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Neuroprotective effect of ethanolic leaf extract of *Commiphora caudata* (Wight &Arn) against lipopolysaccharide-induced neurotoxicity in Wistar rats

Ponmalai Srinivasan, Balasubramanian Arul, Ramalingam Kothai*

Department of Pharmacology, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation (Deemed to be University), Salem-636008, Tamilnadu, India

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ABSTRACT

Introduction: *Commiphora caudata* contains various essential phytoconstituents and is a potential medicinal plant used traditionally to treat various ailments such as neurodegenerative diseases. The present study aimed to evaluate the neuroprotective effect of ethanolic leaf extract of *Commiphora caudata* against the lipopolysaccharides (LPS) induced behavioral changes in rats.

Methods: The *in-vitro* antioxidant potential was evaluated by 1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assay methods. For *in-vivo* studies, the animals were pre-treated with ethanolic leaf extract of *Commiphora caudata* (EECC) at 200 and 400 mg/kg of b.w for 30 days, and neurotoxicity was induced with a single intraperitoneal injection of LPS 1 mg/kg, b.w on day 31. The neurotoxicity was evaluated with a chain of behavioral tests such as Morris water maze test, radial arm maze, and choice reaction time (CRT) tests. At the end of the study, rats were sacrificed, the brain hippocampal region was removed, and the levels of acetylcholinesterase, nitric oxide, and protein were measured.

Results: The IC50 value in the DPPH method was 71.58 ± 15.62 µg, and the total antioxidant activity of EECC was found to be 742.33 ± 14.57 µmol Fe (II)/g extract. In behavioral tests, animals treated with EECC at 200 and 400 mg/kg showed a neuroprotective effect in Morris water maze test, an 8-arm radial maze test, and in CRT test. Both doses reduced acetylcholinesterase, nitric oxide, and protein levels ($P < 0.001$), respectively.

Conclusion: The present study results showed the promising neuroprotective effects of ethanolic extract of leaves of *Commiphora caudata* and its action against the LPS-induced cognitive impairment in rats.

Implication for health policy/practice/research/medical education:
This research work showed the promising neuroprotective effects of ethanolic leaf extract of *Commiphora caudate* against the LPS-induced neurotoxicity in rats. Thus, this plant might be considered a candidate for the isolation of compounds which leads to identifying the novel lead compounds for the development of drugs against neurodegenerative disorders.

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Introduction

Neurodegenerative disorders are common diseases occurring in many developing countries and the second leading cause of death around the world (1). According to World Health Organization, 12% of total deaths globally are due to neurological diseases (2). The prevalence is gradually increasing and may reach 132 million in 2050 (3). The basic feature of many neurological disorders such as Parkinson's disease, Huntington's disease, Alzheimer's disease, and multiple sclerosis is neuro-inflammation. It develops due to the chronic activation of glial cells of the brain, which leads to enhanced neuronal cell death. It causes disturbances in neurotransmitters, loss of neuronal cells, and cognitive deficits, which may eventually result

***Corresponding author**: Ramalingam Kothai,
Email: kothaiarul@yahoo.co.in

in neurological disorders (4). This disorder causes a high degree of morbidity and mortality. It is initiated by many causes like toxic metabolites, autoimmunity, microbes, viruses, traumatic brain injury, and spinal cord injury. The current scenario of therapy depicts that no therapy can cure degenerative disorders, only symptomatic treatments are followed. It includes dopaminergic drugs, cholinesterase inhibitors, antipsychotic drugs, analgesic drugs, and anti-inflammatory agents. These treatments may work well in the beginning, but over the period, producing a lot of side effects (5). In recent times, researchers focused on complementary medicines to protect from degenerative diseases. Many medicinal plants and plant-derived natural compounds show a promising role in the prevention, relief, and delay of neurological diseases. Medicinal plants such as *Ashwagandha, Baccopa monnieri, Centella asiatica, Ginseng, Ginkgo biloba*, and compounds isolated from plant extracts such as celastrol, curcumin, flavonoids, lycopene, resveratrol, sesamol, and trehalose showed antioxidant and neuroprotective effects (6). Many chemicals were examined to develop an animal model for neurodegenerative diseases based on their mode of action towards behavior patterns. Ethanol, colchicine, heavy metals, scopolamine, lipopolysaccharide, streptozotocin, and okadaic acid are some of them (7).

Lipopolysaccharides (LPS), also known as endotoxins, are present in the outer membrane of gram-negative bacteria (8). LPS can enhance the release of inflammatory cytokines (TNF-α, IL-1β, PGE_2, etc) and induces brain inflammation followed by neuronal loss and microglial activation (9). They play a crucial role in the development of neuro-inflammation both *in-vivo* and *in-vitro*. LPS administration elicits clinical, biochemical, and cognitive changes that are comparable to those found in various neurodegenerative disorders associated with neuroinflammation. These include poor spatial memory, increased cytokines, pro-inflammatory transcription factors, astrocytosis, and decreased N-methyl-D-aspartate receptor type 1 (NMDAR1) (10).

Commiphora caudata (Wight & Arn) is a traditional medicinal plant that belongs to the family Burseraceae, commonly known for its antibacterial, antifungal, analgesic, and anti-inflammatory activities. Its resinous exudate is named "myrrh" used in Chinese medicine for trauma, arthritis, fracture, and blood stagnation (11). In Ayurveda, it is used to treat coronary artery ailment, inflammatory disease, neuropharmacological disease, obesity, etc. The following pharmacological activities, such as the antioxidant (12), hepatoprotective (13), anti-ulcer (14), anti-arthritic (15), anti-inflammatory (16), anti-diabetic (17), and antibacterial effects (18) of this plant were reported. In connection with this, the present research aimed to evaluate the neuroprotective effect of leaf extracts of *Commiphora caudata* against LPS induced neuroinflammation in Wistar rats.

Materials and Methods

Drugs and chemicals

LPS, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, and dexamethasone were purchased from the Sigma-Aldrich Chemicals Private Limited, Bangalore, India**.**

Plants materials

The fresh leaves of the *Commiphora caudata* were collected from the Shervaroy hills, Salem, Tamilnadu, in October 2020. The plant materials were identified and authenticated by the Botanical Survey of India, Tamilnadu, Agri University, Coimbatore, Tamilnadu. A voucher specimen (CCKS-1) has been deposited in the Department of Pharmacology, Vinayaka Mission's College of Pharmacy, Salem, Tamilnadu, for future reference CCKS-1

Preparation of extracts

The leaves were then shade dried at room temperature for 10 days and coarsely powdered and stored in an air-tight container. About 500 g of coarsely powdered leaves were taken and subjected to continuous hot percolation with different solvents of increasing order of polarity such as pet ether, chloroform, acetone, ethanol, and aqueous. The extracts were dried under the rotary evaporator and then tested for various phytochemical constituents like alkaloids, flavonoids, glycosides, phenols, saponins, sterols, tannins, proteins, and carbohydrates.

Animals

Healthy adult Wistar male rats of 8 weeks old and weighed 200 gm were used for the study. The animals were procured from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) listed suppliers of Srivenkateshwara Enterprises, Bangalore, India. The animals were kept in well-ventilated polypropylene cages at 12 hours light and 12 hours dark schedule at 25°C and 55%–65% humidity levels. The rats were given a normal diet of pellets and free access to water. Each animal, at the commencement of the experiment, should be between 8 and 12 weeks old.

Preparation of animals

The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days before dosing to allow for acclimatization to the laboratory conditions. Before each test, the animals were fasted for at least 12 hours; the experimental protocols were subjected to the scrutinization of the Institutional Animals Ethical Committee (P.col/26/2021/IAEC/VMCP) and were cleared by the same. All experiments were performed during the morning according to CPCSEA guidelines for the care of laboratory animals and the ethical guideline for investigations of experimental pain in conscious animals.

The standard orogastric cannula was used for oral drug administration in experimental animals.

Determination of in-vitro antioxidant activity

DPPH radical scavenging activity

EECC was tested for antioxidant activity by the DPPH method (19). The extract (20, 40, 60, 80, 100 µg/mL) was mixed with 3 mL of methanolic solution containing DPPH radicals (0.1 mM). After 30 minutes, absorbance was determined at 517 nm. The percent inhibition of activity was calculated by using the formula:

$$\%\,\text{inhibition} = \frac{A_o - A_e}{A_o} \times 100$$

Where, Ao = absorbance without extract; Ae = absorbance with extract.

The results were expressed as IC_{50}, which is the concentration of the sample required to inhibit 50 % of DPPH concentration.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay is a novel method to assess the antioxidant power of the sample. The method performed in this study was described by Prasad et al (20). It is based on the ability of antioxidants to reduce Fe^{+3} to Fe^{+2} in the presence of TPTZ (2, 4, 6-tripyridyl-s-triazine), forming an intense blue Fe^{+2} – TPTZ complex. FRAP solution (3 mL) mixed with 100 ml of the EECC and incubated at 37°C for 10 minutes. Absorbance was measured at 593 nm for different concentrations (0.2, 0.4, 0.8 or 1 mg/mL) of extract in FRAP reagent. The absorbance of the samples was compared to a $FeSO_4$ standard curve and the FRAP values were expressed as mmol Fe (II)/mg extract.

Pharmacological studies

Experimental design

Thirty healthy Wistar male rats weighing 200 g were randomly divided into 5 groups with six animals in each group. Group I served as normal control, which received 0.1 mL of normal saline orally for 30 days. Group II served as disease control, which received a single intraperitoneal dose of LPS (1 mg/kg) (21) on day 31. Group III served as positive control, which received LPS (1 mg/kg) + dexamethasone (0.5 mg/kg) (21) for 30 days. Groups IV and V respectively received LPS (1 mg/kg) + EECC 200 and 400 mg/kg (15) for 30 days. On the last day, after the evaluation of behavioral studies, the animals were sacrificed under light ether anesthesia and the brain was dissected out immediately and subjected to biochemical studies.

Behavioral tests for learning and memory

Morris water maze test

Morris water maze test is a method to assess spatial or place learning (22). It consists of an open circular pool with having a diameter of 100 cm and 50 cm in height with a featureless interior surface. A circular platform was hidden 2 cm below the water level. The circular pool was filled with water maintained at a constant temperature of 23±1°C and camouflaged with 500 mL of milk. All the animals were trained with 3 trials per day with the interval of 5-10 minutes for 5 days a week. Each trial started from one of four assigned polar positions with a different sequence each day. The first step to calculate learning is the escape latency. It is the time taken by the animal to reach the platform. Other parameters like Path length traveled and the time spent in the quadrants were evaluated in the present study.

Radial arm maze test

A radial arm maze is a method to assess the working memory of animals (21). It consists of a wooden apparatus with eight elevated–arm radial maze, which extends from the central platform having a diameter of 26 cm. Each arm is 60 cm long, 2 cm height, and 5 cm width, which holds along the length of the arm. The evaluation was conducted in a well-lighted room, which contained many signs. During the study, all the arms were filled with pellets and the experiment was continued until all the food pellets were collected or 10 minutes passed, whichever occurred first. All the animals were trained daily to collect the pellet for 4 weeks. The trial was ended after 8 choices and the animals had to make more correct choices with fewer errors. The following parameters were checked during the study to assess the performance of the animals, such as the number of correct choices, the number of errors, test duration(s), and the total number of errors before all the food pellets in the eight arms were collected.

Choice reaction time task test

Choice reaction time (CRT) task test (23) is a method to analyze the behavioral and neural mechanisms of stimulus-response affinity in rats. The CRT apparatus consisted of 14 skinner boxes placed in a dark area with a soundless atmosphere. The boxes were equipped with two pressing levers, cue lamps, and a pellet dispenser. When the lever was pressed correctly by the animal, the pellet dispenser released the pellet. In this method, the animals were trained to press any one of the two levers with a continuous reinforcement schedule at a fixed ratio of 1:1. Trials began with differential reinforcement of another behavior (DRO) period (random, 2-5 seconds) during which the animals had to refrain from pressing either of the 2 levers. During the CRT period (maximum 10 seconds), the time between sample presentation with the cue lamp on and pressing the correct lever was defined as the CRT and a food pellet reward would be provided through the pellet dispenser. With further lever-pressing responses, a house lamp was illuminated and intertribal interval (ITI; 20 seconds) begun. One trial

took approximately 30 seconds and each test evaluation consisted of 30 trials. One test was performed every day for 30 days. The parameters measured were the number of incorrect lever pressings during the DRO and ITI periods.

Biochemical studies

At the end of the behavioral studies, all rats were sacrificed under light ether anesthesia and the rat brain hippocampal region was isolated. It was homogenized with 0.1M phosphate buffer (pH 8) at 0°C using Potter–Elvehjem homogenizer. The homogenate was then subjected to centrifugation at 10 000×g for 5 minutes at 4°C to get clear supernatant liquid and the same was used for biochemical estimations like acetylcholinesterase, nitric oxide (NO), and protein.

Estimation of acetylcholinesterase

The brain concentration of acetylcholinesterase was estimated by the method of Ellman et al with slight modification (24). Brain homogenate (0.1 mL) was mixed with 6 ml of sodium phosphate buffer (pH 8), acetylthiocholine iodide (0.2 mL), and 5,5'-dithio-bis-(2-nitrobenzoic acid (DTNB, the Ellman reagent). The changes in the absorbance of the mixture were measured at 412 nm.

Estimation of nitric oxide

The brain concentration of nitric oxide was estimated by the method of Green et al (25). In this method, equal amounts of brain homogenate and Griess reagent (1% sulphanilamide, 2% H3PO4, 0.1% N-(1-naphthyl) ethylenediamine-HCl) were allowed to react at room temperature for 5 min. The resulting bright reddish-purple-colored azo-dye was measured spectrophotometrically at 540 nm.

Estimation of protein

Protein concentrations of the brain homogenates were determined by the standard method of estimation explained by Lowry et al (26).

Statistical analysis

The results were expressed as the mean ± SEM and analyzed statistically by one-way ANOVA followed by Tukey's multiple comparison tests using GraphPad InStat software. Differences were considered statistically significant when $P < 0.001$.

Results

Percentage yield and phytochemical screening

The percentage yield of the extracts was calculated and found to be 2.4, 1.16, 1.20, 12.5, and 6.5 %w/w for pet ether, chloroform, acetone, ethanol, and aqueous extracts, respectively. The glycosides, carbohydrates, phenols, saponins, terpenoids, tannins, and flavonoids were present in the acetone, ethanol, and aqueous extracts. Alkaloids and terpenoids were present in chloroform extract. Gums and fixed oils were present in petroleum ether extract. Thus the phytochemical analysis confirmed the presence of bioactive compounds and this might serve as a potential source in the treatment of neurological disorders. Hence, based on the percentage yield and phytochemical results, ethanolic extract of leaves of *Commiphora caudata* was selected for its neuroprotective studies.

In-vitro antioxidant studies

DPPH radical scavenging activity

The DPPH free radical scavenging activity of the EECC was carried out. The extracts were tested at concentrations of 20, 40, 60, 80, and 100 µg/mL. The EECC showed 91.45% inhibition of the DPPH radical at 100 µg/mL concentration, whereas the standard (ascorbic acid) revealed 95.37% inhibition at the same concentration. The extract showed the DPPH radical scavenging activity even at the lowest concentration of 20 µg/mL. The DPPH radical inhibition was increasing and concentration-dependent as that of ascorbic acid as the standard compound. The IC_{50} values of the EECC and ascorbic acid were found to be 71.58 µg/mL and 86.77 µg/mL, respectively.

Total antioxidant activity by FRAP method

In-vitro total antioxidant activity for EECC was estimated by FRAP method using ascorbic acid as standard. The EECC showed the FRAP activity of 742.33 ± 14.57 µmol Fe (II)/g extract, whereas ascorbic acid showed 1026.67 ± 17.15 /µmol Fe (II)/g.

Morris water maze test

In this test, spatial learning and memory were evaluated by measuring the escape latency, path length, and time spent in the quadrant, which indicated the improvement in cognitive functions. The effect of EECC on spatial learning and memory is shown in Table 1. For escape latency, it was significantly increased in the group II LPS treated animals when compared to normal group animals ($P < 0.001$) and showed the neurotoxicity. Group III animals were treated with dexamethasone, which significantly reduced the escape latency when compared to group II animals ($P < 0.001$). Administration of EECC at 200 and 400 mg/kg, b.w reduced the escape latency in a dose-dependent manner when compared with LPS treated animals ($P < 0.001$). For path length traveled, the LPS treated animals showed a significant increase in the distance traveled when compared to normal group animals. Dexamethasone-treated animals showed a significant reduction in the path length traveled as compared to LPS treated animals ($P < 0.001$). The treatment groups with a low dose (200 mg/kg, b.w) and high dose (400 mg/kg, b.w) exhibited considerable reduction in path length

Table 1. Effect of ethanolic leaf extracts of *Commiphora caudata* (EECC) on behavioral studies in Lipopolysaccharide (LPS) induced neurotoxicity in rats

Groups	Morris water maze			Radial arm maze		Choice reaction time test
	Escape latency (s)	Path length traveled (cm)	Time spent in quadrant (S)	No. of errors	Exploration time (s)	No. incorrect pressing
I (Control)	18.83 ± 0.91	26.50 ± 0.76	70.17 ± 0.75	2.33 ± 0.33	12.17 ± 0.60	40.67 ± 0.88
II (LPS-1 mL/kg)	88.50 ± 0.99[a]	55.67 ± 0.88[a]	28.66 ± 0.84[a]	8.17 ± 0.60[a]	30.5 ± 0.76[a]	88.50 ± 0.76[a]
III (LPS+Dexamethasone 0.5 mg/kg)	28.33 ± 0.76[b]	32.50 ± 0.76[b]	65.50 ± 0.76[b]	3.83 ± 0.95[b]	18.50 ± 0.43[b]	45.17 ± 1.10[b]
IV (LPS+EECC 200 mg/kg)	50.50 ± 0.99[b]	48.33 ± 0.88[b]	38.50 ± 0.43[b]	5.50 ± 0.76[b]	24.17 ± 0.79[b]	66.33 ± 0.76[b]
V (LPS+EECC 400 mg/kg)	32.50 ± 0.76[b]	35.67 ± 0.67[b]	60.33 ± 0.56[b]	4.83 ± 0.60[b]	21.33 ± 0.95[b]	51.17 ± 0.94[b]

Abbreviations: LPS, lipopolysaccharides; EECC, ethanolic leaf extract of *Commiphora caudata.*
Values are expressed as mean ± SEM of 6 animals. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests. [a] ($P < 0.001$) indicates that group II (negative control) was compared with group I (control). [b] ($P < 0.001$) indicates that Group III, IV, and V were compared with group II.

travelled as compared to group II animals ($P < 0.001$). For time spent in the quadrant, the neurotoxicity induced animals showed poor time spent in the quadrant when compared with normal control animals. The positive control animals showed a considerable increase in time spent when compared to group II animals. While EECC treated animals showed a significant increase in the time spent in the quadrant. The EECC at high doses 400 mg/kg, b.w significantly reduced the parameters, such as escape latency ($P < 0.001$), path length travelled ($P < 0.001$) and improved the time spent in quadrant ($P < 0.001$). Thus, the LPS induced long-term memory deficit was improved by the EECC treatment in a dose-dependent manner.

Radial arm maze test

The effect of EECC on working memory was determined using the radial arm maze method. In this, the total number of errors in entry and exploration time was checked to assess its effects (Table 1).

The total number of errors in the entry was significantly increased in LPS treated animals as compared to the control group ($P < 0.001$). Dexamethasone tended to reduce the total number of errors in entry. However, there was a significant ($P < 0.001$) dose-dependent reduction of a total number of errors in the entry on animals treated with EECC 200 mg/kg, b.w compared with those treated with the high dose of 400 mg/kg, b.w. In exploration time, a significant decrease in exploration time was observed in EECC 400 mg/kg, b.w treated animals, as compared to EECC 200 mg/kg, b.w treated animals. Similarly, dexamethasone also reduced the exploration time while, LPS treated animals showed increased exploration time as compared to control group animals ($P < 0.001$). The EECC at high doses (400 mg/kg, b.w) significantly reduced the total number of errors in entry and exploration time ($P < 0.001$) and ameliorated the LPS induced working memory deficit.

Choice reaction time task test

In CRT, incorrect lever pressing was measured to assess the LPS induced abnormal changes in the behavior pattern of animals. The LPS treated animals showed abnormal incorrect pressing compared with the control group animals ($P < 0.001$). Dexamethasone-treated animals considerably reduced the incorrect pressing as compared with LPS treated animals. In the treatment groups, EECC at 200 and 400 mg/kg showed a significant decrease of incorrect lever pressing ($P < 0.001$; Table 1).

Biochemical studies

Acetylcholinesterase enzyme

The results of the inhibitory effect of EECC on acetylcholinesterase enzyme were are in Table 2. In LPS treated neurotoxic animals, the inhibition levels of acetylcholinesterase were high, which denotes the neurodegenerative effects. Treatment with dexamethasone significantly reduced the inhibition levels as compared to LPS treated animals. However, EECC treated groups at 200 and 400 mg/kg, b.w showed a considerable decrease in inhibitory compared to disease control animals ($P < 0.001$). This indicates the neuroprotective effects of EECC compared to disease control animals.

Nitric oxide

The effect of EECC on nitric oxide level is shown in Table 2. A significant increase in NO levels was observed in LPS treated animals as compared to the control animals ($P < 0.001$). Dexamethasone treatment considerably reduced these levels near to normal. Treatment with EECC at 200 mg/kg and 400 mg/kg b.w also reduced the elevated levels of nitric oxide significantly in a dose-dependent manner.

Total protein

The effects of EECC on protein level are shown in Table 2. LPS treatment significantly increased the protein level in the disease control group compared to normal animals. However, pre-treatment with dexamethasone and EECC at 200 and 400 mg/kg b.w significantly reduced these elevated levels as compared to disease control animals.

Table 2. Effect of ethanolic leaf extracts of *Commiphora caudata* (EECC) on biochemical studies in Lipopolysaccharide (LPS)-induced neurotoxicity in rats

Groups	AChe (µg/min/mg protein)	Nitric oxide (µmol/g tissue)	Total Protein (g/dL)
I (Control)	62.33 ± 0.84	0.76 ± 0.02	4.03 ± 0.13
II (LPS-1 mL/kg)	105.33 ± 0.88[a]	5.07 ± 0.14[a]	9.01 ± 0.08[a]
III (LPS+Dexamethasone 0.5 mg/kg)	66.17 ± 0.83[b]	1.51 ± 0.01[b]	5.13 ± 0.15[b]
IV (LPS+EECC 200 mg/kg)	89.16 ± 1.01[b]	3.65 ± 0.07[b]	7.05 ± 0.09[b]
V (LPS+EECC 400 mg/kg)	71.16 ± 0.60[b]	1.86 ± 0.02[b]	6.20 ± 0.10[b]

Abbreviations: LPS, lipopolysaccharides; EECC, ethanolic leaf extract of *Commiphora caudata.*
Values are expressed as mean ± SEM (n = 6). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests. [a] ($P < 0.001$) indicates that Group II (negative control) was compared with group I (control). [b] ($P < 0.001$) indicates that Group III, IV, and V were compared with group II.

Discussion

The present study investigated the protective effect of ethanolic leaf extract of *Commiphora caudata* and its action against the LPS-induced cognitive impairment in rats. The results of the phytochemical analysis showed the presence of sterols, carbohydrates, saponins, tannins, proteins, terpenoids, phenolic compounds, and flavonoids. It was reported that free radicals are key factors for neuronal death in many neuro-disorders such as seizure disorders, schizophrenia, cerebral ischemia, Parkinson's disease, and Alzheimer's disease, etc (27). In this study, the in-vitro antioxidant potential of EECC was evaluated by DPPH and FRAP assay methods and it showed promising antioxidant activity in a dose-dependent manner. In FRAP assay, the ability to reduce Fe^{3+} to Fe^{2+} was also significantly higher at concentrations 0.2–1 mg/mL. It was reported that the phenolic compounds were directly related to the antioxidant property (28).

This study, mainly focused on the effects of EECC on LPS induced behavioral changes in the rat's brain, and the behavioral changes were measured by spatial learning and memory, working memory, and recognition by subjecting them to the Morris Water Maze test, Radial arm maze test and CRT tests, respectively. The Morris water maze specifically tests a hippocampus-dependent memory for spatial memory and learning (29). Administration of intraperitoneal injection of 1 mg/kg LPS produced significant impairment in spatial learning and memory. This impairment was indicated by longer escape latencies to reach the platform and path length traveled and poor time spent in the quadrant by the LPS treated animals as observed in many studies. This may propose that there is a severe degeneration in the brain as a result of exposure to LPS, which contributes to deficits in episodic memory and recognition capacity (30). The rats that received EECC 200 and 400 mg/kg b,w, exhibited faster learning and shorter escape latencies than the LPS treated group. Thus, EECC treatment improved these behavioral abnormalities and restored spatial learning and memory. Thus, the results of the present study confirm our hypothesis that EECC ameliorates spatial memory impairment induced by LPS injection.

Spatial working memory is a critical cognitive function in which information is retained active for a short time after it has been received. The radial-arm maze test is one of the common methods to evaluate working memory in rodents (31). This study was assessed by measuring the number of errors in the entry to arms and exploration time to reach the food pellets. The results of the study depict that the LPS treated animals showed more number errors and an increase in the exploration time when compared to control group animals. This shows the memory impairment changes in disease control animals. However, the EECC treated animals showed a significant reduction in the number of errors in entry and exploration time compared with the LPS-treated animals. These results confirmed that the treatment of EECC improves the LPS-induced impairment of working memory.

CRT tests are used to assess the ability of the animals to maintain attention and vigilance for the target stimulus and not responding to other targets. CRT performances were assessed by the number of correct and incorrect lever pressings during the ITI and DRO periods. It was observed from the results that LPS injection produced abnormal behaviors in CRT performance in terms of the increased number of incorrect lever pressing during the ITI and DRO periods. Pre-treatment with EECC at 200 and 400 mg/kg b.w showed a significant reduction compared to LPS. This suggests the protective effects of EECC against LPS induced behavioral changes.

The central cholinergic system has a major role in neuro-cognition. A significant reduction in acetylcholine leads to marked behavioral changes that have been indicated in many neurological diseases (32). In the present study, LPS induced neurotoxicity caused behavioral changes by escalating the acetylcholinesterase enzyme level. The reduction in the acetylcholinesterase inhibition level due to the treatment with EECC for 30 days suggests the ameliorating effects of EECC against cholinergic changes.

Nitric oxide has important physiological activities and is linked to a variety of neurological illnesses, including Alzheimer's disease and Parkinson's disease. Numerous evidence indicated the excitotoxicity produced by nitric oxide. These activated neurons kill a large number of neurons and thus elevate nitric oxide acts as a key mediator in neurological diseases (33).

Elevated levels of proteins are commonly associated with acute and chronic inflammatory conditions. In our study, LPS treated animals showed high levels of protein in the hippocampus region of the rat brain. Pre-treatment with EECC resulted in a significant reduction in the levels of protein, and this may be due to its antioxidant potential. At the same time, dexamethasone reversed these levels near to normal.

Conclusion

The results of the present study showed the promising neuroprotective effects of ethanolic extract of leaves of *Commiphora caudata* against the LPS-induced neuroinflammation in rats. This may be due to the antioxidant properties of the flavonoids, tannins, and polyphenols present in it. In the future, isolation and characterization studies are required for further evidence of its neuroprotective activity.

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Authors' contribution

RK and BA designed the study, wrote the manuscript with an interpretation of the results. PS carried out the animal study. All authors read and approved the final version and agreed to publish it.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

The experimental protocols were subjected to the scrutinization of the Institutional Animals Ethical Committee (P.col/**26**/2021/IAEC/VMCP) and were cleared by the same. All experiments were performed during the morning according to CPCSEA guidelines for the care of laboratory animals and the ethical guideline for investigations of experimental pain in conscious animals.

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