depression live in South Asian countries including India, where the lifetime prevalence is 24.4%.[4] Nearly 50% of depressed patients are unaware, misdiagnosed, and undertreated. Currently available drugs for the treatment of depression are derived synthetically and associated with several adverse effects and poor patient compliance.[1] Medicinal plants are used since antiquity for the prevention and management of mental disorders. Due to reversal of interest in traditional medicine, people's faith is soaring high for the use of herbal medicines, as they are efficacious and have lesser side effects than that of the prescribed antidepressants. This necessitates the search for novel herbal therapeutic agents as alternative therapy for depression.

Numerous studies have demonstrated that immuno-inflammatory, oxido-nitrosative stress, neurotrophic, and monoaminergic pathways play a significant role in the pathogenesis of depression. Peripheral administration of lipopolysaccharide (LPS) in animals exerts sickness behavior such as loss of appetite, body weight (BW), reduced locomotion, reduced exploratory activity, anhedonia, and depressive-like behavior through a cascade of pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), IL-2, and IL-1β (IL-1β) released in the brain.[1-7] These cytokines induce oxidative as well as nitrosative damage by causing imbalance between oxidant and antioxidant factors in the brain.[7,8] Neuroinflammation increases lipid peroxidation (LPO), DNA damage, and nitrite level, but decreases reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) levels.[8,9] Further, inflammatory cascade reduces neurotrophins such as brain-derived neurotrophic factor (BDNF) in the hippocampus (HC) of animals. BDNF is responsible for the growth, survival, and maintenance of neuron.[9-11] Monoaminergic systems play an important role in the pathophysiology of depression, especially monoaminergic neurotransmitters such as dopamine (DA), serotonin, and norepinephrine (NE).[12-16] Moreover, pro-inflammatory cytokines activate hypothalamic–pituitary–adrenal axis and also alter monoaminergic neurotransmitters such as DA, serotonin (5-hydroxy [5-HT]), and NE.[17] Thus, activation of inflammatory pathway leads to oxido-nitrosative damage, altering synaptic plasticity and catecholamine levels in different brain regions.

Erigeron linifolius (EL) Wild, syn. *Conyza bonariensis,* is a perennial plant belonging to Asteraceae family. It is an erect annual herb of unknown origin but with cosmopolitan distribution. The plant appears in Northeast India as a weed of upland situations in late winter and summer seasons. EL is a synonym of *C. bonariensis* (L.) Cronquist, the nomenclature of which has recently been changed to *E. bonariensis* L. as per the Plant List (www.theplantlist.org) record (gcc-32032) prepared by the International Collaboration of Taxonomic Institutes. The plant is commonly cultivated as an ornamental plant in gardens. Leaves are used as laxative and roots are used in treating diarrhea,[18] while flowers are considered as aphrodisiac, emollient.[19] Phytochemical studies of EL revealed the presence of bioactive constituents such as flavonoids, flavonoid glycosides, cyanin glycoside, taraxeryl acetate, sitosterol, campesterol, stigmasterol, ergosterol,[20] cyclopropenoids,[21] and anthocyanin pigments.[20] It has also been evaluated pharmacologically for anticonvulsant,[18] antitumor,[22] hypoglycemic,[23] and estrogenic[24] activities. Antidiarrheal, antioxidant, anti-inflammatory, antibacterial, antifungal, and cytotoxic activities of EL have also been reported.[25-29] EL showed potent anti-inflammatory activity against carrageenan-induced paw edema model of inflammation in rats, and it is due to the presence of phytochemicals mostly concentrated in the hexane fraction of the extract.[30] Considering the antioxidant and anti-inflammatory effects as well as the presence of flavonoids, the current study was undertaken to evaluate the effect of EL against LPS-induced depressive-like behavior, neuroinflammation, oxido-nitrosative stress, and alteration in tropomyosin receptor kinase (TrkB)-BDNF and monoaminergic pathways in the mouse HC.

MATERIALS AND METHODS

Drugs

LPS from *Escherichia coli,* strain 055:B5, serotonin hydrochloride, DA hydrochloride, (±)-NE (+)-bitartrate salt, and imipramine (IMP) hydrochloride were procured from Sigma-Aldrich Corp., St. Louis, USA. The drugs were prepared fresh on the day of the experiment. All the other chemicals used were of analytical grade.

Animals

Male Swiss Albino mice (weighing 22–27 g) were obtained from the animal facility of the Department of Pharmacology and Toxicology, College of Veterinary Science, Khanapara, Assam, India. They were housed in polypropylene cages and acclimatized for a week under standard conditions of temperature (22°C ± 3°C) and humidity (50% ± 10%) with a 12 h light–dark cycle. The experimental mice had free access to standard pellet diet and water *ad libitum*. All the experimental protocols and methods were approved by the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary Sciences, Assam Agricultural University (770/ac/CPCSEA/FVSc, AAU/IAEC/15-16/367).

Laboratory animal handling and experimental procedures were performed in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India.

Plant material and preparation of extract

Leaves of EL were collected in the month of July 2015 from Jorhat, Assam, India, from the area having a latitude of 26°44'47.47"N and a longitude of 94°12'9.31"E. Plant authentication was done by Dr. I. C. Barua, Principal Scientist, Department of Agronomy, Assam Agricultural University, and a voucher specimen was kept in the herbaria (Acc No: 5189 dated 20.05.2016) of our laboratory for future reference. The leaves were cleaned and air dried for a week at 35°C–40°C and pulverized in an electric grinder. Preparation of EL hydroalcoholic extract (ELHA) was done as per standard methods in Soxhlet extractor and rotary evaporator (BUCHI, ROTAVAPOR, R-210; Switzerland). Percentage yield of powder with respect to dry powder was 17.00% w/v.

Phytochemical analysis: Ultra-high performance liquid chromatography -electrospray ionization orbitrap mass spectroscopy/mass spectroscopy analysis

The ultra-high performance liquid chromatography (UHPLC) system coupled with an electrospray ionization (ESI) orbitrap mass spectroscopy (MS)/MS (UHPLC-DIONEX 3100, Thermo scientific, USA) was utilized for the screening of the phytoconstituents present in the EL extract.[31] The mobile phase of solvent A: water with formic acid (0.01%) and solvent B: 100% acetonitrile was used with a constant flow rate of 0.3 ml/min by following the gradient method. The gradient program began with 95% A for 2 min, and then slowly decreased to 5% A within 6 min and hold at 5% A for 1 min, again to the starting conditions, 95% A for 1 min. Samples (5 µl) were injected onto a Hypersil Gold C_{18} column (150 mm × 3.00 mm, Thermo, USA). Photodiode array detector was used for the identification by simultaneous screening at 275 nm, 366 nm, and 200–400 nm, and mass spectrometer was used for the analysis of the full mass peak and fragmentation pattern of the phytoconstituents in EL extract. The observed mass-to-charge ratio of the sample was compared with the literature and mass databases, the primary tool for characterization of the phytoconstituents.

Acute toxicity study

ELHA (2 g/kg) was administered orally to male Swiss albino mice to evaluate the acute toxicity as per the protocol of Organization for Economic Cooperation and Development guidelines for testing of chemicals 423. They were observed for 24 h for any sign of gross abnormality or mortality and then till 14 days. There was no death at 2 g/kg dose in oral acute toxicity study; therefore, we have selected 1/20th and 1/10th doses of 2 g/kg, i.e., 100 and 200 mg/kg per oral, respectively, for the current study.

Experimental design

The mice were randomly divided into five groups (n = 6 mice/group) as depicted in [Figure 1]: Control (received vehicle, Tween 80, and saline), LPS (negative control – received saline and LPS), and IMP (standard or positive control – received IMP 10 mg/kg BW and LPS), and ELHA (received ELHA 100 and 200 mg/kg BW and LPS). The vehicle control and LPS- and ELHA-treated groups were given respective treatment orally once daily for 14 consecutive days. IMP (10 mg/kg)[9] was injected intraperitoneally (I. P.) for consecutive 14 days. On the last day of treatment, i.e., 14th day, LPS (0.83 mg/kg) was injected intraperitoneally to all groups except the control group 30 min after treatment. After 24 h of LPS challenge, changes in BW, food intake, open-field test (OFT), forced swimming test (FST), tail suspension test (TST), and sucrose preference test were performed to evaluate the depression-like behaviors in mice.[1] Following behavioral studies, mice were sacrificed, and brains were quickly dissected out to isolate HC. All the tissues were stored at −80°C in deep freeze (Thermo Fisher Scientific, Forma 900 series, Waltham, MA, USA) until analysis.

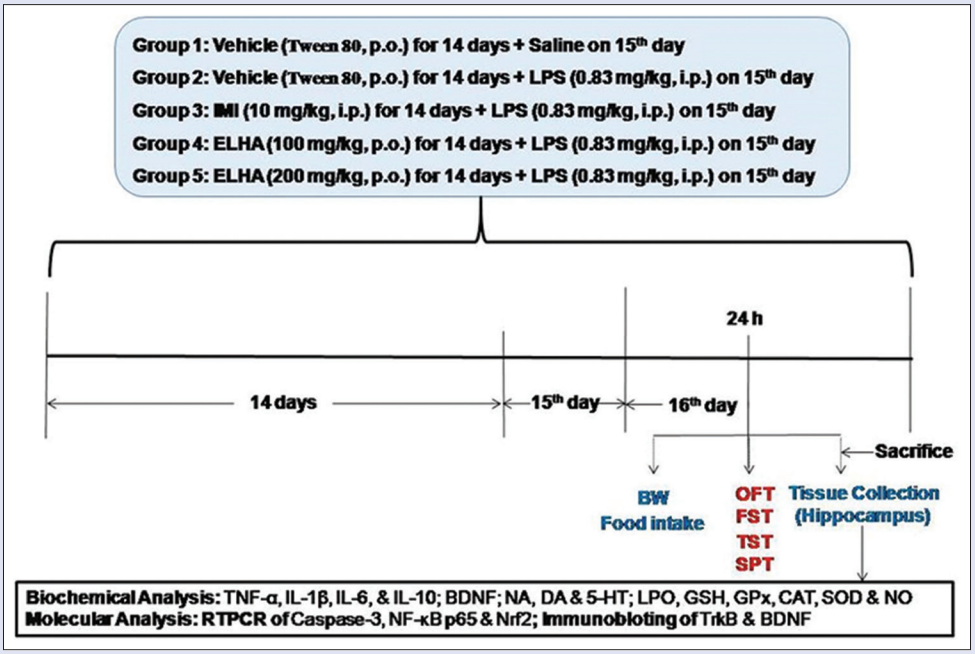

Figure 1: Diagram of experimental design. Imipramine and lipopolysachharide

Behavioral assessments

Food consumption and body mass

Food intake and body mass were recorded once daily at the onset of the dark period. Food containers were filled with 50 g of the pelleted mice chow, and food intake was quantified 2 and 24 h after LPS/saline injection. Consumption of food was recorded by subtracting the food remaining in the food container and on the cage floor from the amount of food measured at the preceding time point. Food spillage in the cage was ignored because it has been previously reported to be similar among rats/mice and generally weigh <1% of the food consumed.[32] BW was also measured at 2 and 24 h after LPS/saline injection. Both food intake and BW were expressed in grams (g).

Open-field test

To investigate the possible effects of the ELHA on locomotor activity, mice were subjected to OFT with minor modifications.[33] They were individually placed into a clean, novel cage similar to the home cage, but devoid of bedding or litter. The cage was divided into 12 virtual quadrants, and locomotor activity was measured by counting the number of line crossings and rearing during 5-min period. The floor of the open-field apparatus was cleaned with 10% ethanol between each mouse.

Forced swimming test

FST was performed to assess the despair behavior of the rodents.[34] The test was performed for mice using FST apparatus (StoeltingCo., IL, USA). Water temperature was maintained at 28°C–30°C ± 1°C. Mice were kept in an inescapable cylinder for 6 min during the test session and video recorded using ANY-maze software (Stoelting Co., USA). Immobility time was counted for the last 4 min. They were considered immobile when ceased struggling, remained floating motionless, and only make those movements necessary to keep their head above the water.

Tail suspension test

TST was conducted using TST apparatus (Stoelting Co., USA) as described by Steru *et al.*[35] Mice were individually suspended in the hook of the TST box, 60 cm above the surface of a table with an adhesive tape placed 1 cm away from the tip of the tail in a dark room. The immobility time of each mouse was video recorded using ANY-maze software (Stoelting Co., USA) for 6 min, and immobility time was counted for the last 4 min of the total 6-min observation period.

Sucrose preference test

The sucrose preference test was done to evaluate anhedonia (response to reward).[36] Before testing, all mice were acclimatized to drinking water and 2% sucrose solution for 5 days before LPS administration to establish a baseline sucrose preference for each mouse. Sucrose solution was filled in a drinking bottle having stopper valve and placed in the home cage of animals. The relative position of bottles was changed daily to avoid the development of a place preference. On the day of testing, mice were deprived of fluid and food for 2 h prior to testing. At the end of the testing, i.e., 48 h post-LPS, fluid content was measured, and sucrose preference was calculated using the following equation:

Sucrose preference (%) = sucrose intake/(sucrose intake + water intake) × 100.

Assessment of oxidative stress and antioxidant status

Lipid peroxidation and nitric oxide

The LPO end product malondialdehyde (MDA) was estimated in the HC by Ohkawa *et al's.*[37] method using the thiobarbituric acid, and the optical density was measured spectrophotometrically at 532 nm. The values are expressed as ηM of MDA/mg of protein. Nitrite, an indicator of the production of nitric oxide (NO), was determined by a colorimetric assay using Griess reagent (Sigma-Aldrich, St. Louis, USA). The concentration of nitrite was determined from a sodium nitrite standard curve and expressed as μM of nitrite/mg of protein.[4]

Antioxidant status

GSH was estimated according to the method described by Ellman.[38] The concentration of reduced GSH was expressed as μM of GSH/mg protein. SOD activity was estimated using SOD assay kit (Sigma-Aldrich, USA) according to the manufacturer's specifications. The SOD activity (units/mg of protein) was calculated by using the standard plot. The CAT activity was determined according to the method of Sinha[39] and expressed as μM of H_2O_2 decomposed/min/mg protein. GSH peroxidase (GPx) was estimated as described by Ahrens *et al.*[40] The total protein was estimated by the method of Bradford.[41]

Neurochemical estimations

Estimation of brain cytokines and brain-derived neurotrophic factor

The levels of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6), anti-inflammatory cytokines (IL-10), and BDNF in the HC were estimated spectrophotometrically (Multiskan Go, Thermofisher Scientific, Waltham, MA, USA) using commercially available ELISA kits (Ray Biotech, USA) according to the manufacturer's instructions.

Estimation of brain catecholamines

Levels of noradrenaline (NA), DA, and 5-HT were estimated using high-performance liquid chromatography (HPLC) (Thermo Scientific, Dionex Ultimate 3000 model and Chromeleon 7 software, MA, USA) coupled with an electrochemical detector (ESA Coulochem® III detector, ESA Biosciences, Inc., Chelmsford, USA.) as described by Kim *et al.*[42] Typically, 0.3N perchloric acid (PCA) solution is added to the tissue sample for preservation and extraction of catecholamines and acid metabolites. The tissue sample was weighed and then placed into a microcentrifuge tube. For every 100 μg of wet weight, a volume of 1.0 mL of 0.3 N PCA is added. Then, the samples were pulse sonicated on ice in this solution to degrade any native enzyme activity and help precipitate the proteins from the sample. The tubes were then centrifuged for 10–15 min (time depends on G-force of the centrifuge) to form a pellet and clear supernatant that is free of particulates. An aliquot of the supernatant was finally diluted 1:2 with readymade MD-TM mobile phase. HPLC chromatographic conditions using a normal bore column approach are as follows: flow: isocratic at 0.60 mL/min; temperature: 32°C; column: MD-150 column, guard column, and holder; injection volume: 10–20 μL partial loop; mobile phase: MD-TM (available from ESA part number 70-1332); Coulochem detector: 5011A cell: E1 at −150 mV: E2 at +220 mV, 5020 cell: EGC at +250 mV.

Reverse transcriptase-polymerase chain reaction analysis

Reverse transcriptase-polymerase chain reaction (PCR) was performed following the methods used by Bodduluru *et al.*[43] cDNA was synthesized using RevertAid First Strand cDNA Synthesis kit (Thermo Scientific) according to the manufacturer's instructions. Oligonucleotide primers used for amplification are shown in Table 1. All PCR samples were denatured at 95°C for 3 min before cycling and were extended for 10 min at 72°C after cycling. The PCR assay using primers was performed for 30 cycles at 95°C for 30 s; annealing temperature varies for different primers for 45 s [Table 1] and 72°C for 45 s (Veriti Thermal Cycler, Applied Biosystems, CA, USA). GAPDH served as internal control to check for equal loading. PCR products were analyzed using the Image Lab 6.0 (Bio Rad Co).

Immunoblotting analysis

Hippocampal tissue samples (30 mg approx.) were homogenized in 5 mL of chilled lysis buffer (RIPA Buffer, Amresco, USA) and centrifuged at 23,000 ×*g* for 20 min at 4°C. The protein concentration of the supernatants was quantified by Bradford reagent (HIMEDIA LABORATORIES, Mumbai, India) with bovine serum albumin (BSA) as the standard. Fifty micrograms of total protein was loaded and separated in 10% polyacrylamide gels containing sodium dodecyl sulfate using Hoefer Midi Gel apparatus (Harvard Apparatus, Holliston, MA, USA).[44] Gels were electrophoresed at 150 V, and the fractionated proteins were visualized by Coomassie blue staining or transferred to nitrocellulose membrane using semi-dry blotting apparatus (Hoefer).[45] The membranes were then blocked using 10 mL of cold blocking buffer containing 3% BSA in tris buffer saline with Tween 20 (TBST) for 1 h and incubated overnight (4°C) with 5 mL of 1% BSA in TBST containing antiserum rabbit/mouse polyclonal IgG (Santa Cruz Biotechnology Inc., Texas, USA) against BDNF and TrkB in 1:500 dilution. After overnight incubation, blots were washed four times (5 min each) with 10 mL of TBST. The blots were then reacted with goat anti-rabbit secondary antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology, Inc.) for 1 h. After rinsing with cold TBST, the color reaction on the nitrocellulose membrane was obtained using commercially available UltraTMB blotting buffer. The membranes were scanned, and band intensities were quantified using Image J software (NIH, Bethesda, MD, USA). Expression of various genes, such as BDNF and TrkB, was studied.

Statistical analysis

Results were expressed as mean ± standard error of mean. Statistical analysis was performed by one-way analysis of variance followed by *post hoc* Tukey's multiple range tests, using GraphPad Prism software version 5.0 (San Diego, CA, USA). Results were considered statistically significant when $P < 0.05$.

RESULTS

Identification of phenolic compounds

The rapid screening of the compounds present in the EL extract was confirmed using chromatographic analysis by using LC-ESI-MS/MS.[31] Initially, different gradient solvents were used for the chromatographic separation. Of these, the results were obtained well using 10-min run time. The gradient method was well optimized for the identification of the phytoconstituents present in EL extract. The chromatogram was recorded at 365 nm. The chromatographic representation is illustrated in Figure 2. The compounds such as ambrosin, isoscutellarein-4-methylester-8-o-glucuronide, friedelin, 6,7,4'-trihydroxyflavonone, scopoletin, and luteolin were identified by using LC-ESI-MS/MS (UHPLC-DIONEX 3100, Thermo scientific, USA) [Figure 3]. The identification of the peaks was executed by the comparison of the retention time, λ_{max}, and mass spectra of the EL extract from the earlier literature and database.[46] The compound, ambrosin, was found to be 247.06 (M + H) + with an empirical formula of $C_{15}H_{18}O_3$;[47] isoscutellarein-4-methylester-8-o-glucuronide was found to be 477.20 (M + H) + with an empirical formula of $C_{22}H_{20}O_{12}$;[48] friedelin was found to be 427.20 (M + H) + with an

Table 1: Oligonucleotide primer sequence for target genes used in reverse transcription polymerase chain reaction

Gene of interest	Primer sequences	Annealing temperature	Product size
NFκB-p65	FP: 5'- AACAAAATGCCCCACGGTTA -3' RP: 5'- GGGACGATGCAATGGACTGT -3'	60°C	115 bp
Caspase-3	FP: 5'- TGGTGATGAAGGGGTCATTTATG -3' RP: 5'- TTCGGCTTTCCAGTCAGACTC -3'	60°C	152 bp
Nrf2	FP: 5'- CAGCATGTTACGTGATGAGG -3' RP: 5'- GCTCAGAAAAGGCTCCATCC -3'	60°C	105bp
GAPDH	FP: 5'-AGGTTGTCTCCTGTGACTTC-3' RP: 5'-CTGTTGCTGTAGCCATATTC-3'	60°C	95bp

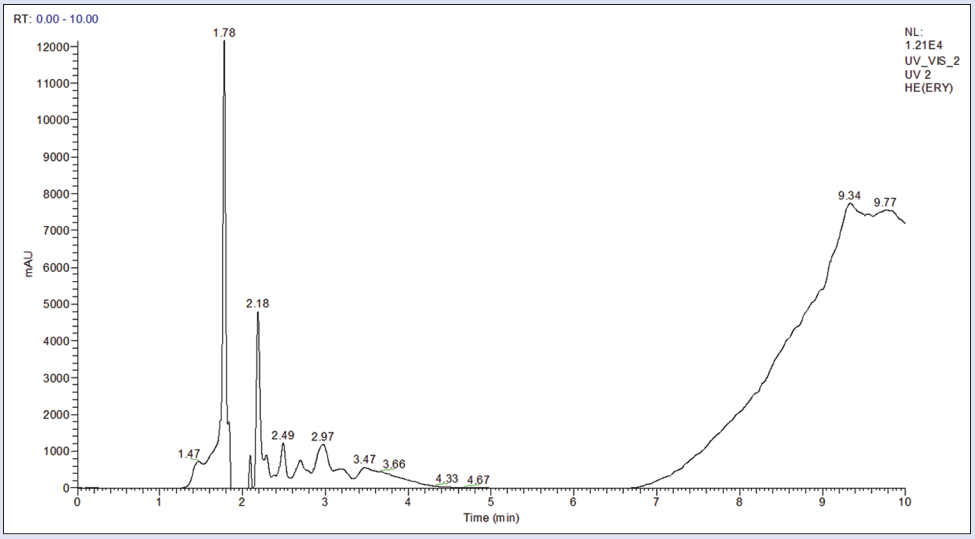

Figure 2: Ultra-high performance liquid chromatography chromatogram of *Conyza bonariensis* extract at 365 nm

empirical formula of $C_{30}H_{50}O$;[46-49] scopoletin was found to be 191.06 $(M-H)^-$ with an empirical formula of $C_{10}H_8O_4$; and[46] luteolin was found to be 287.15 (M + H) + with an empirical formula of $C_{15}H_{10}O_6$.[50]

Acute toxicity study

Acute oral toxicity studies revealed no lethality or any toxic reactions or moribund state up to the end of the study period. ELHA was safe up to a dose level of 2000 mg/kg of BW. Hence the two doses, namely 100 and 200 mg/kg BW, were selected for the study and were also free from toxic effects.

Erigeron linifolius hydroalcoholic extract pretreatment prevented lipopolysachharide-induced body weight loss in mice

LPS administration induced significant loss in BW ($P < 0.001$) and reduction in food intake ($P < 0.05$) when compared to the normal control group. A significant improvement in BW was observed by the pretreatment of IMP ($P < 0.001$) and ELHA in a dose-dependent manner (100 mg/kg; $P < 0.05$ and 200 mg/kg; $P < 0.01$) when compared to that of LPS-challenged mice, but both IMP and ELHA did not show any effect on reduced food intake in LPS-challenged animals [Figure 4a and b].

Erigeron linifolius hydroalcoholic extract pretreatment improved the locomotor activity in open-field test in lipopolysachharide-challenged mice

There was a significant reduction in both number of line crossings ($P < 0.001$) and rearing ($P < 0.05$) in LPS-treated mice, indicating reduced locomotor activity. Number of line crossings improved by treatment with IMP ($P < 0.001$) and ELHA (100 mg/kg; $P < 0.05$ and 200 mg/kg; $P < 0.01$) pretreatment, significantly in a dose-dependent

Table 2: Open-field exploration test (*n*)

Groups	A. Number of line crossings	B. Number of rearing
Vehicle control	98.13±9.31	32.16±4.43
LPS (0.83 mg/kg, i.p.)	40.12±4.23$^{###}$	15.90±1.96$^{\#}$
IMP (10 mg/kg, i.p.) + LPS	89.20±8.79***	28.16±3.61
ELHA (100, p.o.) + LPS	76.21±10.21*	22.08±2.08
ELHA (200, p.o.) + LPS	84.60±8.09**	25.64±4.52

$^{###}P < 0.001$, $^{\#}P < 0.05$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. LPS: Lipopolysaccharide; ELHA: *Erigeron linifolius* hydroalcoholic extract; IMP: Imipramine; i.p: Intraperitoneally; p.o.: Per oral

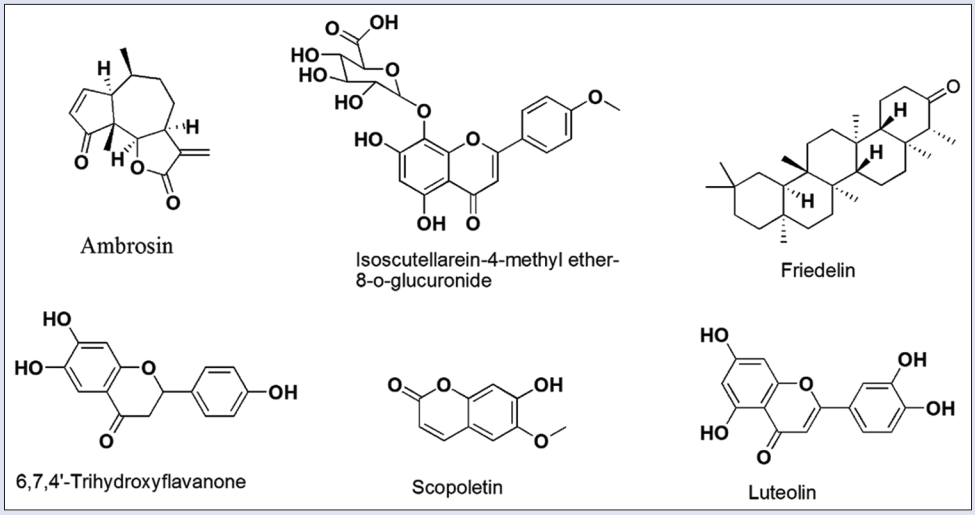

Figure 3: Structure of compounds 1–6 identified from *Conyza bonariensis* by using liquid chromatography-electrospray ionization-mass spectroscopy/mass spectroscopy analysis

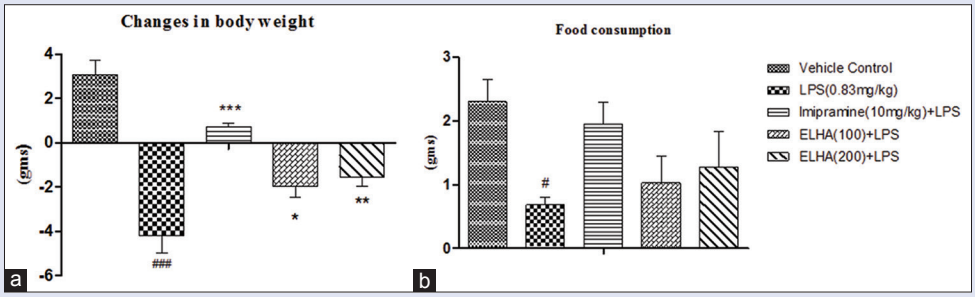

Figure 4: Effect of *Erigeron linifolius* hydroalcoholic extract pretreatment in lipopolysachharide-challenged animals on: (a) Changes in body weight and (b) Food consumption. Pretreated with *Erigeron linifolius* hydroalcoholic extract for 14 days and imipramine was given 30 min prior to lipopolysachharide injection. On the 14th day, lipopolysachharide was administered and both body changes and food intake were measured after 24 h of lipopolysachharide challenge on the 15th day. Values represent the mean ± standard error of mean ($n = 6$). $^{###}P < 0.001$, $^{###}P < 0.01$ compared with normal control. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with lipopolysachharide-challenged group

manner, but no effect was observed in case of number of rearing when compared with LPS-challenged mice [Table 2].

Erigeron linifolius hydroalcoholic extract pretreatment prevented lipopolysachharide-induced increase in immobility time in forced swimming test and tail suspension test

Table 3 shows that both in FST and TST, there was significant ($P < 0.001$) increase in immobility time in LPS-subjected mice when compared to vehicle-treated group. Pretreatment with IMP and ELHA reversed LPS-induced increase in immobility time in FST and TST at both the dosages, i.e., 100 and 200 mg/kg significantly ($P < 0.001$) [Table 3].

Erigeron linifolius hydroalcoholic extract pretreatment did not show any significant effect on lipopolysachharide-induced anhedonic behavior

LPS treatment showed marked reduction in sucrose consumption, inducing anhedonic condition in mice as compared to vehicle-treated mice. Although both IMP and ELHA (100 and 200 mg/kg) pretreatment showed improvement in sucrose consumption in LPS-challenged mice, the effect was not significant [Table 3]. This raised apprehension as to why anti-inflammatory potential is not competent to attenuate anhedonic behavior in LPS-induced depressive behavior. There is possibility of another pathway responsible for anhedonic behavior in animals.

Effect of *Erigeron linifolius* hydroalcoholic extract pretreatment on lipopolysachharide-induced alteration in oxidant and antioxidant parameters

Table 4 shows that LPS administration significantly reduced the GSH level in the HC ($P < 0.05$), whereas IMP ($P < 0.001$) and ELHA (200 mg/kg; $P < 0.05$) pretreatment significantly elevated GSH level when compared with LPS-treated group, but 100 mg/kg pretreatment was ineffective. Results showed significant LPO in the HC ($P < 0.01$) 24 h post-LPS injection in mice when compared with vehicle-treated group. Both IMP (10 mg/kg; $P < 0.05$) and ELHA (200 mg/kg; $P < 0.01$) pretreatment significantly reduced the LPO in the HC. Further, LPS-treated mice showed a decreased CAT (a relevant endogenous antioxidant enzyme responsible for hydrogen peroxide detoxification) activity, which was improved by the pretreatment of IMP ($P < 0.05$), but ELHA pretreatment did not show any significant effect at both the dosages. Furthermore, administration of LPS in animals showed significant decrease ($P < 0.01$) in GPx (important endogenous antioxidant enzymes) content in the HC, whereas significant improvement was observed by IMP ($P < 0.05$) pretreatment but not by ELHA. Results showed that hippocampal nitrite level increased significantly ($P < 0.01$) in mice post 24 h of LPS injection when compared with vehicle-treated group. IMP (10 mg/kg) and ELHA (200 mg/kg) pretreatment prevented the LPS-induced increase in nitrite level in the HC significantly ($P < 0.01$). In addition, lower dose of ELHA (100 mg/kg) also showed protective effect against LPS-induced decrease in hippocampal nitrite level in mice. As depicted in Table 4, LPS evoked significant decrease in SOD (a class of enzymes that catalyzes the reduction of superoxide to hydrogen peroxide) activity. Both IMP and ELHA were ineffective against LPS-induced decrease in hippocampal SOD. Scopoletin is one of the phytoconstituents found in ELHA which might be effective against oxidative stress induced by nitric oxide but in our study we found that scopoletin is devoid of antioxidant property.

Erigeron linifolius hydroalcoholic extract pretreatment rectified lipopolysaccharide-induced alterations in hippocampal pro- and anti-inflammatory cytokines and brain-derived neurotrophic factor level

Results showed that 24 h post-LPS injection, there was a significant increase in the pro-inflammatory cytokines including IL-1β ($P < 0.001$), IL-6 ($P < 0.01$), and TNF-α ($P < 0.001$) levels in the HC of mice. LPS-provoked hippocampal IL-1β was prevented by IMP and ELHA pretreatment significantly ($P < 0.001$) [Table 5]. IMP significantly ($P < 0.001$) attenuated LPS-induced IL-6 level, but ELHA was ineffective at both the dosages. IMP and ELHA at higher dose, i.e., 200 mg/kg, showed significant ($P < 0.001$) protection against LPS-induced elevation in hippocampal TNF-α level, whereas lower

Table 3: Effect of *Erigeron linifolius* hydroalcoholic extract pretreatment in lipopolysaccharide-challenged animals on behavioral parameters: A. Forced swimming test, B. Tail suspension test, C. Sucrose preference test

	Immobility time (s)		Sucrose consumption (%)
Groups	A. Forced swimming test	B. Tail suspension test	C. Sucrose preference test
Vehicle control	45.16±3.07	90.60±4.91	78.01±4.14
LPS (0.83 mg/kg, i.p)	130.30±7.09###	148.20±9.08###	50.91±4.71
IMP (10 mg/kg, i.p) + LPS	58.36±3.86***	73.87±6.68***	68.44±7.01
ELHA (100 mg/kg, p.o.) + LPS	71.10±5.06***	121.10±11.08***	54.96±8.98
ELHA (200 mg/kg, p.o.) + LPS	61.30±4.96***	103.10±8.56***	56.08±6.13

###$P < 0.001$, ***$P < 0.001$. LPS: Lipopolysaccharide; ELHA: *Erigeron linifolius* hydroalcoholic extract; IMP: Imipramine; i.p: Intraperitoneally; p.o.: Per oral

Table 4: Effect on *in vivo* antioxidant enzymes following pretreatment with *Erigeron linifolius* hydroalcoholic extract on lipopolysaccharide-challenged mice

Treatment	GSH (μg/g of tissue)	LPO (nmol/mg of protein)	Catalase (U/min/mg of protein)	GPx (μmol/mg of protein)	NO (μmol/mg of protein)	SOD (U/mg of protein)
Vehicle control	2280±361.3	3.68±0.36	31.90±3.03	34.40±1.56	2.18±0.22	3.65±0.40
LPS (0.83 mg/kg, i.p.)	1280±159.4	6.17±0.78##	16.10±1.97##	22.70±1.86##	4.32±0.24###	1.69±0.40#
IMP (10 mg/kg, i.p.) + LPS	3413±283.2***	4.17±0.28*	27.56±2.81*	33.66±2.49*	1.92±0.10***	3.12±0.30
ELHA (100 mg/kg, p.o.) + LPS	2019±198.6	5.62±0.59	19.08±2.01	28.01±3.01	3.16±0.59	2.38±0.80
ELHA (200 mg/kg, p.o.) + LPS	2286±301.0*	3.91±0.61**	22.01±3.50	30.03±5.08	2.68±0.48**	2.90±0.36

###$P < 0.001$, ##$P < 0.01$, #$P < 0.05$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. LPS: Lipopolysaccharide; ELHA: *Erigeron linifolius* hydroalcoholic extract; IMP: Imipramine; NO: Nitric oxide; LPO: Lipid peroxidation; SOD: Superoxide dismutase; i.p: Intraperitoneally; p.o.: Per oral; GSH: Glutathione

dose of ELHA, i.e., 100 mg/kg, did not show significant protective effect. Compared with the vehicle-treated group, LPS injection showed marked reduction in anti-inflammatory cytokine and IL-10 levels ($P < 0.001$) in the HC. IMP and ELHA (100 and 200 mg/kg) significantly ($P < 0.001$) increased the IL-10 levels, as compared to LPS-treated group. To summarize, ELHA possess anti-inflammatory property as seen from the above results, except IL-6.

Furthermore, results showed that LPS administration significantly reduced the hippocampal BDNF level ($P < 0.001$) when compared to the vehicle-treated group. Pretreatment of ELHA (200 mg/kg) significantly ($P < 0.01$) restored hippocampal BDNF level in LPS-challenged mice [Table 5]. However, IMP and lower dose of ELHA were ineffective to restore LPS-induced decrease in BDNF level in HC.

Erigeron linifolius hydroalcoholic extract pretreatment restored lipopolysaccharide-induced decrease in brain catecholamines level in mice

Table 6 shows significant ($P < 0.001$) reduction in hippocampal NE, DA, and 5-HT concentrations in LPS-treated mice compared to vehicle-treated group. Both IMP (10 mg/kg) and ELHA (100 and 200 mg/kg) restored LPS-induced decrease in catecholamines including NE, DA, and 5-HT concentrations in the HC. The findings of the current study suggested the involvement of dopaminergic, serotonergic, and noradrenergic pathways in the antidepressant-like effect of ELHA. Its antidepressant action resembles more with tricyclic antidepressant IMP.

Table 5: Effect on hippocampal cytokines and brain-derived neurotrophic factor level following pretreatment with *Erigeron linifolius* hydroalcoholic extract in lipopolysaccharide-challenged mice

Treatment	BDNF (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)	IL-10 (pg/mL)
Vehicle control	375±20.12	340±25.95	214±15.09	3453±273.10	712±36.10
LPS (0.83 mg/kg, i.p.)	250±19.78$^{###}$	530±36.10$^{###}$	304±23.11$^{##}$	8023±285.00$^{###}$	342±24.11$^{###}$
IMP (10 mg/kg, i.p.) + LPS	311±28.54	270±14.87***	169±12.99***	4268±310.70***	562±33.10***
ELHA (100 mg/kg, p.o.) + LPS	298±16.39	375±21.67**	273±22.08	7263±387.60	465±28.30*
ELHA (200 mg/kg, p.o.) + LPS	362±23.78**	364±15.08***	234±19.20	4354±277.80***	496±30.60**

$^{###}P < 0.001$, $^{##}P < 0.01$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ LPS: Lipopolysaccharide; ELHA: *Erigeron linifolius* hydroalcoholic extract; IMP: Imipramine; i.p: Intraperitoneally; p.o.: Per oral; BDNF: Brain-derived neurotrophic factor; IL: Interleukin; TNF-α: Tumor necrosis factor-alpha

Table 6: Changes in hippocampal NE, dopamine, and 5-HT levels following pretreatment with *Erigeron linifolius* hydroalcoholic extract in lipopolysaccharide-challenged mice

Groups	Norepinephrine (ng/g of tissue)	Dopamine (ng/g of tissue)	5-HT (ng/g of tissue)
Vehicle control	110.8±6.29	169.4±5.96	115.3±8.92
LPS (0.83 mg/kg, i.p.)	63.25±4.10$^{###}$	88.10±8.38$^{###}$	61.2±4.35$^{###}$
IMP (10 mg/kg, i.p.) + LPS	114.8±5.54***	181.6±6.37***	139.1±6.82***
ELHA (100 mg/kg, p.o.) + LPS	152.5±5.18***	60.0±3.57*	73.5±3.96
ELHA (200 mg/kg, p.o.) + LPS	194.6±8.01***	147.5±6.01***	87.5±5.01*

$^{###}P < 0.001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. LPS: Lipopolysaccharide; ELHA: *Erigeron linifolius* hydroalcoholic extract; IMP: Imipramine; i.p: Intraperitoneally; p.o.: Per oral

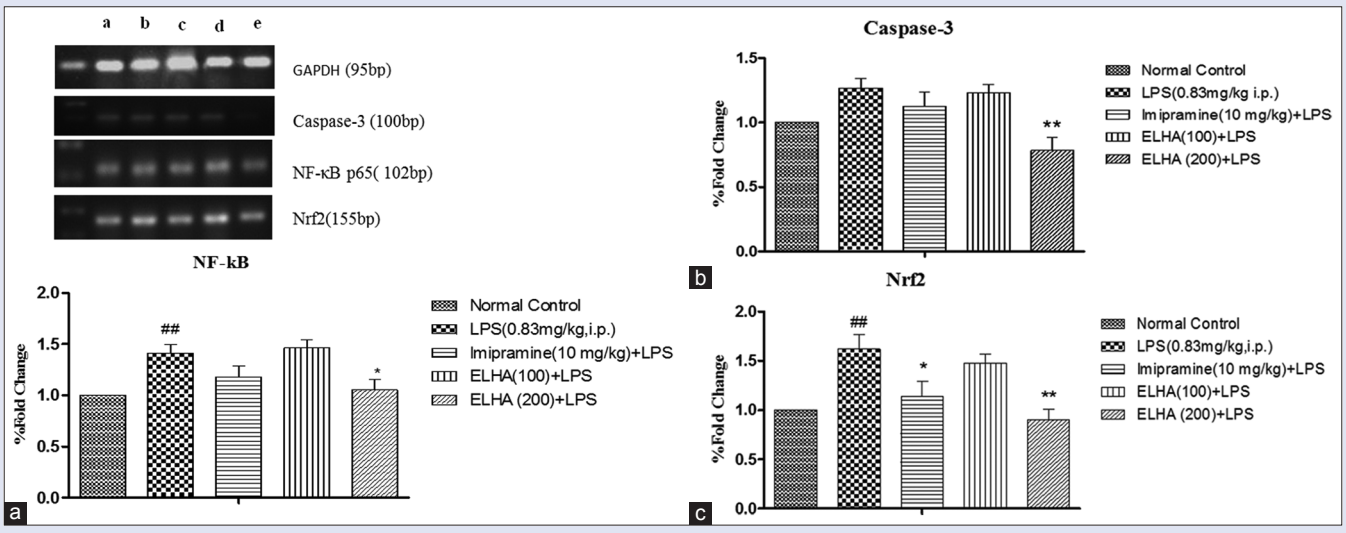

Figure 5: Effect of *Erigeron linifolius* hydroalcoholic extract in lipopolysachharide-challenged animals on hippocampal mRNA expression of (a) Nuclear factor-kappa B phosphor 65 (b) Caspase-3, (c) Nuclear-related factor 2, and GAPDH (d). (a) Lane 1 – marker, (b) Lane 1 – normal control, (c) Lane 2 – lipopolysachharide, (d) Lane 3 – imipramine + lipopolysachharide, (e) Lane 4 – *Erigeron linifolius* hydroalcoholic extract 100 + lipopolysachharide, (f) Lane 5 – *Erigeron linifolius* hydroalcoholic extract 200 + lipopolysachharide. Data are expressed as "fold changes" as compared with normal control. Values represent mean ± standard error of mean ($n = 3$) in each group. Statistical significance was determined by one-way analysis of variance followed by Tukey's *post hoc* test. ##$P < 0.01$ compared with normal control. *$P < 0.05$; **$P < 0.01$ compared with lipopolysachharide-challenged group

Effect of *Erigeron linifolius* hydroalcoholic extract on reverse transcriptase polymerase chain reaction analysis of caspase-3, nuclear factor-kappa B phosphor 65, and nuclear-related factor 2

Reverse transcriptase PCR analysis of caspase-3, nuclear factor-kappa B phosphor 65 (NF-κB p65), and nuclear-related factor 2 (Nrf2) is depicted in Figure 5a-c. There was upregulation of the Caspase-3 gene in the LPS-treated group, when compared to vehicle control group. The standard drug IMP-treated group showed downregulation in Caspase-3 mRNA level which was followed by the ELHA (200 mg/kg, $P < 0.01$) extract-treated group when compared with LPS-treated group. ELHA (100 mg/kg) did not show any effect on LPS-induced upregulation of Caspase-3 mRNA level. An upregulation in the expression of the NF-κB p65 gene was observed in LPS-challenged group when compared to normal control ($P < 0.01$), whereas ELHA (200 mg/kg) extract-treated group showed significant downregulation ($P < 0.05$) in the expression of the gene which implicates that the plant extract might have anti-inflammatory property which indirectly influences the antidepressant activity of the plant. The mRNA expression level of Nrf2 upregulated significantly ($P < 0.01$) in LPS-treated group when compared to control group which was downregulated significantly by the treatment of ELHA (200 mg/kg, $P < 0.01$) and IMP ($P < 0.05$). Downregulation in Nrf2 mRNA level by ELHA could be attributed to its antioxidant phytoconstituents. In this study also, its anti-apoptotic, anti-inflammatory, and antioxidant properties were visible like biochemical studies.

Immunoblotting analysis of tropomyosin receptor kinase and brain-derived neurotrophic factor

The immunoblotting analysis of TrkB and BDNF is represented in Figure 6A and B. There was significant ($P < 0.001$) suppression of TrkB and BDNF expression in LPS-challenged mice. Pretreatment with

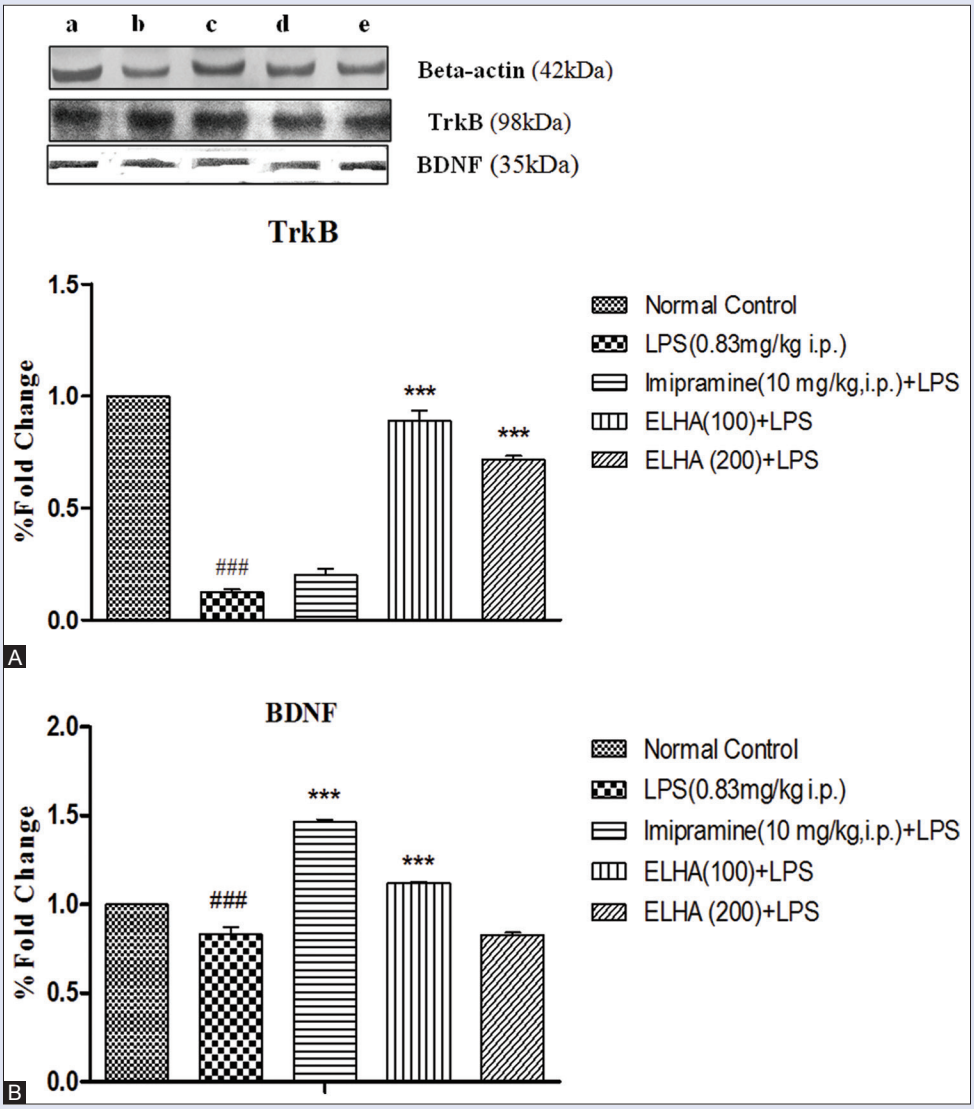

Figure 6: Effect of *Erigeron linifolius* hydroalcoholic extract in lipopolysachharide-challenged animals on hippocampal protein expression of (A) Tropomyosin receptor kinase and (B) brain-derived neurotrophic factor. (a) Lane 1 – normal control, (b) Lane 2 – lipopolysachharide, (c) Lane 3 – imipramine + lipopolysachharide, (d) Lane 4 – *Erigeron linifolius* hydroalcoholic extract 100 + lipopolysachharide, (e) Lane 5 – *Erigeron linifolius* hydroalcoholic extract 200 + lipopolysachharide. Values are expressed as percent fold change represented as mean ± standard error of mean ($n = 3$). ###$P < 0.001$ compared with normal control. *** $P < 0.001$ compared with lipopolysachharide-challenged group

standard drug, IMP, did not show any improvement in downregulated TrkB level, while a significant ($P < 0.001$) upregulation in TrkB expression was observed in ELHA (100 mg/kg and 200 mg/kg)-treated mice when compared with LPS-treated mice. Furthermore, BDNF expression level in HC of LPS-treated mice was also decreased significantly ($P < 0.001$) by the LPS injection. A significant increase in the expression of BDNF in standard drug-treated mice ($P < 0.001$) when compared to LPS-treated group was observed. Similarly, an upregulation of hippocampal BDNF expression was also observed in ELHA (100 mg/kg)-treated mice. ELHA treatment significantly upregulated BDNF and TrkB translational level in the target tissue, i.e., HC. BDNF, the hallmark for depression, unregulated in both the studies, indicating that this pathway could be responsible for its antidepressant-like action.

DISCUSSION

This study demonstrated the potential therapeutic application of ELHA in the treatment of depressive-like behavior by modulating neuroinflammation, oxidative stress, BDNF-TrkB signaling, and monoaminergic pathway. Several studies revealed that EL has potential impact for the treatment of various diseases. Studies suggested that friedelin possessed potent anti-inflammatory, analgesic, and antipyretic activities.[50,51] According to findings of an earlier research, scopoletin (at a concentration of 10 μM) significantly preserved GSH content by 50% and the activity of superoxide dismutase by 36% and also inhibited the production of MDA to the degree as seen in the control.[52] Luteolin has been used for treating various diseases such as hypertension, inflammatory disorders, and cancer.[53]

Earlier studies have shown that depression is often accompanied with sickness behavior including loss of food and water intake, decrease in BW, fever, and anxiety. These symptoms occur due to the production of inflammatory cytokines and reactive oxygen species (ROS).[4] This study also supports previous findings that LPS causes weight loss and anorexia in mice,[4-7] and it might be due to the increase of pro-inflammatory cytokines (IL-1, IL-6, and TNF-α) affecting hypothalamic region of mouse brain.[7] Attenuation of LPS-induced BW loss by ELHA pretreatment may be due to its potent antioxidant and anti-inflammatory activities.[26,27]

Locomotor activity is affected in depression both in animals and humans, which may be due to ROS, peroxide, and pro-inflammatory cytokine production.[4] These results are in accordance with the earlier studies which showed that LPS challenge reduces the locomotor activity in mice.[7] ELHA pretreatment also improved the locomotor activity in LPS-challenged animals, which may be attributed to its antioxidant and anti-inflammatory activities.[26,27] In our study, HPLC analysis demonstrated the presence of freidelin and scopoletin as phytoconstituents in ELHA which probably responsible for anti-inflammatory and antioxidant properties of ELHA.

Multiple lines of evidences suggested the involvement of inflammatory pathway in the pathogenesis of depression.[1,9] Pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α activate oxido-nitrosative stress pathway to damage brain tissues, leading to depressive-like behavior in both humans and animals.[8] In animals, LPS is used to induce depressive-like behavior that can be assessed by increased immobility time in FST and TST.[1-9] Furthermore, pro-inflammatory cytokines including IL-1β and IL-6 upregulate indoleamine 2, 3-dioxygenase (IDO) enzyme which is responsible for immobile behavior in FST and TST.[54] Findings of the present study are in accordance with earlier studies that LPS increased immobility duration in FST and TST after 24 h of its injection when compared with that of vehicle-treated animals.[1,9] ELHA pretreatment significantly attenuated the depressive-like behavior by reducing the immobility duration in FST and TST which may be due to the suppression of inflammatory cytokines and ROS by its phytoconstituent friedelin.[26,27]

Pro-inflammatory cytokines play an important role in the anhedonic behavior in animals which can be induced by LPS injection. It elevates the cytokine level which further augments serotonin transporters and upregulates IDO enzyme, leading to behavioral alterations including anhedonia.[55] The present results are not in accordance with that of earlier studies and raise the questions in the possible involvement of another pathway in mediating LPS-induced anhedonic behavior in animals.

Neuroinflammation, which is accompanied by brain immune response and glial cell activation has been shown to play an important role in depression. Moreover, antidepressant agents have anti-neuroinflammatory properties. *In vivo* studies using animal models have demonstrated that different types of antidepressants modulated the expression of inflammatory mediator, such as cytokines, microgliosis, and astrogliosis in the nervous system. *In vitro* studies on rodent glial cells have also demonstrated that some antidepressants decrease glial generation of inflammatory molecules.[56] In our study, we have found that ELHA attenuated inflammation by inhibiting the nuclear factor-κB (NF-κB) pathway and the enzymatic activity of the pro-inflammatory cytokine macrophage inhibitory factor in the peripheral system and central nervous system [Figure 5a].

The nuclear factor erythroid 2-related factor 2 (Nrf2) is an upstream transcription factor modulating Phase II enzyme activity, which interacts with the antioxidant response element (ARE) in the nucleus to induce ARE-dependent gene expression. During oxidative stress, Nrf2 translocates into the nucleus to induce the expression of hemeoxygenase-1,[57] which plays an essential role in maintaining cellular redox homeostasis against ROS generation and oxidative stress.[57] Pretreatment with ELHA showed significant increase in Nrf2 gene expression, exhibiting its antioxidant property [Figure 5c]. Caspase-3 is an important executer of apoptosis, was elevated in the LPS-induced HC, and this increase was prevented by ELHA pretreatment, from which we could infer that ELHA may intervene in LPS-induced cell death [Figure 5b].

Various studies have indicated that monoamines including DA, serotonin, and NE play significant role in the pathogenesis of depression, and currently available antidepressants act through monoaminergic pathway.[13,14] Moreover, decreased synthesis of these biogenic amines resulted in the development of clinical relapse and prevention of antidepressant effects of administered medication.[58] This monoaminergic theory of depression is developed 30 years ago, which demonstrates the role of serotonergic, noradrenergic, and dopaminergic systems.[14] Effect of antidepressant agents on behavioral symptoms through investigation of particular neurotransmitter level is significant to assess the direct correlation of neurotransmitters and antidepressant effects.[59] In this study, LPS injection depleted hippocampal monoamines (DA, 5-HT, and NE) and showed depressive-like behaviors tested in TST and FST. The results are in accordance with the earlier studies which suggested that various compounds showed antidepressant effect by improving the DA, 5-HT, and NE levels in the HC.[60] Considering these results, we conclude that monoamine level should be maintained at an optimum level to exert antidepressant effect by ELHA. Currently available antidepressants selectively increase the level of only one monoamine; therefore, limited antidepressant effect can be observed. Therefore, development of triple reuptake inhibitors is of great importance to produce rapid antidepressant action as well as safe and efficacious therapeutics. Accordingly, ELHA could be a potential antidepressant agent because it produces antidepressant effects by modulating monoaminergic systems, and it might be due

to phytoconstituents present in it. Further investigation is needed to perform to find out the exact mechanism behind its antidepressant action.

Multiple lines of evidence suggest that BDNF is integral to both the pathophysiology of depression and the therapeutic mechanism of antidepressants.[61-63] Loss of BDNF in the brain attenuates the action of antidepressants,[64] while responses typically elicited by antidepressants were lost in mice with either reduced brain BDNF or inhibited TrkB signaling.[63,64] In order to understand the molecular mechanism of the ELHA extract on its antidepressant effect, we investigated the expression of BDNF and TrkB protein levels in the HC of mice induced by LPS.

CONCLUSION

Findings of the current study suggest that ELHA showed ameliorating effect against LPS-induced depressive-like behavior, which might be due to the suppression of pro-inflammatory cytokines, oxidative and nitrosative stress, and upregulation of BDNF and monoaminergic pathways in the HC of mice. Moreover, chemical constituents present in this plant may be responsible for the protective activity of ELHA. Thus, ELHA could be a putative candidate against neuropsychiatric disorders associated with neuroinflammation and oxido-nitrosative stress.

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Conflicts of interest

There are no conflicts of interest.

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