Curcumin prevents sickness behavior and fever by the modulation of Nrf2 activity in a model of endotoxemia

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Abstract

Lipopolysaccharide (LPS) is a potent inducer of inflammation, triggering behavioral changes and fever. The present study aimed to evaluate whether pretreatment with curcumin prevents the behavioral changes and fever induced by LPS through the modulation of nuclear factor-erythroid 2 related factor 2 (Nrf2). Male Wistar rats were pretreated for 2 consecutive days with vehicle or curcumin at doses of 25, 50, or 100 mg/kg by oral gavage. The animals intraperitoneally received either vehicle or LPS at a dose of 500 µg/kg. After 2 h, the behavioral responses were assessed through open field test (OFT), social interaction test, forced swim test (FST), and food intake assessment. The febrile response was assessed by telemetry after vehicle or LPS injection to evaluate the effect of curcumin on the thermoregulatory response during the immunological challenge. The pretreatment with curcumin at doses of 50 and 100 mg/kg prevented the reduction of distance traveled on OFT, increased the immobility time of FST, impaired social withdrawal, decreased food intake, and induced fever. In addition, at these doses, it was possible to observe a significant decrease in the plasma levels of TNF- α and IL1- β and an increase in Nrf2 translocation to the cell nucleus during the immunological challenge. Our data provide further evidence of curcumin's ability to prevent LPS-induced sickness behavior and fever possibly by a mechanism related to the modulation of Nrf2 translocation.

1. Introduction

Communication between the immune system and the brain is important for the host organism to engage in (Sylvia et al., 2018) temporary and specific defense behaviors (Mazuco et al., 2019), such as reduced locomotor and exploratory activities, social withdrawal, anorexia, and anhedonia, which together are known as sickness behavior (Dantzer, 2004; Poon et al., 2015; Nilsson et al., 2017; Oliveira et al., 2020). In addition, inflammation is often accompanied by thermal manifestations, such as fever (Poon et al., 2015; Blomqvist and Engblom, 2018). Although inflammation is an adaptive reaction to different harmful stimuli, in chronic conditions such as sepsis, it can cause severe multiple organ dysfunction and, consequently, neurological disorders and death (Barichello et al., 2019). Therefore, it is important to discover new multi-targeted, non-toxic, and highly potent agents for therapeutic use in different human diseases related to inflammation.

Curcuma longa (curcumin), popularly known as turmeric, has gained considerable attention due to its therapeutic potential for various diseases, owing to its anti-cancer, antioxidant, anti-inflammatory, and neuroprotective properties (Pulido-Moran et al., 2016; Chen et al., 2018; Zhou et al., 2020). In particular, it exerts anti-inflammatory effects through the downregulation of different pro-inflammatory cytokines (Gupta et al., 2014; Kumar et al., 2017) and modulation of transcription factors (Hassan et al., 2019; Zhou et al., 2020), including elevation of the nuclear factor-erythroid 2 related factor 2 (Nrf2) (Hassan et al., 2019). Under physiological conditions, Nrf2 is anchored with the cytoplasmic Kelch-like ECH-associated protein 1 (Keap1), which mediates degradation by ubiquitination (Bellezza et al., 2018; Rahman et al., 2021). In response to stress, Nrf2 dissociates from Keap1 and translocates into the nucleus, where it binds to the antioxidant-response element, regulating the expression of various defensive genes involved

in anti-oxidation and anti-inflammation (Bellezza et al., 2018; Rahman et al., 2021; Yang et al., 2021). Therefore, our study aimed to determine whether pretreatment with curcumin attenuates sickness behavior and fever by modulating Nrf2 activity in a model of endotoxemia induced by the systemic administration of lipopolysaccharide (LPS), a cell wall component of gram-negative bacteria.

2. Methods

2.1. Animals

Adult male Wistar rats weighing 200–300 g were obtained from the Central Animal Facility of the Federal University of Alfenas, Brazil. The animals were housed under controlled light (12 h light-dark cycle; lights turned on at 07:00 a.m.) and temperature ($22 \pm 1^\circ\text{C}$) conditions with ad libitum access to water and chow. The animals were allowed to habituate to the housing facilities for at least one week before the experiments began. The behavioral studies were performed in a quiet room between 10:00 a.m. and 12:00 p.m. to minimize circadian variation. All experiments were approved by the Ethics Committee of the Federal University of Alfenas and conducted in accordance with their guidelines (protocol #39/2018).

2.2. Behavioral Experimental Protocol

The rats were pretreated with vehicle or curcumin (25, 50, or 100 mg/kg) by oral gavage once a day for 2 consecutive days. One hour after pretreatment on day 2, the control group rats were administered saline (1 ml/kg) or LPS from *Escherichia coli* (serotype 026:B6; 500 $\mu\text{g/kg}$) to establish a sickness behavior model (Orlandi et al., 2015). Subsequently, sickness behavior was evaluated by open field test (OFT), forced swim test (FST), social interaction test, or food intake assessment 2 hours after the administration of LPS. This time point was chosen based on previous behavioral, endocrinal, and neurochemical studies (Orlandi et al., 2015; Munshi et al., 2019; Oliveira et al., 2020). LPS was dissolved in a sterile saline solution (0.9% NaCl), and curcumin was suspended in 1% carboxymethylcellulose. LPS and curcumin were purchased from Sigma-Aldrich (São Paulo, Brazil).

Immediately after the behavioral tests, the animals were euthanized, and their blood was collected in heparinized tubes. In addition, their brains were collected, and the mediobasal hypothalami were microdissected for protein expression analysis, as described previously (Enes-Marques et al., 2020). The blood was centrifuged, and the plasma was aliquoted and stored at -20°C . The plasma samples were analyzed for tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) using commercially available enzyme-linked immunosorbent assay kits (PeproTech Funpec-RP, Brazil) according to the manufacturer's guidelines.

For Nrf2 quantification, extraction of cytoplasmic and nuclear proteins from the mediobasal hypothalamus was performed according to the guidelines of the Cytoplasmic and Nuclear Protein Extraction Kit (Boster Biological Technology, Pleasanton, CA, USA). After extraction, the samples were separated and stored in a -80°C freezer for further analysis.

2.2.1. Open Field Test (OFT)

Locomotor activity was quantified for 5 min in a circular acrylic apparatus (diameter of 60 cm and walls of 50-cm height) with a black background. Each rat ($n = 8$ per group) was gently placed in the center of the apparatus, and the parameters “distance traveled” and “immobility time” were automatically measured by the software EthoVision XT version 9.0. The apparatus was cleaned with a 5% ethanol solution before the placement of each animal to avoid possible bias effects of odor cues left by previous rats (Orlandi et al., 2015).

2.2.2. Forced Swim Test (FST)

Rats ($n = 8$ per group) were placed in a vertical acrylic cylinder (diameter $\times$ height: 24 $\times$ 60 cm) filled with 40 cm of water (25°C). This water volume allowed the animals to swim or float without touching the bottom of the apparatus with their paws or tails. One day before the test, the animals were subjected to 15 min of swimming. After the swimming session, they were dried with towels and placed under a lamp (32°C) for 15–30 min. On the day of the test (24 h after adaptation), the animals were placed in the cylinder for 5 min and their behavior was recorded. The parameters “immobility,” “swimming,” and “climbing” were evaluated. Immobility was considered when the rats made no effort to escape, barring the necessary movements that allowed them to keep their heads above the water (Detke et al., 1997).

2.2.3. Social Interaction Test

Three days before the test, the animals were allowed to individually explore the square open field apparatus (60 $\times$ 60-cm wide and 60-cm high) for 15 min/day to familiarize themselves with it, but not with their partner. Each experimental rat was randomly assigned to an unknown partner of the same sex (juvenile visiting rat), with lower age and weight (at least 100 g less). The pair of rats was placed in the apparatus for 10 min, and the total amount of time spent engaging in social interaction (including sniffing, enticing, following, mounting, and crawling on or direct contact with the visiting animal) was recorded (Der-Avakian et al., 2010; Orlandi et al., 2015).

2.2.4. Food Intake

To assess food consumption, the rats ($n = 8$ per group) were allocated to individual cages with free access to water and chow. On the day of the test, the animals were deprived of chow but not water for 2 h after treatment. After food restriction, the animals were offered previously weighed pelletized chow, and the chow intake pattern was monitored for 24 h by weighing the animals at 2, 4, 6, and 24 h after reintroduction (Rorato et al., 2012).

2.3. Body Temperature Measurement

Animals were anesthetized using 2,2,2-tribromoethanol (Aldrich, Milwaukee, WI, USA; 250 mg/kg, intraperitoneally). A paramedian laparotomy (1.5 cm) was performed to insert a biotelemetry probe device (model G2 E-Mitter®, STARR Life Sciences Corp., Oakmont, PA, USA) into the abdominal cavity. The incision was then sutured, and the implanted device was used for body temperature measurements.

Immediately after surgery, the animals were treated with penicillin (100,000 UI). Once fully conscious, the intervened rats were individually housed in cages for 5 days before the experiments for complete recovery. Body temperature was recorded at 10-min intervals for 8 h after the injection of saline or LPS (500 µg/kg, intraperitoneally) in animals previously treated with curcumin (25, 50, or 100 mg/kg) or vehicle for 2 consecutive days. The cages were placed under a telemetry receiver (model ER-4000 Energizer/Receiver Mini-Mitter®, STARR Life Sciences Corp., Oakmont, PA. USA) connected to a personal computer. Data were collected using appropriate software (Vital View®, Mini-Mitter). To determine the basal temperature, the mean temperature measured 30 min before the first administration was calculated, and the difference between the mean basal temperature and the temperature obtained at each interval was calculated to obtain the temperature variation. The temperature response to LPS was monitored during the light phase to minimize activity-related changes in body temperature. Only animals with basal body temperatures below 37.5°C were subjected to the experimental procedure (Crunfli et al., 2015; Silva and Giusti-Paiva et al., 2015).

2.4. Western Blotting

Aliquots of the lysates containing 30 µg of protein were denatured in Laemmli sample buffer (4% sodium dodecyl sulfate [SDS], 20% glycerol, 0.02% bromophenol blue, Tris 1 M [pH: 6.8], and dithiothreitol 0.1 M) at 95°C for 5 min and subjected to SDS-polyacrylamide gel electrophoresis on a 1.5-mm thick 12% electrophoresis gel. Electrophoresis and subsequent transfer of the proteins to a 0.45-mm nitrocellulose membrane were performed. Subsequently, the membrane was incubated in a 5% skim milk blocking solution with Tris–HCl buffer (1.5 M, pH: 7.4) plus 0.1% Tween 20 (TBS-T) for 2 h. The membranes were incubated overnight with anti-Nrf2 (1:1000, Abcam #ab51163) primary antibodies at 4°C in a solution containing TBS-T. The membranes were then washed and incubated at room temperature for 1 h with a horseradish peroxidase-conjugated anti-mouse-IgG antibody (1:2000, Abcam #ab51163) in a solution containing TBS-T, followed by incubation with a mixture of chemiluminescence reagents (Clarity Western ECL Substrate; Bio-Rad Laboratories, Hercules, CA, USA). Detection by chemiluminescence was performed using a ChemiDoc XRS Imaging System (Bio-Rad). Each membrane was stripped and re-probed with anti-β-actin (1:4000; Sigma-Aldrich #A4700) to ensure equal protein loading. Gel digital images were used to quantify optical density. Nrf2 expression was normalized by β-actin and expressed as a percentage of the control group.

2.5. Statistical Analysis

Data were analyzed using GraphPad Prism 8.0. The results are expressed as mean ± standard error of the mean. The symmetry of the data was tested using the Kolmogorov–Smirnov, Shapiro–Wilk, and D'Agostino-Pearson Omnibus normality tests. Data was considered symmetric if approved by at least one of the three tests. The effects of pretreatment (vehicle or curcumin) and immunological challenge factors (treatment with saline or LPS) were analyzed using a two-way analysis of variance followed by Tukey's post-hoc test. The significance level was set at $P < 0.05$.

3. Results

Figure 1 shows the effect of oral pretreatment with curcumin at doses of 25, 50, or 100 mg/kg 2 h after LPS-induced inflammation. In the OFT, vehicle + LPS-treated animals presented a reduction in the distance traveled (Fig. 1A) and an increase in the immobility time (Fig. 1B) when compared to the control group. Pretreatment with curcumin at doses of 50 and 100 mg/kg did not significantly change the locomotor activity in the OFT 120 min after intraperitoneal saline injection, but it did prevent the reduction in travel distance (Fig. 1A) and immobility time (Fig. 1B) induced by LPS, reaching the same levels exhibited by the control group. The total social interaction time in animals immunologically challenged by LPS was significantly reduced when compared to the control group (Fig. 1C). Pretreatment with curcumin at 50 or 100 mg/kg prevented the reduction of social behavior induced by LPS (Fig. 1C). Regarding feeding behavior, the animals treated with LPS presented a reduction in food intake 24 h after injection when compared to the control group. However, when pretreated with curcumin at 50 or 100 mg/kg, the animals presented an increase in the feeding pattern after 24 h when compared to animals treated with vehicle + LPS (Fig. 1D). Pretreatment with curcumin at 25 mg/kg did not prevent the decrease in locomotor activity (Fig. 1A and 1B), social interaction (Fig. 1C), or hypophagia (Fig. 1D) induced by LPS.

The analysis of the FST revealed that the immunological challenge caused by LPS increased the immobility time (Fig. 2A) and reduced the swimming (Fig. 2B) and climbing times (Fig. 2C) when compared to the control group. Similar to the OFT results, pretreatment with curcumin at 25 mg/kg did not prevent the increase in immobility time (Fig. 2A) and, consequently, did not prevent the reduction in swimming time (Fig. 2B) induced by LPS. Only pretreatments with curcumin at 50 or 100 mg/kg were able to change the immobility and swimming times (Fig. 2A and 2B), that is, only at these doses, curcumin was able to prevent depressive-like behavior. Regarding climbing, we observed a significant difference only in animals pretreated with curcumin at 100 mg/kg when compared to the vehicle + LPS-treated group (i.e., these animals presented an increase in climbing time; Fig. 2C).

Figure 3A shows that animals in the vehicle + LPS-treated group developed a febrile response after LPS injection when compared to the control group. The thermal index (area under the curve, Fig. 3B) from 2 to 8 h after LPS, observed in rats pretreated with curcumin at 25 mg/kg, was not able to prevent fever; however, pretreatment with curcumin at 50 or 100 mg/kg prevented the LPS-induced increase in body temperature.

Plasma levels of TNF- α (Fig. 4A) and IL1- β (Fig. 4B) significantly increased 2 h after LPS injection. Pretreatment with curcumin at all tested doses prevented the increase in TNF- α and IL1- β when compared to the vehicle + LPS-treated group. Since the well-established activation pattern of Nrf2 is a translocation from the cytoplasm to the nucleus, we investigated the cytoplasmic and nuclear Nrf2. The results showed that when compared to the vehicle group, curcumin reduced cytoplasmic Nrf2 and increased nuclear Nrf2 with both saline and LPS treatment, suggesting that curcumin administration induced Nrf2 nuclear translocation. The statistical data are presented in Table 1.

Table 1

Two-way analysis of variance in groups pretreated with vehicle or curcumin (pretreatment factor) and subjected to an injection of vehicle or LPS (immunological challenge factor) in the open field test, social interaction test, food intake assessment, forced swim test, body temperature measurement (thermal index), plasma cytokine measurements, and Nrf2 expression on hypothalamus.

Parameters	Pretreatment factor	Immunological challenge factor	Interaction
Open field test			
Distance traveled	$F_{(3,56)} = 4.77, P < 0.01$	$F_{(1,56)} = 37.6, P < 0.001$	$F_{(3,56)} = 6.17, P < 0.01$
Immobility time	$F_{(3,56)} = 3.24, P < 0.05$	$F_{(1,56)} = 38.1, P < 0.001$	$F_{(3,56)} = 5.31, P < 0.01$
Social interaction test			
Interaction time	$F_{(3,56)} = 20.8, P < 0.001$	$F_{(1,56)} = 167, P < 0.001$	$F_{(3,56)} = 34.7, P < 0.001$
Food ingestion			
Food intake	$F_{(3,56)} = 25.5, P < 0.001$	$F_{(1,56)} = 140, P < 0.001$	$F_{(3,56)} = 26.4, P < 0.001$
Forced swim test			
Floating time	$F_{(3,56)} = 14.7, P < 0.001$	$F_{(1,56)} = 21.0, P < 0.001$	$F_{(3,56)} = 6.08, P < 0.01$
Swimming time	$F_{(3,56)} = 6.85, P < 0.001$	$F_{(1,56)} = 15.3, P < 0.001$	$F_{(3,56)} = 4.65, P < 0.01$
Climbing time	$F_{(3,56)} = 19.6, P < 0.001$	$F_{(1,56)} = 3.69, P = 0.060$	$F_{(3,56)} = 8.36, P < 0.05$
Body temperature			
Thermal index	$F_{(3,43)} = 18.8, P < 0.001$	$F_{(1,43)} = 33.5, P < 0.001$	$F_{(3,53)} = 14.6, P < 0.001$
Cytokines			
TNF- α	$F_{(2,40)} = 16.7, P < 0.001$	$F_{(1,40)} = 26.0, P < 0.001$	$F_{(2,40)} = 16.8, P < 0.001$
IL-1 β	$F_{(2,40)} = 15.4, P < 0.001$	$F_{(1,40)} = 25.3, P < 0.001$	$F_{(2,40)} = 15.9, P < 0.001$

F: F value; P: P value; TNF- α : tumor necrosis factor- α ; IL 1- β : interleukin1- β ; Nrf2: nuclear factor-erythroid 2 related factor 2

Parameters	Pretreatment factor	Immunological challenge factor	Interaction
Nrf2 expression			
Cytoplasmic Nrf2	$F_{(2,25)} = 4.05, P < 0.05$	$F_{(1,25)} = 10.9, P < 0.01$	$F_{(2,25)} = 0.33, P = 0.720$
Nuclear Nrf2	$F_{(2,25)} = 3.65, P < 0.05$	$F_{(1,25)} = 3.99, P = 0.056$	$F_{(2,25)} = 0.05, P = 0.945$
F: F value; P: P value; TNF- α : tumor necrosis factor- α ; IL 1- β : interleukin1- β ; Nrf2: nuclear factor-erythroid 2 related factor 2			

4. Discussion

In our study, LPS induced a decrease in exploratory behavior in rats in OFT, depressive-like symptoms in FST, social interaction, and anorexia, allowing us to verify the ability of this endotoxin to produce characteristic sickness behavior. Sickness behavior accompanied by fever constitute a highly regulated and adaptive strategy to fight infections (Quan and Banks, 2007; Harden et al., 2015; Oliveira et al., 2020). Our results show that curcumin, when administered prior to an immunological challenge, may counteract a loss of motivated behaviors and fever during illness, suggesting a potential effect of curcumin in modulating sickness behavior.

LPS is recognized by CD14–MD2 receptors present in monocyte membranes, and it sends intracellular signals via toll-like receptor 4 (TLR-4), culminating in the activation of NF- κ B and release of pro-inflammatory mediators (Engblom et al., 2002; Bellezza et al., 2018; Blomqvist and Engblom, 2018). The production of TNF- α and IL1- β induces the synthesis of cyclooxygenase enzyme-2 (COX-2) in the hypothalamic vascular network, resulting in an increase in the cerebral levels of prostaglandin E2 via COX-2 (Engblom et al., 2002; Kawahara et al., 2015; Blomqvist and Engblom, 2018), sickness behavior (Dantzer, 2004; de Paiva et al., 2010; Oliveira et al., 2020), and onset of LPS-induced fever (Evans et al., 2015; Garami et al., 2018).

Intracerebroventricular and intraperitoneal administration of pro-inflammatory cytokines induces a complete sickness state in rodents, manifested by a reduction in locomotor/exploratory behavior and food intake, and an increase in immobility time, anhedonia, social withdrawal, and fever (Dantzer, 2004; Palin et al., 2007; de Paiva et al., 2010). In our study, we evaluated the plasma levels of cytokines to investigate the mechanisms responsible for the effects of curcumin on the prevention of sickness behavior and fever. We observed that pretreatment with curcumin prevented the peripheral synthesis of TNF- α and IL-1 β 2 h after the immunological challenge with LPS. Previous studies have shown that curcumin negatively regulates the synthesis of different cytokines by competitively binding to myeloid differentiation protein 2, which is the LPS-binding component of the MD-2/TRL4 complex (Mohan and Gupta, 2018). In addition, curcumin also has the ability to modulate LPS-induced TLR-4 receptor

signaling by inhibiting MyD88-dependent pathways and TRIF proteins (Wang et al., 2015; Boozari et al., 2019).

In vivo studies have shown that the transcription factor Nrf2 plays an important role in neuroinflammatory response (Nedzvetsky et al., 2017; Rahman et al., 2021). Under basal conditions, Nrf2 is bound to its repressive protein Keap1 in the cytoplasm and is degraded through the ubiquitin-proteasome pathway. However, exposure to stressors and inducers, such as cytokines, releases Nrf2 from Keap1 and, consequently, induces its translocation to the nucleus of the cell, culminating in the expression of cytoprotective genes (Mohan and Gupta, 2018; Rojo et al., 2018; Rahman et al., 2021). In our study, we explored the possible effects of curcumin on Nrf2 translocation during endotoxemia. The pretreatment with 50 or 100 mg/kg of curcumin induced a higher dose-dependent increase in Nrf2 translocation 2 h after LPS administration. As for the effect of curcumin on Nrf2 activity, it has been demonstrated that this compound exerts an exogenous agonist action on Nrf2 (Liu et al., 2016).

In conclusion, our data indicate that pretreatment with curcumin prevents the LPS-induced inflammatory response and, consequently, sickness behavior and fever. The present study suggests that curcumin is a potential substance capable of preventing or reducing systemic inflammation by modulating the translocation of Nrf2.

Credit authorship contribution statement

Dr. L. Reis was involved in the study conception and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content. Dr. M.K. Oliveira, Dr. V.C.T. Rojas, Dr. T.H. Batista, Dr. E.S. Estevam, and Dr. F. Vitor-Vieira were involved in the study conception and design, acquisition of data, and analysis and interpretation of data. Dr. F.C. Vilela and Dr. A. Giusti-Paiva were involved in the study conception and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; administrative, technical, or material support; and study supervision.

Abbreviations

FST

forced swim test

IL-1 β

interleukin-1 β

Keap1

Kelch-like ECH-associated protein 1

LPS

Lipopolysaccharide

Nrf2

nuclear factor-erythroid 2 related factor 2

OFT
open field test
TNF- α
tumor necrosis factor- α

Declarations

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Availability of data and materials

This study's data are included in the article, and the corresponding author can provide the primary data.

Consent to participate

All authors are aware of their participation in this work.

Consent for publication

All authors are aware of the publication of this work.

Declaration of competing interest

The authors report no biomedical financial interests or other potential conflicts of interest.

Code availability

Not applicable.

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Figures

Figure 1

Pretreatment with curcumin prevented lipopolysaccharide (LPS)-induced sickness behavior assessed by traveled distance (A) and mobility time (B) in open field test, time of interaction in social interaction test (C), and cumulative food intake (D). Columns and error bars represent the mean $\pm$ standard error of the mean. *** $P < 0.001$ compared with the vehicle + saline-treated group. # $P < 0.05$, ## $P < 0.01$, and ### $P < 0.001$ compared with the vehicle + LPS-treated group.

Figure 2

Pretreatment with curcumin prevented lipopolysaccharide (LPS)-induced depressive-like behavior assessed by floating time (A), swimming time (B), and climbing time (C) in forced swim test. Columns and error bars represent the mean $\pm$ standard error of the mean. *** $P < 0.001$ compared with the vehicle + saline-treated group. # $P < 0.05$, ## $P < 0.01$, and ### $P < 0.001$ compared with the vehicle + LPS-treated group.

Figure 3

Time course of the effects of pretreatment with curcumin on the change in body temperature (A) and fever index (area under the curve) (B) evaluated after the administration of either lipopolysaccharide (LPS) or saline. Each point or column represents the mean $\pm$ standard error of the mean. Symbols denote the significance levels: * $P < 0.001$ compared with the vehicle + saline-treated group. ### $P < 0.001$ compared with the vehicle + LPS-treated group.

Figure 4

Pretreatment with curcumin prevented lipopolysaccharide (LPS)-induced increase in the plasma levels of tumor necrosis factor- α (TNF- α) (A) and interleukin (IL)-1 β (B). Pretreatment with curcumin induced Nrf2 translocation from the cytoplasm to the nucleus, as evidenced by the reduction of nuclear factor-erythroid 2 related factor 2 (Nrf2) expression in cytoplasmic fraction (C) and increase in the nuclear fraction (D) in both saline and LPS-treated groups. Columns and error bars represent the mean $\pm$ standard error of the mean.