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Fustin alleviates lipopolysaccharide-induced anxiety-depression-like performances by modulation of oxidative stress/neuroinflammatory markers/ NF-κB/caspase-3/BDNF expression in rodents

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Abstract. – OBJECTIVE: Anxiety and depression are common psychiatric disorders that affect millions of people worldwide. Lipopolysaccharide (LPS) is a bacterial endotoxin that has been demonstrated to cause depression and anxiety-like behaviors in animal models. Fustin is a flavonoid found in various plant species that have been reported to have neuroprotective effects. The study proposed the evaluation of fustin's impact on anxiety and depression in LPS-injected rats.

MATERIALS AND METHODS: The efficacy of fustin in higher and lower doses was studied by administering a single dose of LPS-injected anxiety/depression in rodents. Behavioral models like the elevated plus maze test, open field test, marble burying test, force swimming test, tail suspension test, and hyperemotionality behavior were performed to evaluate anxiety/depression in rodents. The neuroinflammatory markers such as interleukin-6 (IL-6), interleukin-1β (IL-1β), nuclear factor-κB (NF-κB), tumor necrosis factor-α (TNF-α), apoptosis marker caspase-3, and brain-derived neurotrophic factor (BDNF) were also measured as a part of the study. Additionally, biochemical markers of oxidative stress, such as malonaldehyde (MDA) and antioxidants, including superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), and nitric oxide (NO), were also evaluated.

RESULTS: LPS administration resulted in significant ($p<0.001$) changes in behavior tests and biochemical markers including IL-1β, IL-6, NF-κB, TNF-α, NO, caspase-3, BDNF, MDA, CAT, SOD, and GSH. In contrast, treating the rats with fustin significantly improved the behavior tests and restored the changes in biochemical markers.

CONCLUSIONS: The current work established the efficacy of fustin with its therapeutic impact on depression and anxiety-like behaviors in rodent experimental models through its modulation of apoptosis markers, oxidative stress, and neuroinflammation.

Key Words:
Anxiety, Depression, Fustin, Lipopolysaccharide, Neuroinflammatory markers.

Introduction

Disorders of mental health like anxiety and depression can have a significant effect on the life quality of a person. Anxiety is marked by excessive concern, nervousness, and fear, while depression is recognized by feelings of despondency, helplessness, and disinterest in daily activities. The development of depression can result from a combination of genetic, environmental, and psychological elements[1]. An imbalance of neurotransmitters in an individual's brain can negatively impact mood and lead to anxiety. Anxiety involves dysregulation of the body's normal fear response. Stressful experiences and environmental factors can also play a role in anxiety development[2]. The amygdala region in the brain is involved in processing emotions, including fear and anxiety. In individuals with anxiety disorders, the amygdala may be hype-

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ractive or oversensitive, leading to exaggerated fear responses and heightened anxiety. Inflammation developing in the central nervous system (CNS) has been linked with several psychiatric ailments such as schizophrenia, depression, anxiety, epilepsy, and Alzheimer's disease[3]. Altered serotonin levels or dysfunction in the serotonin system have been implicated in anxiety disorders. Low serotonin levels may contribute to the increased anxiety symptoms. Chronic stress and anxiety can activate the hypothalamic-pituitary-adrenal (HPA) axis, leading to the release of stress hormones, such as cortisol. Prolonged activation of the HPA axis can disrupt the normal stress response and contribute to the development of anxiety disorders. Anxiety and depression are termed critical neuropsychiatric illnesses that occur in a comorbid state. Studies[4] have shown that over-anxiety is observed in half of the depressed population. Depression is known to be a prevalent psychological illness that has a close association with inflammation. Disturbances in circadian rhythms, the internal biological processes that regulate sleep-wake cycles, mood, and other physiological functions, are commonly observed in depression. Disruptions in the circadian system can contribute to sleep disturbances and mood dysregulation. The production of elevated cytokines that are inflammatory in nature by the brain and microglial activation are the intimations of dysfunctionality in cerebral signaling and depression[5]. This relation between the neurological system and the immune system is crucial for the development of neuropsychiatric conditions[6]. Reduced levels of serotonin and norepinephrine may lead to disturbances in mood, sleep, appetite, and other depressive symptoms. Inflammatory markers like interleukin-6 (IL-6), interleukin-1β (IL-1β), nuclear factor-κB (NF-κB), tumor necrosis factor-α (TNF-α), caspase-3 show higher level in the depression[4,7]. Inflammation affects the neurotransmitter function, disrupts neuroplasticity, and contributes to the development of depressive symptoms. A downregulation in brain-derived neurotrophic factor (BDNF) results in a disruption of communication between nerve cells in specific regions of the brain that are important for controlling mood, like the hippocampus and prefrontal cortex. Reduced levels of BDNF and impaired neuroplasticity have been observed, and it potentially contributes to the structural and functional changes observed in the brain[8]. This decrease also leads to a downregulation in the growth of new neurons that leads to depression[9,10]. The elevated levels of malondialdehyde (MDA) and decreased antioxidant levels, such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT), have been observed in brain tissue during depression and anxiety[11,12]. Elevated levels of nitric oxide (NO) produced during neuroinflammation can also result in brain dysfunction and contribute to depression and anxiety[2,13]. There is growing evidence that supports and shows that oxidative stress and neuroinflammation play an important part in inducing depression and anxiety[14,15].

The use of lipopolysaccharide (LPS) as an inducer of cytokines is majorly considered in the studies of animal models and helps to explore the correlation among the impairment of memory, neuroinflammation, depression, and anxiety[16,17]. LPS triggers the inflammatory responses by activating the cells of the immune system and stimulating the inflammatory cytokines release, such as IL-6, IL-1β, NF-κB, and TNF-α[2,3]. The rapid response of neuroinflammation towards LPS administration results in elevated peroxide levels and reactive oxygen species (ROS) in CNS that cause oxidative stress-mediated damage when antioxidant defenses are overwhelmed[18]. In this study, a natural approach was evaluated for managing neuroinflammation and anxiety-like symptoms[3]. Flavonoids, including flavanonols, often have antioxidant properties. Oxidative stress has been implicated in mood disorders, and antioxidants may help reduce this stress, potentially improving mood[19]. Flavonoids may support brain health by protecting neurons from damage and inflammation, which could indirectly affect mood regulation[20].

Fustin is an active chemical constituent found in the heartwood of Rhus Verniciflua Stokes, a medicinal herb belonging to Anacardiaceae family used in traditional medicine[21]. It has a flavone group structure and is a 3′,4′,7-trihydroxyflavanol[22]. The heartwood of R. Verniciflua has antimutagenic and anti-rheumatoid properties due to the antioxidant characteristics of fustin. The higher concentration of polyphenolic constituents in plants also contributes to its anti-inflammatory properties[21,23]. As previously reported, fustin protects against gastric ulcer[24], streptozotocin (STZ) injected hyperglycemic[25], cognitive damage in STZ-injected diabetic[26], high-fat diet/STZ evoked diabetes[27], 3-nitropropionic acid impact Huntington's disease in rats[28]. The present study proposed the

assessment of efficacy of fustin against LPS induced anxiety-depression in rats.

Materials and Methods

Animals

Male Wistar albino rats weighing 180±20 g (10 weeks old) were used for testing. They were kept in a lab with regulated temperature (24±3°C) and humidity (50-60%). The rats in the experimental model were provided with unlimited water and pellet food during the entire course of the experiment. They were housed in cages made of opaque polypropylene. The acclimatization period for the rats was 10 days before the start of the study, and they were treated humanely. The experimental design maintained the regular circadian cycle to minimize its impact on the results. The Institutional Animal Care and Use Committee (IACUC/TRS/PT/23/28) granted the approval to conduct the study. The entire investigation was directed in accordance with ARRIVE guidelines29. The rats used in the study had not undergone any previous procedures.

Chemicals

LPS (Sigma-Aldrich, St. Louis, MO, USA) and fustin (>98 %, stability ≥4-year, MSW Pharma, M.S., India) were used in the study. The various evaluations in the recent study utilized high-grade chemicals.

Experimental Design

To induce depression and anxiety in laboratory animals, the administration of LPS was given intraperitoneally with a concentration of 0.5 mg/kg and freshly dissolved with 0.9 % saline (pH 7.4) for 7 days. The rats were grouped into 5, each group consisting of 6 rats, and the groups were selected randomly to ensure fairness. A total of 30 rats received the following treatments:

- Group I was the control, treated with saline (1 mL/kg i.p.).
- Group II was the LPS control, injected with 0.5 mg/kg.
- Group III was provided with LPS along with fustin at 10 mg/kg.
- Group IV was also provided with LPS along with a higher dose of fustin at 20 mg/kg.
- Group V was fustin per se group at 20 mg/kg.

Fustin was orally given at doses of 10 and 20 mg/kg/day, soluble with dimethyl sulfoxide (DMSO) in the morning time post-LPS administration. Two hours following the LPS administration, animal behavioral evaluations were conducted in accordance with the given treatment.

Behavioral Analysis

Elevated plus-maze test

The elevated plus maze comprises a central platform along with four elevated arms extending out from it. Two of the arms were enclosed by walls, while the other two arms were open except for the platform, entrance, exit points, and ceiling. In the proposed test, a mouse was kept at the center and given a set amount of time to explore the maze. The time spent inside the walled arms, when compared with the arms that are open, leads to the identification of fear or anxiety. This test is based on rodents' natural tendency to avoid elevated or open areas and their curiosity to visit unfamiliar areas. According to the theory, the rat with low anxiety tends to spend more duration in the arms of the maze that is open, whereas the rats with high anxiety will spend more duration in closed arms[30].

Open field test

The purpose of the open field test was to measure a rat's investigative drive, curiosity, fear or anxiety, and motor movement. The platform was divided into 16 equal squares and further separated into peripheral area and central area. The rat was kept at the field's center and permitted to explore it for 5 minutes, with its behavioral activities captured by a digital camera. Rats with higher anxiety levels observed in the open field test tend to enter the central zone less frequently, move farther, and spend less time there[31].

Marble-burying behavior

The rats were kept in Plexiglas cages. The bottom of the cage was filled with about 3 cm of wood chips, and 20 marbles were placed in a grid pattern. The marbles used in the study were clear, except for one computerized supplement where black marbles were used. After the 20-minute duration of the test, subjects were taken out with utmost care to avoid any disturbance to the bedding. The marbles were considered buried if they were covered with bedding by at least 75%[32].

Forced swim test

The forced swim test was performed to analyze depressive behavior in rats. It was usually conducted using two transparent Plexiglas barrels, each measu-

ring a height of 45 cm and a diameter of 20 cm, containing water at 24±1°C. The barrels were divided by a hazy plastic screen to prevent the rats from seeing each other. The rats were moved to the testing area and given an hour to get accustomed to the surroundings before the test was conducted. The testing room was illuminated with white light (600 lux). At the time of performing the test, the rodents were positioned at the center of the tank and monitored by a camera system and software as they swam around. After the test, the rats were quickly taken out of the tank, a paper towel was used to make them dry, and they were placed back in their cages[33].

Tail suspension test

The tail suspension test in rats evaluates depressive behavior. The rats were suspended in the air with their tails hanging down 50 cm from the level of the ground. The idea behind the proposed test was that the animals would try to get themselves free from the stressful situation. Each rat was suspended for the duration of 6 minutes, with the last 4 minutes of immobility being noted. Prolonged periods of immobility, where the animal stops trying to escape and becomes still, were seen as an indication of depressive behavior[5,34].

Hyper-emotionality behavior

The hyper-emotionality of rats was evaluated based on their behavior in response to stimuli related to struggle and fighting. It was a method to study how early social isolation affects aggression and anxiety in rodents. The experimenters classified the rat's response to being touched with gloves as a fight response, and the response to having its tail pinched with blunt forceps as a struggle response. The response towards the external stimuli was rated on a scale of 0 to 4, with 0 showing no response and 4 indicating a strong response. The hyper-emotionality score was measured by summing up the scores obtained from the assessment[35].

Brain tissue homogenization

After the behavioral test, the rats were anesthetized with 80 mg/kg ketamine and 10 mg/kg xylazine intraperitoneally. Complete brains were removed from sacrificed animals and kept at -50°C. The wash brain tissue was prepared in isotonic saline (ice-cold), the tissue homogenate was prepared with phosphate buffer (0.1 M, pH 7.4, ice-cold), and biochemical analysis was done with the supernatant.

Biochemical parameters assessment

MDA in brain homogenates was measured using the Wills method. The absorbance at 535 nm was measured using an ultraviolet (UV) spectrophotometer. MDA formation in wet tissue was measured as nmol/mg[36].

GSH was measured using a method described by Ellman previously[37]. A 412 nm absorbance measurement was conducted on the finished product to determine GSH content in nmol//mg.

The SOD was measured using the method developed by Misra and Frodvich[38]. SOD activity was calculated as the percentage of control. A measurement of the mixture's absorbance was done at 560 nm. SOD formation was measured as U/mg.

The CAT activity was assessed by mixing 0.1 ml of solution of supernatant with 1.9 mL of phosphate buffer (pH 7.0, 200 mM) in a cuvette. After the reaction, 10 mM of newly formed H_2O_2 (1.0 mL) was added. A spectrophotometric method was used to quantify the variations in absorbance at 240 nm. The breakdown of 1 μmol(e) of H_2O_2/min at a pH of 7.0 was expressed as a single unit of CAT. The CAT activity was expected to be U/mg[24,39].

The neuroinflammatory markers such as IL-6, IL-1β, NF-κB, TNF-α, NO, BDNF, and apoptosis marker-caspase-3 in the homogenate of brain tissue samples of rats were analyzed using enzyme-linked immunosorbent assay (ELISA) kits (MBS, USA) following the instructions provided by the manufacturer. Concentrations of such neuroinflammatory markers (TNF-α, IL-6, and IL-1β) were measured in pg/mg, NO in μM/mg, NF-κB concentrations in ng/mg caspase-3 concentrations in ng/ml, BDNF concentrations in pg/mg.

Statistical Analysis

The examination was shown in the form of standard error mean (SEM) and it was performed using the software named GraphPad Prism. A one-way analysis of variance (ANOVA) was taken into consideration to determine the data, and the Shapiro-Wilk normality test was used to confirm its validity and distribution of data. One-way ANOVA followed by Tukey's post hoc test was conducted to compare groups for the analysis of the data. The *p*-value lower than 0.05 was considered significant.

Results

Elevated Plus Maze Test

The rats provided with LPS represented a remarkable lowering of the entries in the elevated

plus maze test with open arms, as shown in Figure 1 A-B. However, the treatment with fustin at the dosage of 10 and 20 mg/kg significantly prevented this change and seemed to restore the open arm entries [F (4, 25)=38.65; p<0.0001]. The entry number of rats to the closed arms increased with LPS treatment but decreased after treatment with both doses of fustin [F (4, 25)=9.296; p<0.0001]. Per se fustin group did not show any significant changes.

Open Field Test

The results of fustin administration on rats in the open field test, i.e., the number of squares and rears, are depicted in Figure 2 A-B. The test measures the spontaneous movement of the rats. The number of squares decreased in the LPS-treated group, but it significantly improved by increasing the dose of fustin to 10 or 20 [F (4, 25)=69.25; p<0.0001]. Similarly, the number of rears by the rats lowered in the doses of fustin [F (4, 25)=135.9; p<0.0001]. Fustin significantly enhanced the mobility of the rats provided with LPS. Notable changes were not observed in the fustin group.

Marble-Burying Behaviour

The treatment of rats by providing fustin at the amount of 10 and 20 undergoing marble-burying behavior is shown in Figure 3. The outcomes demonstrated that the administration of LPS resulted in a rise in the total number of marbles buried by the rats, which was remarkably reduced by fustin therapy at the administered doses [F (4, 25)=87.51; p<0.0001]. Administration of fustin in the per se group did not show any impeccable changes.

Forced Swim Test and Tail Suspension Test

The study outcomes on the impact of fustin on rats subjected to the forced swimming test are depicted in Figure 4A. To measure depression-like behavior following LPS administration, the forced swim test was used. A considerable rise in the immobility time in rats was noticed after LPS treatment, and this effect was partially reversed by administering both doses of fustin [F (4, 25)=73.09; p<0.0001]. There were no notable changes observed in the fustin per se group.

The results of the tail suspension test on rats treated with LPS and fustin are presented in Figure 4B. This test was used to measure the rats' ability to cope with stress caused by LPS and the amount of time they spent in an immobile state. All the rats in different groups were employed for the tail suspension test, and the results depicted that the LPS-treated group had a greater duration of immobility compared to the group administered with fustin at administered doses of 10 and 20 mg/kg [F (4, 25)=45.47; p<0.0001]. Fustin's administration helped to normalize the condition. Fustin per se group did not exhibit any notable alterations.

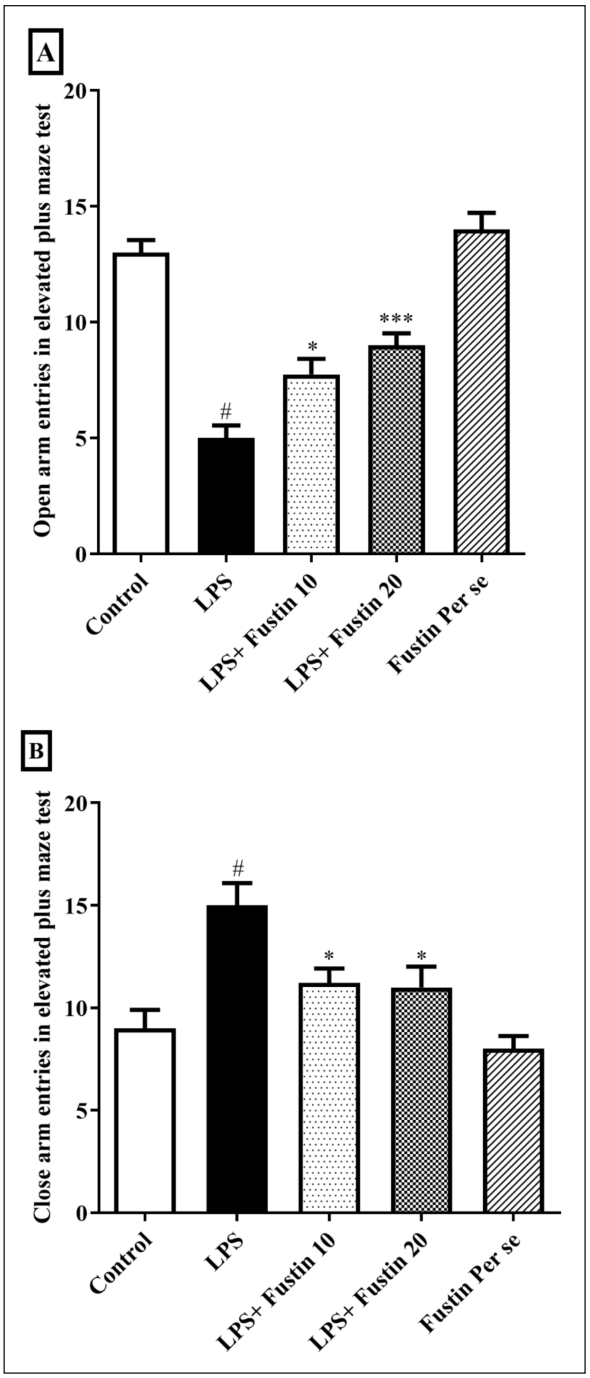

Figure 1. A-B, Effect of fustin on elevated plus maze test. **A**, Number of open arm entries and (**B**) Number of close arm entries. ***p<0.001 shows statistically significant results *vs.* #p<0.05 LPS cluster.

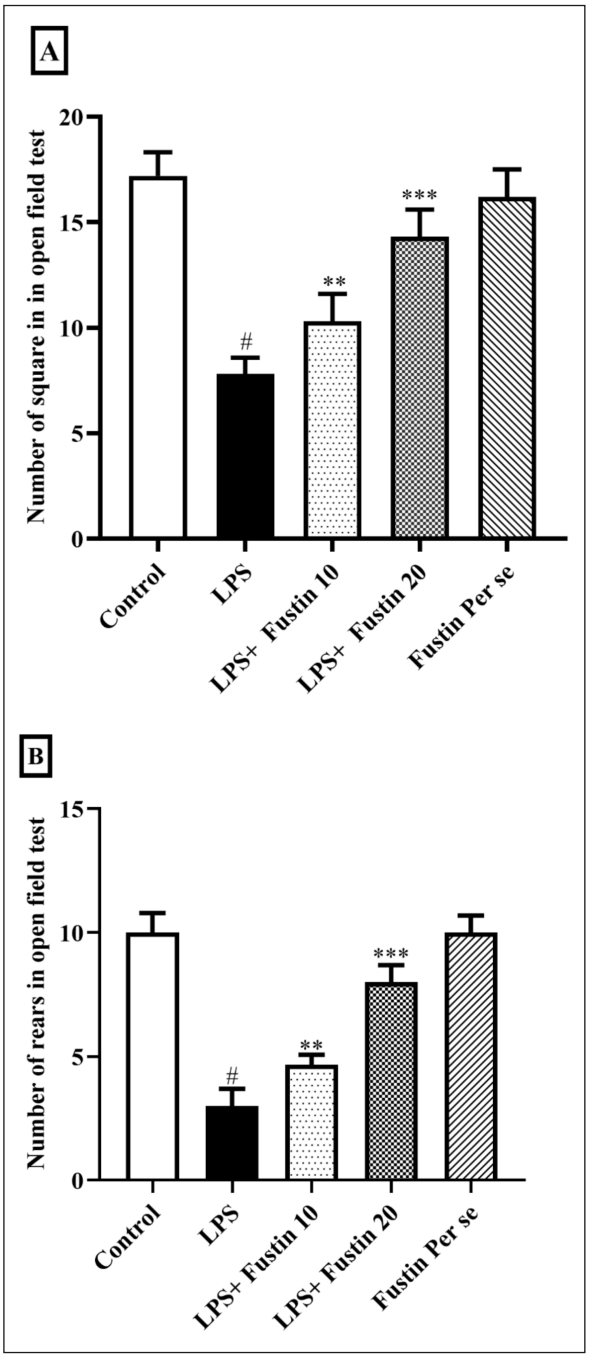

Figure 2. A-B, Outcomes of fustin on open field test. **A**, Number of squares (**B**) Number of rears. ***p<0.001 shows statistically significant results *vs.* #p<0.05 LPS cluster.

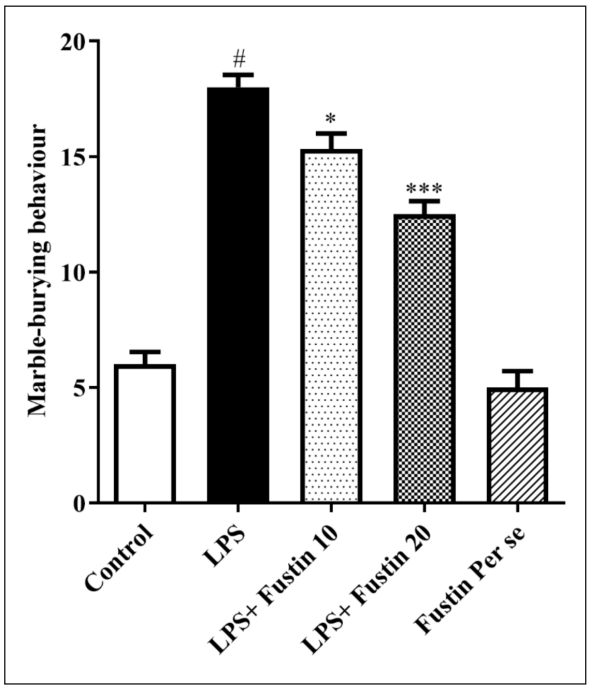

Figure 3. Outcomes of fustin on marble-burying behavior. ***p<0.001 shows statistically significant results *vs.* #p<0.05 LPS cluster.

Hyper Emotional Behavior

The outcome of the hyper-emotional behavior test is illustrated in Figure 5. LPS administration caused an increase in hyper-emotional behavior, indicating heightened emotional behavior, which was improved after treatment with fustin. The results indicated treatment with fustin significantly improved the condition in hyper-emotional behavior [F (4, 25)=32.13; p<0.0001]. The per se group provided with fustin showed no changes.

Oxidative and Antioxidant Parameters

The data presented in Figure 6 A-D showcases the impact of fustin on several key oxidative and antioxidant parameters. The analysis demonstrated that the LPS treatment group had noticeably elevated (p<0.001) MDA levels in comparison to the control group. However, administering fustin at the dose of 10 and 20 mg/kg resulted in a remarkable decrease [F (4, 25)=12.49; p<0.0001] in MDA levels. The LPS control group also experienced a notable drop (p<0.001) in the level of GSH, SOD, and CAT. Conversely, fustin treatment led to a significant restored level of GSH [F (4, 25)=19.21; p<0.0001], SOD [F (4, 25)=11.93; p<0.0001] and CAT level [F (4, 25)=58.08; p<0.0001]. Fustin in the per se group showed no discernible change.

Markers Estimation

The levels of markers such as NO (Figure 7), IL-6, IL-1β, NF-κB, TNF-α (Figure 8 A-D), BDNF, and caspase-3 (Figure 9 A-B) were remarkably increased among the group treated with the

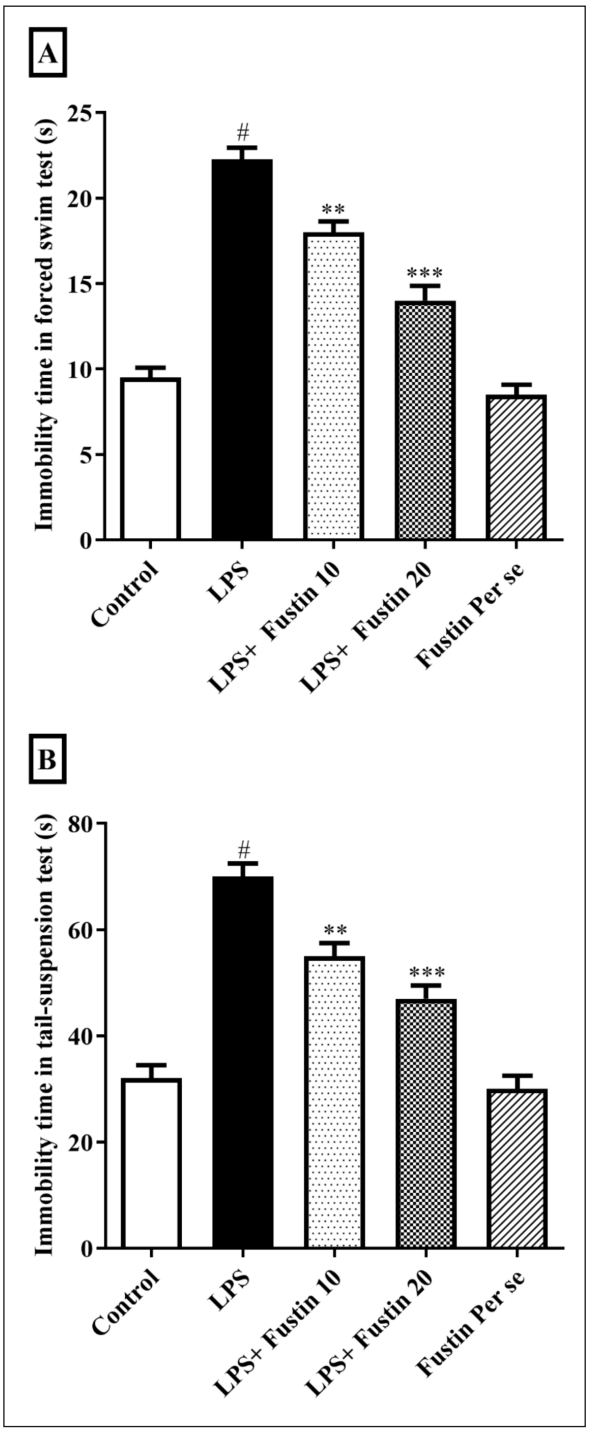

Figure 4. A-B, Outcomes of fustin on (**A**) Forced swim test (**B**) Tail-suspension test. ***p<0.001 shows statistically significant results *vs.* #p<0.05 LPS cluster.

LPS and control group (p<0.001). Furthermore, administering fustin at the dose of 10 and 20 mg/kg led to a remarkable lowering in the level of IL-6 [F (4, 25)=54.02; p<0.0001], IL-1β [F (4, 25)=12.53; p<0.0001], TNF-α [F (4, 25)=48.19; p<0.0001], NO [F (4, 25)=29.42; p<0.0001], NF-κB [F (4, 25)=29.94; p<0.0001], BDNF [F (4, 25)=62.06; p<0.0001] and caspase-3 markers [F (4, 25)=80.47; p<0.0001]. The fustin per se group did not exhibit any remarkable alterations.

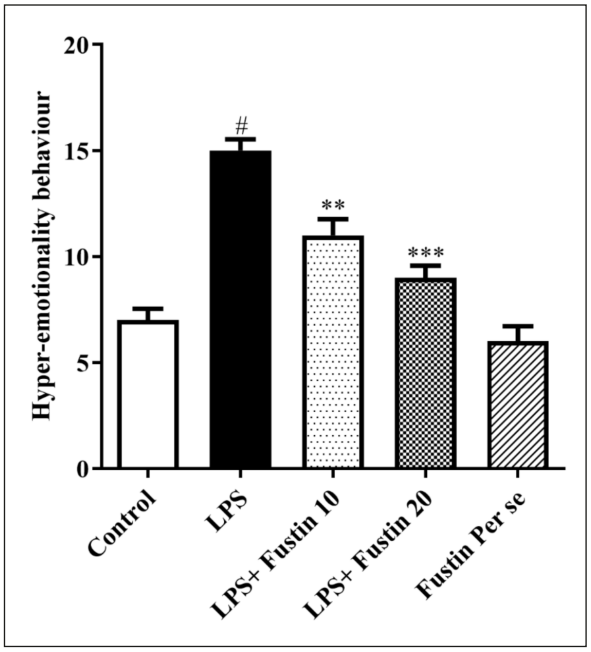

Figure 5. Outcomes of fustin on hyper-emotionality behavior. ***p<0.001 shows statistically significant results *vs.* #p<0.05 LPS cluster.

Discussion

Anxiety serves as a warning signal for the potential dangers in the environment and prompts actions to restore balance to the body's homeostatic state. The constant exposure to stress, also known as chronic stress, can have biological effects because of the body's continual adaptations, which can eventually lead to stress-related illnesses like anxiety and depression[40]. The theory behind depression caused by inflammatory activities suggests that the body's inflammatory immune system can impact neurochemicals or cause harm to neurons and lead to depressive activity. Excessive amounts of cytokines or long-term exposure to these signaling molecules can also affect the brain in a harmful manner[41]. The administration of LPS created a difference between the generation of ROS and the capability of the body to detoxify, resulting in cellular damage and oxidative stress[42]. The use of LPS administration in animal models is a common approach

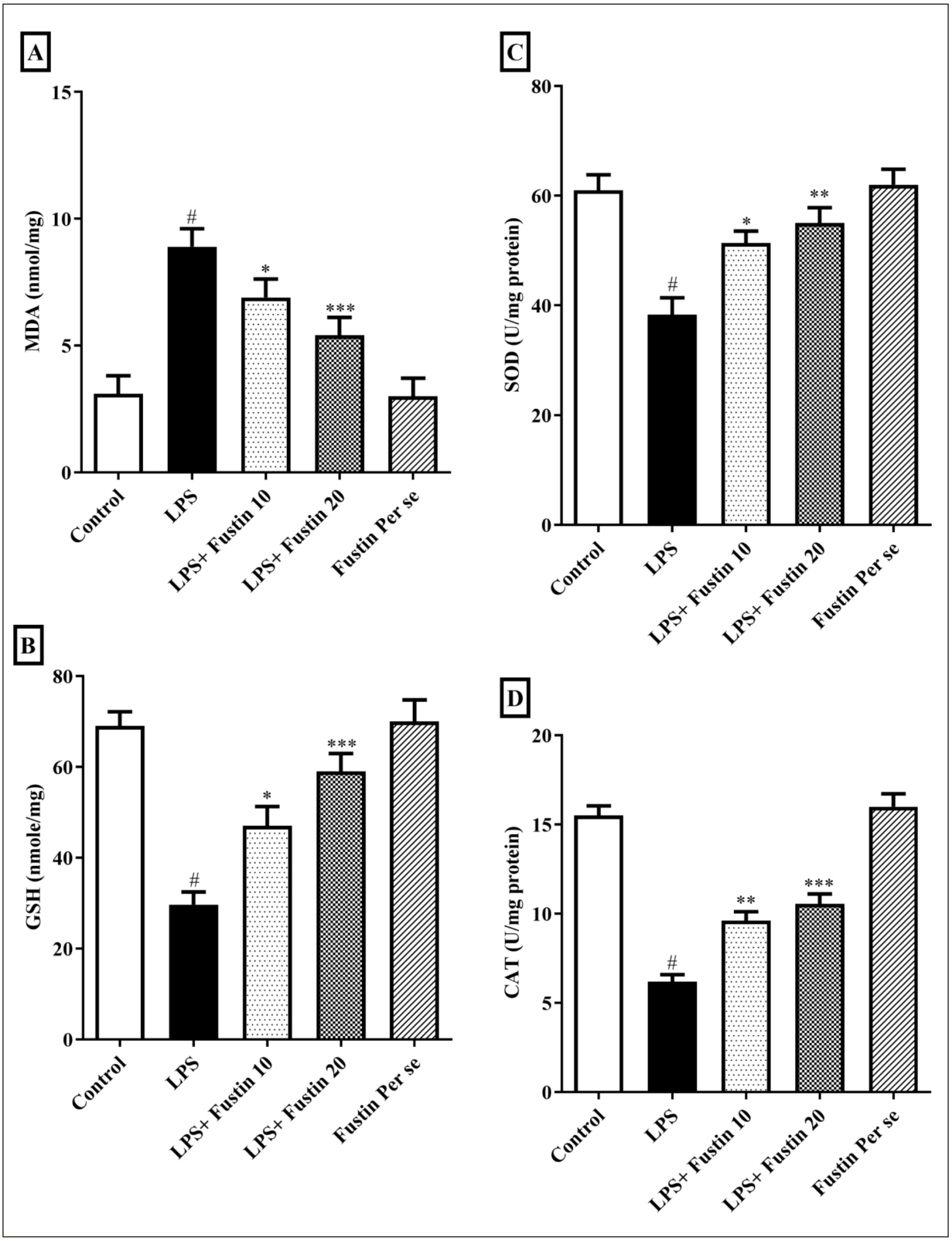

Figure 6. **A-D**, Outcomes of fustin on oxidative and antioxidant parameters: (**A**) MDA - Malonaldehyde; (**B**) GSH – Glutathione; (**C**) SOD - Superoxide dismutase; (**D**) CAT - Catalase activity. *p<0.05, **p<0.001, ***p<0.0001 shows statistically significant results *vs.* #p<0.05 LPS cluster.

to induce inflammation-associated depression in rodents[7]. The administration of LPS, a bacterial endotoxin, has been shown to cause significant damage to multiple organs like the heart, kidney, gastrointestinal tract, liver, lungs, and brain. The application of flavonoids and other polyphenolic compounds derived from plants has demonstrated positive results in reducing the harmful effects of LPS[43]. Fustin was studied in the present research as a potential antioxidant medication for managing anxiety and depression caused by oxidative stress in rats that were triggered by LPS. In this study, it was found that administering fustin to rats leads to behavioral and biochemical markers that increase upon administering LPS.

Additionally, we compared the control rodents with the LPS-treated rodents in our study and found that the latter group showed significant changes, including a marked decrease in certain behavioral markers and biochemical markers[44]. In addition, our testing revealed that rodents treated with 10 or 20 mg/kg doses of fustin exhibited an observable improvement in their performance on various behavioral paradigms compared to the LPS-evoked rats.

LPS can induce an acute inflammatory response that has been shown to affect behavior in various parameters, including the open field test and marble-burying behavior, and elevated plus maze test[7]. In the elevated plus maze test, LPS administration has led to anxiety-like behaviors, such as less time spent by the rats in the arms of the maze that are open and more in its closed arms. This shows that the administration of LPS led to increased anxiety in rats[45]. The post-treatment of fustin with LPS administration reduced the behavioral changes induced by LPS by spending greater time and entering the open arms more frequently than closed arms and suggests that fustin may have an effect against LPS-injected anxiety in rats that are protective in nature. Similarly, LPS administration causes decreased exploration and increased anxiety-like behaviors, such as decreased box crossing numbers, duration in the nearness to the central area, and rearing in the open field. This suggests that LPS can lead to decreased spontaneous activity in rats. After administering fustin, the increase in crossing numbers and rearing in open field tests confirms its ability to reduce anxiety. In the marble-burying behavior test, LPS administration has been reported to cause a rise in the number of marbles buried. This elevation in burying behavior is thought to reflect an increase in repetitive and compulsive behaviors, which is indicative of anxiety or stress in rats[46].

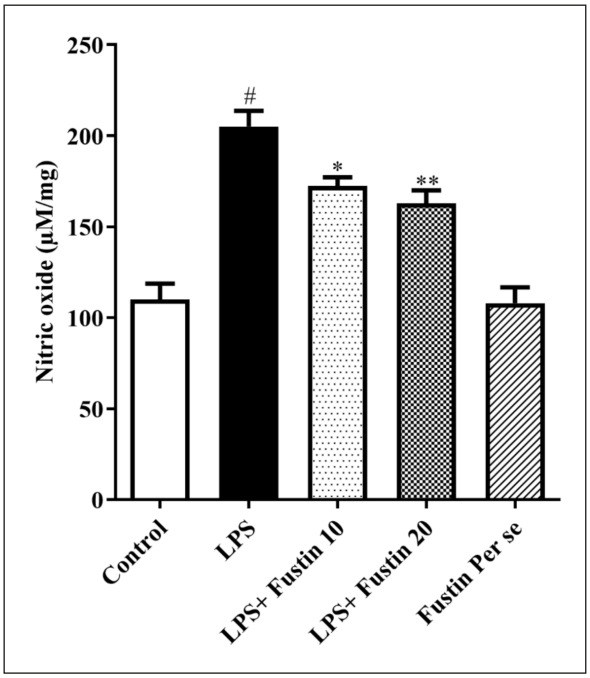

Figure 7. Outcomes of fustin on nitric oxide. ***p<0.001 shows statistically significant results *vs.* #p<0.05 LPS cluster.

In this study, the tail-suspension test and forced swim test were taken into consideration to study the depression type of behavior in rats exposed to LPS. The results showed that these rats were less active and showed signs of immobility, which are hallmarks of depression. The forced swim test and tail-suspension test measure the level of dejection and depression by measuring the progressive immobility observed in the rats. Antidepressant drugs have been shown to shorten the duration of immobility in these tests, allowing the animals to escape the drowning-like situation[6]. According to prior research, the immobility of rats in the tail-suspension test and forced swim test is an indicator of depression-like behaviour[1,47]. Based on these findings, the results showed that providing the rats with fustin reduced the depression-type behavior induced by LPS. Additionally, the results of the analysis of hyper-emotional behavior in rats induced with LPS showed an increase in response. The elevated response during handling with gloved hands and tail pinching with non-sharp forceps indicated depression behavior. Treatment with fustin then showed outcomes comparable to the LPS-evoked rats, reducing the hyper-emotional behavior. The outcomes of the study showed that

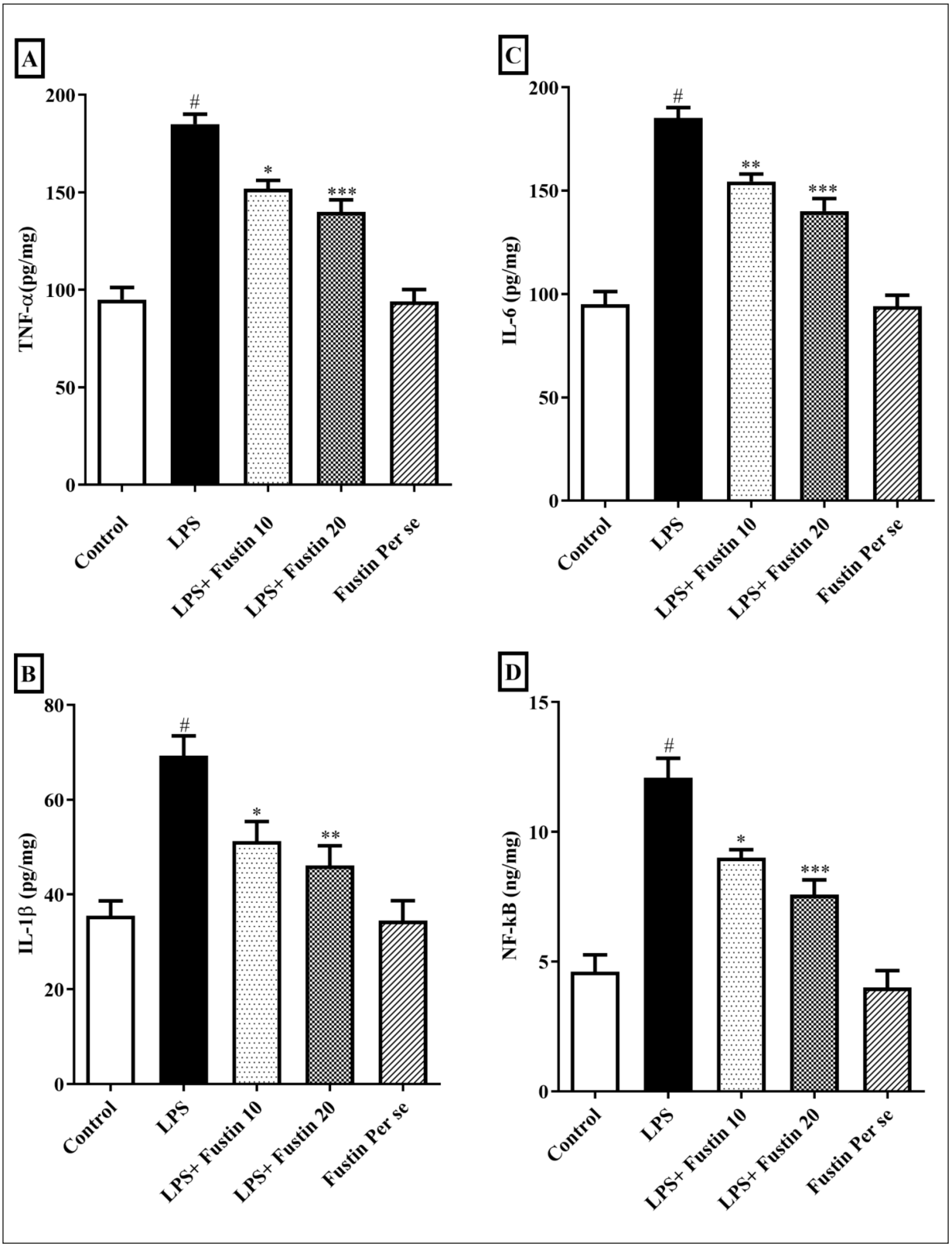

Figure 8. A-D, Outcomes of fustin on markers (**A**). Tumor necrosis factor-α (TNF-α) (**B**). Interleukin-1β (IL-1β) (**C**). Interleukin-6 (IL-6) (**D**). Nuclear factor-kappa B (NF-κB). *p<0.05, **p<0.001, ***p<0.0001 shows statistically significant results *vs.* #p<0.05 LPS cluster.

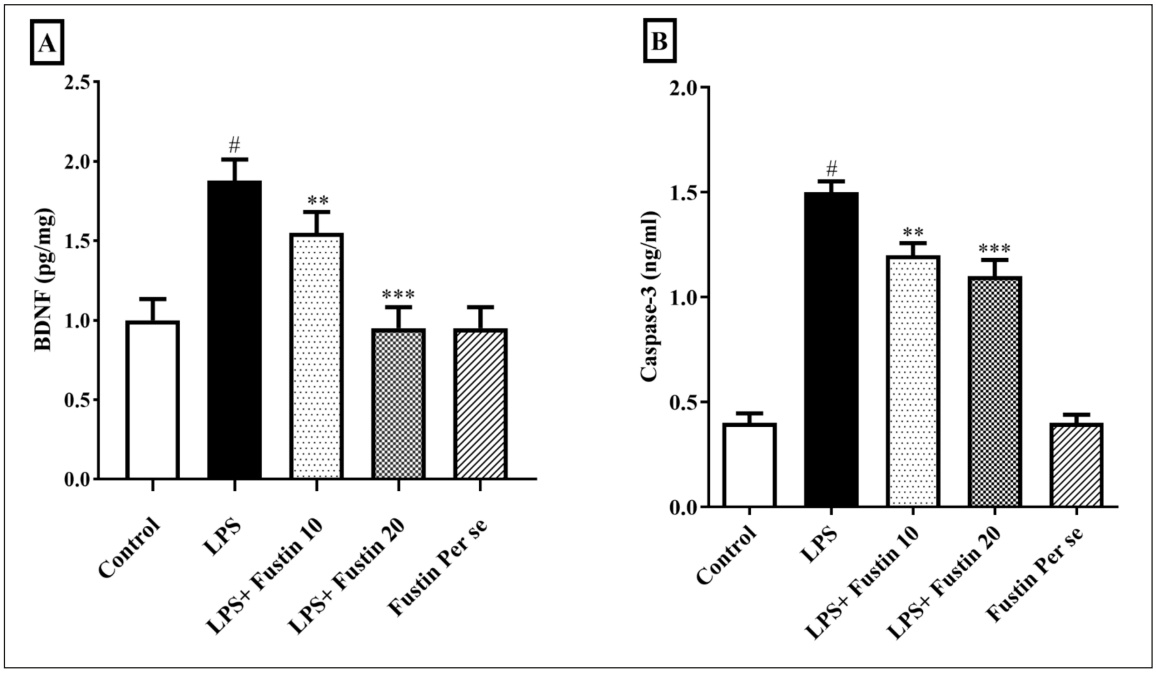

Figure 9. A-B, Outcomes of fustin on inflammatory and apoptosis markers. BDNF, caspase-3. *p<0.05, **p<0.001, ***p<0.0001 shows statistically significant results *vs.* #p<0.05 LPS cluster.

significant changes that occur with behavioral tests are considerable aspects of depression and anxiety.

The outcomes of the present work depicted that the activation of IL-1β, IL-6, NF-κB, TNF-α, and BDNF are all associated with the inflammatory reaction to LPS and have been implicated in the occurrence of anxiety and depression-like responses in rodents. BDNF is a protein that is essential for the growth, survival, and function of neurons. There has been an association between depression and anxiety and reduced levels of BDNF. Post hoc test exposed that treatment with fustin rat both doses reduced the levels of IL-1β, TNF-α, IL-6, and BDNF in the rat's brain. Fustin also inhibited the activation of NF-κB, a key transcription that controls the level of cytokines that are pro-inflammatory in nature[48]. Increased activation of caspase-3 is associated with neuronal damage and cell death in the brain. This has been implicated in the pathogenesis of mood disorders such as anxiety and depression[29]. The elevated caspase-3 level on the administration of LPS was significantly moderated by fustin. Fustin may have anti-inflammatory effects and be useful in mood disorder treatment.

In the present study, the rodents treated with LPS have remarkably lower grades of GSH, CAT, and SOD in the brain of rodents, along with the raised amount of MDA. The post-treatment with fustin for 7-day administration of LPS substantially maintained the level of evaluated markers, demonstrating fustin as an anti-oxidant agent in anxiety and depression type of changes[25].

The changes in cytokines that are pro-inflammatory in nature and the markers of oxidative stress could participate in the molecular causes of anxiety and depression-type behaviors[16]. Fustin appears to modulate a variety of molecular pathways and parameters involved in the progression of depression and anxiety in rats caused by LPS. Its effects on pro-inflammatory markers, oxidative stress, antioxidants, BDNF, NF-κB, and caspase-3 expression propose that it may have a therapeutic impact useful in the treatment of mood dysfunction due to its flavonoid factors and its antioxidant properties (Figure 10). However, more research is needed to better understand how these interactions work and to determine if fustin could be useful in curing anxiety and depression. In future studies, other genetic models, and molecular and immunohistochemistry will be employed to verify the mechanism of fustin. However, this study has several limitations due to its brief duration and the small number of animals employed for behavioral tests and analysis of biochemical markers. Moreover, additional studies are also required to assess the long-term studies of fustin administration, including the optimal dose and duration of treatment.

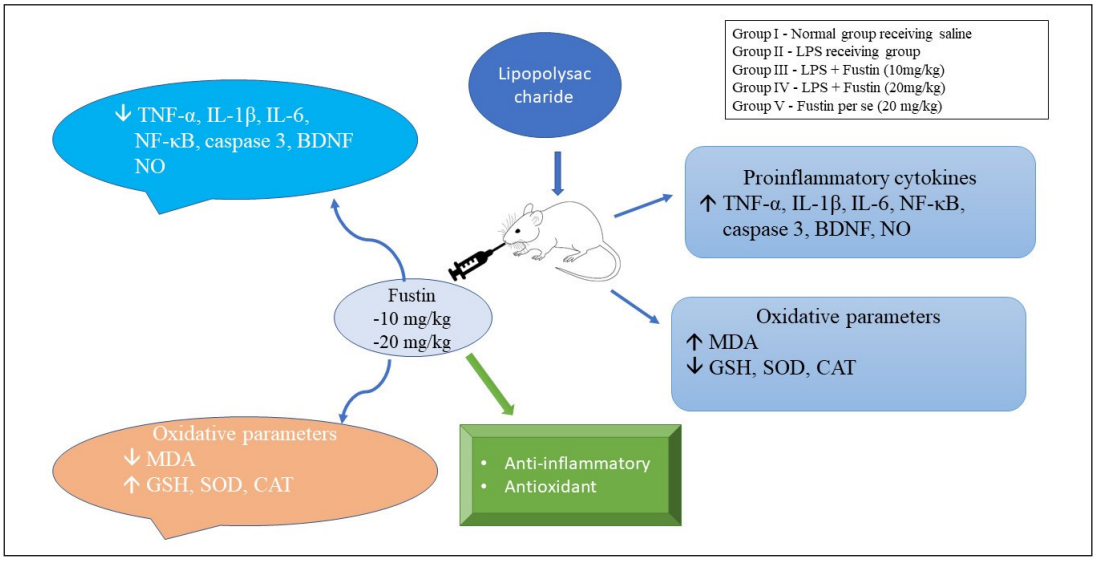

Figure 10. Hypothetical mechanism of fustin against LPS-induced anxiety-depression.

Conclusions

The outcomes of the proposed study show that fustin effectively ameliorates LPS-induced anxiety and depression type of behaviors in rodents, likely by regulating the behavioral parameters along with restored biochemical markers. These results may encourage the prospective therapeutic use of fustin in the management of mood disorders.

Authors' Contributions

Imran Kazmi: Conceptualization; methodology and first draft of manuscript: Nadeem Sayyed and Azizah Bawadood; Fund acquisition: Fahad A. Al-Abbasi; Critical Revision of manuscript: Amira M. Alghamdi, May M. Alqurashi, Ryan A. Sheikh, Sami I. Alzarea. All authors read and approved the manuscript.

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Conflict of Interest

No conflict of interest.

Informed Consent

Not applicable.

Ethics Approval

The research design applied in our study meets Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. The Institutional Animal Care and Use Committee, M.S., India (IACUC/TRS/PT/23/28) granted the approval to conduct the study.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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