Neuroimmune modulator role of of standardized *Ocimum sanctum* Linn. (Holy Basil) leaf extract in endotoxemic-driven behavioral- sickness deficits in female rodents

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Abstract

Activation of the innate immune system by bacterial endotoxins such as lipopolysaccharide (LPS) produces marked neurophysiological, neuroendocrine, and behavioural changes in animals, manifesting as sickness behaviours like hypophagia, weight loss, reduced locomotion, and depressive-like symptoms. This study evaluated chronic administration of ethanol extract of *Ocimum sanctum* Linn. leaves (EEOS) against LPS-induced sick behavioral deficits in rats. LPS (1 mg/kg i.p.) significantly reduced ambulation (10.67 vs. 50.50), grooming (2.83 vs. 11.0), social interaction duration, locomotor activity, and spatial memory performance, while elevating immobility and anxiety-like behavior. EEOS treatment (50, 100 & 200 mg/kg) dose-dependently improved these measures. In the open-field, EEOS 200 mg/kg restored ambulation (61.17), grooming (11.50), and lowered immobility (38.58), comparable to indomethacin. Elevated-plus maze tests showed increased open-arm entries and time (3.17 entries, 65.81 s) in EEOS 200 mg/kg rats versus LPS controls. Social interaction improved markedly, with reduced passive interaction time and restored exploratory contacts. EEOS also normalized body temperature (from >39 °C post-LPS to ~37.4 °C), mitigated weight loss (other groups 168 g vs 182 g control), and improved food and water intake. Radial-arm maze trials indicated fewer errors and shorter completion times in EEOS-treated animals, supporting cognitive benefits. Overall, chronic EEOS administration attenuated sickness and depressive-like behaviors induced by LPS, likely through immunomodulatory and antioxidant actions suppressing pro-inflammatory cytokines and modulating neurotransmitter systems. These findings support *Ocimum sanctum* as a promising natural intervention for inflammation-associated neurobehavioral disorders.

Keywords: *Ocimum sanctum*, LPS, endotoxemia, sickness behavior, cognition, neuroinflammation.

1. Introduction

Lipopolysaccharide (LPS) is regarded as a complex glycolipid localized in the outer membrane of gram-negative bacteria, either injected or generated during the course of infections, induces through various mechanisms, several pathophysiological conditions producing a constellation of hemodynamic, hematological, metabolic and neuroendocrine changes, called "sickness behavior" in humans or laboratory animals.[1-3] and is a key molecule in the pathogenesis of gram-negative endotoxemia, sepsis and septic shock.[4] These include regulated increases in body temperature[5,6] sleep[7] as well as depressive like signs,[8] activation of the hypothalamo-pituitary-adrenocortical-axis (HPA-axis),[9,10] suppression of body weight gain,[1,8] feeding,[8,11] drinking,[8,12] locomotor and exploratory activity and reduced social behavior[8] and alterations in brain neurotransmitter.[3,13] The effects of LPS, which has been under experimental research for several years, are due to the peripheral release of pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6 and tumor necrosis factor-alpha (TNFα) by activated monocytes and macrophages in the CNS.[14] The same effects can be obtained by systemic administration of the cytokine-inducer LPS, a component of the cell wall of Gram-negative bacteria.[8,15,16] LPS induces the expression of not only pro-inflammatory but also anti-inflammatory cytokines such as IL-10 and IL-13 in the brain.[17] Another important anti-inflammatory cytokine IL-4, although not synthesized in the normal brain is strongly expressed during brain injury, infection, experimental autoimmune encephalomyelitis, and neurodegenerative processes.[17,18] Thus it has been suggested that immunological activation with LPS or cytokines themselves may be interpreted by the CNS as a stressor, and that the immune system may act as a sensory organ for non-cognitive stimuli such as bacteria, tumors, viruses etc.[19,20] Many laboratories have reported that LPS treatment induces neurotoxicity via microglia activation in mixed neuron/glia cultures. Activated microglia produce large amounts of prostanoids, ROS, NO and proinflammatory cytokines such as TNF- α, IL-1, IL-6 and IL-8, AA metabolites, and quinolinic acid which are capable of sustaining inflammatory state and in turn, cause neuronal damage.[21,22] *Ocimum sanctum* (*O.sanctum* Linn. Holy basil, Family: *Labiatae*) is a well known, widely distributed and highly esteemed and a sacred medicinal herb especially for Hindus in the Indian subcontinent. Indian Materia Medica[23] describes the use of various extracts of *O.sanctum* leaves in a variety of disorders, like bronchitis, rheumatism and pyrexia.[24] Several recent investigations of *O.sanctum* indicating neuroprotective, antidepressive, antianxiety, antistress, antiulcer, adaptogenic, analgesic,

antipyretic, anti-inflammatory, immunomodulatory, cardioprotective, hyolipedemic, hypoglycemic, hepatoprotective, diuretic, radioprotective, anticarcinogenic and antioxidant properties have been reviewed by various authors[25-28] There has been a recent surge of interest in the behavioral effects of pro-inflammatory cytokines in behavioral and neuropsychopharmacology. Since *O.sanctum* is a well-known rasayan whose influence on sickness behavior has received very little attention especially about its effect on the feeding pattern and feeding rhythms that underlie the hypophagia, general behavior, locomotor activity, changes in body weight and pyrexia following LPS administration in female rats. If sickness behavior is the ineluctable result of the brain action of those proinflammatory cytokines that are released at the periphery during the course of an innate immune response or even in response to exteroceptive stressors, it becomes important to find out how this behavior is regulated. The present study was undertaken to elucidate the role of EEOS to the behavioral and pyrogenic responses induced by systemic LPS challenge. We anticipated that behavioral tests used in the present study could contribute to the evaluation of potential drugs effective in the prevention of sickness syndrome

.2. Materials and Methods

2.1. Animals

Adult 3-4-month-old female *Sprague-Dawley* rats weighing between 120-160 g were used. Animals were procured from the Central Animal House of the institute, housed in colony cages at an ambient temperature of 25 ± 2°C and 45-55% relative humidity with 12-hour light / dark cycles. They had free access to pellet chow (Brook Bond, Lipton, India) and water *ad libitum.* Animals were exposed only once to the experiments on any day of the study, performed between 0900 to 1700 hours to avoid the influences of circadian rhythm. A protocol for the use of animal studies was approved by the Institutional Animal Ethical Committee, under the regulation of CPCSEA, New Delhi (JSS/IAEC//PhCology/02/2004-05).

2.2. Plant material and extraction

The aerial parts of the plant *O.sanctum* were collected from Bhavani, Erode district, Tamil Nadu, India. It was taxonomically identified by the Survey of Medicinal Plants and Collection Unit, Ooty, Tamil Nadu, India, and a herbarium of the plant is preserved in the Department of Pharmacognosy,. The whole plant was washed, and leaves were separated from other aerial parts, freed from earthy material and shade dried with occasional sifting at room temperature.

Dried leaves were coarsely powdered (1.9 kg ± 0.5 dry basis) and subjected to extraction by cold maceration with 90% ethanol (17.38 % yield) at room temperature with continuous stirring (300 rpm) for 7 days, after defatting with pet ether (60-80°C). The solvents were evaporated with rotary vacuum and stored in a desiccator, and then made into a fine suspension using 0.5% Tween 80. The principle chemical constituents (rosmarinic and ursolic acid) of the extract were previously quantified using HPLC and LCMS.[27,28] The ethanol extract of *O.sanctum* leaves (EEOS) was then employed against LPS-induced deficits in rats to evaluate the effect on 'sickness behavior.'

2.3. Drug treatment

Escherichia Coli LPS (*serotype 055:B5*, Sigma Chemical Company, New Delhi, India) was dissolved in sterile 0.9% NaCl to give a concentration of 100 µg/ml and was used. Our experiment was designed to assess the effect of EEOS (50, 100 and 200 mg/kg; p.o) and indomethacin (10 mg/kg; i.p), administered 1 hour after a single intraperitoneal injection of LPS (1.0 mg/kg) on water and food intake, body temperature, locomotor activity and anxiety levels in non-fasted rats. The rats were divided into 6 groups, and except for the vehicle-treated group, all other groups were administered EEOS and indomethacin up to 21 days. The dose of LPS was determined in view of the results of a pilot study and our preliminary experiments that assessed the ability of EEOS to attenuate the LPS-induced sickness behaviour. The animals were subjected to the following behavioural studies immediately after a single LPS administration from day 1 to day 21.

2.4. Behavioural tests

The animals were observed for 45 minutes immediately after LPS injection on day 1. They were observed for any changes in behaviour and scored 5 when they exhibited any of the following behaviour, and scored 0 for normal behaviour. The psychological and physiological effects of immune activation following LPS injection resemble the characteristics of depression. The essential features of depression are depressed mood and loss of interest or pleasure in all, or almost all, activities (anhedonia). The following depressive-like symptoms were diagnosed and observed for scoring, which included: (1) appetite disturbance, (2) sleep disturbances, (3) psychomotor disturbance, (4) self-care behaviours, (5) aggression, (6) ambulation, (7) grooming and (8) faecal counts. These behavioural symptoms are collectively termed 'sickness behaviour', and may be an adaptive response to an infectious agent attack, rather than secondary to the

disease process itself and the fever that accompanies it. The general behaviour is scored relative to the 'sickness behaviour' exhibited by the animals.

2.5. Food intake

The measurement of food intake was studied by presenting pre-weighed food to the animals in all the groups immediately following LPS and drug treatment. The amount of food consumed by the animals (food intake g/g weight of rat) was evaluated by weighing the remaining amount of food, 24 hours after food presentation, with an accuracy of ± 0.1 g for 21 days. Spillage of food pellets was rare, but any obvious spillage was noted, and those data were excluded from the analysis. The food pellets were placed at a height accessible to the experimental animals (5cm from the floor of the cage), so they did not need to rear up to reach water and food.

2.6. Water intake

The animals in all the groups had free access to water during the entire duration of the study. Water intake was studied by measuring the volume of water (water intake mL/g body weight of rat) consumed over 24 hours for 21 days, following injection of LPS, in all the groups. Clean water was provided in graduated burettes with drinking spouts, allowing direct volumetric measurements of intake to the nearest 0.1 mL. The drinking spouts were placed at a height accessible to the experimental animals (5cm from the floor of the cage), so that they did not need to rear up to reach water.

2.7. Body weight

The body weight of the animals was monitored daily by weighing on a top-loading balance with an accuracy of ± 0.1 g. All measurements were made every day between 0830 and 0915 hours, immediately before administration of LPS or drug treatment, starting from the day of injection (day 1) and continued for 21 days thereafter. Changes in body weight were calculated by subtracting the weight of the animal obtained on every day from that of the animal's weight immediately before the first LPS injection and expressed as g% changes (changes in body weight per 100 g).

2.8. Measurement of body temperature

In addition to the above-mentioned behaviour parameters, the changes in body temperature were measured for 21 days following LPS and drug treatment. Rectal temperature was recorded using a digital thermometer. The thin probe of the thermometer was inserted about 5 mm into the rectum of the rats. The temperature was allowed to equilibrate for 15-30 seconds before readings

were taken. All the measurements were made at an ambient temperature of 25ºC ± 2ºC. The rectal temperature was recorded every 2 hours up to 8 hours (0900 hours), immediately after a single i.p. injection of LPS on day 1, as described earlier. Thereafter, the rectal temperature was recorded every 24 hours, immediately before the administration of EEOS from day 1 to day 21.

2.9. Anxiety

2.9.1. Open-field exploratory behaviour test

An open-field apparatus similar to that of Bronstein, 1972[29] made of plywood and consisting of a square (61×61×61cm), was used. The entire apparatus is painted black except for 6mm white lines that divide the floor into 16 squares. The open-field was lit by a 100W bulb focusing onto the field from a height of about 100cm above the floor. The entire room was kept dark during the experiment. Each animal was centrally placed in the test apparatus for 5 min, and the behavioural aspects of anxiety, such as ambulation, rearing, self-grooming, defecation and activity in central squares were recorded. The open-field apparatus was then cleaned using 5% ethanol before introducing the next animal to preclude the possible cueing effects of odours left by previous subjects. To minimise the possible influences of circadian changes on rat open-field behaviour, control and experimental animals were intermixed.

2.9.2. Elevated-plus maze behaviour test

The apparatus was made from wooden material. The floor in the maze was covered with a plastic mat, the maze compartments consisted of two open arms, 50×10cm and two enclosed arms of the same dimensions with 40cm high plastic walls. The arms extended from the central platform of 10×10cm to give the apparatus a plus sign appearance. The maze was mounted on a plexiglass base, 50cm above the floor. Experiments were carried out in a darkened and quiet room with a constant light of 15W, directed towards the apparatus. The light levels on the open and enclosed arms were equal. Animals were brought into the room 1 h before the start of the experiments. The rats were individually placed on the central square of the plus maze facing the open arm for a 5-minute test.[30] The control and experimental rats were intermixed. During that time, the number of entries in open and closed arms and the time spent in the open and closed arms were scored by direct observation. Arm entries were defined as the entry of all four paws into the arm.

2.9.3. Social interaction test

Rats were isolated singly for 5 days before the test. The social interaction arena was a dimly lit plexiglass box (60×60×35cm) with a solid floor. The rats received two 10-minute familiarization

sessions individually in the test arena, at an interval of 1 h, 24 h before final testing. On the next day, rats were paired on a weight basis and placed in the test area for 10 min. The time spent by the rat pair in active social interaction, characterized by sniffing, following, grooming, kicking, boxing, biting, wrestling or crawling over or under the partner, time of contact, duration of social interaction, locomotor and passive immobility were recorded.[31] Control and experimental rats were intermixed.

2.9.4. Ambulatory behaviour test

The spontaneous motor activity was determined in an automated cage rack photo beam activity system (Techno). This operates on photoelectric cells, which are connected in a circuit with a counter. When a beam of light falling on the photocell is cut off by the animals, a count is recorded. The rats were placed individually into a clear 45.7 cm X 23.5 cm X 20.3 cm activity cage with 4 photo beams spaced 11 cm apart on either side of the cage. Photo beams were positioned 52 mm above the cage floor. Testing occurred in a room with dim illumination, and the movement was studied for the next 5 minutes.[32] In order to reduce any neophobic response to the test conditions, the cages had been previously dirtied by rats other than those used for the test, and there was no cleaning between trials.

2.10. Depression

2.10.1 Forced-swim test

Rats were forced to swim individually in a glass jar (25 X 12 X 25cm^3) containing fresh water of 15 cm height and maintained at 25ºC (±3 ºC). After an initial 2-minute period of vigorous activity, each animal assumed a typical immobile posture. A rat was considered to be immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 min of a total 6 min test.[33] The changes in immobility duration were studied after administering drugs in separate groups of animals. Each animal was used only once.

2.11. Cognitive function

2.11.1. Radial-arm maze test

The effect of LPS, EEOS and indomethacin on learning and memory was evaluated in an eight-arm radial arm maze made of wood and elevated 50 cm above the floor level. The radial-arm maze consisted of an octagonal central platform, a 35 cm diameter arena, from which eight arms, each 80 cm long, 10 cm wide and 4 cm high, radiated at equal angles. The maze was located in

the center of the room. Each arm contained a food cup (1 cm deep) and was centered 2 cm from the terminal end of each arm. The testing room contained many extra maze cues and was dimly lit while sessions were in progress. At the beginning of each trial, the animals were given drug-free training for three days, for 10 min. On day 4, the rats were tested one session per day, with all eight arms baited with 1 pellet of food each. Rats were allowed to run freely in the maze until they collected all 8 pellets of food or 10 min had elapsed, whichever occurred first. The following parameters were recorded: (i) total number of errors, i.e., re-entry into baited arms that had already been visited during the session; (ii) total arm entries; and (iii) the number of days to learn the task.[34] The rats were declared learnt after reaching the performance of committing one mistake on three consecutive days.

2.12. Statistical analysis

The data are expressed as mean ± SEM from six observations in each group. The behavioural data were subjected to one-way analysis of variance (ANOVA), followed by Dennett's multiple comparison posttests. A probability level (p) of value of less than 0.05 was considered to be statistically significant. The statistical analysis was carried out using GraphPad Prism for Windows (GraphPad Prism Software; version 4.03; San Diego, California, USA).

3.1. Results & Discussions

3.1. Behavioural tests

General behavior

Treatment of LPS resulted in sickness, poverty in behavior and invariably all the animals exhibited calmness after the administration of LPS. Rats treated with LPS exhibited a prolonged sickness behaviour in comparison to the control rats (1.5 ± 0.91). While the LPS-treated group scored 4.5 ± 0.68, the scores of animals treated with EEOS were 4.0 ± 0.51, 3.5 ± 0.2 and 3.25 ± 0.32 for 50, 100 and 200 mg/kg, respectively. Indomethacin-treated animals exhibited little or no obvious change in behaviour (2.0 ± 0.36).

3.2. Food intake

LPS caused a significant decrease in food consumption in all the groups except the vehicle group Maximum suppression in food intake was observed, in all the groups in the first 24 hours following LPS injection (LPS group: -0.151 ± 0.0001 g/g body wt, $p<0.001$; EEOS 50 mg/kg: -0.114 ± 0.002 g/g body wt, $p<0.001$; EEOS 100: -0.133 ± 0.003 g/g body wt, $p<0.001$; EEOS 200 mg/kg: -0.122 ± 0.001 g/g body wt, $p<0.001$;) in comparison to control animals, which

consumed 0.162 g of food/gram body weight. The quantity of food intake recovered gradually over the course of study, in all the groups, and reached the values, equivalent to that of control rats (food intake: 0.243 ± 0.006 g/g body wt, $p<0.001$ on day 21), except in LPS- treated rats (food intake: 0.136 ± 0.002 g/g body wt, $p<0.01$ on day 21). Similarly, indomethacin-treated rats consumed 0.219 ± 0.005 g / g body wt ($p<0.01$ in comparison to the control and LPS group) on day 21.

3.3. Water intake

LPS insult significantly reduced (0.074 ml/g/day; $p<0.001$) water intake, indicating that parenteral administration of LPS caused prompt but transient suppression in water intake, in comparison to control rats (0.150 ml/g/day; $p<0.01$). The suppression of water intake was evident for the first 6 days. Post-treatment with EEOS (50, 100 and 200 mg/kg b.w.) significantly ($p<0.01$) attenuated the LPS-induced loss of water intake. Similarly, in comparison to LPS-treated rats, indomethacin-treated rats significantly consumed more water (0.110 ml/g/day; $p<0.01$, after 24 hours). The results of the EEOS-treated group are significantly ($p<0.01$) comparable with control rats

3.4. Body weight

Body weight changes monitored for 3 weeks following LPS challenge demonstrated a significant effect of EEOS treatment on body weight gain. Post-hoc analysis demonstrated that chronic EEOS treatment produced a dose-dependent significant increase in body weight gain. LPS caused a significant reduction in body weight (-19.34 ± 1.24 g and -15.13 ± 1.79 g, in the LPS-treated group), in comparison to the control rats after 24 and 48 hours, respectively. The body weight of the rats recovered slowly over the second and third day, and the body weight was found to be equivalent (in gram weight) to the mean body weight of the control rats, on day 9. However, the indomethacin-treated group displayed a complete attenuation of LPS-induced reduction in body weight gain (-5.8 ± 1.98g and -3.10 ± 0.68 g weight loss, after 24 and 48 hours, respectively, following LPS-treatment). At the end of 21 days of experimental period, the mean body weight of the LPS-treated rats was found to be 168.10 ± 1.04 g, while that in groups EEOS 50, 100 and 200 mg/kg treated rats were 172.32 ± 3.91, 180.36 ± 2.67 and 168.19 ± 2.56 g, respectively

3.5. Body temperature

Rats disturbed by handling due to injection responded with an increase in body temperature that lasted for about 120 minutes, which is regarded as a stress-induced rise in mean body temperature The mean body temperature was higher in LPS-treated animals, evoking a significant monophasic increase in mean body temperature, as early as at 4^{th} hour (39.52°C ± 0.003, $p<0.01$), whereas, at the same time, the mean body temperature observed in the control group was 37.42°C ± 0.003. Significant decrease in the mean body temperature was observed with varying doses of EEOS-treated groups, in comparison to control and LPS-treated groups (EEOS 50: 39.02°C ± 0.004, $p<0.0$; EEOS 100: 38.50°C ± 0.006, $p<0.01$; EEOS 200: 38.20°C ± 0.004, $p<0.01$). The least disturbance in mean body temperature was observed in the indomethacin-treated group, which showed 38.22°C ± 0.014 ($p<0.01$) after 4 hours of LPS injection. No further significant increase in body temperature was observed in any of the groups after 4 hours. Compared with the LPS-induced fever, the febrile responses of the other groups were abrogated almost completely. Injection of EEOS was found to significantly suppress the LPS-induced fever in rats.

3.6. Behavioural studies

The results of the experiment on the various doses of EEOS and LPS on the behaviour in the open-field test revealed significant differences among the groups in line crossings, rearing and grooming episodes. Post-hoc comparisons showed that each one of the groups administered with LPS manifested significantly fewer line crossings and rearing than saline-injected controls. Animals injected with high doses of EEOS crossed significantly more lines in comparison to the lower dose levels. Administration of indomethacin significantly attenuated the effects of LPS in the open field test. The data were obtained on the cage locomotor activity on days 1 and 21. In comparison to the controls (48.50 ± 2.33), the locomotion of LPS–treated rats (15.67 ± 1.20, $p<0.01$) was significantly decreased. The effects of LPS were significantly attenuated in rats following treatment with EEOS in doses employed (50 mg/kg: 21.67 ± 1.05; 100 mg/kg: 30.67 ± 2.17, and 200 mg/kg: 43.00 ± 1.21). Indomethacin (52.67 ± 1.99) significantly increased the ambulatory behaviour in rats. Our results confirmed that systemic LPS administration inhibits the consumption of water[12,35] and food,[8,11] reduces locomotor activity,[8,35] increases anxiety levels[36,37] and has pyrogenic properties.[5,38,39]. These findings are in accord with the results of previous experiments demonstrating that LPS treatment induces a response in brain neurotransmission[40,41] and activation of the HPA-axis.[40] This finding complements work showing

that the NSAIDs attenuated the decrease in body weight and sickness behaviour induced by LPS.[8,11,42,43] Despite extensive study, the mechanism of action of LPS in the brain has yet to be fully elucidated.[44,45] Several lines of investigation now suggest that its primary action in the brain may be mediated by an increase in the concentrations of pro-inflammatory cytokines and several autacoid factors.[46-49] Moreover, it has also been proposed that LPS may stimulate cerebral lipid peroxidation and oxidative damage through an increased production of reactive oxygen intermediates.[44,50,51] Based on this evidence, many of the tissue injuries induced by LPS could be mediated by an overproduction of reactive oxygen, free radicals, proteases and pro-inflammatory cytokine.[52]

The effect of LPS on line crossings and rearings may be viewed as a general depressive effect on locomotion as well as suppression of exploratory behaviour. These resorts are consistent with a recent report that LPS decreases locomotor and exploratory activity in different paradigms.[3,8] The effect of LPS on grooming represents the reduction in the self-care behaviours associated with sickness. Reduced grooming has been anecdotally documented,7 but to our knowledge but quantitatively has not been demonstrated before. LPS-induced depression of motor, exploratory and self-care behaviours may be a model for the adaptive behavioural response to infection and sickness.[2,7] Reduced locomotor and exploratory behaviours save body energy reserves that are required for the increased metabolic costs of fever and reduce heat loss that occurs from exposure of the body surface during locomotion. The effect of LPS on grooming may be secondary to its effect on general motor activity. Alternatively, it has been suggested that reduced grooming may be selectively adaptive during illness since it prevents heat loss from exposure of the skin surface and loss of water through saliva used in grooming.[7] The mechanism by which LPS produces its effect on behaviour could involve the production of cytokines such as IL-1, IL-6, TNF and/or production of other immune- or neural-derived factors, such as endogenous opioids. Indeed, exogenous administration of IL-1 was found to decrease exploratory behaviour in a multi-compartment chamber[3] as well as exploration of a juvenile conspecific and food-motivated operant behaviour.[53] Moreover, several investigations have demonstrated that some of the behavioural effects of LPS are mediated by IL-1 secretion. For example, administration of IL-1 receptor antagonist (IL-1 ra) was found to block the effect of LPS on social exploration and body weight.[1,2] Ford & Klugman (1980)[54] and Matsuzek & Ishikawa (1981)[55] reported that perturbations in neurotransmitter activities such as 5-HT and NA

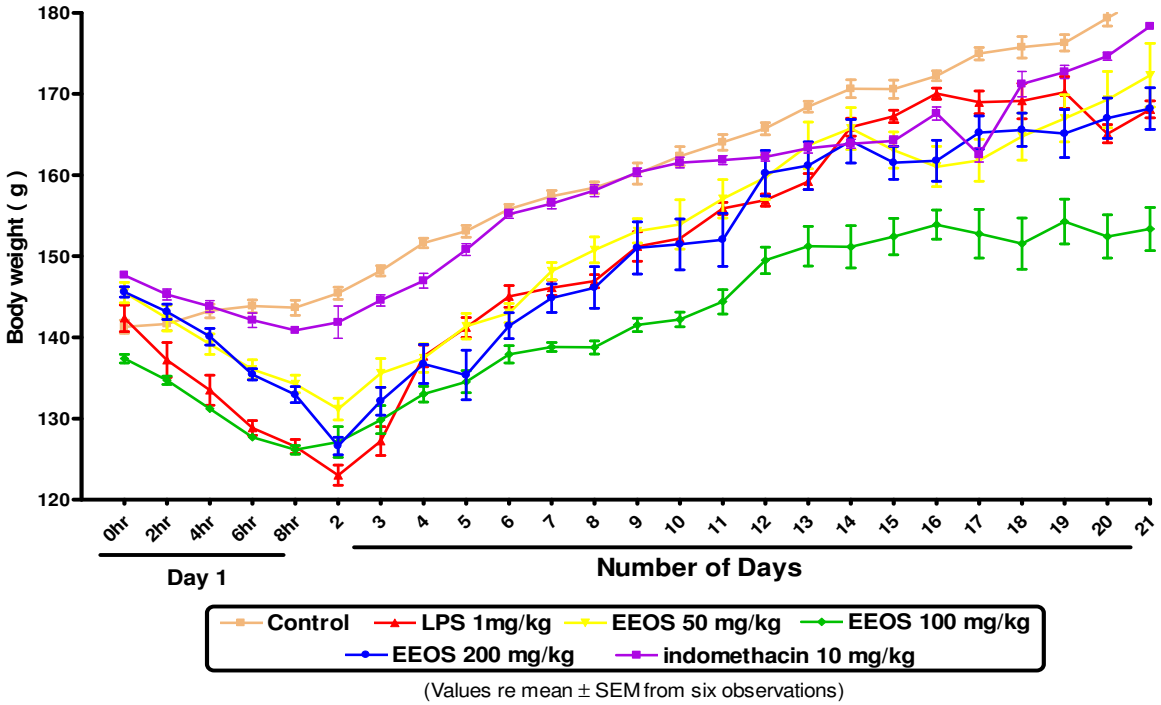
could account for the elevated body temperature following LPS administration. Other intermediates that could play a role in LPS-induced fever include PGs[56] and NO[57]. Cytokines such as IL-1α and ß, IL-6 and TNF-α and neuropeptides such as vasopressin and MSH are also believed to act as mediators of LPS-induced fever[5]. Sakina et al., (1990)[58] and Maity et al., (2000)[59] have reported on the antidepressant action of *O.sanctum* and further suggested that antidepressant drugs are clinically effective after chronic, but not acute treatment.[60] Similarly, in the present study, chronic EEOS treatment (for 21 days) may have produced adaptive changes in several neural systems, particularly monoaminergic systems and the hypothalamus-pituitary-adrenal axis (HPA-axis),[9,10,61,62] causing attenuation of LPS-induced suppression of food and water consumption and body weight gain, hyperthermia and general behaviour parameters of the rats. These findings provide further support for the similarity between LPS-induced immune activation, anxiety and depression. Activation of the HPA-axis alters neurotransmitter, immune, and behavioural functions and may contribute to the development of depressive symptoms in humans.[10,61,63,64.] In a report, De La Garza et al., (2004)[65] showed that LPS administration strongly increased corticosterone release, affecting behavioural parameters, which was significantly lower in diclofenac-treated rats. LPS-induced changes in the concentrations of biogenic amines in the hypothalamus are without a doubt significant contributors to the other central effects of LPS, such as anorexia, sleep and fever[1,5,8,11,35,38]. Thus, EEOS is considered to antagonise the effects of LPS on the HPA-axis, thereby normalising the behavioural parameters observed in this study. EEOS has been found to prevent stress-induced increase in plasma corticosterone and leukocyte count,[66] and normalise the stress-induced changes in central ACh and biogenic amines like NA, Adr, 5-HT, and 5-HT turnover in the brain following.[67] Hence, the inhibition of the rise in plasma corticosterone and its effect on central neurotransmitter levels by EEOS, in response to several stressors, may be considered to be responsible for normal behavioural parameters observed with EEOS-treated animals. PGE_2 levels of brain interstitial fluid rise following peripheral injection of LPS. Pharmacological blockade of PGE_2 synthesis attenuates many peripheral LPS-induced responses, such as fever,[68] brain *c-fos* expression, HPA-axis activation,[9] increased splenic sympathetic activity,[69] activation of serotonergic and noradrenergic neurotransmission in the hippocampus and increased blood-brain barrier permeability.[70] Increased production of PGE_2 in the brain, therefore, is critically involved in these CNS-linked responses to peripheral LPS on behavioural parameters. Hence, the positive

behavioural effects produced by EEOS may be attributed to its ability to inhibit and downregulate arachidonic acid metabolites, PGE_2 and COX activity, peripherally. Another possible explanation of the present result is that chronic treatment with EEOS suppressed LPS-induced activation of cytokine systems, which normally mediate the behavioural effects of LPS. Thus, it could be possible that the protective actions of EEOS might be through the suppression of free radicals. Ravindran et al., (2005)[71] and Samson et al., (2006)[72] have demonstrated that administration of EEOS has prevented the sub-acute noise stress-induced increase in the levels of neurotransmitters (NA, Adr, DA, 5-HT and 5-HT turnover, respectively) and a D_2 receptor agonistic action, in discrete rat brain regions. It is also known that stressful situations stimulate various areas of the hypothalamus and activate the HPA-axis. The changes in the brain neurotransmitter levels after the noise exposure are well protected and were brought towards normal levels in the EEOS-treated groups, indicating that some of the active principles present can cross the blood-brain barrier. Active principles present in *Ocimum* species, such as rosmarinic acid, lithospermic acid, and other phenolics, terpenoids, etc[73] have been attributed to be responsible for their diverse medicinal activities. Therefore, it can be assumed that the activity of *O.sanctum* in reducing the elevated neurotransmitter levels may depend on one or many of its active principles, such as roamarinic acid, ursolic acid, eugenol, methyleugenol, α,ß-caryophyllene, methylchavicol, linalool, 1,8-cineol, orientin, vicenin, etc which may act on the synthesis or reuptake of brain neurotransmitters. This hypothesis is well supported by the reports that *O.sanctum* can act through D_2 receptor and alleviate neurological disturbances. Moreover, it is also further reported that *O.sanctum* normalized the stress-induced membrane changes in the hippocampus and sensorimotor cortex. These reports also indicate that *O.sanctum* is a non-specific anti-stressor.[74] Therefore, this activity of *O.sanctum* and the probable interactions with the D_2 receptors can be assumed to play a major role in the normalisation of the brain neurotransmitter levels. The suppression of food and water intake, body weight and body temperature is considered to be symptoms associated with depression and termed as ‘sickness behaviour.’ The sickness behaviour is in some ways similar to depressive behaviour following LPS treatment.[75] Sakina et al., (1990)[58] and Maity et al., (2000)[59] have reported on the antidepressant action of *O.sanctum* and further suggested that antidepressant drugs are clinically effective after chronic, but not acute treatment.[61] Similarly, in the present study, chronic EEOS treatment (for 21 days) may have produced adaptive changes in several neural systems,

particularly monoaminergic systems and the HPA-axis[9,10,61,63] causing attenuation of LPS-induced suppression of food and water consumption and body weight gain, hyperthermia and general behaviour parameters of the rats. These findings provide further support for the similarity between LPS-induced immune activation, anxiety and depression. *O.sanctum* has been found to prevent stress-induced increase in plasma corticosterone and leukocyte count,[66] and normalise the stress-induced changes in central ACh and biogenic amines like NA, Adr, 5-HT, and 5-HT turnover in the brain.[67] Hence, the inhibition of the rise in plasma corticosterone and its effect on central neurotransmitter levels by *O.sanctum*, in response to several stressors, may be considered to be responsible for normal behavioural parameters observed with EEOS-treated animals. PGE_2 levels of brain interstitial fluid rise following peripheral injection of LPS. Pharmacological blockade of PGE_2 synthesis attenuates many peripheral LPS-induced responses, such as fever,[68] brain *c-fos* expression, HPA-axis activation,[9] increased splenic sympathetic activity,[69] activation of 5-HT and NA neurotransmission in the hippocampus,[14] and increased blood-brain barrier permeability.[70] Increased production of PGE_2 in the brain, therefore, is critically involved in these CNS-linked responses to peripheral LPS on behavioural parameters. Hence, the positive behavioural effects produced by EEOS may be attributed to its ability to inhibit and downregulate arachidonic acid metabolites, PGE_2 and COX activity, peripherally. Another possible explanation of the present result, is that chronic treatment with EEOS suppressed LPS-induced activation of cytokine systems, which normally mediate the behavioural effects of LPS. The role of receptors in the reduction of corticosterone levels was also suggested by Wilson et al., (1980).[76] This could fulfil the criteria suggested by Brekhman & Dardymov (1969)[77] that the adaptogenic agents could possess the normalising action irrespective of the direction of preventing pathological changes. The concepts of Brekhman & Dardymov (1969)[77] regarding the adaptogenic properties of *O.sanctum* have been accepted by several researchers in their investigations on the herb, like Sakina et al., (1990)[74] and Singh et al., (1991).[78] *O.sanctum* was found to prevent inflammation, generation of ROS against various stressors (stress-induced increase in plasma corticosterone[66,79] and leukocyte count, organ weight[80] and neutrophil function,[81] plasma lipid profiles.[82] This reduction in neurotransmitter level and other blood profile strongly suggests that *O.sanctum* may be responsible for attenuating the behavioural sickness-like parameters. Hence, it is now evident that ethanol extract of *O.sanctum* prevented the changes in stress-induced indices like plasma corticosterone level, leukocyte count,

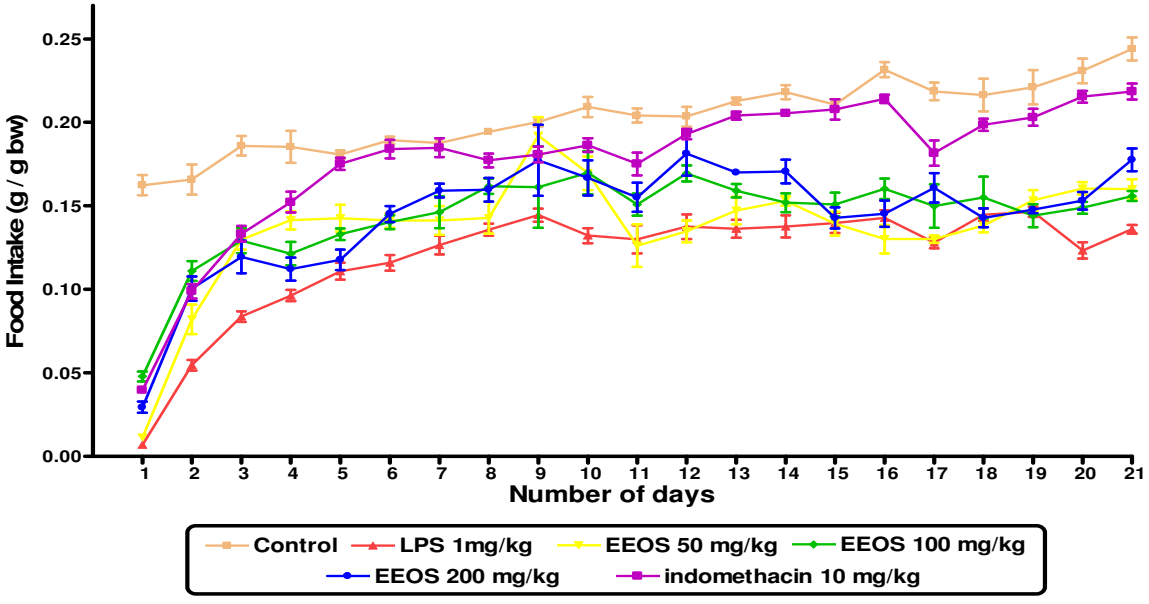
neutrophil function and plasma lipid profiles, and thus acted peripherally as well. The CNS plays an important role in the development of stress-induced ulcers, and *O.sanctum* was found to prevent the development of ulcers during stressful conditions. In the light of all these observations, it could be speculated that the CNS might be one of the important sites of action of *O.sanctum* extract. Although the protective effect of *O.sanctum* on CNS against several stressors[83-89] has been well documented and established, the mechanism of action of *O.sanctum* extract on IL-1 receptor antagonist has to be investigated and elucidated. Much attention had been focused on immuno-regulatory changes during stress, and various reports indicate that *O.sanctum* had prevented the stress-induced immunosuppression in rats. In summary, LPS produced significant locomotor decrements and other general behaviour parameters more robustly. In addition, LPS caused significant changes in body weight, food and water intake and body temperature. The behavioural effects were significantly attenuated by EEOS in a dose-dependent manner. Since treatment with EEOS antagonised the behavioural and pyrogenic effects induced by LPS, we could believe in an interaction between LPS administration and various apparatus involved in sickness behaviour.

Figure 43. Effect of EEOS, indomethacin on body weight changes in LPS- treated rats



(Values re mean ± SEM from six observations)

Figure 44. Effect of EEOS, indomethacin on food intake in LPS-treated rats



(Values are mean ± SEM from six observations)

Figure 45. Effect of EEOS, indomethacin on water intake in LPS-treated rats

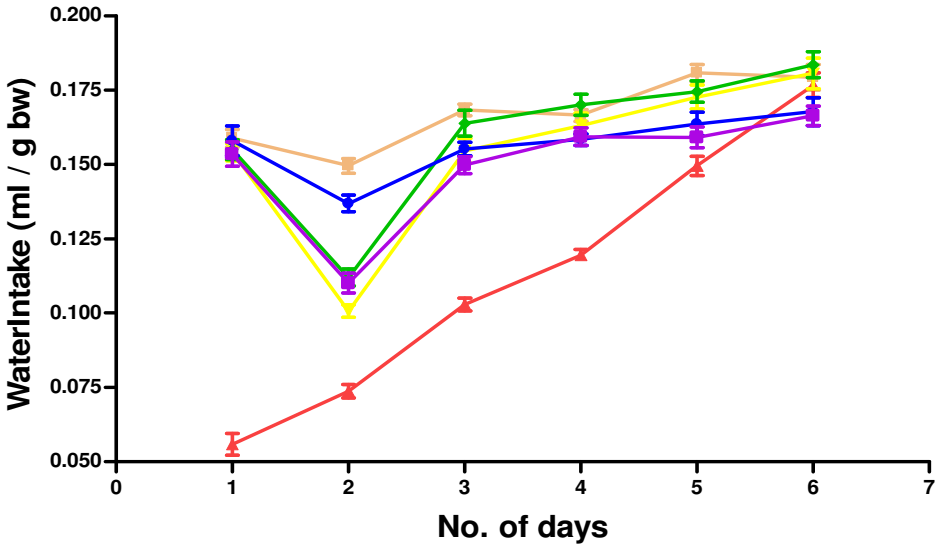
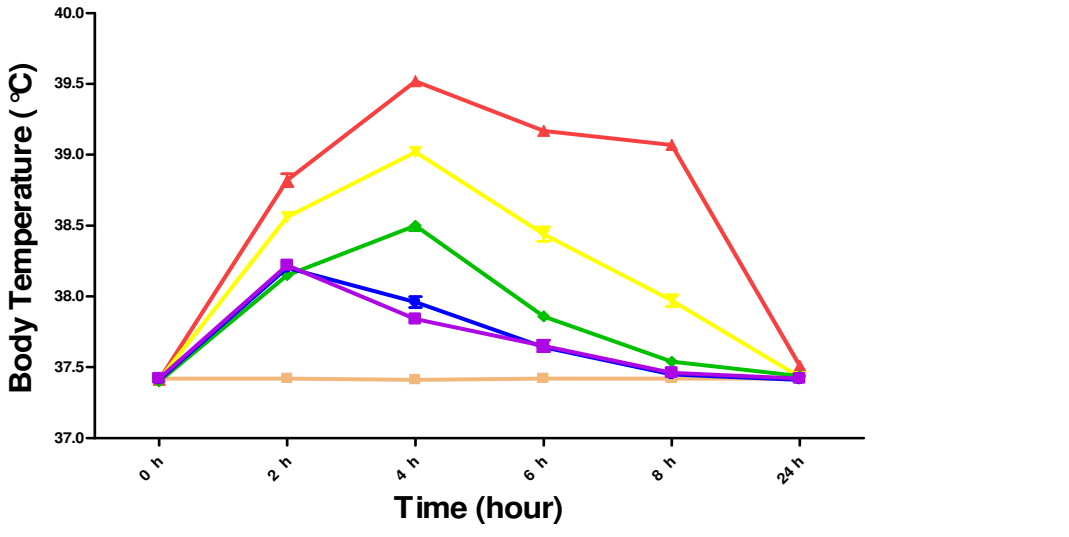


Figure 46. Effect of EEOS, indomethacin on body temperature in LPS-treated rat



(Values are mean ± SEM from six observation)

Table 1: Effect of EEOS on various drug treatments on Open-field exploratory behaviour in LPS-treated rats on day 1 and day 21

Groups	Day	Ambulation	Rearing	Grooming	No. of FP	Central Sq	Immobility
Control	01	50.50^{dgk} ±2.39	17.50^{dg} ±1.38	11.00^{dg} ±0.51	1.83^{dh} ±0.16	1.17^{n} ±0.16	31.05^{dgk} ±1.57
	21	53.83^{dg} ±2.91	18.50^{dgk} ±1.17	12.33^{dgn} ±0.61	1.67^{e} ±0.21	2.00^{fj} ±0.44	34.76^{dgk} ±1.54
LPS	01	10.67^{agk} ±0.55	4.50^{ajk} ±0.22	2.83^{ajk} ±0.40	3.67^{an} ±0.33	0.33^{m} ±0.21	114.1^{agk} ±6.26
	21	15.50^{agk} ±0.88	2.83^{agk} ±0.30	3.17^{ajk} ±0.30	3.00^{bjk} ±0.36	0.17^{cm} ±0.16	105.3^{agk} ±4.01
EEOS 50 mg/kg	01	20.17^{adk} ±0.70	8.17^{aek} ±0.30	5.83^{afn} ±0.47	3.17^{b} ±0.30	0.50^{m} ±0.22	82.63^{adn} ±3.13
	21	31.33^{adk} ±1.66	7.33^{adk} ±0.42	6.33^{afn} ±0.55	2.00^{fm} ±0.25	2.00^{f} ±0.25	84.20^{adk} ±2.88
EEOS 100 mg/kg	01	39.51^{adg} ±0.80	14.67^{dg} ±0.76	8.83^{dj} ±1.04	2.33^{e} ±0.21	2.83^{ceh} ±0.30	69.80^{adj} ±3.54
	21	48.83^{dg} ±1.01	12.00^{adg} ±0.57	9.17^{cdj} ±0.87	0.83^{dh} ±0.16	2.50^{e} ±0.34	55.47^{adg} ±1.46
EEOS 200 mg/kg	01	54.50^{dgk} ±1.33	19.83^{dgk} ±0.74	9.50^{dh} ±0.76	2.17^{ej} ±0.30	4.50^{adgn} ±0.76	43.84^{dgk} ±1.44
	21	61.17^{cdgk} ±1.35	15.67^{cdgm} ±0.88	11.50^{dg} ±0.88	0.33^{bdg} ±0.21	3.83^{cdj} ±0.65	38.58^{dgk} ±1.80
Indomethacin 10 mg/kg	01	60.17^{adgk} ±1.85	21.00^{cdgk} ±0.68	10.17^{dg} ±0.79	2.33^{e} ±0.21	3.00^{cdh} ±0.51	50.79^{bdgm} ±1.81
	21	63.67^{bdgk} ±1.78	15.83^{dgm} ±0.65	11.67^{dg} ±0.71	1.50^{d} ±0.22	3.17^{d} ±0.54	48.22^{bdg} ±2.14

Values are expressed in mean±SEM; $^{a}p<0.05$ $^{b}p<0.01$, $^{c}p<0.001$ compared with control and $^{x}p<0.05$ $^{y}p<0.01$, $^{z}p<0.001$ compared with LPS

Figure 1: Effect of EEOS in Open-Ffeld exploratory behaviour in LPS-treated rats on day 1 and day 21

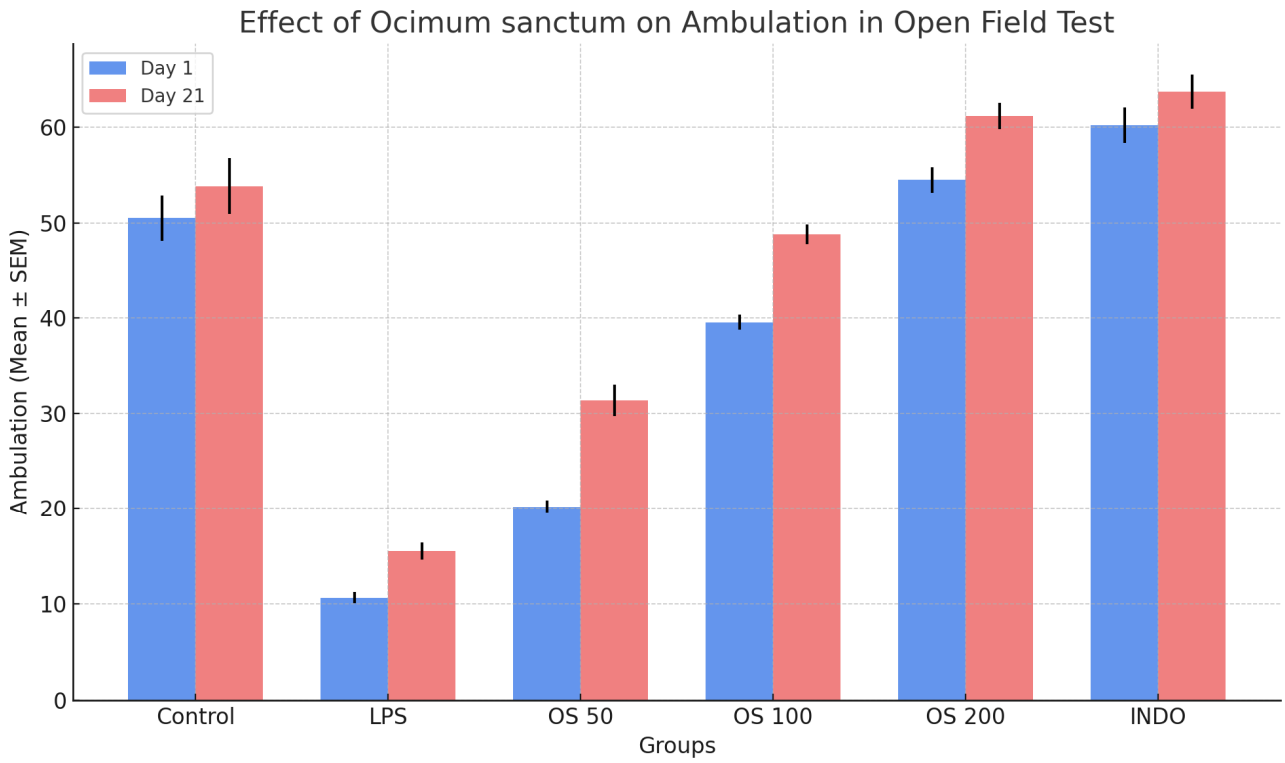


Table 2: Effect of EEOS on various drug treatments on Elevated-plus maze behaviour in LPS-treated rats on day 1 and day 21.

Groups	Day	No. of Entries		Time Spent		Rearing	No. of FP
		Open	Closed	Open	Closed		
Control	01	2.67^{dgm} ±0.33	6.17^{dhk} ±0.47	72.27^{dg} ±3.50	227.3^{dg} ±4.04	11.67^{dgk} ±1.08	1.33^{d} ±0.21
	21	3.50^{dgn} ±0.34	6.33^{dgk} ±0.49	70.44^{dg} ±1.13	229.5^{dg} ±1.13	11.67^{dgk} ±1.08	1.33^{d} ±0.21
LPS	01	0.50^{an} ±0.22	8.83^{agk} ±0.30	29.97^{ahk} ±1.19	270.0^{agk} ±1.19	1.50^{ak} ±0.22	5.67^{agk} ±0.55
	21	0.50^{ak} ±0.22	9.17^{agk} ±0.16	15.80^{agk} ±1.33	284.3^{agk} ±1.25	1.50^{ajk} ±0.22	5.67^{agk} ±0.55
EEOS 50 mg/kg	01	1.17^{a} ±0.16	4.50^{bd} ±0.22	50.71^{adm} ±1.60	249.3^{adm} ±1.59	2.33^{am} ±0.21	1.17^{d} ±0.30
	21	1.33^{am} ±0.21	3.83^{ad} ±0.30	48.88^{adn} ±2.77	250.9^{adn} ±2.70	3.83^{afm} ±0.30	1.50^{d} ±0.22
EEOS 100 mg/kg	01	1.50^{bf} ±0.22	3.67^{ad} ±0.21	68.24^{dh} ±4.33	231.7^{dh} ±4.33	5.17^{adh} ±0.30	1.83^{d} ±0.30
	21	2.50^{cdh} ±0.22	4.17^{ad} ±0.16	61.23^{dj} ±3.97	238.7^{dj} ±3.97	6.83^{adh} ±0.30	0.83^{d} ±0.16

EEOS 200 mg/kg	01	1.83^{e} ±0.16	3.00^{adj} ±0.25	77.68^{dg} ±2.45	222.3^{dg} ±2.45	6.50^{adg} ±0.42	2.67^{cdj} ±0.21
	21	3.17^{dg} ±0.16	3.00^{adn} ±0.25	65.81^{dg} ±1.92	234.1^{dg} ±1.93	7.00^{adh} ±0.36	0.67^{d} ±0.21
Indomethacin 10 mg/kg	01	1.00^{a} ±0.25	4.17^{bd} ±0.47	62.33^{d} ±5.97	232.6^{dh} ±3.76	4.33^{ae} ±0.55	2.33^{d} ±0.33
	21	1.50^{afn} ±0.22	4.50^{ad} ±0.22	59.06^{cdj} ±3.62	238.9^{dj} ±3.62	4.67^{aen} ±0.42	1.67^{d} ±0.33

Figure 2: Effect of EEOS on Elevated-plus maze behaviour in LPS-treated rats on day 1 and day 21.

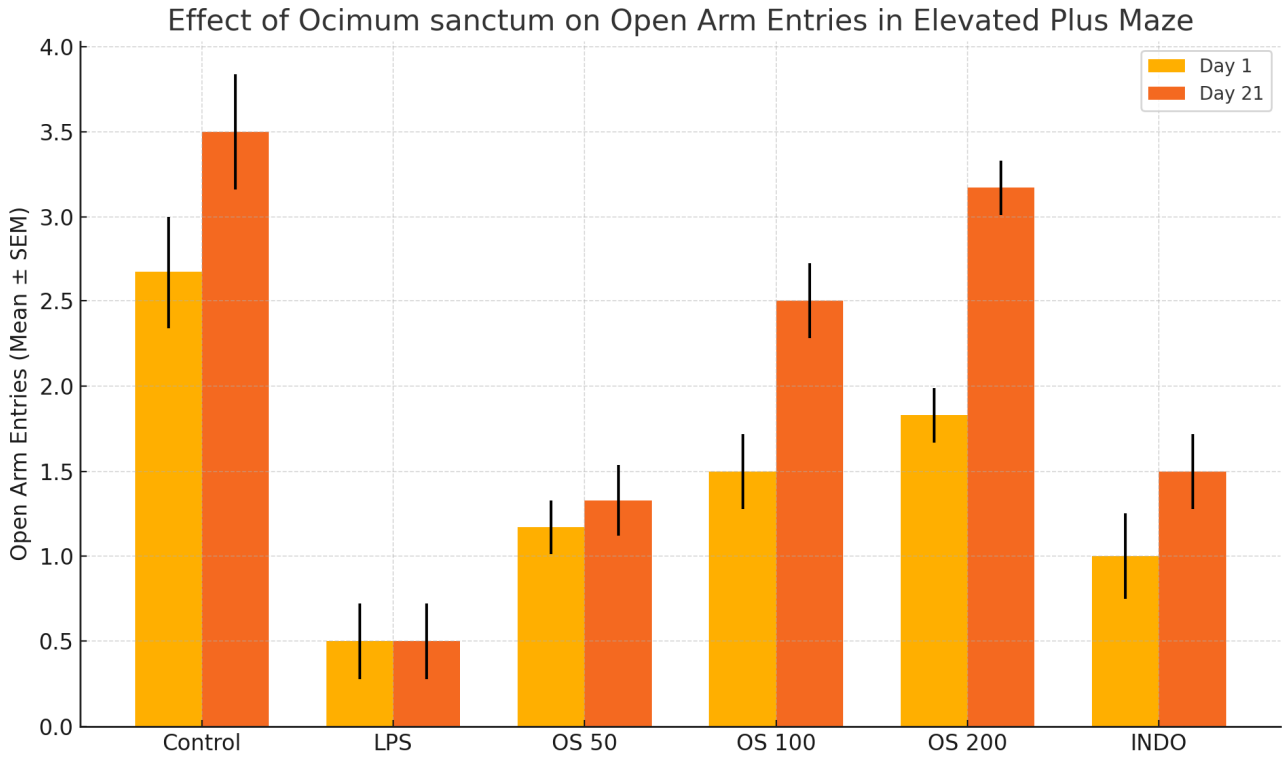


Table 3: Effect of EEOS on various drug treatments on Social Interaction Tests in LPS-treated rats on day 1 and day 21.

Groups	Day	Time of Contact	Duration of SIT	Duration of PIT	# of FP
Control	01	5.97^{dgk} ±0.78	424.9^{dgn}±24.27	25.37^{dgm} ±2.83	4.33^{d} ±0.61
	21	6.02^{dgn} ±0.55	420.2^{dgk} ±7.85	26.37^{dgk} ±1.60	4.50^{dk} ±0.34

LPS	01	28.55^{agk} ±1.45	250.9^{ahk} ±2.75	92.05^{agk} ±4.05	9.33^{agk}±0.49
	21	23.63^{agk} ±1.18	271.8^{agk} ±4.03	92.95^{agk} ±1.85	8.00^{agk}±0.25
EEOS 50 mg/kg	01	21.33^{adk} ±0.58	308.6^{aek} ±3.84	70.87^{adk} ±2.76	5.67^{dm}±0.55
	21	14.65^{adk} ±0.86	334.4^{adk} ±2.70	70.15^{adk} ±3.08	5.33^{dk} ±0.42
EEOS 100 mg/kg	01	11.75^{adg} ±0.68	375.5^{cdg} ±4.00	39.35^{bdg} ±2.22	3.00^{dh} ±0.63
	21	9.08^{cdg} ±0.56	369.0^{adg} ±2.72	41.72^{adg} ±1.48	2.33^{adg}±0.42
EEOS 200 mg/kg	01	8.78^{dg} ±0.31	401.3^{dg} ±2.29	29.47^{dg} ±0.96	1.67^{bdg}±0.33
	21	8.10^{dg} ±0.23	383.6^{adg} ±2.57	29.42^{dgk} ±1.29	1.17^{adg}±0.16
Indomethacin 10 mg/kg	01	14.07^{adg} ±0.52	362.0^{bdh} ±8.05	53.38^{adgm}±1.97	3.17^{dh} ±0.40
	21	10.97^{adh} ±0.50	356.2^{adj} ±7.23	52.23^{adgm}±1.86	1.83^{adg}±0.30

Figure 3: Effect of EEOS on Social Interaction tests in LPS-treated rats on day 1 and day 21.

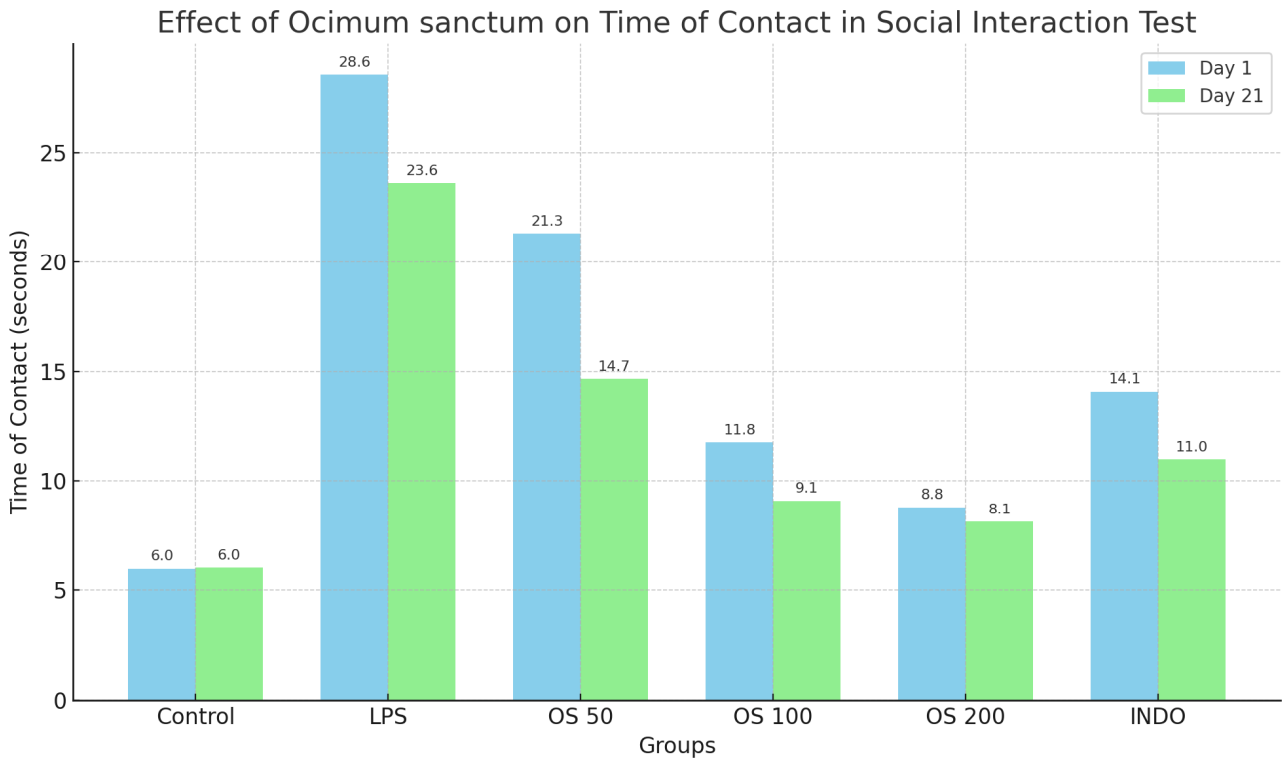


Table 4: Effect of EEOS on various drug treatments on locomotor activity in LPS-treated rats on day 1 & day 21.

Groups	Locomotor activity (Day 1)	Locomotor activity (Day 21)
Control	47.67^{dgk} ±1.78	45.17^{dgk} ±1.97
LPS	6.00^{agk} ±0.57	19.17^{ak} ±1.01
EEOS 50 mg/kg	20.67^{adm} ±1.14	23.00^{am} ±0.81
EEOS 100 mg/kg	28.17^{adh} ±1.04	30.50^{adh} ±1.28
EEOS 200 mg/kg	36.17^{adgm} ±1.24	34.50^{adg} ±1.17
Indomethacin 10 mg/kg	35.00^{adgm} ±2.14	33.67^{adg} ±1.76

Figure 4: Effect of EEOS on Locomotor activity in LPS-treated rats on day 1 & day 21.

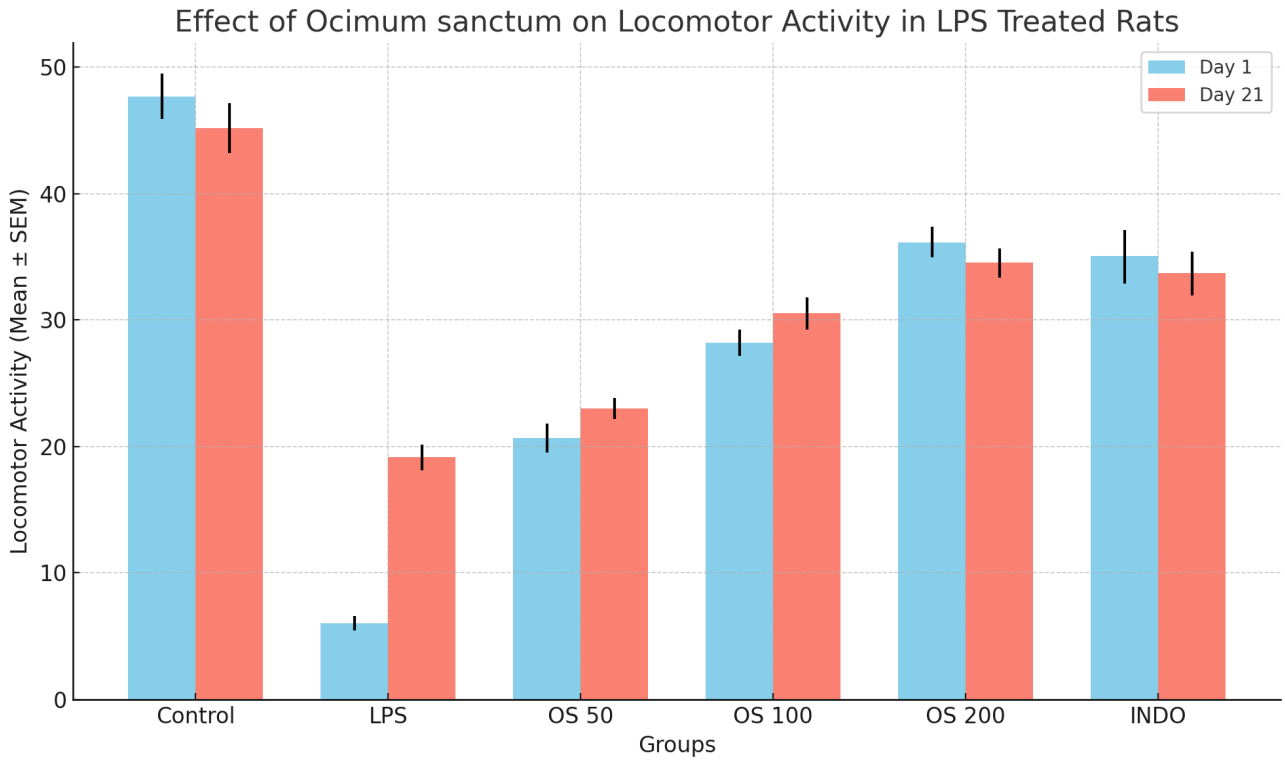


Table 5: Effect of EEOS on various drug treatments on Radial Arm Maze activity in LPS-treated rats on day 1

Groups	Trials	Errors	Time	Days
Control	$11.50^{j} \pm 0.22$	$2.67^{dgk} \pm 0.21$	$183.36^{d} \pm 4.68$	$5.17^{dgk} \pm 0.30$
LPS	11.83 ± 0.40	$6.17^{agk} \pm 0.16$	$257.62^{agk} \pm 6.69$	$11.67^{agk} \pm 0.33$
EEOS 50 mg/kg	$12.66^{c} \pm 0.33$	$4.67^{ad} \pm 0.33$	$200.38^{d} \pm 6.15$	$9.17^{ad} \pm 0.30$
EEOS 100 mg/kg	12.17 ± 0.30	$4.17^{ad} \pm 0.16$	$200.27^{d} \pm 6.32$	$8.17^{ad} \pm 0.30$
EEOS 200 mg/kg	$11.33^{j} \pm 0.20$	$3.66^{cdj} \pm 0.21$	$180.66^{d} \pm 5.50$	$8.17^{ad} \pm 0.47$
Indomethacin 10 mg/kg	$11.33^{j} \pm 0.21$	$3.50^{dj} \pm 0.34$	$195.07^{d} \pm 6.25$	$7.33^{adh} \pm 0.21$

Table-6: Effect of EEOS on LPS-Body Weight

Groups	**0hr**	**2hr**	**4hr**	**6hr**	**8hr±**
Control	141.3 ± 0.81	141.6 ± 0.78	143.3 ± 0.85y	143.8 ± 0.79y	143.6 ± 0.94y
LPS	142.3 ± 1.64	137.2 ± 2.17	133.5 ± 1.84b	128.8 ± 0.92b	126.5 ± 0.87b
EEOS 50 mg/kg	145.5 ± 1.28a	142.3 ± 1.57x	139.2 ± 1.27y	136.1 ± 1.18by	134.2 ± 1.07by
EEOS 100 mg/kg	137.4 ± 0.54ay	134.7 ± 0.51b	131.2 ± 0.28b	127.7 ± 0.36b	126.1 ± 0.51b
EEOS 200 mg/kg	145.6 ± 0.64a	143.1 ± 0.94y	140.1 ± 1.02y	135.4 ± 0.69by	132.9 ± 0.99by
Indomethacin 10 mg/kg	147.7 ± 0.30by	145.3 ± 0.68y	143.8 ± 0.72y	142.1 ± 0.88y	140.9 ± 0.38y

4. Summary and Conclusion

The study investigated the endotoxemia-induced neuroprotective and behavioural effects of chronic treatment with ethanol extract of *O.sanctum* (EEOS) Linn. (Holy Basil) in female rats subjected to lipopolysaccharide (LPS)-induced sickness behaviour, compared across behavioural assays and biochemical parameters:

(1) Open-field exploratory behaviour: LPS group showed marked behavioural suppression (↓ ambulation, ↓ rearing, ↑ immobility) on both days, indicating sickness-like behaviour (Table 1 & Fig. 1). Treatment with EEOS at all doses significantly improved exploratory activity in a dose-dependent manner, with EEOS 200 mg/kg showing near-normalisation, comparable to indomethain (standard drug 10 mg/kg).

(2) Elevated-plus maze behaviour: LPS rats had fewer open arm entries and less time spent in open arms, consistent with increased anxiety-like behaviour (Table 2 & Fig. 2). EEOS treatment improved entries and time in open arms, particularly with EEOS 100 and 200, indicating anxiolytic effects. Rearing behaviour and faecal pellet count (FP), indicators of emotional reactivity, were normalised with higher EEOS doses.

(3) Social interaction test behavior: LPS significantly disrupted social behaviour (↑ time of contact, ↓ SIT duration, ↑ PIT), suggesting social withdrawal and fatigue (Table 3 & Fig. 3). EEOS, especially at 100 and 200 mg/kg, significantly restored social behaviours toward normal values, indicating anti-sickness and pro-social effects.

(4) Ambulatory behavior: LPS induced a dramatic reduction in spontaneous locomotor activity (Table 4 & Fig. 4). EEOS treatment improved locomotion in a dose-dependent fashion, again with EEOS 200 mg/kg showing the best effect, similar to indomethacin.

(5) Radia-arm maze behavior: LPS rats showed increased errors, longer time, and delayed learning, indicating memory impairment (Table 5). EEOS treatment significantly reduced errors and task completion time, particularly at 200 mg/kg, suggesting cognitive improvement.

(6) Body weight: The LPS group experienced significant and progressive body weight loss, consistent with systemic illness (Table 6). EEOS 200 mg/kg and indomethacin groups prevented significant weight loss, suggesting systemic protection and reduced inflammatory burden.

Though further research may establish the exact role of various biochemical apparatus in attenuating the endotoxemic effects of LPS, it may be concluded, that chronic administration of EEOS (Holy Basil) extract significantly attenuated LPS-induced behavioural impairments, including reduced locomotion and exploration, increased anxiety and social withdrawal, memory deficits and cognitive dysfunction, systemic sickness behaviours including weight loss. Among the doses tested, 200 mg/kg of EEOS showed the most profound protective effects, often comparable to the standard anti-inflammatory drug, indomethacin. These findings support the anti-inflammatory, anxiolytic, cognitive-enhancing, and pro-social effects of *O.sanctum*, making it a potential therapeutic agent against sickness behaviour induced by inflammatory stimuli.

5. References

1. Bluthé RM, Michaud B, Kelley KW, Dantzer R. Vagotomy blocks behavioral effects of interleukin-1 in rats. *Brain Behav Immun.* 1992;6(3):258–265.
2. Kent S, Bret-Dibat JL, Kelley KW, Dantzer R. Mechanisms of sickness-induced decreases in food-motivated behavior. *Neurosci Biobehavioral Rev*. 1996; 20(1):171-175.
3. Dunn AJ. Systemic interleukin-1 administration induces activation of cerebral catecholaminergic systems: Comparison with stress responses. *Psychopharmacol (Berl).* 1992;108(1-2):25–33.

4. Bone F. Phytotherapy for the immune system. *J Aust Coll Nutr Environ Med.* 1991;10(1):3–7.
5. Kluger MJ. The role of fever in disease. *Psychoneuroendocrinol*. 1991;16(1–3):53–70.
6. Kozak W, Kluger MJ, Soszynski D, Conn CA, Rudolph K, Leon LR. Cytokine receptors and fever: Old views and new thoughts. *Clin Infect Dis.* 1994;19(5):970–977.
7. Hart BL. Biological basis of the behavior of sick animals. *Neurosci Biobehav Rev.* 1988;12(2):123–137.
8. Yirmiya R. Endotoxin produces a depressive-like episode in rats. *Brain Res.* 1996;711(1–2):163–174.
9. Parrott AC, Hindmarch I. The hypothalamic-pituitary-adrenal (HPA) axis and the effects of psychotropic drugs. *Hum Psychopharmacol*. 1995;10(5):373–381.
10. Lenczowski MJ, Van Dam AM, Poole S, Larrick JW, Tilders FJ, Berkenbosch F. Effects of interleukin-1 on central monoaminergic systems and the hypothalamic-pituitary-adrenal axis. *J Neurosci*. 1997;17(14):5176–84.
11. O'Reilly CA, Pappenheimer JR, Fencl V. Role of interleukin-1 in regulating sleep in rabbits. *Am J Physiol*. 1988;255(1 Pt 2):R94–102.
12. Nava F, Carta G, Colombo G, Gessa GL. Lipopolysaccharide causes selective changes in dopamine and serotonin metabolism in different rat brain areas. *J Neurochem.* 1997a;69(5):2031–2035.
13. Linthorst AC, Flachskamm C, Müller-Preuss P, Holsboer F, Reul JM. Chronic stress and dexamethasone increase interleukin-1β mRNA expression in rat hypothalamus and pituitary. *J Neuroendocrinol.* 1996;8(12):887–893.
14. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci.* 2008;9(1):46–56.
15. Ma W, Dumont Y, Vercauteren F, Quirion R. Cytokines modulate serotonin and corticotropin-releasing factor receptors in the rat brain. *Neuropsychopharmacol.* 2000;22(5):522–532.
16. Yirmiya R, Winocur G, Goshen I. Brain interleukin-1 is involved in spatial memory and passive avoidance conditioning. *Neurobiol Learn Mem.* 2002;78(2):379–389.

17. Kelley KW, Bluthé RM, Dantzer R, Zhou JH, Shen WH, Johnson RW, Broussard SR. *Brain Behav Immun.* 2003;17(Suppl 1)S112-118. doi: 10.1016/s0889-1591(02)00077-6.
18. Arsenijevic D, Girard J, Seydoux J, Chang HR, Dulloo A, Challet E, et al. Altered energy balance and resistance to obesity in mice lacking 5-HT1B receptors. *Nature.* 1998;391(6666):793–796.
19. Anisman H, Merali Z. Cytokines, stress and depressive illness: brain–immune interactions. *Ann Med.* 2003;35(1):2–11.
20. Shen Y, Connor TJ, Kelly JP, Leonard BE. Differential effect of chronic imipramine and desipramine administration on IL-1β and IL-6 concentrations in rat brain and serum. *Neuroimmunomodulation.* 1999;6(6):330–336.
21. LeMay LG, Vander AJ, Kluger MJ. The effects of psychological stress on plasma interleukin-6 activity in rats. *Am J Physiol.* 1990;258(3 Pt 2):R638–R641.
22. Zuckerman SH, Shellhaas J, Butler LD. Differential regulation of lipopolysaccharide-induced interleukin 1 and interleukin 6 production in murine macrophages by prostaglandin E2. *Cell Immunol.* 1989;122(1):305–313.
23. Nadkarni KM, Indian Materia Medica, Mumbai, India: Popular Prakashan, 1976:671.
24. Singh N, Hoette Y, Miller R. Tulsi: The mother medicine of nature. Lucknow: International institute of herbal medicine; 2002.
25. Uma Devi P. Radioprotective, anticarcinogenic and antioxidant properties of the Indian medicinal plant *Ocimum sanctum* (Tulsi). *Indian J Exp Biol.* 2001;39(3):185–190.
26. Jha NK, Pandey IK, Jha AK. *O.sanctum*: Tulsi. *Phytopharm*. 2005;6:3-18.
27. Shanmuga Sundaram R, Srinivasan R, Natarajan D, Murugesan S. HPLC analysis and in vitro antioxidant activity of *O.sanctum* Linn. leaf extract. *Asian J Pharm Clin Res*. 2011;4(3):118–122.
28. Shanmuga Sundaram R, Ramanathan M, Rajesh R, Satheesh B, Saravanan D. LC-MS quantification of rosmarinic acid and ursolic acid in the *O.sanctum* Linn. leaf extract (Holy Basil, Tulsi). *J Liquid Chromatogr Relat Technol*. 2012;35(5):634-650. doi:10.1080/10826076.2011.606583.
29. Bronstein PM. Aggression in the rat: Effects of frustrative nonreward and environmental rearing conditions. *J Exp Anal Behav.* 1972;18(1):17–24.

30. Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods*. 1985;14(3):149-167. https://doi.org/10.1016/0165-0270(85)90031-7.
31. Johnson MK. Reality Monitoring: An Experimental Phenomenological Approach. *J Exp Psychol:General.* 1988;117(4):390-394.
32. Ramanathan M, Sivakumar S, Anandvijaykumar PR, Saravanababu C, Rathnavel Pandian P. Neuroprotective evaluation of standardized extract of *Centella asiatica* in MSG-treated rats. *Indian J Exp Biol.* 2007;45:425–431.
33. Porsolt RD, Le Pichon M, Jalfre M. Depression: A new animal model sensitive to antidepressant treatments. *Nature.* 1977;266(5604):730–732.
34. Olton DS, Collison C, Werz MA. Spatial memory and radial arm maze performance of rats. *Learn Motiv.* 1977;8(3):289–314.
35. Nava F, Carboni S, Battolla G. LPS-induced fever, anorexia and neurochemical changes in the hypothalamus. *Physiol Behav*. 1997;61(5):659–64.
36. Maier SF, Goehler LE, Schmidt ED, Watkins LR. The role of the vagus nerve in cytokine-to-brain communication. *Ann N Y Acad Sci.* 1998;840:289–300.
37. Leonard BE, Song C. Stress and the immune system in the etiology of anxiety and depression. *Pharmacol Biochem Behav.* 1996;54(1):299–303.
38. Klir JJ, Sgoifo A, Canon F, Vachon P, Tache Y. Central prostaglandins in LPS-induced fever and anorexia. *Am J Physiol*. 1993;265(6 Pt 2):R1239–45.
39. Elmquist JK, Scammell TE, Saper CB. Mechanisms of CNS response to systemic immune challenge: The febrile response. *Trends Neurosci.* 1997;20(12):565–570.
40. Linthorst AC, Flachskamm C, Holsboer F, Reul JM. Local administration of dexamethasone modulates interleukin-1β-induced activation of the hypothalamic-pituitary-adrenal axis. *Brain Res.* 1995;673(2):242–250.
41. Okamoto S, Kaul M, Lipton SA. Pathogenesis of oxidant stress in immunodeficiency virus-associated neurodegeneration. *J Neurovirol.* 2002;8(2):205–214.
42. Sugino H, Yamamoto S, Muramatsu S, Kakinuma K. Induction of interleukin-1 by tumor necrosis factor in murine peritoneal macrophages. *Immunology.* 1989;68(2):250–254.
43. Ulevitch RJ, Tobias PS. Recognition of lipopolysaccharide by the innate immune system. *Curr Opin Immunol.* 1995;7(1):20–26.

44. Laye S, Parnet P, Goujon E, Dantzer R. Peripheral administration of lipopolysaccharide induces the expression of cytokine transcripts in the brain and pituitary of mice. *Brain Res Mol Brain Res.* 1994;27(1):157–162.
45. Hopkins SJ, Rothwell NJ. Cytokines and the nervous system. *I. Expression and recognition. Trends Neurosci.* 1995;18(2):83–88.
46. Rothwell NJ, Hopkins SJ. Cytokines and the nervous system II: Actions and mechanisms of action. *Trends Neurosci.* 1995;18(3):130–136.
47. Merrill JE, Benveniste EN. Cytokines in inflammatory brain lesions: helpful and harmful. *Trends Neurosci.* 1996;19(8):331–338.
48. Sakaguchi S, Saito H, Sasazuki T. Altered thymic T-cell selection due to a mutation of the *H-2 I-A beta* gene leads to autoimmune disease. *Nature.* 1981;280(5724):327–329.
49. Yoshikawa T, Yoshida N, Manabe H, Kondo M. Cytokines and free radicals in sepsis: Pathophysiological roles and therapeutic strategies. *Surg Today.* 1994;24(1):1–8.
50. Lipton JM, Zhang C, Wiemann B. Advances in understanding regulation of body temperature by lipopolysaccharide. *Am J Physiol.* 1993;265(5 Pt 2):R883–R894. Dwivedi Y. Adaptogenic properties of *Withania somnifera* (Ashwagandha): A review. *Indian Drugs.* 1997;34(10):571–576.
51. Sembulingam K, Sembulingam P, Namasivayam A. Effect of *Ocimum sanctum* Linn. on noise-induced changes in rats. *Indian J Physiol Pharmacol.* 1999;43(4):479–484.
52. Sehic E, Fitch CA, Dantzer R, Kelley KW. Regulation of the hypothalamic–pituitary–adrenal axis by cytokines: Role of prostaglandins. *Ann N Y Acad Sci.* 1996;780:62–77.
53. Kristin Andrews The animal mind: An introduction to the philosophy of animal cognition. I Ed 1971, Published by by Routledge (Taylar & Francis), Oxon.
54. Ford CW, Klugman KP. The role of neurotransmitters in lipopolysaccharide-induced pathophysiology. *J Med Microbiol*. 1980;13(2):153–60.
55. Matsuzek K, Ishikawa K. Perturbations in neurotransmitter activities such as 5-HT and noradrenaline (NA) under pathological conditions. *Neuropharmacology*. 1981;20(5):515–21. Feldberg W, Saxena PN. Fever induced by bacterial pyrogens and its relation to prostaglandin synthesis. *J Physiol*. 1975;250(3):581–600.
56. Lin MT, Lin CH. Cytokines such as IL-1α, IL-1β, IL-6, and TNF-α and neuropeptides in the regulation of fever and inflammation. *Neuroimmunomodulation*. 1996;3(3):123–33.

57. Lin MT, Lin CH. Nitric oxide as a mediator of fever: involvement of cyclic GMP and hypothalamic prostaglandins. *J Pharmacol Exp Ther*. 1996;277(2):737–44..
58. Sakina MR, Dandiya PC, Vohora SB. Central histaminergic and serotonergic system involvement in *Ocimum sanctum*-induced psychotropic effects. *Indian J Pharmacol*. 1990;22(2):139–4
59. Maity TK, Mandal SC, Saha BP, Pal M. Evaluation of antidepressant activity of *Ocimum sanctum* Linn. in rodents. *Indian J Exp Biol*. 2000;38(1):72–4.
60. Montgomery SA. Why do antidepressants take so long to work? A review of the delay in onset of antidepressant efficacy. *Br J Psychiatry Suppl*. 1994;(22):50–8... Rivier C, Vale W. Influence of cytokines on the hypothalamic-pituitary-adrenal axis in the rat. *J Neurosci*. 1989;9(9):3525–31
61. Rivier C, Vale W. Influence of cytokines on the hypothalamic-pituitary-adrenal axis in the rat. *J Neurosci*. 1989;9(9):3525–31.
62. Tilders FJH, Schmidt ED, DeRijk RH, Oitzl MS, Shipston MJ. Differential effects of bacterial endotoxin (LPS) on neuroendocrine and behavioral responses: attenuation by adrenal hormones. *Ann N Y Acad Sci*. 1994;741:378–96
63. Pariante CM, Miller AH. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. *Biol Psychiatry*. 2001;49(5):391–404.
64. De La Garza R, Asnis GM, Fabrizio M, Tobe RH. LPS administration increases corticosterone levels and alters behavioral responses: relevance to depression. *Brain Res*. 2004;1023(2):162–70.
65. Dwivedi C. Immunomodulatory and anti-inflammatory effects of *Ocimum sanctum* Linn. (*Tulsi*). *Indian J Physiol Pharmacol*. 1997;41(4):387–93.
66. Sembulingam K, Sembulingam P, Namasivayam A. Effect of *Ocimum sanctum* on adrenocortical function during noise stress. *Indian J Physiol Pharmacol.* 2005;49(1):125–128.
67. Sehic E, Blatteis CM, Li S, Li Z. Pyrogenic tolerance to lipopolysaccharide is associated with reduced synthesis of prostaglandin E2 in rat brain. *Brain Res Bull*. 1996;39(3):189–95.

68. MacNeil JG, Michael GJ, Fagan SC, Maixner W, Goehler LE. Lipopolysaccharide increases splenic sympathetic nerve activity: implications for neuroimmune communication. *Am J Physiol*. 1997;273(5 Pt 2):R1757–61..
69. de Vries HE, Kuiper J, de Boer AG, Van Berkel TJ, Breimer DD. The blood-brain barrier in neuroinflammatory diseases. *Pharmacol Rev*. 1996;48(4):465–87.
70. Ravindran R, Rathinasamy SD, Samson J, Senthilvelan M. Lipid peroxidation and antioxidant status in rat brain regions after chronic noise exposure. *Pharmacol Res*. 2005;52(5):467–72.
71. Samson J, Sheeladevi R, Ravindran R, Senthilvelan M. Effect of *Ocimum sanctum* on noise-induced changes in free radical scavenging enzymes in rat brain. *Environ Toxicol Pharmacol*. 2006;21(1):25–9.
72. Phippen WB, Simon JE. Anthocyanins and phenolic compounds in *Ocimum* species: their roles in plant color and medicinal properties. *J Agric Food Chem*. 2000;48(6):2454–60.
73. Sakina MR, Dandiya PC, Vohora SB. Evidence for the involvement of the dopaminergic system in the psychotropic effects of *Ocimum sanctum* Linn. *Indian J Pharmacol*. 1990;22(2):139–43.
74. Maity TK, Mandal SC, Saha BP, Pal M. Effect of *Ocimum sanctum* roots extract on swimming performance in mice. *Phytother Res*. 2000;14(2):120–1.
75. Wilson JR, Wurtman RJ, Axelrod J. Regulation of corticosterone secretion by central adrenergic receptors. *Endocrinology*. 1980;106(5):1633–6.
76. Brekhman II, Dardymov IV. New substances of plant origin which increase nonspecific resistance. *Annu Rev Pharmacol*. 1969;9:419–30.
77. Brekhman II, Dardymov IV. New substances of plant origin which increase nonspecific resistance. *Annu Rev Pharmacol*. 1969;9:419–30.
78. Singh N, Nath R, Lata A, Singh SP, Kohli RP, Bhargava KP. Withania somnifera (Ashwagandha), a rejuvenating herbal drug which enhances survival during stress (an adaptogenic effect). *Indian J Exp Biol*. 1991;29(5):459–62.
79. Sembulingam K, Sembulingam P. *Essentials of Medical Physiology*. 2nd ed. New Delhi: Jaypee Brothers Medical Publishers; 1997,387-397
80. Sembulingam K, Sembulingam P. Effect of *Ocimum sanctum* on neutrophil function and organ weight under stress. *Indian J Physiol Pharmacol*. 1998;42(4):473–6.

81. Archana R, Namasivayam A. Effect of *Ocimum sanctum* on noise-induced changes in neutrophil functions. *J Ethnopharmacol*. 2000;73(1–2):81–5.
82. Sarkar A, Pandey DN, Pant MC. A report on the effect of *Ocimum sanctum* (Tulsi) leaves on blood lipid profile in normal albino rabbits. *Indian J Physiol Pharmacol*. 1994;38(4):311–2.
83. Abdel-Gawad MM, Khalifa WA. Neuroprotective activity of *Ocimum sanctum* in chemical and noise-induced stress: review of CNS effects. *J Ethnopharmacol*. 2001;75(2–3):123–35.
84. Sembulingam K, Sembulingam P, Namasivayam A. Protective effects of *Ocimum sanctum* on central neurochemical adaptations induced by noise stress: modulation of acetylcholine and biogenic amines. *Phytother Res*. 2005;19(4):318–24.
85. Ravindran R, Rathinasamy SD, Samson J, Senthilvelan M. Antioxidant and neuroprotective actions of *Ocimum sanctum*: mitigation of stress-induced lipid peroxidation in brain regions. *Pharmacol Res*. 2005;52(5):467–72.
86. Samson J, Sheeladevi R, Ravindran R, Senthilvelan M. Chronic noise stress, antioxidant enzyme activity, and *Ocimum sanctum*: reversal of oxidative damage in rat brain. *Environ Toxicol Pharmacol*. 2006;21(1):25–9.
87. Uma Devi P. Effects of *Ocimum sanctum* on neural antioxidant systems under psychological stress. *Indian J Physiol Pharmacol*. 2001;45(2):207–12.
88. Yanpallewar SU, Pant AB, Godbole MM. Tulsi extract prevents stress-induced changes in brain neurotransmitters: implications for anxiolytic and adaptogenic effects. *Life Sci*. 2004;75(8):763–71
89. Prakash P, Gupta N. Adaptogenic and neuroenhancing effects of *Ocimum sanctum* in rodents under acute restraint stress. *Pharmacog Rev*. 2000;4(7):95–105.