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Original Article

Mitragyna speciosa Korth. downregulates macrophage inflammatory responses by inhibiting TLR-4 and increasing IL-10 production

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ABSTRACT

Background and Aims: *Mitragyna speciosa* Korth. is a tropical plant native to Asia with various medicinal properties. This study examined the immunotherapeutic potential of *M. speciosa* methanolic extract (MSME) against lipopolysaccharide (LPS)-stimulated activation of macrophages via the expression of *Toll-like receptor 4 (TLR-4)* and *CD14* and downstream signalling cascades leading to the activation of *nuclear factor kappa B (NF-κB)*, which potentially affects macrophage immune responses.

Methods: The expression of *TLR-4/CD14* and *NF-κB* genes in macrophages was determined in total RNA by qRT-PCR. Subsequently, the macrophage phagocytic activities and secretion of immune mediators such as reactive oxygen species (ROS) and cytokines were evaluated by fluorescent latex beads uptake assay, 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA), and cytometric bead array, respectively, in LPS-activated RAW264.7 cells.

Results: MSME significantly reduced macrophage-mediating inflammation by inhibiting *TLR-4/CD14* signalling and subsequently suppressed the *NF-κB* expression. Inhibition of *TLR-4* by MSME attenuated macrophage phagocytic activity, which consequently reduced the production of ROS and pro-inflammatory cytokines such as IL-6, MCP-1, and TNF-α. Interestingly, MSME significantly increased the production of IL-10, which supports the anti-inflammatory properties of *M. speciosa.*

Conclusion: Our findings suggest the therapeutic potential of MSME through the suppression of macrophage inflammatory responses mediated by IL-10 secretion.

Keywords: *Mitragyna speciosa*; Macrophages; Anti-inflammatory; IL-10, TLR-4.

INTRODUCTION

Mitragyna speciosa Korth. (*M. speciosa*) locally known as Kratom, belongs to the Rubiaceae family and has a rich history of medicinal use for centuries (Jansen & Prast, 1988). The leaves have medicinal value in treating chronic pain, as morphine substitutes in the treatment of addiction, and as an energy booster. *M. speciosa* has also been featured in folk remedies aimed at addressing various ailments, such as coughing, diarrhoea, diabetes, and hypertension (Assanangkornchai, Muekthong, Sam-angsri, & Pattanasattayawong, 2007). *M. speciosa* possesses noteworthy therapeutic attributes, including anti-inflammatory (Tohar, Shilpi, Sivasothy, Ahmad, & Awang, 2019), antioxidant (Parthasarathy et al., 2009), antibacterial (Juanda, Andayani, & Maftuch, 2019), and anti-diabetic (Zailan, Sarchio, & Hassan, 2022), antidepressant (Buckhalter et al., 2021), anti-pain and analgesic (Kruegel et al., 2019), and antipyretic effects (Annas, Mastura Shaik Mossadeq, & Abdul Kadir, 2020). Plant leaves have yielded a plethora of bioactive phytochemicals, including alkaloids like mitragynine, speciogynine, paynantheine, and 7-hydroxymitragynine, along with flavonoids, polyphenols, flavonoids, saponins, triterpenoid saponins, glycoside derivatives, roseoside, vogeloside, and epivogeloside (Firmansyah, Sundalian, & Taufiq, 2020; Kafo, Mahayidin, et al., 2023; Zailan et al., 2022).

Mitragynine is a well-characterised alkaloid found in *M. speciosa* that has emerged as a potential reservoir for natural anti-inflammatory agents (Shaik Mossadeq et al., 2009). It has demonstrated anti-inflammatory properties by impeding the secretion of inflammatory mediators, including nitric oxide (NO), interleukin (IL)-6, tumour necrosis factor (TNF)-α, and IL-1β (Kafo, Elsalami, et al., 2023; Sornsenee, Chimplee, & Romyasamit, 2023). In addition, *M. speciosa* extract was found to alleviate the inflammatory response of macrophages through the attenuation of phagocytosis activity and downregulation of

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proinflammatory cytokine gene expression (Kafo, Elsalami, et al., 2023).

Macrophages are vital components of the immune system and have diverse functions, particularly antigen presentation, phagocytosis, wound repair, and embryonic tissue remodelling. Macrophages are classified into two phenotypes, M1 and M2. M1 is a classical macrophage activation that typically mediates pathogen elimination through phagocytosis and the production of inflammatory mediators such as NO, reactive oxygen species (ROS), and cytokines (Ahmad, Jantan, Kumolosasi, Haque, & Bukhari, 2018). M2 macrophages undergo alternative activation in response to anti-inflammatory stimuli and play a pivotal role in processes related to tissue restitution, wound healing, and the resolution of inflammatory responses. The M2 functional repertoire includes the secretion of anti-inflammatory cytokines, notably IL-10 and transforming growth factor-beta (TGF-β) (Dalmas, Tordjman, Guerre-Millo, & Clément, 2012). Moreover, M2 macrophages actively participate in tissue remodelling, angiogenesis, and immune response modulation, highlighting their multifaceted involvement in maintaining tissue homeostasis and resolving inflammatory conditions (Atri, Guerfali, & Laouini, 2018). Dysregulation in the equilibrium of macrophage polarisation between M1 and M2 phenotypes is frequently correlated with various pathological states or chronic inflammatory manifestations, including conditions like rheumatoid arthritis and systemic lupus erythematosus (SLE). The modulation of distinct macrophage phenotypes plays a pivotal role in governing the onset, progression, and resolution of inflammatory disorders (Dalmas et al., 2012). Consequently, the directional differentiation of macrophages into either the M1 or M2 phenotype has emerged as a prospective therapeutic strategy for managing inflammatory disorders. Notably, prevalent approaches to mitigating inflammation involve augmentation of M2 polarisation and/or attenuation of M1 polarisation (Y. Wang, Smith, Hao, He, & Kong, 2019).

M. speciosa potentially mitigates inflammatory responses by attenuating excessive NO production in RAW264.7 cells (Kafo, Elsalami, et al., 2023; Tohar et al., 2019). Additionally, Mitragynine isolated from *M. speciosa* has been shown to suppress prostaglandin E2 (PGE2) production by inhibiting *cyclooxygenase-2 (COX-2)* expression in lipopolysaccharide (LPS)-stimulated macrophages (Utar, Majid, Adenan, Jamil, & Lan, 2011). The current study complements our previous investigation that elucidated the impact of MSME on macrophages (Kafo, Elsalami, et al., 2023). In the current phase, we assessed the immunotherapeutic effects and mechanisms of *M. speciosa* methanolic extract (MSME) on LPS-stimulated macrophage immune response. This study encompasses the assessment of *Toll-like receptor 4 (TLR-4)* and *CD14* expression, the downstream signalling cascade through NF-κB, and the evaluation of macrophage activities.

MATERIALS AND METHODS

Mitragyna speciosa methanolic extract (MSME)

M. speciosa leaves were collected from Kedah, Malaysia, and the plant sample (KM 0024/22) was authenticated by the Institute of Bioscience (IBS) at the Universiti Putra Malaysia (UPM). The extraction of *M. speciosa* and characterisation of its bioactive compounds were described in detail in our previous study (Zailan et al., 2022). Briefly, a quantity of 100 g of the powdered leaves were extracted in 100 mL of methanol (v/v) using an Ace Soxhlet Extractor 6730 and Condenser 6740 (Quick Fit, England) for 4 h at 60°C. The resulting extract was concentrated using a rotary evaporator. The dried extract was dissolved in methanol and further diluted in dH20 (the maximum concentration of methanol was estimated as ≤ 0.1% in the cell culture).

RAW264.7 cell culture

RAW264.7, a murine-derived monocyte-macrophage cell line from the American Type Culture Collection (ATCC® TIB-71™) was cultivated in Dulbecco's Modified Eagle Media (DMEM) (Capricorn, Germany) containing 10% Foetal Bovine Serum, 1% glutamine, and 1% Penicillin-Streptomycin at 37°C in a 5% CO_2 incubator.

Lipopolysaccharide (LPS) stimulation and MSME treatment

RAW264.7 cells were stimulated with LPS (E. coli O111: B4, Merck, Germany) 1 µg/mL following our previous study (Kafo, Elsalami, et al., 2023). The evaluation of the cytotoxicity of MSME was performed in our previous study in which the working concentrations of MSME (25, 50, and 100 µg/mL) on RAW264.7 cells were determined based on IC20 (≥80% cell viability) to limit the degree of cell death due to the toxicity of the extract (Kafo, Elsalami, et al., 2023). Dexamethasone (10 µM) (Solarbio, China) is an immunosuppressive drug used as a positive control, and its concentration was determined in a previous study (George, Shyni, Abraham, Nisha, & Raghu, 2021). In contrast, 0.1% (final concentration) methanol was used as the cell-only control.

Evaluation of phagocytosis by latex beads

Red fluorescent latex beads (2.0 µ, carboxylate-modified polystyrene) (Sigma-Aldrich, Cat. no: L3030, Louis, USA) were used to evaluate the phagocytic capacity of RAW264.7 cells, as described by (Feng et al., 2021). RAW264.7 cells (1×10^5 cells/well) were treated with MSME or dexamethasone, along with the presence or absence of LPS. After 24 h of incubation, the culture medium was replaced with a suspension of 0.1% latex beads and incubated for 30 min. The cells were

washed three times with phosphate buffer saline and fixed with 10% paraformaldehyde (Solarbio, China), followed by imaging using a fluorescent microscope (Avio Vert A1, Germany).

Measurement of Reactive Oxygen Species (ROS)

The pre-treated cells were stained with 20 µM 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) (Invitrogen, Carlsbad, CA) for 30 min at 37°C. Flow cytometry (BD FACS Aria) was used to quantify the generation of ROS (Liu, Chen, Zheng, Yu, & Wei, 2022). The percentage of ROS-expressing cells was determined by H2DCFDA+ cells in the FL-1 channel via comparison with the unstained control.

Cytokine production

The BDTM Cytometric Bead Array (CBA) Mouse Inflammation Kit (Becton Dickinson, Holdrege, NE, USA) was used to assess cytokine production, including IL-6, IL-10, MCP-1, and TNF, from the cell culture supernatant following the manufacturer's instructions. Each sample was acquired via flow cytometry using FACSDiva software (BD FACS LSR Fortessa) followed by data analysis using FCAP Array software.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total cellular RNA was extracted from macrophages using Trizol reagent (Thermo Fisher Scientific, USA) following the manufacturer's instructions. The RNA was then converted to cDNA by reverse transcription using a HiScript III 1st Strand cDNA Synthesis Kit (Vazyme, China). Second, a gDNA wiper mixture was prepared by combining RNA with 2 µL of 5× gDNA wiper mix and RNase-free water to a final volume of 10 µL and incubating at 42°C for 2 min. Next, the first-strand cDNA synthesis mixture was prepared with 2 µL of 10× RT mix, 2 µL of HiScript III Enzyme Mix, 1 µL of Oligo (Dt)20 VN, 1 µL of Random hexamer, and 4 µL of RNase-free water. Finally, both mixtures were combined and incubated at 37°C for 15 min, followed by inactivation at 85°C for 5 s. qRT-PCR was performed using the SYBR Green master mix kit on an Eppendorf realplex4 qPCR Real-Time PCR Thermocycler, as outlined by (Srisuwan, Tongtawe, Srimanote, & Voravuthikunchai, 2014) and All-in-One qPCR primers including *TLR*-4 (5'-CTAAGGCCAACCGTGAAAAG-3' and 5'-ACCAGAGGCATACAGGGACA-3'), CD14 (5'- CCTCCAAGTTTTAGCGCTGC-3' and 5'-CAGCATCCCGCAGTGAATTG-3'), *NF-κB* (5'-GGACCTATGAGACCTTCAAGAG-3' and 5'-AGAAGTTGAGTTTCGGGTAGG -3'), and *β*-actin (5'- CTAAGGCCAACCGTGAAAAG-3' and 5'-ACCAGAGGCATACAGGGACA3') (Gene Copoeia, Rockville, MD, USA). The qPCR protocol included 40 amplification cycles, starting with an initial step at 95°C for 10 min, followed by denaturation at 95°C for 5 s, annealing at 65°C for 10 s (except for TLR-4, which annealed at 60°C), and extension at 72°C for 15 s. The primers were validated by Gene Copoeia (Rockville, MD, USA). In addition, a standard curve of each primer was generated to validate the primer efficiency. Gene expression levels of genes were normalised to β-actin and quantified using the $^{\Delta\Delta}$CT method to determine fold changes in gene expression ($2^{-\Delta\Delta}$CT). The results of each sample were expressed as $\log^2$ fold-change in gene expression (Liu et al., 2022).

Statistical analysis

The GraphPad Prism software (version 9.0) was used for data analysis. A one-way ANOVA was applied to assess variations in the treatment control and sample groups. The values presented are means ± standard error mean (SEM) of two or three independent experiments.

RESULTS AND DISCUSSION

MSME downregulates the expression of *TLR-4, CD14*, and *NF-κB* in LPS-stimulated macrophages

Macrophages play a pivotal role in orchestrating the inflammatory immune response. Concurrently, the liberation of the cytokines and mediators from macrophage cells relies on the synergistic interplay between *TLR-4* and *CD14* (Kawasaki & Kawai, 2014). This collaboration facilitates the recognition and binding of microbial components such as LPS (Elisia et al., 2018). The activation of macrophages initiates the stimulation of *NF-κB* and mitogen-activated protein kinases (MAPKs) through diverse signal transduction pathways. Subsequently, this activation triggers the expression *of inducible nitric oxide synthase (iNOS), COX-2*, and production of proinflammatory cytokines, such as TNF-α, IL-1*β*, and IL-6 (Ahmad et al., 2018; Kim et al., 2018). TLR ligands are responsible for the classical activation of macrophages, inducing a phenotypic shift towards the M1 proinflammatory state (Wang et al., 2019). However, dysregulated TLR signalling can perturb immune homeostasis, characterised by the sustained secretion of pro-inflammatory cytokines and chemokine. Such disruption can frequently be implicated in the pathogenesis of various inflammatory and autoimmune disorders, including SLE, sepsis, atherosclerosis, and asthma (Gao, Xiong, Li, & Yang, 2017). Consequently, the modulation of the functionality of these pattern recognition receptors holds the potential to serve as a pivotal determinant in treating these disorders and may contribute to polarising the macrophage activation to M2 (anti-inflammatory response).

In the present study, the effects of MSME on *TLR-4, CD14*, and *NF-κB* gene expression were evaluated using qRT–PCR. The expression of *TLR-4, CD14*, and *NF-κB* in macrophages

increased by stimulation of LPS. Figure 1 shows that MSME (100 µg/mL) significantly reduced *TLR-4* (by 2-fold changes; P=0.0076), *CD14* (by 1.6-fold changes; P=0.0356), and *NF-κB* (by 2.25-fold changes; P=0.0008) in a dose-dependent manner. In addition, the expression of *TLR-4, CD14*, and *NF-κB* is also significantly inhibited by dexamethasone (by 2.28, 1.8, and 2.3-fold changes, respectively). The suppression of TLR/NF-κB signalling activation in macrophages by bioactive natural compounds can downregulate inflammatory responses, which is useful for managing or treating inflammatory diseases (Kim et al., 2018; Liao et al., 2021; Limtrakul, Yodkeeree, Pitchakarn, & Punfa, 2015; Wang et al., 2022).

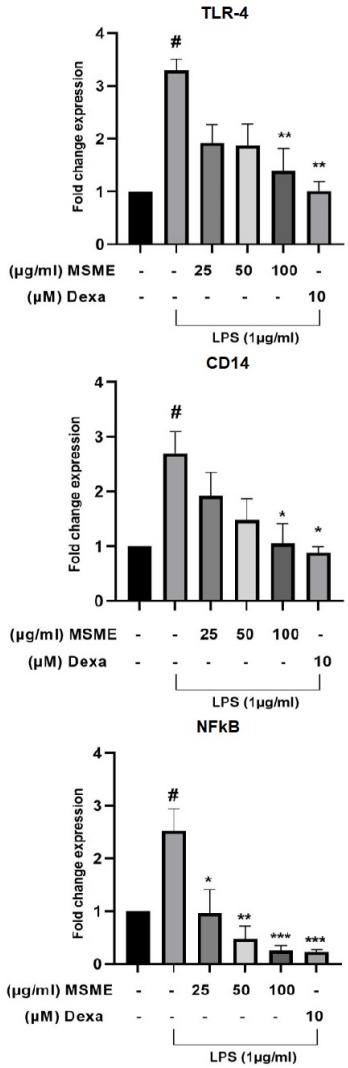


Figure 1. The effect of MSME on the expression of *TLR-4, CD14*, and *NF-κB* in LPS-stimulated RAW264.7 cells. Total RNA was isolated from RAW264.7 cells cultured with LPS (1 µg/mL), MSME, and/or dexamethasone (Dexa). *TLR-4, CD14*, and *NF-κB* expression levels were quantified using qRT–PCR. All data were normalised with the housekeeping gene β-actin. The presented values are the mean ± SEM from three independent experiments. #($p<0.05$) signifies statistical significance compared with the unstimulated control, whereas *($p<0.05$), ** ($p<0.01$), and *** ($p<0.001$) indicate statistical significance compared with the LPS control.

MSME reduces macrophage phagocytic capacity

Phagocytosis is a cellular process characterised by the ingestion and internalisation of particles by phagocytic cells. It constitutes a multifaceted phenomenon encompassing diverse cellular behaviours, in which the orchestrated execution of this process is imperative for effective host defence mechanisms (Ryu et al., 2016). Phagocytosis is potentiated through the involvement of TLR-4, a pivotal component that plays crucial roles in initiating signal transduction pathways. These pathways ultimately result in the effective eradication and removal of pathogens, highlighting TLR-4's integral role in the immune system's capacity to combat microbial threats (Doyle et al., 2004). However, the regulation of TLR-4 receptors can affect phagocytosis activity.

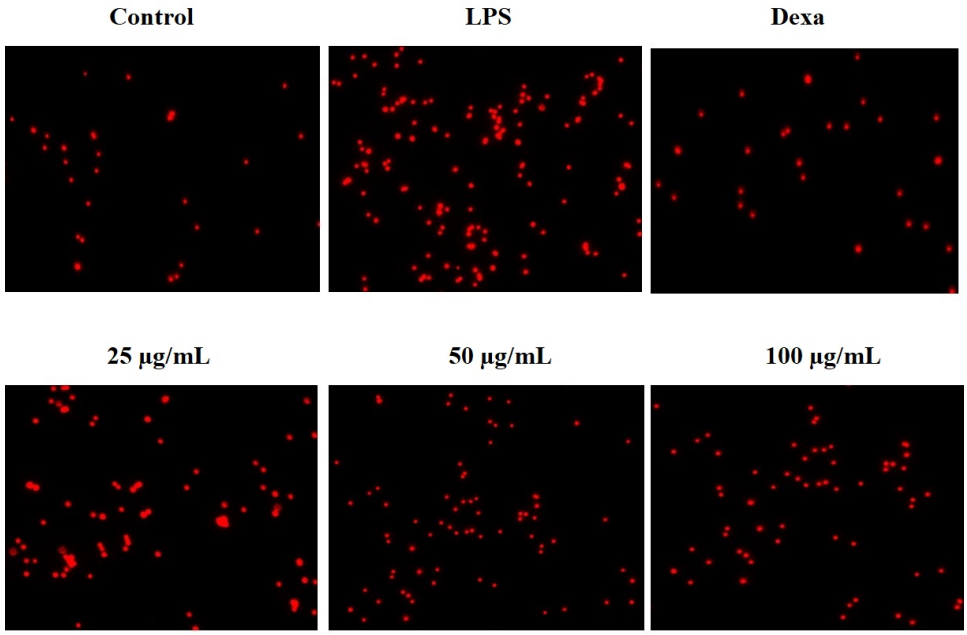
Latex beads are frequently employed in phagocytosis assays and function as representative particles that replicate the properties of pathogens or other foreign entities for experimental purposes (Gu et al., 2014). As indicated in Figure 2, stimulation of RAW264.7 cells with LPS increased the number of macrophages that engulfed the beads. Conversely, the number of cells treated with MSME exerted a reduction in engulfed beads. This finding suggests the attenuation of the phagocytosis activity of macrophages by MSME. Similarly, reduced capacity of macrophage phagocytic activity was also observed following treatment with dexamethasone.

Extracts derived from certain medicinal plants have exhibited the capacity to hinder the phagocytic activity of immune cells, which is consistent with the findings of this investigation. Specifically, the study elucidated those extracts from *Phyllanthus amarus* and *P. urinaria* exhibited a moderate inhibitory effect on E. coli uptake by monocyte cells (Jantan, Ilangkovan, Yuandani, & Mohamad, 2014). Moreover, the aqueous and methanolic extracts of *Ixora coccinea* have the ability to inhibit the phagocytic activity of neutrophils. This inhibitory effect is ascribed to the influence of these extracts on the activity and intracellular killing mechanism of neutrophils, thereby contributing to the modulation of immune cellular responses in inflammatory contexts (Wickramasinghe, Kumara, De Silva, Ratnasooriya, & Handunnetti, 2014).

MSME reduces ROS production in LPS-stimulated macrophages

Reactive Oxygen Species (ROS) are oxygen-containing molecules with chemical reactivity produced as inherent byproducts of cellular metabolism. ROS delicately influences various physiological and pathological processes in living organisms. Although they are necessary for various cellular functions, an imbalance between ROS production and the cellular antioxidant defence mechanisms can lead to oxidative stress, which is implicated in tissue damage, a range of diseases, and the ageing process (Finkel, 2011; Qi et al., 2013).

Upon exposure to bacterial LPS, macrophages exhibit a no-



Test	Phagocytosis rate
Control	+
LPS	++++
LPS + Dexamethasone 10 µM	+
LPS + MSME 25 µg/mL	+++
LPS + MSME 50 µg/mL	+++
LPS + MSME 100 µg/mL	++

Figure 2. MSME reduces macrophage phagocytic capacity by inhibiting the internalisation of fluorescent latex beads. RAW264.7 cells were cultured with MSME or dexamethasone in the presence or absence of LPS for 24 h. 0.1% latex beads were added for 30 min, and the cells were fixed with 10% paraformaldehyde. A representative field of macrophage phagocytic activity towards fluorescent latex beads was captured under 4x magnification from three independent experiments. The table shows qualitative phagocytosis scores.

table elevation of ROS production by 70% from RAW264.7 from RAW264.7 (p=0.0071). Our findings showed significant inhibition of ROS by MSME, suggesting antioxidant properties of MSME (Figure 3). This data was in line with a previous study that demonstrated the antioxidant activity of *M. speciosa* through its free radical-scavenging activity (Parthasarathy et al., 2009; Zailan et al., 2022). Therefore, ROS production may be suppressed by downregulation of *TLR-4* and *NF-κB*. As previously demonstrated by Meng et al., pre-treatment of macrophages with curcumin extract diminished excessive ROS generation. This inhibitory effect on the inflammatory response is posited to be contingent on the suppression of *TLR-4* activation, the prevention of *NF-κB* nuclear translocation, and the reduction in NADPH-mediated intracellular ROS production (Meng et al., 2013). Additionally, the methanol extract of *Caragana rosea Turcz* exhibits a dose-dependent reduction in ROS levels, which is achieved through the regulation of upstream *NF-κB* proteins and the TLR-4-mediated *NF-κB* signalling pathway (Meng et al., 2013).

MSME inhibits proinflammatory cytokine expression in LPS-stimulated macrophages

Cytokines play a crucial role in facilitating communication and networking among cells of the immune system in maintaining or re-establishing a state of equilibrium through the coordination of various types of cells such as haematopoietic, inflammatory, and lymphoid cells (Ahmad et al., 2018). Activated macrophages generate and sequentially release a diverse array of pro-inflammatory cytokines, including IL-6, IL-β, and TNF-α. Within the context of the inflammatory response, macrophages are the most highly responsive components of the innate immune system. They take on the roles of both initiators and detectors to regulate the course of both inflammatory and immunological reactions. To intensify and deliver this immune reaction, RAW 264.7 cells markedly enhance the production and transcription of TNF-α, IL-1β, IL-6, iNOS, and MCP-1 following LPS induction (Ahmad et al., 2018; Liu et al., 2022; Zailan et al., 2022).

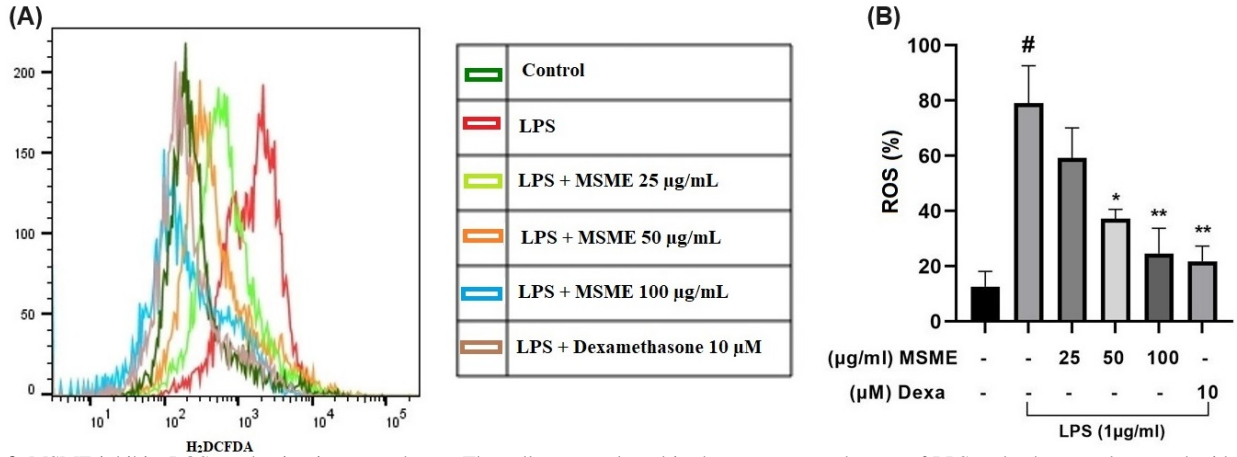


Figure 3. MSME inhibits ROS production in macrophages. The cells were cultured in the presence or absence of LPS and subsequently treated with MSME. Following a 24-h incubation, the cells were stained with 20µM of H2DCFDA and acquired on the flow cytometry. Cells expressing ROS+ were quantified according to fluorescence intensity. (A) Each overlayed histogram is representative of three independent experiments. (B) Bar graph presented as means ± SEM of ROS from three independent experiments. #(P<0.05) indicates statistical significance compared with the untreated control, whereas *(P ≤ 0.05), **(P < 0.01), and ***(P < 0.001) indicates statistical significance compared with LPS alone.

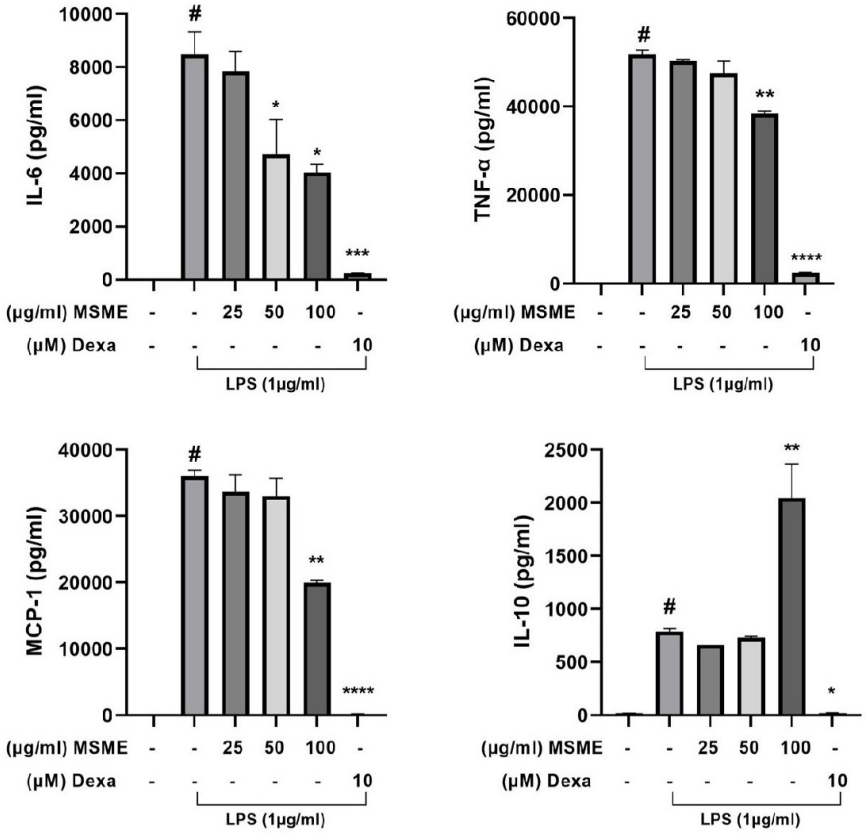


Figure 4. MSME reduces proinflammatory cytokine production in macrophages. RAW264.7 cells were treated with MSME or dexamethasone in the presence or absence of LPS. The production of secreted cytokines was evaluated using the BD Mouse Inflammatory Cytometric Bead Array Kit via flow cytometry. The data is represented as mean ± SEM from three independent experiments. #(p<0.05) indicates statistical significance in comparison with the unstimulated control. *(p<0.05), **(p<0.01), ***(p<0.001), and ****(p<0.0001) indicate statistical significance in comparison with the LPS stimulation without MSME treatment.

In this study, the secreted cytokines in the cell culture supernatants were quantified. Our findings showed that LPS-stimulated RAW264.7 cells with LPS significantly increased TNF-α, IL-6, IL-10, and MCP1, which principally indicated inflammatory responses (Figure 4). However, this phenomenon is counteracted by MSME treatment. It shows that MSME significantly inhibits the production of macrophage pro-inflammatory cytokines including TNF-α, IL-6, and MCP1 ($p<0.05$) in a dose-dependent manner. Expectedly, the production of these cytokines was also significantly inhibited by dexamethasone ($p<0.001$). These findings align with our earlier investigation demonstrating that MSME downregulates the expression of proinflammatory cytokines, including IL-6, TNF-α, and IL-1β (Kafo, Elsalami, et al., 2023). Similarly, fermented *M. speciosa* leaves exhibit anti-inflammatory properties by markedly suppressing the production of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6 (Sornsenee et al., 2023). Furthermore, the methanol extract derived from the fruits of K*ochia scoparia* exerted inhibitory effects on LPS-induced proinflammatory cytokines, including PGE2 and TNF-α, as well as on the expression of iNOS and COX-2. These effects are attributed to the suppression of NF-κB activation (Shin et al., 2004). This supports the assertion that the observed inhibition of cytokine production is linked to the downregulation of cytokine gene expression. On the other hand, the secretion of the anti-inflammatory cytokine, IL-10, was significantly elevated by MSME (100 µg/mL; $p<0.001$) but not with dexamethasone treatment. The increase in IL-10 production showed the anti-inflammatory effect of the extract, which is in line with a study showing an increase in IL-10 levels by Moringa oleifera flower extract (Tan, Arulselvan, Karthivashan, & Fakurazi, 2015). However, the suppression of IL-10 by dexamethasone is associated with the mechanism by which glucocorticoids alleviate inflammation in allergic diseases (Fushimi, Okayama, Seki, Shimura, & Shirato, 1997; Tan et al., 2015). Dexamethasone exerted varying effects on LPS-induced TNF-α and IL-10 secretion. Unlike TNF-α which was inhibited in a dose-dependent manner, the dexamethasone effect on IL-10 was biphasic, whereby it increased IL-10 secretion at lower concentrations but inhibited it at higher concentrations (Franchimont et al., 1999).

CONCLUSION

The present study investigated the efficacy and mechanism of MSME in downregulating immune responses in an *in vitro* LPS-induced inflammation model in macrophages. The anti-inflammatory effect of MSME may be attributed to the up-regulation of IL-10, which concomitantly reduced the expression of pro-inflammatory cytokines (IL-6, TNF-α, and MCP-1) through the inhibition of *TLR-4* and *CD14* and the attenuation of *NF-κB* expression. In addition, a reduction in the phagocytosis capacity of macrophages and ROS production by MSME exerts the attenuation of inflammatory responses. Our findings suggest that MSME exhibits potent anti-inflammatory properties and is a potential candidate for the prevention and treatment of inflammatory diseases.

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