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EFFECT OF *CYMBOPOGON CITRATUS* LIPOSACCHARIDE INDUCED SICKNESS BEHAVIOR IN MICE

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ABSTRACT

Cymbopogen citratus also known as lemongrass is a rich source of phenolics and flavonoids that has been used for centuries to cure a variety of illness. The current study was designed to evaluate the protective effect of hot water extract of *Cymbopogen citratus* on LPS-induced sickness behaviour in mice**.** Swiss albino mice were pre-treated for 3 days with hot water extract of *Cymbopogen citratus* (AECC) 50,100 and 200 mg/kg, p.o, and Dexamethasone (1 mg/kg, i.p.) one hour prior to LPS (0.83 mg/kg, i.p.) administration. After 2 hours, sickness behaviour in the mice was assessed using a variety of behavioural assays such as plus maze, open field, light–dark box, forced swim, social behaviour tests, sucrose preference, thermal hyperalgesia, and food and water intake at various time intervals following with LPS challenge. Oxidative stress markers such as reduced glutathione and lipid peroxidation, and nitrate were also measured in the brain tissue homogenate of mice. Pre-treatment with (AECC) 50,100 and 200 mg/kg, p.o, significantly attenuated the behavioural alterations, anhedonia, and anorexia ameliorated the changes in brain oxidative stress markers due to LPS induced sickness. *Cymbopogen citratus* showed protective effect against LPS induced sickness behaviour in mice and the observed effect could be due to its potential antioxidant activity.

KEYWORDS: *Cymbopogen citratus*, lipopolysaccharide, sickness behaviour, anorexia.

1. INTRODUCTION

Sickness is a synchronized set of behavioral changes that arise during infection which includes symptoms such as fever, fatigue, loss of appetite, disinterest in the social and physical environment, and anhedonia. The effect of pro-inflammatory cytokines on the brain, which is regulated by neurohormonal mechanisms, is called functional homeostatic adaptation. In response to infections, the process is initiated by pro-inflammatory cytokines, which are released by activated monocytes and macrophages. The released cytokines can act by 2 mechanisms i) they alter the cerebral activity directly ii) they lead to an increase in cytokine synthesis in the brain.[1,2] To produce sickness behavior in rodents, lipopolysaccharide (LPS) is administrated which is a cell wall component of gram negative bacteria. Due to LPS administration there will be peripheral stimulation of the innate immune system, which results in an exaggerated neuro inflammatory response by high secretion of inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α)[3,4], which results in prolonged sickness behavioral conditions. Depression is one of the condition which results in reduced locomotor activity and anhedonia in sickness behavior.[5,6] Pathophysiological changes which are caused by administration of LPS in animals are similar to that of human. The first-line drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), tricyclic anti-depressants (TCAs), and selective serotonin reuptake inhibitors (SSRIs) are used to treat depression disorders which are caused by sickness, but they also have side effects such as gastritis, high resistance, relapse rates, and recurrence of depression.[7] As a result, there is a need to invent and create drugable candidate or nutraceuticals products, both existing and newer ones that are free of this side effect for improved treatment of sickness-related depression.

The herb lemongrass (*Cymbopogon citratus*) is widely used as a spice in Indonesia. These are known to contain phenolic compounds, which are powerful antioxidants. *Cymbopogon citratus* is a popular herb in tropical areas, particularly Southeast Asia which belongs to family

Poaceae. Its oil is used in Aromatherapy which includes essential oils like citral, nerol, geraniol, citronellal, terpinolene, geranyl acetate, myrcene. The leaves of *C. Citratus* include two triterpenoids, cymbopogone and cymbopogonol, as well as flavones which are luteolin and its 6-C-glucoside, which have been extracted. Studies indicate that it possesses various pharmacological activities such as analgesic, anti-convulsant, anti-amoebic, anti-bacterial, anti-depressant, anti-anxiety, antifungal and anti-inflammatory properties. Various other effects like anti-malarial, anti-mutagenicity, antioxidants, hypoglycemic and neurobehavioral have also been studied.[8] Owing to antioxidant and anti inflammatory potential it is anticipated that aqueous extract of *Cymbopogen citratus* reduces the symptoms of LPS induced sickness behaviour in mice. Therefore the present study was undertaken to evaluate the effect of *Cymbopogen citratus* on LPS induced sickness behaviour in mice.

2. MATERIALS AND METHOD

CHEMICALS:LPS (Sigma–Aldrich, St. Louis, USA), dexamethasone (Cadila Healthcare Ltd., Ahmadabad, India), 5, 5′-dithiobis-2-nitrobenzoic acid, thiobarbituric acid and trichloroacetic acid (Hi-Media Laboratories Pvt. Ltd., Mumbai, India) were procured.

COLLECTION AND AUTHENTICATION

The leaves of *Cymbopogen citratus* (family: Poaceae) were collected locally in month of June from nearby areas of Tumkur, Karnataka were identified and authenticated by professor Chidananda, S.S.W.C Tumkur (SSCP/2022-P-12).

2.1. EXTRACTION

Fresh *Cymbopogon citratus* leaves were collected. The leaves were cleaned, dried in the shade and then pulverized using a pulverizer. The aqueous leaf extract of *Cymbopogon citratus* was made by soaking 600 grams (g) of the powdered, dried leaf material in distilled water for 24 hours. Then, 3mm thick Whatman filter paper was used to filter the solution. The filtrate was concentrated at 40^0C in a rotary evaporator, and the remaining material was then dried in a dessicator before being stored in a sterilized glass. The *Cymbopogon citratus* was dissolved in normal saline to create the aqueous extract and were given in doses of 50,100 and 200 mg/kg, p.o.[9]

2.2 EXPERIMENTAL ANIMALS

In the current study male Swiss albino mice of weight 25-30 g were bred in the animal house of Sree Siddaganga College of Pharmacy (SSCP), Tumakuru, India were kept in temperature-controlled environments with 12-hour light-dark cycles. They had free access to normal pellets and unlimited water. The studies were approved by the Institutional Animal Ethical Committee (IAEC) of Sree Siddaganga College of Pharmacy, Tumakuru, Karnataka (approval No. SSCP/IAEC. Clear/195/21-22), in accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments Animals (CPCSEA), Government of India.

2.3 EXPERIMENTAL DESIGN

Cymbopogen citratus was dissolved in distilled water and administered to the mice orally via oral gavage for three days with the respective doses. LPS is derived from *Escherichia coli* which was administered intraperitoneally (i.p.) at a dose of 0.83 mg/kg after being diluted with saline (pH 7.4).

The mice were randomly divided into six groups consisting of 8 animals in each group. Groups I and II received, normal saline and LPS respectively where as Groups III, IV and V received a suspension of *Cymbopogon citratus* and normal saline in three doses (50, 100 and 200mg/kg p.o.). Group VI served as standard control and was treated with dexamethasone (1 mg/kg, i.p.). All these treatments were given regularly for 3 days. On day 3, after 1hour of administration of above treatments, all the groups were challenged with LPS (0.83mg/kg i.p.) except Group I. Two hours post-LPS administration, animals were evaluated for behavioural tests like elevated plus maze, light–dark box, forced swim, social behaviour and open field, food and water intake and sucrose preference. Animals were sacrificed and brain was isolated and used for assessing oxidative stress parameters and nitrate.

3) LIPOPOLYSACCHARIDE-INDUCED SICKNESS BEHAVIOR IN MICE

Elevated Plus Maze Test

The elevated plus maze are connected to the two identically sized closed arms with a 12-cm-high wall and opposite open arms (30 X 5 cm). A central square (5 cm) connects these arms, and the entire maze is elevated 50 cm from the ground. Each mice was placed on the center square with its head facing to the open arm during the test, and evaluated for 5 minutes, the number of entries and the amount of time spent in the open arm were recorded.[11]

3.1 LIGHT–DARK BOX TEST

The light-dark box is a device comprised of a wooden box with an open top and two distinct chambers: a bright chamber (30x30x35 cm) painted with white and a dark chamber (25x35x35 cm) painted with black. A tiny open doorway (7.5 x 5 cm), located in the center of the floor-level divider which connects the two sections. The mice were placed in the light chamber, which were free to move between the two chambers. The number of movement between the light and dark compartments, as well as the number of entries and time spent in the light compartment, were all noted for 5 mins.[12]

3.2 FORCED SWIM TEST.

The swim test was carried out for mice according to the method developed by Porsolt et al. (1977). Mice were placed in a vertical glass cylinder with a diameter of 12

cm and a height of 26 cm it was filled with water to a depth of 16 cm and temperature was maintained at 25°C. For testing, each mouse was submerged in the cylinder for six minutes, and the floating time (i.e., only moving enough to keep its head above water) was noted.[13]

3.3 SOCIAL INTERACTION

Juvenile mice were placed in the home cage of an experimental animal for 10 minutes test. The interaction between the subject and juvenile's was monitored (e.g., smelling, crawling under or climbing over, genital inspection), and the duration was determined by a trained observer who was blind to the experimental treatments. Mice were tested for social behaviour immediately following LPS injection, and then in interval of 2, 4, 8, and 24 hours using a different juvenile each time. The results are given as a percentage decrease in time spent with other mice when compared to the respective baseline measurements.[14]

3.4 BIOCHEMICAL ESTIMATIONS

The animals were sacrificed by overdose of anesthesia after the open field test. The brain was removed, homogenized using cold 10% w/v phosphate buffered saline solution, and was centrifuged at 12000 rpm for 15 minutes at 4°C (Remi Motors Ltd., Mumbai, India). The supernatant liquid was used for biochemical analysis. According to Lowry, the total protein content of the brain homogenate was determined using bovine serum albumin as a reference (1964). Malondialdehyde (MDA) production was calculated according to Gelvan and Saltam to determine the amount of LPO, and the results were expressed as nmol MDA/mg protein. Using Ellman's (1959) method, the GSH content was measured, and the outcomes were as nmol/mg protein.[15]

3.5 REDUCED GLUTATHIONE (GSH)

The natural defense against oxidative damage was assessed by measuring glutathione (GSH) levels. The method is based on the reaction of Ellman's reagent (DTNB) with free thiol groups (Ellman, 1959). Before being introduced to a solution which has 50% trichloroacetic acid, the brain sections (HC and PFC) were diluted in EDTA 0.02 M buffers (10% w/v). After centrifugation (10,000 rpm, 5 minutes), the homogenate supernatant was collected and combined with 0.4 M tris-HCl buffer, at pH 8.9, and 0.01 M 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB). The GSH was measured using a spectrophotometer at 412 nm resulting in yellow color. The outcomes were calculated utilizing a standard glutathione curve.[16]

3.6 LIPID PEROXIDATION LEVEL

lipid peroxidation was evaluated according to Esterbauer and Cheeseman technique which was given by the development of thiobarbituric acid reactive compounds (TBARS). 250 mL of tissue homogenate were added to 1.5 mL of 1% phosphoric acid (pH 2.0) and 1 mL of 0.6 % TBA in air light tubes and the samples were frizzed before extracting MDA (malondialdehyde). TBA was extracted with 2.5 mL of butanol was centrifuged for 5 minutes at 2000 rpm and analysed at 532 nm. Because MDA accounts for 99 % of TBARS, the TBARS concentration in the samples was determined using the extinction coefficient of MDA, which is 1.56 x 105 M1 cm^1. Lipid peroxidation is measured in terms of nmol TBARS/mg protein.[17]

3.7 PROTEIN ESTIMATION

The protein content was measured according to the method of Lowry et al. using bovine serum albumin as standard.[18]

3.8 NITRITE ASSAY

Nitrite was measured in the brain of mice using the Griess reagent as an indicator of nitric oxide production. Post mitochondrial supernatant (100 L) was treated with Griess reagent (1:1 solution) 1% sulphanilamide in 5% phosphoric acid and 0.1 naphthylamine diamine Di hydrochloric acid in water were added and absorbance was measured at 542 nm. A standard curve for sodium nitrite was used to calculate nitrite concentration.[19]

4) LIPOPOLYSACCHARIDE-INDUCED SUCROSE PREFERENCE AND THERMAL HYPERALGESIA IN MICE

In order to measure sucrose preference, mice were given with two solutions: water and a 10% sucrose solution in bottles with stoppers with ball-type sipper tubes. All mice were familiarized to the two-bottle prior to testing. All mice drank both solution; however sucrose was favoured over water. Food was restricted for 2 hours before testing and on the day of testing, i.e., the final day. Each group of mice received the last doses of vehicle, *Cymbopogen citratus*, and dexamethasone 30 minutes before the LPS injection. For the next 24 hours after LPS injection, mice were given access to water and a 10% sucrose solution. The fluid intake was calculated by measured the water and sucrose bottles at the end of the experiment at 2 and 24 hours.

4.1. LIPOPOLYSACCHARIDE-INDUCED THERMAL HYPERALGESIA IN MICE

4.2. Hot-plate test

The animals were individually placed on a hot-plate (Eddy's hot-plate) with the temperature set to 55 °C. The first evidence of paw licking or a leap response to avoid the heat was used as a measure of pain threshold; the cut-off time was 10 s to avoid paw injury.[20]

4.3. Tail immersion (hot water) test Tail

The animal's tail was submerged in a hot water bath 52° ± 0.5 °C until tail withdrawal (flicking reaction) or a struggle indication was noticed (cut-off 12 sec). Hyperalgesia is indicated by a decrease in tail withdrawal time.[21]

4.4. Cold allodynia

The tail immersion test was used to assess cold allodynia. Each animal's tail flick latency was assessed

by submerging the tail in a cup filled with water at a constant temperature of 10° C and monitoring the tail withdrawal latency in seconds (cut off time: 15 seconds) using a manual stop watch. Allodynia is indicated by a shorter time of immersion.[22]

5. LIPOPOLYSACCHARIDE-INDUCED ANOREXIA IN MICE

Feeding behaviour (Food and water intake)[23]

Measurement of changes in body weight, food and water intake, were assessed two days prior to the experiment by keeping the animals in individual cages. 50 g of food pellets and 100 ml of water were given to each animal cage along with the injection of saline or LPS (0.83 mg/kg, i.p.) in order to determine the amount of food and water consumed. Body weight, food and drink intake were measured prior to treatment as well as after 6 and 24 hours.

5.1. Body weight[24]

Body weight was measured, 24 h post lipopolysaccharide or vehicle challenge.

5.2. Body Temperature[25]

Thermo probe was inserted into the rectum of mice to a depth of 2 cm initial rectal temperatures were recorded on first day, as well as on third day, after LPS injection. The changes in rectal temperature before and after treatment of drugs were measured in all groups.

5.3. Foot-shock induced aggression by agressometer[26]

After 2h of the lipopolysaccharide administration, equipment was turned on and each pair of mice was observed for a period of 3 min. It was evaluated by delivering a current of 60 Hz to the foot of the animal for 3 min. A period of relaxation of 5 sec was given. After two successive shocks. The fighting behaviour was observed.

5.4. Locomotor activity by using actophotometer[27]

After 2 h of the lipopolysaccharide administration, equipment was turned on. Mice were placed individually in the activity cage for 5 min and the activity scores of all animals were recorded.

5.5. STASTICAL ANALYSIS

The data was presented as mean SEM. The data was statistically analysed using one-way analysis of variance (ANOVA), followed by the Turkeys post hoc test for grading aggression and other data. GraphPad prism 5.0 software was used to perform the Tukey's multiple comparison tests (GraphPad, San Diego, CA).

RESULTS

Behavioural tests

The elevated plus maze

When compared to the normal control group, peripheral LPS administration causes decrease in both number of open arm entries (0.75 ± 0.66) and the time spent (3.87 ± 1.05). however Pretreatment with *Cymbopogen citratus* (50, 100 and 200 mg/kg, p.o.) and Dexamethasone (1 mg/kg, i.p.) significantly increased both the number of open arm entries (1.72 ±0.66, 2.125± 0.59, 2.50±0.50 and 2.62±0.69) and time spent in open arm (13.5±0.52, 14.2±0.36, 15.12 ± 0.38 and 18.12±0.45) when compared to the LPS treated group.(Table 1)

Table 1: Effects of pre-treatment of *Cymbopogen citratus* on LPS induced sickness behaviour of mice in the Elevated plus maze (EPM). (Table 1)

Groups	Treatment	Number of entries		Total time spent in Sec	
		Open arm	Closed arm	Open arm	Closed arm
1	Normal saline (1 ml/kg,i.p.)	2.75 ± 0.66	7.0 ± 0.28	15.2 ± 0.46	224 ± 2.96
2	LPS alone (0.83 mg/kg,i.p.)	0.75 ± 0.66$^{###}$	1.8 ± 0.19$^{###}$	3.8 ± 0.35$^{###}$	285.1 ± 5.1$^{###}$
3	Dexamethasone (1mg/kg,i.p.) + LPS (0.83 mg/kg, i.p.)	2.62 ± 0.69****	5.1 ± 0.35***	18.1 ± 0.45***	238 ± 3.13*
4	AECC (50 mg/kg, p.o) + LPS (0.83 mg/kg, i.p.)	1.72 ± 0.66***	4.75 ± 0.52*	13.5 ± 0.52***	241.1 ± 2.90**
5	AECC (100mg/kg p.o) + LPS (0.83 mg/kg i.p.)	2.125 ± 0.59**	5.5 ± 0.37	14.2 ± 0.36**	2.34.7 ± 2.77
6	AECC (200 mg/kg, p.o) + LPS (0.83 mg/kg, i.p.)	2.50 ± 0.50*	6.5 ± 0.28**	15.1 ± 0.38***	223.2 ± 1.52*

Values are given as mean ± S.E.M. for group of eight animals each. The intergroup variation was measured by One-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test. $^{\#}p<0.05$, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ v/s saline treatment; $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ when compared with LPS alone group.

Effect of *Cymbopogen citratus* on behaviour of mice in light-dark box test

LPS injection to mice resulted significantly ($p<0.01$) $p<0.001$) decreased the number of entries (1.12 ± 0.30), time spent (38.62 ± 2.47) and transition (0.87 ± 0.199) in light compartment compared to normal control respectively. Treatment with *Cymbopogen citratus* (50, 100 and 200 mg/kg, p.o.) and Dexamethasone (1 mg/kg i.p.) prior to LPS shot significantly ($p<0.05$; $p<0.01$; $p<0.001$) increased the time spent in light compartment

(103.5 ± 1.97, 109.6 ±2.41, 117.37 ± 2.11, and 106.62 ± 1.57) and the number of transitions (1.75 ± 0.22, 3.5 ± 0.37, 4.12 ± 0.35 and 3 ± 0.28) with the significance (p<0.05; p<0.01) in comparison with the LPS treated group. (Fig:1)

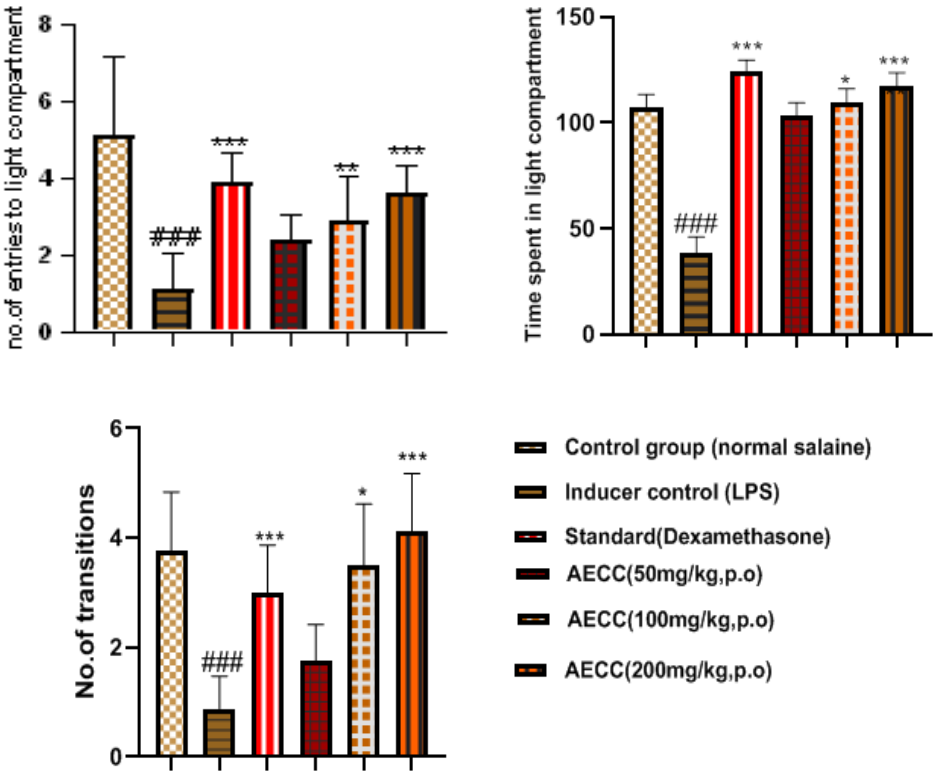

FIG. 1: Effects of pre-treatment of *C.citratus* on LPS-induced sickness behaviour of mice tested in the light-dark box.

3.3. OPEN FIELD TEST

The animals spontaneous locomotor activity was measured in an open field. LPS significantly (p<0.01 and p<0.05) decreased the peripheral (25.0 ±0.84) and central (2.5 ± 0.23) number of line crossings in comparison with the normal control group. However on pre treatment of *C. citratus* (50, 100 and 200mg/kg, p.o.).) inhibited the LPS induced decrease in line crossings and thus showed increased (74.62 ± 0.72, 82.25 ± 0.59 and 85.62 ± 0.59) and rear up climbs (6.25 ±0.80, 4.12 ± 0.38,and 2 ± 0.23). The lower dose of *C.citratus* was slightly more effective than the higher dose. (Table 2)

Table 2: Effects of pre-treatment of *Cymbopogen citratus* on LPS induced sickness behaviour in the open field test (OFT) (Table 2).

Groups	Treatments	Line crossings		Climbs	Rears
		Central		Peripheral	
1	Normal saline (1 ml/kg, i.p.)	7.75 ± 0.27	75.62 ± 0.59	10.5 ± 0.28	4.37 ± 0.28
2	LPS alone (0.83mg/kg,i.p.)	2.5 ± 0.23$^{###}$	25.0 ± 0.84$^{###}$	2 ± 0.23$^{###}$	1.25 ± 0.44$^{###}$
3	Dexamethasone (1 mg/kg, i.p.) + LPS (0.83 mg/kg, i.p.)	6.12 ± 0.30**	95.87 ± 0.34**	5.12 ± 0.42**	4.25 ± 0.22*
4	AECC (50 mg/kg,p.o) + LPS (0.83 mg/kg, i.p.)	4.75 ± 0.27**	74.62 ± 0.72	6.25 ±0.80***	5.12 ± 0.26***
5	AECC (100 mg/kg, p.o) + LPS (0.83 mg/kg, i.p.)	5.12 ± 0.19	82.25 ± 0.59**	4.12 ± 0.38	4.87 ± 0.26
6	AECC (200mg/kg, p.o.) + LPS (0.83 mg/kg i.p.)	6 ± 0.37	85.62 ± 0.59	2 ± 0.23	2.87 ± 0.26

Values are given as mean ± S.E.M. for group of eight animals each. The intergroup variation was measured by One-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test. The symbols denote the significance levels: #p<0.05, ##p<0.01 and ###p<0.001 v/s saline treatment; *p<0.05, **p<0.01 and ***p<0.001 when compared with LPS alone group.

3.4. SOCIAL INTRACTION AND FORCED SWIM TEST

Social exploration was measured two hour post LPS treatment. LPS associated social behaviour such as sniffing, crawling, and genital investigation was attenuated by pre-treatment with Cymbopogen citratus and Dexamethasone of C. citratus (50, 100 and 200mg/kg, p.o.) and Dexamethasone (1 mg/kg) showed significantly ($p<0.001$) increased (74.62 ± 0.86, 65 ± 0.62, 63.62 ± 0.89) and (72.12 ± 0.53) social interaction time respectively when compared to LPS alone group (Fig 2)

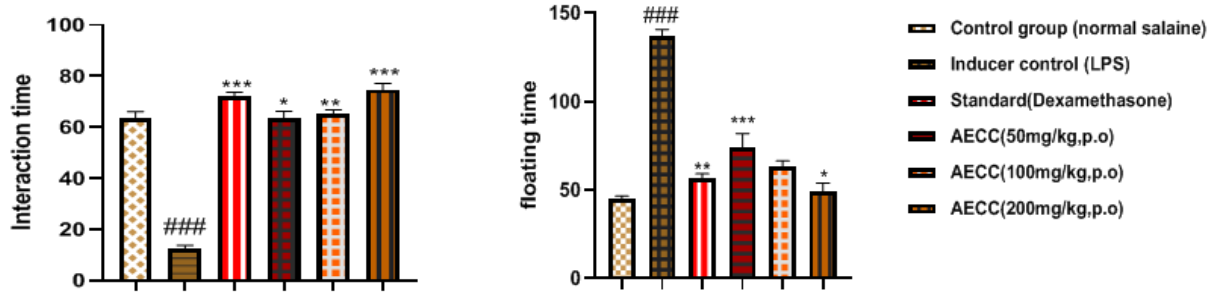

Fig. 2: Effects of pre-treatment of C.citratus on LPS-induced sickness behaviour on social interaction and forced swim test.

(A) Social interaction time and (B) Floating time; Values are given as mean ± S.E.M. for group of eight animals each. Results are shown as mean ± SEM. $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ v/s saline treatment; $^{***}p<0.001$ when compared with the LPS alone group.

BIOCHEMICAL ESTIMATION

Reduced glutathione (GSH)

GSH levels in the brain homogenates of LPS-treated mice were significantly lower ($p<0.001$) when compared to the control group. GSH levels were significantly ($p<0.001$) preserved by pretreatment with Dexamethasone (1 mg/kg). *C. citratus* (50, 100 and 200mg/kg, p.o.) showing enhanced GSH levels, but the results were insignificant. (Fig :3)

Lipid peroxidation (LPO)

MDA levels in LPS alone treated mice were significantly ($p<0.01$) higher than in the normal control group. Dexamethasone (1 mg/kg) and *C. citratus (*50, 100 and 200mg/kg, p.o.).) pre treatments significantly ($p< 0.01$) inhibited the LPS-mediated effects on MDA and thus showed reduced levels. (Fig :3)

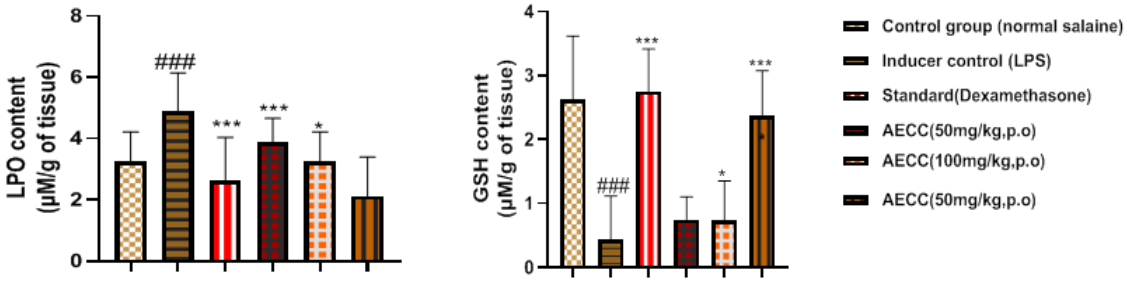

Fig. 3: Effects of pre-treatment of *C.citratus* on (LPS) on brain oxidative stress parameters in mice.

(A) Reduced glutathione and (B) Lipid peroxidation; Values are given as mean ± S.E.M. for group of eight animals each. Results are shown as mean ± SEM. $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ v/s saline treatment; $^{**}p<0.01$ and $^{***}p<0.001$ when compared with the LPS alone group.

Measurement of Total protein in brain tissue

The amount of protein in brain tissue was found to be lower than in the LPS treated inducer control ($p<0.001$). Whereas pretreatment with Dexamethasone and all doses of showed *C. citratus* (50, 100 and 200mg/kg, p.o.) ($p<0.01$) decreased protein content in brain tissues. (Fig:4)

Nitrite assay

Nitrite levels in the brain were higher after 24 hours of LPS exposure when compared to the vehicle control

group (p<0.001). Whereas, pretreatment with *C. citratus* (50, 100 and 200mg/kg, p.o.) prevented the LPS-induced increase in nitrite level in the brain and***p<0.001 when compared with the LPS alone group (Fig :4)

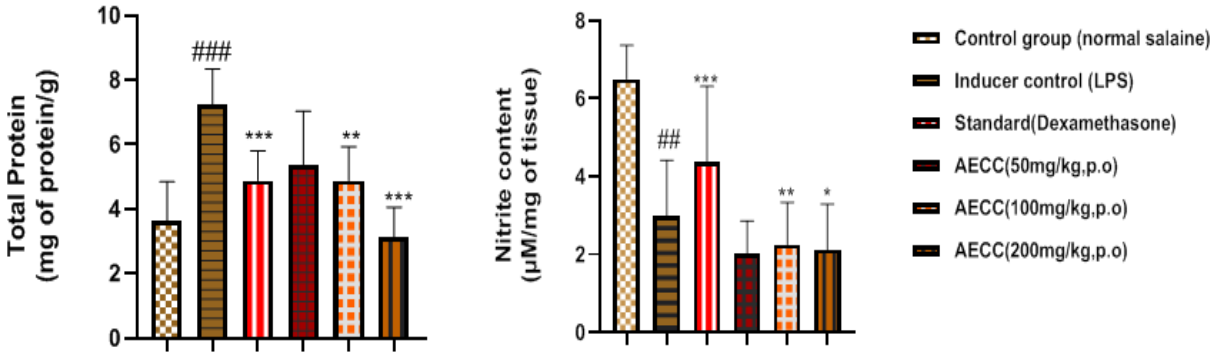

Fig. 4: Effects of Cymbopogen citratus on protein and nitrate content in LPS induced sickness behaviour in mice
(A) Total Protein and (B) Nitrate content; Values are given as mean ± S.E.M. for group of eight animals each. Results are shown as mean ± SEM. ##p<0.01 and ###p<0.001 v/s saline treatment; **p<0.01 and ***p<0.001 when compared with the LPS alone group.

POPOLYSACCHARIDE INDUCED ANOREXIA IN MICE

The mice model of LPS-induced anorexia has been widely used for evaluating potential anti- inflammatory compounds. The effect of *C.citratus* in LPS-induced anorexia was assessed by measuring the food and water intake, body temperature, body weight, aggression test and locomotor activity.

Effect of *Cymbopogen citratus* on food intake

LPS significantly (p<0.001) depressed food intake at 6h (0.37 ± 0.16) as well as 24 h (1.37 ± 0.23) food intake. Pre-treatment with *C.citratus* (50, 100 and 100 mg/kg) and Dexamethasone (1 mg/kg) significantly (p<0.01, p<0.001) and (p<0.05, p<0.01) reversed LPS induced anorexic effects and increased the food intake at 6 h (0.25 ± 0.14, 0.5 ± 0.16, 1 ± 0.10) and (1.5±0.16) 12 h (1.25±0.271.5±0.16, 2.62 ± 0.40) in comparison with the LPS alone treated group.

Effect of *Cymbopogen citratus* on water intake

LPS significantly (p<0.001, p<0.01) depressed water intake at 6 h (0.75±0.22) as well as overnight (1.2 ± 0.14) water intake. Prior treatment with *C.citratus* (50, 100, 200 mg/kg) and Dexamethasone (1 mg/kg) significantly (p<0.05, p<0.01 and p<0.001) reversed LPS induced effects and substantially increased the water intake at 6 h (0.87 ± 0.19, 1.37 ± 0.16, 1.75 ± 0.22 and 2 ± 0.28) and 24 h (6.0 ± 0.72, 6.5 ± 0.76, 7.4 ± 0.50 and7.2 ± 0.43) when compared to LPS treated group. (Table: 3)

Table: 3 Effects of pre-treatment of *Cymbopogen citratus* on food intake and water intake in LPS treated mice.

Group	Treatments	Food intake (g)		Water intake (ml)	
		6 h	12 h	6 h	12 h
1	Normal saline (2 ml/kg, i.p)	1.87 ± 0.19	3.5 ± 0.28	2.12 ± 0.199	6.3 ± 0.59*
2	LPS alone (0.83 mg/kg,i.p.)	0.37 ± 0.16###	1.37 ± 0.23###	0.75 ± 0.22###	1.2 ± 0.14###
3	Dexamethasone (1mg/kg, i.p.) + LPS (0.83 mg/kg, i.p.)	1.5 ± 0.16***	2.65 ± 0.37	2 ± 0.28***	7.2 ± 0.43***
4	AECC (50mg/kg, p.o.) + LPS (0.83 mg/kg, i.p.)	0.25 ± 0.14	1.25 ± 0.27*	0.87 ± 0.19	6.0 ± 0.72*
5	AECC (100 mg/kg, p.o.) + LPS (0.83 mg/kg, i.p.)	0.5 ± 0.16**	1.5 ± 0.16*	1.37 ± 0.16***	6.5 ± 0.76
6	AECC (200 mg/kg, p.o.) + LPS (0.83 mg/kg,i.p.)	1 ± 0.10**	2.62 ± 0.40**	1.75 ± 0.22*	7.4 ± 0.50*

Values are given as mean ± S.E.M. for group of eight animals each. The intergroup variation was measured by One-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test. The symbols denote the significance levels: ##p<0.01 and ###p<0.001 v/s saline treatment; *p<0.05, **p<0.01 and ***p<0.001, when compared with LPS alone group.

Effect of *Cymbopogen citratus* on body weight

There was a significant ($p<0.001$) decreased % (-11.62 ± 0.49) of body weight in mice treated with LPS when compared to control mice. Pre-treatment with *C.citratus* (50, 100 and 200 mg/kg) and Dexamethasone (1 mg/kg) showed Significant ($p<0.001$) increased % of body weight (-5 ± 0.33, -7 ± 0.23 and -6.12 ± 0.35) when compared to LPS treated mice. (Table:4)

Effect of *Cymbopogen citratus* on body temperature

LPS treated mice showed significantly ($p<0.001$) increased % of rectal temperature (4.87 ± 0.38) recorded on digital thermometer when compared to normal control mice. Pre-treatment with *C.citratus* (50, 100 and 200 mg/kg) and Dexamethasone (1 mg/kg) showed Significant ($p<0.05$, $p<0.001$) drop off in body temperature (2.6 ± 0.40, 2.3 ± 0.49, 2 ± 0.23 and 0.5 ± 0.16). (Table:4)

Table: 4 Effects of pre-treatment of *Cymbopogen citratus* on body weight and body temperature in LPS treated mice.

Groups	TREATMENTS	% Decrease in body weight	% Increase in Body temperature
1	Normal saline (1 ml/kg, i.p.)	-6.62 ± 0.40	0.37 ± 0.16
2	LPS alone (0.83 mg/kg, i.p.)	-11.62 ± 0.49$^{\#}$	4.87 ± 0.38
3	Dexamethasone (1 mg/kg, i.p.) + LPS (0.83 mg/kg, i.p.)	-4.75 ± 0.22***	0.5 ± 0.16**
4	AECC *CC* (50 mg/kg, p.o.) + LPS (0.83 mg/kg, i.p.)	-5 ± 0.33	2.6 ± 0.40
5	AECC (100 mg/kg, p.o) + LPS (0.83 mg/kg, i.p.)	-7 ± 0.23*	2.3 ± 0.49
6	AECC (200 mg/kg, p.o.) + LPS (0.83 mg/kg, i.p.)	-6.12 ± 0.35	2 ± 0.23*

Values are given as mean ± S.E.M. for group of eight animals each. The intergroup variation was measured by One-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test. The symbols denote the significance levels: $^{\#\#\#}p<0.001$ v/s saline treatment; $^{*}p<0.05$ and $^{***}p<0.001$ v/s LPS alone group

EFFECT OF *CYMBOPOGEN CITRATUS* IN AGGRESSION TEST

Spontaneous aggressive posturing was observed in animals treated with LPS (0.83 mg/kg). LPS treated groups showed significant ($p<0.01$) aggressiveness when compared to normal control mice (5.5 ± 0.40). Pre-treatment with *C.citratus* (200 mg/kg) and Dexamethasone (1 mg/kg), preceded diminished aggressive behaviour (2.7 ± 0.27and 2.8 ± 0.42) respectively, with significance ($p<0.05$) when compared to LPS treated group. (Table 5)

Effect of *Cymbopogen citratus* in Locomotor Activity

LPS treated groups showed significant ($p<0.001$) CNS depressant activity when compared to control mice (45.5 ± 0.74). However this depression was significantly ($p<0.00$, $p<0.05$) less with pre-treatment with *C.citratus* (200 mg/kg p.o) and Dexamethasone (1 mg/kg) (179±1.34) and 136.87±1.39) respectively, when compared with LPS treated group. (Table 5)

Table 5 Effects of pre-treatment of *Cymbopogen citratus* on Aggression test and locomotor activity LPS treated mice.

Groups	Treatments	Aggression (grading)	Number of beam Crossings
1	Normal saline (1 ml/kg, i.p.)	3 ± 0.44	312 ± 1.82
2	LPS alone (0.83 mg/kg, i.p.)	5.5 ± 0.40$^{\#\#\#}$	45.5 ± 0.74$^{\#\#\#}$
3	Dexamethasone (1 mg/kg, i.p.) +LPS (0.83 mg/kg, i.p.)	2.8 ± 0.42	136.87 ± 1.39*
4	AECC (50 mg/kg, p.o.) + LPS (0.83 mg/kg, i.p.)	3.5 ± 0.50*	104.62 ± 1.04
5	AECC (100 mg/kg p.o.) + LPS (0.83 mg/kg, i.p.)	3 ± 0.33	150.37 ± 1.90**
6	AECC (200 mg/kg p.o.) + LPS (0.83 mg/kg, i.p.)	2.7 ± 0.27**	179 ± 1.34

Values are given as mean ± S.E.M. for group of eight animals each. The intergroup variation was measured by One-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test. The symbols denote the significance levels. $^{\#\#}p<0.05$, $^{\#\#\#}p<0.001$ v/s saline treatment; $^{**}p<0.05$ and $^{***}p<0.001$ when compared with the LPS alone treated group

3.9. LIPOPOLYSACCHARIDE INDUCED SICKNESS BEHAVIOUR IN MICE.

Effect of *Cymbopogen citratus* on percentage preference for sucrose solution

The purpose of this study was to determine the effect of on LPS-induced anhedonia. The sucrose preference was evaluated after 2 and at 24 hours. Mice pre-treated with LPS showed a significant ($p<0.001$) decrease in sucrose preference at 2h (6.12 ± 0.58) and 24 h (5.12 ± 0.25) compared to the control group. (Fig:5)

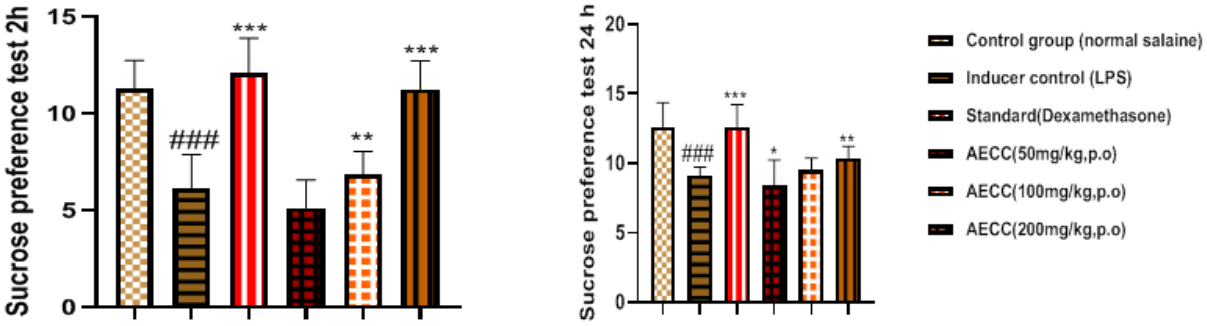

Fig: 5: Effects of pre-treatment of *Cymbopogen citratus* on sucrose preference test in LPS treated mice.
2h (A) and 24 h (B) time interval for preference of 10% sucrose. Values are given as mean ± S.E.M. for group of eight animals each. Results are shown as mean ± SEM. ###p<0.001v/s saline treatment; *p<0.05, **p<0.01 and ***p<0.001 when compared with the LPS alone group

3.10. EFFECT OF *CYMBOPOGEN CITRATUS* ON THERMAL HYPERALGESIA

C. citratus effect on LPS-induced thermal hyperalgesia was evaluated using the hot plate test, cold allodynia, and tail immersion tests. *C. citratus* (50, 100 and 200mg/kg, p.o.) administration abolished the hyperalgesia induced by LPS (0.83 mg/kg) and partially prevented the hyperalgesia

3.11. Effect of *Cymbopogen citratus* on hot plate test

LPS (0.83 mg/kg i.p.) treatment resulted in a significant (p<0.001) reduction in latency response (0.62 ± 0.16 sec) compared to the normal control (4.75 ± 0.54 sec). Pre-treatment with Dexamethasone, on the other hand, had a significant (p<0.001) influence on the animals' tail flicking time to the hot plate (2.62 ± 0.33). When compared to the LPS group, pre-treatment with *C. citratus* (50, 100 and 200mg/kg, p.o.) significantly influenced the increased reaction time (p<0.05). (Table: 6)

3.12. Effect of *Cymbopogen citratus* on tail immersion test

When compared to the normal control group, there is a decrease in tail withdrawal time in the hot water tail immersion test (1.25 ± 0.22) . Pre-treatment with *C. citratus* (50, 100 and 200mg/kg, p.o) and Dexamethasone (1 mg/kg) significantly (p<0.001) prevented the actions of LPS,(3.25 ± 0.27, 2.12 ± 0.26 , 2.75 ± 0.27, and 3.5 ± 0.16) thereby indicating the cessation of LPS-induced hyperalgesia. (Table: 6)

3.13. Effect of *Cymbopogen citratus* on cold allodynia

There is a significant (p<0.001) decrease in tail withdrawal time when administered with LPS alone (2.5 ± 0.16) in the cold allodynia test when compared to the normal control (3.75 ± 0.32). *C. citratus* (50, 100 and 200mg/kg, p.o) and Dexamethasone (1 mg/kg) pre-treatment significantly (p<0.001; p<0.001) inhibited LPS action (3.12 ± 0.2 , 4 ± 0.33, 4.25 ± 0.22and 5.62 ± 0.37 sec, respectively). These findings suggest that pre-treatment with all doses of *C. citratus* reversed the hyperalgesia caused by LPS injection. (Table: 6)

Table 6: Effect of pretreatment of *Cymbopogen citratus* on thermal hyperalgesia in LPS treated mice.

Groups	Treatments	Hot plate test	Tail immersion test (5 min)	
			Hot water	Cold water
1	Normal saline (1 ml/kg, i.p.)	4.75 ± 0.54	4 ± 0.23	3.75 ± 0.32
2	LPS alone (0.83 mg/kg, i.p.)	0.62 ± 0.16###	1.2 ± 0.22###	2.5 ± 0.16###
3	Dexamethasone (1 mg/kg, i.p.) + LPS (0.83 mg/kg, i.p.)	3 ± 0.23***	3.5 ± 0.16***	5.62 ± 0.37***
4	AECC (50 mg/kg, p.o) + LPS (0.83 mg/kg, i.p.)	1.37 ± 0.16	2.12 ± 0.26	3.12 ± 0.26
5	AECC (100mg/kg, p.o.) + LPS (0.83 mg/kg, i.p.)	1.75 ± 0.22	2.75 ± 0.27**	4 ± 0.33
6	AECC (200mg/kg, p.o) + LPS (0.83 mg/kg, i.p.)	2.62 ± 0.33	3.25 ± 0.27**	4.25 ± 0.22**

Values are given as mean ± S.E.M. for group of eight animals each. The intergroup variation was measured by One-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test. The symbols denote the significance levels: ##p<0.05, ###p<0.001 v/s saline treatment; *p<0.05, **p<0.01 and ***p<0.001, when compared with LPS alone group.

DISCUSSION

Numerous conditions, including chronic disease and depressive-like behavior, are linked to neuroinflammation. Sickness behavior is a normal homeostatic response triggered by the release of proinflammatory cytokines which produces devastating side effect of infections. If this proinflammatory activity is not stopped after healing from infectious illnesses, it may result in severe emotional, cognitive, and physical problems that impair quality of life. Animals can be made to exhibit disease symptoms by giving them cytokine-inducing substances like LPS. When LPS binds to immune cells, signaling cascades are activated, enhancing the expression of a number of genes, including those that code for the pro-inflammatory cytokines IL-1, IL-6, and TNF-α. In reaction to the released cytokines, CNS macrophages and microglia produce identical cytokines that target neuronal substrates and generate an infection.[28]

Dexamethasone, a potent synthetic glucocorticoid, exerts immunosuppressive and anti-inflammatory actions by preventing the synthesis of TNF- α and inflammatory cytokines. Dexamethasone was used as a standard reference to evaluate the efficacy of *C. citraus* against LPS-induced sickness behavior and anorexia.[29]

Inflammatory cytokines, that are responsible for of sadness, social anxiety, anorexia, and weight loss, are modulated by LPS-induced ROS. Weight loss is a known side effect of TNF-α and IL-6's centrally mediated influence on food intake suppression. This may be because peripheral cytokines directly reduce food intake by acting on glucose-sensitive neurons in hypothalamic nuclei such as the lateral hypothalamic arc.[30]

Symptoms of sickness reactions during sickness behavior include lethargy, hypersomnia, hyperalgesia, changes in body temperature, lack of concentration, decreased motivation and altered mood, decrease in locomotor activity, exploratory behavior, anorexia, adipsia, and anhedonia. The findings from experiments using the elevated plus maze, light-dark box, social behavior, forced swim, open field, food and water intake, and sucrose preference in LPS-challenged mice are corroborated by the results of these tests. Pre-administration of *Cymbopogen citratus* to LPS-challenged mice, on the other hand, reduces exploratory behaviour, locomotor abnormalities, anorexia, and anhedonia, implying that it has a protective effect against LPS-induced sickness and anorexia.

The effect of *Cymbopogen citratus* on LPS-induced hypoactivity and exploratory behaviour in the mice was studied using an open field test. When compared to LPS-alone-treated mice, pre-treatment with *Cymbopogen citratus* (50, 100 and 200 mg/kg p.o) resulted in a two to three times increase in peripheral, central, and total number of line crossings, climbs, and rears When compared to *Cymbopogen citratus* administration, pre-treatment of LPS-challenged mice with dexamethasone protected against LPS-induced hypoactivity to a similar extent.

Increased number of entries and time spent in the open arm in the plus maze test, and increased time in light compartment, number of transitions between compartments in the light and dark box tests indicate anxiolytic like activity of *Cymbopogen citratus* against LPS-induced anxiety like effect in the animals. Similar effects were seen in dexamethasone-treated animals, but they were more significant than with *Cymbopogen citratus*.

As previously stated, sickness behaviour exhibits many common characteristics with depression. As a result, when forced swim test was carried out to determine the effect of *Cymbopogen citratus* on LPS-induced depression-like behaviour, Pre-treatment with both doses of *Cymbopogen citratus* significantly reduced LPS-induced depression in the mice, by exhibiting reduction in floating time.

According to the literature review, LPS causes anhedonia, which is a major symptom of depression. In LPS-treated mice, pretreatment with dexamethasone had a strong antidepressant effect and was compared to *Cymbopogen citratus* at higher doses. The sucrose preference test revealed that *Cymbopogen citratus* had an anti-anhedonia effect against LPS challenged mice , which was validated by increase in sucrose preference in *Cymbopogen citratus* pre-treated animals compared to the LPS alone group. [11]

One of the most common indications of immune system activation is a decrease in food intake. Injections of LPS or cytokines cause a reduction in food intake. When compared to LPS-alone-treated mice, pre-treatment with *Cymbopogen citratus* dramatically limited LPS-mediated anorexia and demonstrated an increase in food and water intake, confirming *Cymbopogen citratus* anti-anorexic actions against LPS. Dexamethasone pre-treatment to LPS-treated mice resulted in total elimination of LPS-mediated anorexia;

In LPS injected animals, a social behaviour test was established to determine the subject's motivation to participate in social interaction with young conspecifics at various time intervals. The test revealed that LPS is linked to a decrease in social behaviour, which was minimized by pre-treatment with *Cymbopogen citratus*, indicating that *Cymbopogen citratus* has a protective effect. Peroxides and reactive oxygen species (ROS) are produced in large quantities by LPS, mainly by macrophages and infiltrating neutrophils [4]. ROS and peroxides are essential components of the host defence system and may impact sickness behaviour through the generation of NFBκB dependent cytokines[5]. Pre-administration of *Cymbopogen citratus* to LPS-injected mice dramatically inhibited LPS-induced increase in

LPO and decreased in GSH content in the brain these results further support the antioxidant activities of *Cymbopogen citratus*. When compared with dexamethasone *Cymbopogen citratus* showed good antioxidant activity against LPS-induced oxidative stress in mice brain Hence, the beneficial effects obtained in the present study might also because of the antioxidant activities of *Cymbopogen citratus*.

In summary, the authors conclude that *Cymbopogen citratus* holds shielding effects against LPS-induced sickness behaviour and anorexia in mice, which was evidenced by attenuation of behavioural and biochemical changes. Further studies are required to elucidate the exact mechanisms of protective assets of *Cymbopogen citratus*.

CONCLUSION

The authors conclude that *Cymbopogen citratus* exerts protective effects against LPS-induced sickness behaviour and anorexia in mice, which was evidenced by attenuation of behavioural and biochemical changes. This effect could be attributed to its anti-inflammatory, antioxidant properties due to presence of vital phytoconstituents like flavonoids in it. Further studies are required to elucidate the exact mechanisms of protective assets of *Cymbopogen citratus*.

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