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Research Article

COUMARIN DERIVATIVE CITROPTEN AMELIORATES INFLAMMATION-INDUCED DEPRESSION BY SUPPRESSING PRO-INFLAMMATORY CYTOKINES AND RESTORING ANTIOXIDANT DEFENSE: AN *IN VIVO* STUDY

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ABSTRACT

Objectives: Citropten (CP), a naturally occurring coumarin with reported antioxidant and anti-inflammatory effects, has not yet been comprehensively evaluated for antidepressant activity. The present investigation is designed to evaluate the antidepressant-like ability of CP in mice challenged with lipopolysaccharide (LPS) by evaluating behavioral performance, brain neuroinflammatory cytokines, and markers of oxidative stress.

Methods: Swiss albino male mice were pre-treated orally for 15 days with vehicle, CP with 25 and 50 mg/kg, or fluoxetine with 20 mg/kg. On day 15, they received a LPS injection intraperitoneally (0.83 mg/kg). Functional behavioral responses were tested on day 16 using the forced swim test (FST), tail suspension test (TST), and open field test (OFT). Furthermore, mice brain tissues were collected to analyze cytokines, such as interleukin (IL)-6, tumor necrosis factor-α, and IL-1β, and stress markers (glutathione [GSH], malondialdehyde [MDA], and catalase [CAT]).

Results: LPS treatment led to a significant rise in immobility time in the FST (164.2±9.3 s vs. 115.5±4.5 s; $p<0.001$) and TST (172.0±7.6 s vs. 120.7±3.9 s; $p<0.001$) and reduced OFT exploration. CP produced clear dose-dependent improvement with a 50 mg/kg dose, reducing immobility to 91.2±5.8s (FST) and 104.8±7.9s (TST; $p<0.001$), comparable to fluoxetine. LPS increased brain cytokines by ~4–5 fold and caused oxidative imbalance (↑MDA, ↓GSH, ↓CAT), while CP suppressed cytokine levels and restored antioxidant defenses (reduced MDA; increased GSH and CAT).

Conclusion: In mice exposed to LPS, CP demonstrated robust antidepressant-like efficacy through simultaneous suppression of central inflammatory responses and mitigation of oxidative stress, indicating promise for treating inflammation-linked depression.

Keywords: Citropten, Coumarin, Depression, Neuroinflammation, Lipopolysaccharide, Oxidative stress.

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INTRODUCTION

Major depressive disorder (MDD) is a chronic and relapsing psychiatric condition marked by persistent low mood, loss of interest, and disturbances in sleep, appetite, and psychomotor activity, and an increased risk of suicide [1]. Globally, it is a primary factor in disability, associated with considerable individual, social, and financial burdens. Recent analyses indicate a steady global rise in MDD prevalence, particularly among aging populations, with projections suggesting further escalation through 2050, especially in low-income regions and among men [2]. The COVID-19 pandemic further amplified this burden: Global prevalence of depressive disorders rose by nearly 28% in 2020, adding more than 53 million cases compared to pre-pandemic levels [3]. These trends reflect an urgent challenge for public health and suggest biological links among psychosocial stress, immune activation, and depressive pathology.

According to the inflammatory hypothesis, immune dysregulation plays a central role in depression pathogenesis. Evidence shows that systemic infections, chronic stress, or metabolic disorders can activate peripheral immune responses, leading to elevated pro-inflammatory cytokines that influence central nervous system (CNS) function [4-6]. Meta-analyses have demonstrated that individuals with MDD exhibit significantly elevated circulating levels of interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), and C-reactive protein compared to healthy controls [7,8]. These mediators interfere with serotonergic, dopaminergic, and glutamatergic neurotransmission, disrupt the "hypothalamic–pituitary–adrenal axis", and downregulate brain-derived neurotrophic factor (BDNF), thereby impairing neuroplasticity [9,10]. Parallel to immune activation, oxidative and nitrosative stress contribute to neuronal dysfunction by generating excessive reactive oxygen species (ROS), causing lipid peroxidation, mitochondrial damage, and depleting antioxidant defenses, such as glutathione (GSH), catalase (CAT), and superoxide dismutase [11]. Inflammatory and oxidative processes form a self-perpetuating cycle that underlies the persistence of depressive symptoms.

Within the CNS, this pathology manifests as neuroinflammation. Microglia, the resident immune cells, respond to chronic stressors by releasing pro-inflammatory mediators and ROS, leading to synaptic and neuronal alterations [5,12]. Dysfunctional astrocytes further compromise antioxidant and metabolic support, while activation of the nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (NLRP3) inflammasome enhances cytokine release, linking stress to depressive phenotypes [13,14]. These mechanisms justify targeting neuroinflammation and oxidative stress as therapeutic strategies for MDD. Rodent models, especially lipopolysaccharide (LPS)-induced depression, are widely used to study these mechanisms. LPS, a bacterial endotoxin, activates toll-like receptor-4 (TLR4) signaling, driving nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation, cytokine release, oxidative stress, and behavioral changes resembling depression [15-17]. Studies show that LPS-treated mice develop anhedonia, reduced locomotion, and memory impairment, alongside elevated TNF-α, IL-6, IL-1β, and malondialdehyde (MDA), with depleted GSH and CAT [18-22]. Importantly, this model parallels human conditions in which infection-related immune activation contributes to depression, such as in post-COVID-19 neuropsychiatric manifestations [23,24]. Thus, LPS models

offer robust translational value for identifying novel anti-inflammatory and antioxidant therapies.

Natural phytochemicals have attracted growing scientific interest as safer alternatives for managing neuroinflammation-associated depression. Coumarins, a diverse group of benzopyrone derivatives abundant in plants, exhibit a broad pharmacological spectrum, including antioxidant, anti-inflammatory, antimicrobial, anticancer, hepatoprotective, and neuroprotective properties [25-27]. Their neuroprotective effects are mediated by ROS scavenging, inhibition of NF-κB and mitogen-activated protein kinase (MAPK) signaling, regulation of monoamine oxidase activity, and modulation of neurotransmitter systems [28-30]. Citropten (CP) (5,7-dimethoxycoumarin), also called limettin, is a naturally occurring polymethoxylated coumarin mainly present in citrus fruits, such as lemon (*Citrus limon*), bergamot (*Citrus bergamia*), and in *Carica papaya* [31,32]. Pharmacological studies report its antioxidant, anti-inflammatory, anticancer, hepatoprotective, and neuroprotective properties [33-36]. Recent *in vitro* findings show that CP protects neuronal cells against oxidative stress by attenuating mitochondrial dysfunction, reducing ROS production, and suppressing NF-κB and cytokine release [37]. It has also alleviated stress- and pain-related depression-like behaviors through regulation of monoaminergic and inflammatory pathways [36,38]. Despite promising findings, no comprehensive study has evaluated CP in LPS-challenged, depression-like models that are driven principally by inflammatory and oxidative mechanisms.

Accordingly, the current study was conducted to examine the antidepressant activity of CP using an LPS-triggered murine model of depression. Specifically, it examined whether CP could attenuate behavioral deficits while reducing pro-inflammatory cytokines and restoring antioxidant balance. These results may offer new perspectives into the therapeutic role of CP in inflammation-associated depression.

METHODS

Chemicals

High-purity, analytical-grade reagents and chemicals were used in all experiments, procured from approved suppliers. CP (5,7-dimethoxycoumarin, ≥98% purity) and LPS (serotype O127:B8) were procured from Sigma-Aldrich (USA). Fluoxetine hydrochloride (≥99% purity, CAS No.: 56296-78-7) was obtained from Vivan Life Sciences (India). All substances were stored under recommended conditions and prepared freshly before use according to experimental requirements.

Animals

Adult male Swiss albino mice (7–8 weeks old, 20–30 g) were housed in sterile polycarbonate cages under standard laboratory conditions, which included a 12-h light/dark cycle, a temperature of 22±3°C, and a relative humidity of 50–70%. The animals were given unlimited access to clean water and a regular pellet meal. The mice were acclimated to the laboratory environment for one week before the commencement of the experiments. All procedures were carried out in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines and approved by the Institutional Animal Ethics Committee (IAEC) of the Biocyte Institute of Research and Development, Sangli (Approval No.: IAEC/BiRD/Sangli/2024-25/13). The light phase, which lasted from 10:00 to 17:00 h, was when the experiments were conducted.

Preparation of doses

CP doses 25 and 50 mg/kg were selected based on previous reports of its pharmacological effects [35-36]. For administration, drug suspensions were freshly prepared in a vehicle of Tween 80 (1%) and carboxymethyl cellulose (0.5%), and given orally (p.o) at a dose of 10 mL/kg. Fluoxetine 20 mg/kg was used as a reference antidepressant and prepared similarly. The reference dose of fluoxetine (20 mg/kg, p.o.) was selected based on prior studies demonstrating robust antidepressant-like efficacy in the LPS-induced depression model [39-43]. LPS (0.83 mg/kg) was dissolved in sterile, endotoxin-free 0.9% saline and administered intraperitoneally (i.p.) at a dose volume of 5 mL/kg to induce neuroinflammation and depression-like behaviors in all groups except the vehicle control [21,44,45].

Experimental design

Upon commencement of the study, the animals were distributed into five groups, each comprising six mice [19,22,44,46-48]: Group I (Vehicle control; VEH+SAL): Vehicle 10 mL/kg; p.o. + On day 15 Saline, 5 mL/kg; i.p. Group II (LPS control; VEH+LPS): Vehicle 10 mL/kg; p.o. + On day 15 LPS, 0.83 mg/kg; i.p. Group III (CP 25 + LPS): CP 25 mg/kg; p.o. + On day 15 LPS, 0.83 mg/kg; i.p., Group IV (CP 50 + LPS): CP 50 mg/kg; p.o. + On day 15 LPS, 0.83 mg/kg; i.p., Standard Group V (FLU 20 + LPS): Fluoxetine 20 mg/kg; p.o. + On day 15 LPS, 0.83 mg/kg; i.p.

From Day 1 onwards, mice received once-daily oral treatment with Vehicle, CP, and Fluoxetine. On Day 15, 45 min after oral dosing, all groups excluding the vehicle control (Group I) were given a single intraperitoneal injection of LPS with 0.83 mg/kg to induce neuroinflammation and depression-relevant behavioral changes. This LPS dose is widely used in mouse models of inflammation-associated depression and has been shown to reliably induce depressive-like behavior when evaluated 24 h after administration [16,21,39-45].

On Day 16, animals were orally treated with the respective compound and behavioral tests included the tail suspension test (TST), forced swim test (FST), and open field test (OFT) were executed to assess antidepressant potential. To minimize potential interference of behavioral testing on biochemical outcomes, behavioral and biochemical assessments were conducted in separate sets of animals. Post ~24 h of the LPS or Saline intraperitoneal administration animals from a separate set were sacrificed by cervical dislocation and entire brain samples were rapidly collected for biochemical analysis (Fig. 1). Behavioral assessments and brain tissue sampling for biochemical analyses were performed on day 16, 24 h after LPS administration. This time point is commonly used in LPS-induced depression models to minimize the influence of acute sickness responses and allow reliable evaluation of depressive-like behaviors [21,44,45].

FST

The FST was conducted as described previously with slight modifications [45-49]. For this test, each animal was positioned individually within a glass vessel (25 cm tall, 15 cm in diameter), which containing 15 cm of water at a temperature of 25±1°C. Following a 2-min acclimatization, immobility behavior was noted over the past 4 min of the 6-min test period. Immobility was characterized as the active behaviors absence, such as swimming or climbing, with minimal required movements necessary merely to prevent submersion of the head. The water was replaced after each trial to prevent olfactory cues or contamination effects. After the completion of the test, mice were carefully back to their home cages, dried, and placed below a warming lamp.

TST

The TST was employed to estimate depression-like behavior in mice, following established behavioral paradigms. Mouse individually suspended the tails of the mice were carefully fixed to adhesive tape fixed roughly 1 cm from the tip, elevating the animal 50 cm above the surface. To prevent external disturbances, the testing environment ensured both visual and auditory isolation. Following an initial 2-min habituation phase, immobility duration was recorded over a 4-min observation window. Mice were deemed immobile when all voluntary motion stopped, allowing only those movements necessary to sustain posture. Immobility was scored only when the mouse remained completely motionless, exhibiting passive hanging behavior. The experiment was conducted under low illumination, with the observer blinded to treatment conditions to eliminate bias [44-47,50].

OFT

The OFT was conducted to assess spontaneous locomotor activity and exploratory behavior, following previously established protocols with slight modifications [1]. Mice were introduced one mouse at a time was

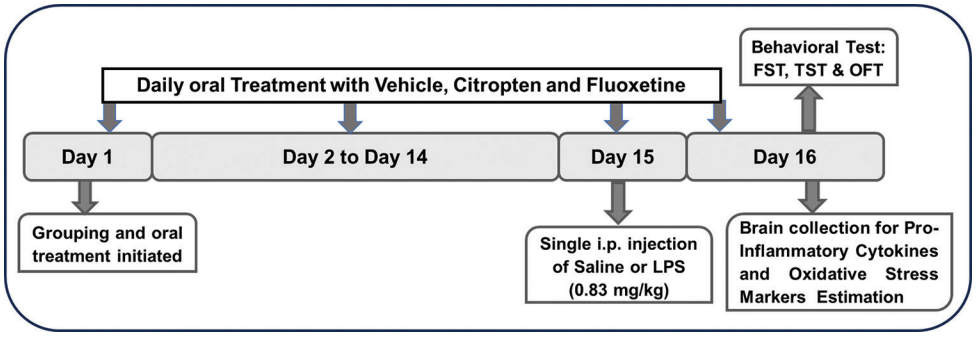

Fig. 1: Overview of the experimental protocol and timeline. Animals were administered vehicle, citropten, or fluoxetine orally once daily for 16 days. On day 15, animals received a single intraperitoneal injection of lipopolysaccharide or saline. Behavioral assessments and subsequent biochemical analysis of brain tissues were performed on day 16

gently positioned in the center of the open-field arena (45×45×40 cm), with the central zone area (20×20 cm). Each trial lasted 5 min, during which the animals' behavior was recorded under ambient illumination (100 lux). Parameters assessed included total distance travelled, frequency of rearing (vertical activity), and number of center crossings. The test chamber was thoroughly cleaned with 70% ethanol between sessions to prevent olfactory interference [44,45].

Brain homogenate preparation

Brain tissues were quickly removed and placed on a pre-chilled metal surface before being homogenized (10% w/v) in ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenates were then centrifugate at 12,000 × g for 20 min at 4±1°C, and the received supernatants were carefully collected for subsequent biochemical analyses of oxidative stress and neuroinflammatory markers.

Pro-inflammatory cytokine estimation

Proinflammatory cytokine levels (IL-1β, IL-6, and TNF-α) in brain tissue supernatants were measured using an enzyme-linked immunosorbent assay method employing cytokine-specific primary antibodies and HRP-conjugated secondary antibodies. To ensure accurate and reliable comparisons, brain tissue homogenates were normalized for total protein concentration before analysis, and cytokine levels were normalized relative to protein content. Cytokine concentrations were determined using corresponding cytokine standards. Concisely, 100 µL of each prepared supernatant was dropped to 96-well plates and re-incubated overnight at 38±2°C. Plates were then cleaned twice with PBS and pre-treated with 200 µL of buffer for 1 h at ambient temperature. After completing the washing process, 100 µL of antibodies (Primary) specific for TNF-α, IL-6, and IL-1β were dropped and re-incubated for 2 h at ambient temperature. Wells were washed again, subsequently re-incubated with 100 µL of secondary antibody (HRP-conjugated) for 1 h. Color changes were achieved utilizing 200 µL of o-dianisidine hydrochloride substrate, and the processed reaction was ended by dropping 50 µL of HCl (5N). Absorbance was recorded at 415 nm using a microplate reader [37].

Evaluation of oxidative stress biomarkers

MDA levels, indicative of lipid peroxidation in brain tissues, were measured utilizing the thiobarbituric acid (TBA) assay with spectrophotometric detection of TBA–MDA adducts according to previously established methods. MDA levels were normalized to total protein content and expressed as µM MDA/mg protein [45,51]. Reduced GSH was quantified by 5,5'-dithiobis(2-nitro benzoic acid) method and results expressed as µM GSH/mg protein [45,52]. CAT activity was determined based on Claiborne's method (1995) and reported as µM of H_2O_2 decomposed per minute per mg of protein (µM/min/mg protein) [52,53]. Protein strength was confirmed utilizing the bicinchoninic acid assay to normalize all estimated biomarker values.

Statistical analysis

Data are presented as mean±standard error of the mean, which represents the precision of the estimated group mean and is commonly used for comparison of treatment effects across experimental groups. Statistical comparisons versus the LPS-treated group were performed using one-way analysis of variance followed by Dunnett's *post hoc* test for multiple comparisons. No formal outlier detection tests were applied, and no data points were excluded from the analysis. All statistical analyses were confirmed with version 9 GraphPad Prism (GraphPad Software, La Jolla, CA, USA). A $p<0.05$ was considered statistically significant. Significance levels are indicated in the corresponding figures.

RESULTS

Effect of repeated CP dosing on LPS-triggered behavioral modifications

The effects of repeated CP administration were assessed in an LPS-induced depression model. Mice receiving intraperitoneal injections of LPS at 0.83 mg/kg exhibited a pronounced depression-like phenotype, evidenced by a significant increase in immobility time in both the FST and TST compared to the vehicle control. In the FST, immobility time increased to 164.17±9.32 s in LPS-treated mice versus 115.50±4.48 s in the vehicle group ($p<0.001$). Similarly, in the TST, immobility rose to 172.00±7.59 s compared with 120.67±3.86 s in controls ($p<0.001$), confirming the successful induction of depression-like symptoms (Fig. 2a and b).

Administration of CP before LPS exposure significantly reversed these alterations. In the FST, immobility was reduced to 117.33±6.84 s (25 mg/kg) and 91.17±5.75 s (50 mg/kg), corresponding to 29% and 44% reductions, respectively, relative to the LPS alone treated group ($p<0.001$ value). Fluoxetine (20 mg/kg) exerted the most pronounced response, lowering immobility time to 71.17±5.57 s ($p<0.001$), corresponding to a 53% reduction. A comparable trend was observed in the TST, where CP reduced immobility to 127.00±8.83 s (25 mg/kg) and 104.83±7.90 s (50 mg/kg), equating to 26% and 39% decreases, respectively ($p<0.001$), while fluoxetine lowered immobility to 93.67±5.61 s (46% reduction) (Fig. 2b).

In OFT, LPS challenge resulted in suppressed exploratory behavior, evident from reduced rearings (14.50±5.71) and crossings (98.83±8.20) compared to controls (21.00±2.33 and 119.50±6.80, respectively). CP produced a clear, dose-dependent recovery from LPS-induced behavioral impairments. At 25 mg/kg, mice exhibited 18.33±6.60 rearings and 106.83±8.14 crossings, while the higher possible dose (50 mg/kg) restored performance to near control levels (21.17±3.40 rearings; 124.33±6.99 crossings), suggesting reversal of LPS-induced suppression without causing hyperactivity. Fluoxetine elicited the greatest improvement among all groups, validating the model and treatment paradigm (28.00±1.81 rearings and 131.83±7.58 crossings; $p<0.05$ vs. LPS alone group) (Fig. 2c). Collectively, these results demonstrate that sub-chronic CP treatment effectively mitigates LPS-induced behavioral alterations, where the 50 mg/kg dose exhibited antidepressant-like effects similar to those of Fluoxetine.

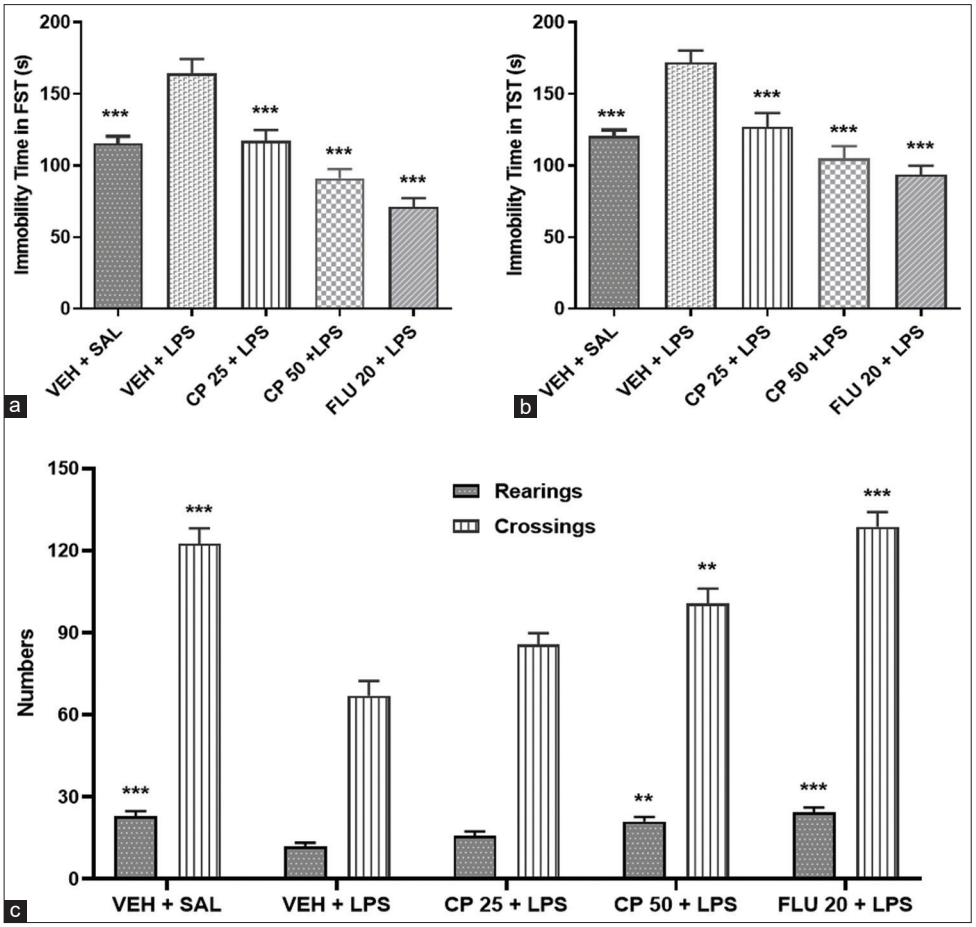

Fig. 2: Impact of repeated Citropten administration on LPS-triggered behavioral responses in mice assessed using (a) forced swim test (FST), (b) tail suspension test (TST), and (c) open field test (OFT). Immobility time in mice was evaluated using the FST and TST, while exploratory activity (rearing and crossing counts) was assessed in the OFT. Data are presented as mean±SEM (n=6 per group). Statistical significance versus the LPS-treated group: *p<0.05, **p<0.01, ***p<0.001 as determined by one-way analysis of variance followed by Dunnett's *post hoc* test. VEH: Vehicle, SAL: Saline, CP 25: Citropten 25 mg/kg, CP 50: Citropten 50 mg/kg, FLU: Fluoxetine, LPS: Lipopolysaccharide

Effect of CP on brain neuroinflammatory markers (IL-6, TNF-α, and IL-1β)

The ability of CP pre-treatment to modulate LPS-driven neuroinflammation was investigated by analyzing pro-inflammatory cytokine expression in brain tissue.

Following LPS injection (0.83 mg/kg, i.p.), brain concentrations of IL-6, IL-1β, and TNF-α, were markedly increased relative to control animals, as illustrated in Fig. 3, confirming strong induction of neuroinflammation. The fold increases observed in the LPS alone group relative to vehicle alone group were approximately 4.1-fold for TNF-α (58.67±6.79 vs. 14.33±2.56 ng/mL), 5.2-fold for IL-6 (82.67±6.94 vs. 16.00±6.00 ng/mL), and 2.2-fold for IL-1β (46.33±3.11 vs. 21.33±1.61 ng/mL), all highly significant (p<0.001; Fig. 3).

Pre-treatment with CP at 25 mg/kg led to modest numerical reductions in cytokine levels (TNF-α: 47.67±4.33; IL-6: 67.00±6.05; IL-1β: 39.00±3.49 ng/mL), corresponding to 19%, 19%, and 16% reductions, respectively, compared to the LPS group. However, these reductions were not statistically significant. In contrast, CP administration at 50 mg/kg significantly attenuated LPS-induced elevations of cytokines, TNF-α by 39%, IL-6 by 35%, and IL-1β by 25%, with respective levels of 36.00±3.97, 54.00±5.46, and 34.67±2.57 ng/mL (p<0.05; Fig. 3).

Fluoxetine, administered at 20 mg/kg as a reference drug, demonstrated significant suppression of LPS-induced cytokine elevations, reducing TNF-α by 45% (32.33±3.48 ng/mL; p<0.01), IL-6 by 38% (51.67±5.09 ng/mL; p<0.05), and IL-1β by 30% (32.33±2.08 ng/mL; p<0.01) relative to the LPS group, as shown in Fig. 3. These findings suggest that CP at 50 mg/kg effectively suppresses neuroinflammatory cytokine overproduction in response to LPS, though its efficacy is slightly lower than that of fluoxetine.

Effect of CP on oxidative stress markers in brain tissue

Oxidative stress factor, namely, MDA levels, GSH content, and CAT activity, were assessed in brain tissue 24 h following LPS injection. LPS significantly increased oxidative stress biomarkers in brain homogenates, as proved by higher lipid peroxidation and reduced antioxidant defenses (Fig. 4).

As depicted in Fig. 4a, LPS significantly elevated MDA levels against to vehicle-treated animals (2.07±0.16 vs. 0.92±0.14 μM/mg protein, p<0.001), confirming enhanced lipid peroxidation. Pre-treatment with CP at 50 mg/kg significantly reduced MDA concentrations to 32% (1.42±0.14 μM/mg protein; p<0.05) in LPS-challenged mice, while the 25 mg/kg dose showed only a moderate 20%, non-significant decrease (1.67±0.14 μM/mg protein). Fluoxetine (20 mg/kg) showed the strongest effect, significantly reducing MDA elevation to 36% (1.32±0.15 μM/mg protein; p<0.01), demonstrating a protective antioxidant effect.

LPS-treated mice exhibited a notable decline in GSH levels compared with vehicle-treated controls (3.47±0.64 vs. 8.63±0.75 μM/mg protein;

p<0.001; Fig. 4b). Administration of CP at 50 mg/kg significantly reversed this LPS-induced decrease, restoring GSH levels to 7.13±0.62 μM/mg protein (p<0.05), whereas the lower dose (25 mg/kg) produced a modest, non-significant increase (6.03±0.69 μM/mg protein). Treatment with Fluoxetine also led to a noteworthy rise in GSH levels (7.87±0.68 μM/mg protein; p<0.01) relative to the LPS-treated animal group.

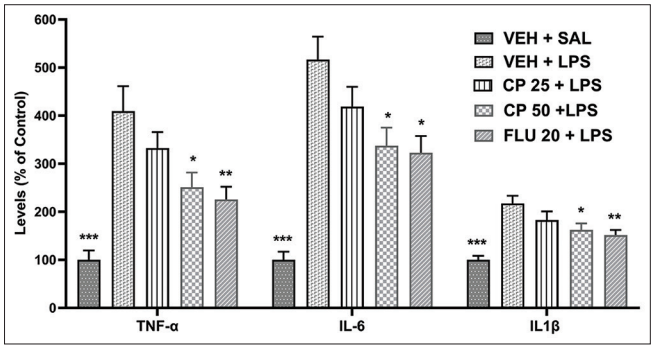

Fig. 3: Effect of Citropten on lipopolysaccharide (LPS)-induced elevation of brain pro-inflammatory cytokines (interleukin-6 [IL-6], tumor necrosis factor-α, IL-1β). Levels were measured using an enzyme-linked immunosorbent assay and normalized to total protein content. Data are presented as mean±SEM (n=6 per group). Statistical significance versus the LPS-treated group: *p<0.05, **p<0.01, ***p<0.001 as determined by one-way analysis of variance followed by Dunnett's *post hoc* test

LPS exposure also significantly reduced CAT activity in brain tissue (16.83±1.95 vs. 34.33±3.45 μM/min/mg protein; p<0.001), indicating impairment of enzymatic antioxidant defenses (Fig. 4c). A dose-related enhancement in CAT activity was noted following CP treatment, with 50 mg/kg significantly raising CAT levels (26.33±1.59 μM/min/mg protein; p<0.05) and 25 mg/kg showing a moderate but non-significant increase (22.67±1.92 μM/min/mg protein). CAT activity was significantly elevated following fluoxetine treatment (29.00±2.25 μM/min/mg protein; p<0.01), supporting its known antioxidant role. Collectively, these results demonstrate that CP, particularly at 50 mg/kg, effectively reduces oxidative stress and restores antioxidant defenses in LPS-challenged mice, with efficacy approaching that of fluoxetine.

DISCUSSION

Depression is a complex, multifactorial disorder in which both neuroinflammatory and oxidative processes play pivotal roles in its underlying pathophysiology [5,7,54]. The LPS-induced systemic inflammation model in rodents is widely accepted as a clinically relevant paradigm to study immune-related depression [16]. By activating TLR4 and NF-κB, LPS triggers excessive cytokine release and oxidative imbalance, which are key drivers of mood disturbances [17,55]. This model recapitulates the neuroinflammatory hypothesis of depression, as elevated pro-inflammatory mediators, such as TNF-α, IL-6, and IL-1β, contribute directly to the manifestation of depressive-like

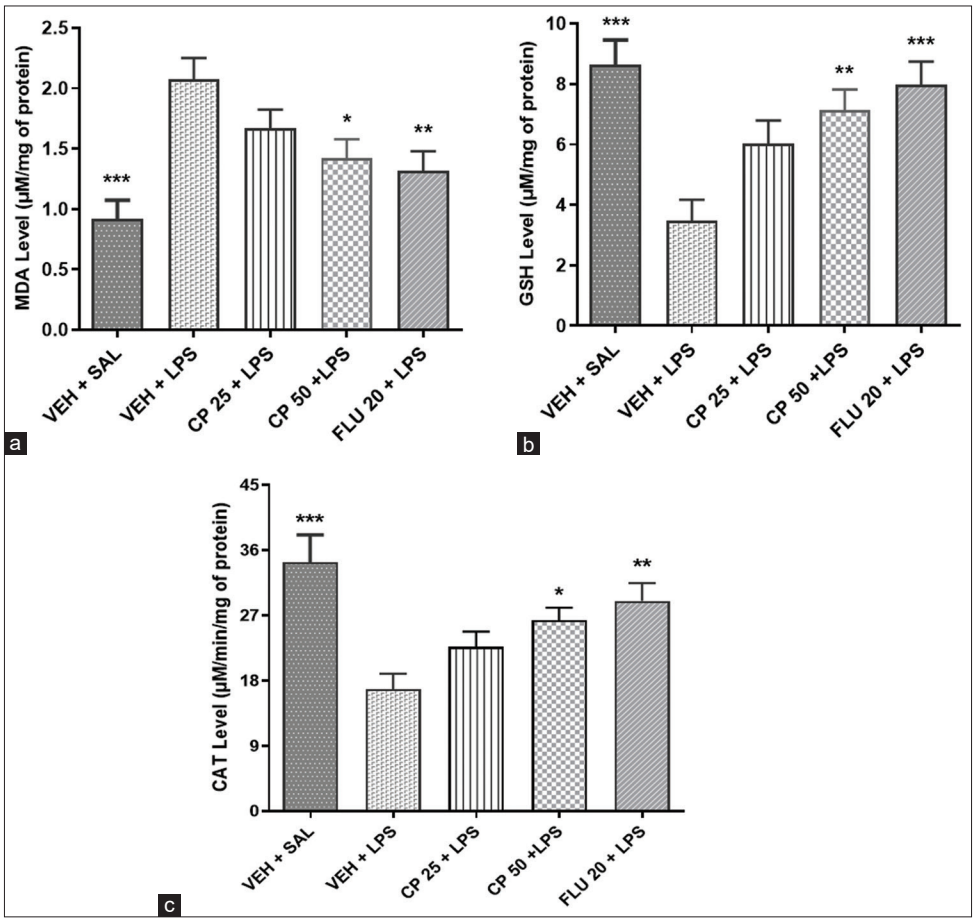

Fig. 4: Outcome of Citropten on brain oxidative stress markers—(a) malondialdehyde (MDA), (b) glutathione (GSH), and (c) catalase (CAT)—following LPS administration in mice. MDA levels were determined using the thiobarbituric acid assay, reduced GSH levels were estimated by the DTNB method, and CAT activity was evaluated by monitoring the breakdown of hydrogen peroxide (H_2O_2). All measurements were normalized to total protein content. Data are presented as mean±SEM (n=6 per group). Statistical significance versus the LPS-treated group: *p<0.05, **p<0.01, ***p<0.001 as determined by one-way analysis of variance followed by Dunnett's *post hoc* test

phenotypes [16,17]. In the present study, we employed this validated paradigm to evaluate the antidepressant potential of CP, a naturally occurring coumarin, hypothesizing that its effects are mediated through anti-inflammatory and antioxidant mechanisms.

Behavioral testing confirmed that LPS exposure caused a depressive-like phenotype in Swiss albino mice, characterized by increased immobility in both the FST and TST and reduced exploratory behavior in the OFT. These outcomes are well established as reliable indicators of behavioral despair in rodent models and are consistent with previous findings [20,21,44,45]. CP administration produced a significant, dose-dependent reversal of LPS-induced behavioral deficits, markedly reducing immobility across both despair paradigms. Notably, OFT results indicated that CP did not alter baseline locomotor activity, thereby confirming that its antidepressant-like effect was specific and not due to non-specific psychostimulant actions. Findings from the behavioral analyses imply that CP demonstrates antidepressant-like properties, which might occur through the regulation of neuroinflammatory pathways and oxidative stress processes.

A hallmark of the LPS-induced depression model is excessive neuroinflammation, driven by the overproduction of pro-inflammatory cytokines [15-17]. In the present study, LPS administration significantly increased the levels of TNF-α, IL-6, and IL-1β in brain tissue, consistent with previous findings that link cytokine surges to depressive-like phenotypes [18,19]. Treatment with CP effectively attenuated this cytokine elevation, maintaining levels comparable to control animals. These results indicate that modulation of the neuroinflammatory cascade represents a key mechanism underlying CP's antidepressant-like effect, in line with the growing recognition of inflammation as an essential pathway in the onset and progression of MDD. In addition to neuroinflammation, oxidative stress constitutes a major contributing factor to LPS-induced neuronal impairment and depressive behavior [4,11]. In our study, LPS exposure elevated MDA, a marker of lipid peroxidation, while reducing antioxidant defenses, such as GSH and CAT, reflecting an impaired redox state. CP treatment not only suppressed lipid peroxidation but also restored GSH and CAT levels, thereby reinforcing endogenous antioxidant capacity. These findings demonstrate that CP demonstrates a robust antioxidant effect in addition to its anti-inflammatory action. The combined regulation of inflammatory and oxidative pathways suggests a multi-target mechanism of action, which may underlie the broad therapeutic efficacy noted in the present investigation. However, present biochemical assessments were performed using whole-brain homogenates; region-specific alterations in key depression-related areas, such as the pre-frontal cortex and hippocampus, may not have been fully captured, highlighting the need for targeted regional analyses in future studies may offer more precise mechanistic insights.

Our findings reinforce growing evidence that natural compounds capable of modulating neuroinflammation and oxidative stress possess considerable promise for treating neuropsychiatric disorders. The findings for CP are consistent with similar studies on other coumarin derivatives. For example, coumarins, such as esculetin and its derivative 4-methylesculetin have been shown to ameliorate LPS-induced depression by inhibiting the NLRP3 inflammasome pathway and reducing pro-inflammatory cytokines [21,56]. Similarly, bergapten, another coumarin, demonstrated antidepressant effects by inhibiting cyclooxygenase-2 expression [57]. CP, being a polymethoxylated coumarin, shares structural similarities with these compounds but exhibits additional lipophilicity, potentially enhancing its brain penetration [34,37]. Its ability to simultaneously modulate oxidative stress and inflammatory cascades positions it as a promising coumarin derivative with therapeutic advantages.

These findings with CP align with studies showing similar antidepressant-like responses from various non-coumarin natural compounds in LPS- triggered depressive models. Honokiol has been reported to reverse depressive-like behaviors by simultaneously attenuating neuroinflammation and oxido-nitrosative stress [39]. Similarly, sodium butyrate, mangiferin, tauroursodeoxycholic acid (TUDCA), the selective peroxisome proliferator-activated receptor-α agonist WY-14643, agmatine, and geniposide have all demonstrated protective effects against LPS-induced behavioral deficits by suppressing pro-inflammatory cytokines and restoring redox balance [20,45,58-61]. The recurrence of this therapeutic pattern across structurally diverse agents highlights a unifying mechanism in which modulation of the immune-inflammatory axis and oxidative stress pathways provides a robust strategy for alleviating inflammation-associated depression. Fluoxetine produced stronger behavioral and biochemical effects, likely due to its well-established pharmacokinetics, efficient brain penetration, and additional anti-inflammatory and antioxidant actions. In contrast, CP is less well characterized, and further pharmacokinetic, dose-optimization, and pharmacodynamic relationships studies are needed to fully define its therapeutic potential.

Although the current study provides strong evidence supporting CP's antidepressant potential, certain limitations should be acknowledged. The acute LPS model may not fully reflect the chronic and multifactorial characteristics of MDD, which typically involves prolonged neuroinflammatory and oxidative processes. Moreover, the precise molecular targets of CP were not delineated in this study. Future work should therefore investigate its interaction with critical signaling pathways, including NF-κB, MAPK, NLRP3 inflammasome, Nrf2, and BDNF, which play central roles in regulating inflammatory and antioxidant responses [62-65]. Extending the evaluation to more clinically relevant chronic models, such as the chronic unpredictable mild stress paradigm and depression models involving comorbid metabolic inflammation, will also be essential for assessing its sustained efficacy and translational value [66-68]. Long-term safety and tolerability studies are equally important to establish its therapeutic feasibility. In addition, combining CP with conventional antidepressants may yield synergistic benefits by enhancing therapeutic efficacy and reducing adverse reactions. Because of its significant anti-inflammatory and antioxidant activity, CP might serve as a potential therapy for other neurodegenerative and neuroinflammatory diseases, namely, Parkinson's and Alzheimer's disease. Ultimately, validation in higher animal models and rigorously designed clinical studies will be necessary to determine whether CP can be advanced as a safe and effective novel antidepressant candidate.

CONCLUSION

CP demonstrated notable antidepressant-like effects in mice following LPS administration. Our findings confirm that this therapeutic action is primarily mediated through its capacity to suppress neuroinflammation and counteract oxidative stress markers. Treatment with CP markedly reversed LPS-induced behavioral despair, as indicated by improved outcomes in the FST and TST. These changes coincided with lowered pro-inflammatory cytokine levels, enhancement of the antioxidant defense system, and diminished MDA-mediated lipid peroxidation. These findings offer a solid scientific basis for considering CP as a possible new therapeutic option for depression, especially in cases associated with inflammation. Its multi-target mechanism of action—simultaneously addressing both neuroinflammation and oxidative stress—suggests a promising approach to treating this complex disorder. In summary, CP shows potential as a pharmaco-therapeutic agent for depression, acting through modulation of neuroinflammation and oxidative stress, and may help overcome limitations of existing treatments.

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AUTHORS' CONTRIBUTION

Vikram P Jadhav: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software,

Validation, Visualization, Writing - Original Draft. Pradeep Kumar Mohanty: Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing - Review and Editing. All authors have read and agreed to the published version of the manuscript

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The authors have declared that no competing interests exist.

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