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Research Article
Inhibition of Microglia Synaptic Pruning Mediates the Anti-Neuroinflammatory Activity of Diosgenin

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Abstract

Background and Objective: Neuroinflammation, driven by immune system dysregulation, impairs central nervous system functionality, often leading to synaptic alterations. This study explored the efficacy of Diosgenin in mitigating neuroinflammation, with a particular focus on its capacity to hinder synaptic pruning processes. **Materials and Methods:** The BV2 microglial cells and ICR mice were pretreated with Diosgenin, followed by the induction of a neuroinflammatory response via lipopolysaccharide (LPS) in both *in vitro* and *in vivo* settings. **Results:** The *in vitro* findings revealed that LPS exposure diminished BV2 cell functionality and escalated apoptosis, triggering the secretion of proinflammatory cytokines such as IL-1β, IL-6 and TNF-α. Conversely, Diosgenin enhanced cell viability, diminished apoptosis and reduced the levels of these cytokines. *In vivo* analyses demonstrated that LPS administration provoked neuroinflammation in mice, evidenced by heightened inflammatory markers and cytokines in the hippocampus, activation of the C3/C3aR signaling cascades and consequent synaptic pruning. The administration of Diosgenin attenuated these effects by curtailing proinflammatory cytokine production, suppressing the activation of the mentioned signaling pathways and notably obstructing the LPS-induced synaptic pruning by microglia within the hippocampus. **Conclusion:** Current results indicated that Diosgenin ameliorates LPS-induced neuroinflammation by modulating hippocampal cytokine profiles, inhibiting specific neuroinflammatory pathways and preventing complement C3-driven synaptic pruning, underscoring its potential as a therapeutic agent in neuroinflammatory disorders.

Key words: Diosgenin, neuroinflammation, complement C3, microglia, synaptic pruning

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Neuroinflammation is increasingly acknowledged as a pivotal element in the pathology of mental illnesses. Studies employing positron emission tomography have shown a 30% higher prevalence of neuroinflammation in patients with depression than in healthy counterparts, establishing a direct relationship between the intensity of depression and the extent of inflammatory activity[1]. Autopsies have consistently found increased inflammatory markers in the brains of depressed individuals[2], highlighting the profound connection between neuroinflammation and psychiatric disorders. Microglia, the central immune cells within the central nervous system, play a key role in mediating neuroinflammatory responses. These cells transition from a resting state, characterized by small nuclei and thin branches, to an activated state with larger nuclei and thicker branches upon encountering external stimuli, such as pathogens. This transformation is accompanied by the production of pro-inflammatory cytokines, leading to detrimental outcomes like impaired neuroregeneration, synaptic anomalies and neuronal cell death[3]. Lipopolysaccharide (LPS), a component of bacterial cell walls, is known to induce strong neuroinflammatory reactions by interacting with cellular receptors, thereby triggering adverse behavioral changes in animal models, including memory and cognitive deficits[4].

Microglial activation prompts astrocytes to generate complement proteins, exacerbating the inflammatory state within the central nervous system. This cascade results in the formation of inflammatory fragments that compromise the blood-brain barrier, intensifying immune-related damage[5,6]. Moreover, the interaction of complement proteins with microglial receptors amplifies local inflammation and leads to dysregulated synaptic pruning, further impairing neuronal function[7].

Diosgenin, a steroidal saponin found in various legumes and yams, has garnered attention for its immunomodulatory, anti-inflammatory and anticancer capabilities[8]. This compound has been shown to modulate cellular signaling and apoptosis, particularly in the context of cancer therapy, by selectively targeting and inducing apoptosis in tumor cells[9,10]. Notably, in osteosarcoma models, Diosgenin has demonstrated a significant reduction in NF-κB signaling pathway activity and its associated pro-inflammatory genes, thereby inducing apoptosis in osteosarcoma cells[11].

Given these properties, our study investigates the role of Diosgenin in counteracting neuroinflammation, with a specific focus on its effect on synaptic pruning. We established an *in vitro* model of neuroinflammation using LPS-treated BV2 microglial cells to assess the effects of Diosgenin on cellular health, inflammatory responses and apoptotic processes. Furthermore, an *in vivo* model of LPS-induced neuroinflammation in mice was utilized to ascertain the therapeutic efficacy of Diosgenin. The investigation into the mechanism of Diosgenin in alleviating neuroinflammation was conducted through PCR, ELISA and immunofluorescence assays, aiming to elucidate its potential as a treatment for neuroinflammatory conditions.

MATERIALS AND METHODS

Study area: The *in vitro* experiments were performed at Southern Ocean Research Centre, Xiamen, China in 2019. The *in vivo* experiments were performed at Huaqiao University, Xiamen, China in 2020.

Cell and reagents: Mouse microglia BV2 (CL-0493) was purchased from Wuhan Procell (Wuhan, China). The DMEM medium (C3110-0500) and fetal bovine serum (C04001-500) were purchased from Vivacell (Denzlingen, Germany). Diosgenin (M7747) was purchased from Abmole (Houston, USA). The LPS (L2880) was purchased from Sigma-Aldrich (St. Louis, USA). The TNF-α ELISA kit (BP80317), IL-6 ELISA kit (BP80293) and IL-1β ELISA kit (BP80277) were purchased from Purdue Bioscience (New York, USA). The CCK-8 kit (HY-K0301) was purchased from MCE (Dallas, USA). The C3 antibody (PA5-21349) was purchased from Thermofisher (Waltham, USA). The C3aR antibody (sc-133172) was purchased from Santa Cruz (Fremont, USA). The Iba1 antibody (ab5076) was purchased from Abcam (Cambridge, USA). Synaptophysin antibody (36406) was purchased from Cell Signaling Technology (Danvers, USA).

***In vitro* cell culture and passaging:** The BV2 microglial cells, known for their intermediate adherence and suspension growth characteristics, were maintained in a controlled environment at 37°C with 5% CO_2. The culture media was refreshed bi- to tri-weekly, with subculturing occurring every two to four days at dilution ratios between 1:2 and 1:4. Upon reaching an approximate confluency of 80%, the medium was removed and the cells were washed twice using sterile phosphate-buffered saline (PBS). To facilitate cell detachment, 1 mL of trypsin was applied, allowing approximately one minute for the cells to contract and assume a rounded shape. The enzymatic reaction was halted by the addition of 3 mL of specialized BV2 medium, followed by gentle shaking to dislodge any adhering cells. The resulting cell suspension was centrifuged at 1000 rpm for 5 min. The supernatant was then discarded and the cell pellet was reconstituted in fresh BV2 medium to achieve a homogeneous single-cell suspension.

This suspension was subsequently seeded into new culture flasks at a 1:3 split ratio, filled with the required volume of medium. Flasks were lightly shaken to distribute the cells uniformly before being incubated again at 37°C with 5% CO_2. Only cells in their logarithmic phase of growth were utilized for the ensuing experimental assays.

Drug treatment in BV2 microglia

Initial exploration: Concentration-dependent effects of Diosgenin on BV2 cells. The initial segment of the study was designed to ascertain how different concentrations of Diosgenin affect normal BV2 microglial cells. A range of Diosgenin concentrations was examined, specifically 2.5, 5, 10, 20, 40, 80 and 160 μM, to determine its cellular impact under standard conditions.

Subsequent analysis: The effects of Diosgenin on LPS-induced inflammatory response in BV2 cells. In the following phase, we assessed the modulatory effects of Diosgenin on BV2 cells under inflammatory stress induced by LPS. For this purpose, the cells were initially exposed to varying concentrations of Diosgenin for a duration of 3 hrs. Subsequently, the culture was supplemented with 10 μg/mL of LPS and the cells were incubated for an additional 6 hrs to evaluate the response to combined Diosgenin and LPS treatment.

CCK-8 kit to detect BV2 cell activity: Logarithmic phase BV2 microglial cells were enzymatically detached, centrifuged and resuspended in the culture medium to achieve a density of 5×10^4 cells/mL. This suspension was seeded into 96-well plates, allocating 100 μL per well, with five replicates established for each experimental condition. The cells were then cultured in a 37°C incubator with 5% CO_2 for 24 hrs to facilitate complete adherence. Post-adhesion, the old medium was aspirated and replaced with 100 μL of medium containing Diosgenin at predetermined concentrations and the cells were incubated for 3 hrs. After this Diosgenin exposure, the medium was replaced with one containing 10 μg/mL LPS and the cells were further incubated for 6 hrs under the same conditions. Subsequent to this incubation, 10 μL of CCK-8 solution was introduced to each well and the plates were incubated for an additional 3 hrs at 37°C. The development of a slight yellow coloration in the medium indicated the time to measure the absorbance at 450 nm using a Multiskan FC microplate reader (Thermofisher, Lenexa, Kansas, USA).

ELISA for IL-1β, IL-6 and TNF-α levels: The BV2 microglial cells, in their logarithmic phase of growth, were plated onto 24-well plates at a density of 1×10^6 cells/mL. Each experimental condition was replicated in three wells. Once the cells had adhered, the old medium was removed and the cells underwent treatment with varying concentrations of Diosgenin for 3 hrs. After this period, the cells were exposed to 10 μg/mL of LPS, followed by a further incubation of 6 hrs. The supernatant was then harvested from each well for analysis. The concentrations of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α were measured using ELISA kits, with the procedures carried out according to the guidelines provided by the kit manufacturers.

Detection of apoptosis by flow cytometry: Upon achieving a confluency exceeding 80%, the cells were harvested through trypsinization, omitting EDTA in the process. These cells were then suspended and centrifuged to achieve a concentration of 1×10^6 cells/mL. Two milliliters of this suspension was allocated into each well of a 6-well plate and incubated for 24 hrs, allowing cell attachment before the commencement of various experimental treatments. Following these treatments, the supernatant was removed and trypsinization without EDTA was repeated for cell detachment. The cells were then gathered by centrifugation at 2000 rpm for 5 min, washed twice with chilled PBS at 4°C and resuspended in 500 μL of binding buffer, ensuring homogeneity through gentle pipetting. For apoptotic and necrotic analysis, 5 μL of Annexin V-FITC was added to the cell suspension and mixed carefully, followed by the addition of 5 μL of propidium iodide (PI) and another gentle mixing. The cells were incubated at room temperature away from light for 5-15 min before immediate flow cytometric analysis to determine apoptotic and necrotic cell fractions.

Animals: Male ICR mice, weighing 25±2 g and aged between 8 weeks, of clean grade, were procured from the Shanghai Slaughter Animal Center. They were housed five per cage, under a 12 hrs light/dark cycle (light phase from 09:00 to 21:00). A week-long acclimatization period was allowed for the mice to adapt to their new surroundings before the onset of the experimental procedures. The housing conditions were maintained at a temperature of 22±2°C and a relative humidity of 55±5%. Throughout the study, the mice were given *ad libitum* access to food and water.

Ethical consideration: The protocol for the animal study was reviewed and approved by the Experimental Animal Ethics Committee of Huaqiao University, conforming to the institutional guidelines for the care and use of experimental animals (Approval No. A2020002).

Drug administration: In this study, the mice were randomly divided into five separate groups, each comprising ten animals. These included a normal control group, an LPS-induced model control group treated with 0.83 mg/kg LPS and three experimental groups treated with Diosgenin at doses of 20, 40 and 60 mg/kg, respectively. Except for the normal control group, mice in all other groups were given their respective treatments through daily intraperitoneal injections for seven consecutive days. On the final day, one hour after the last treatment dose, the normal control group was given a PBS injection intraperitoneally, whereas the LPS model control and the Diosgenin-treated groups received an intraperitoneal injection of LPS.

Immunofluorescence: Mice were anesthetized with an intraperitoneal injection of sodium pentobarbital, ensuring deep anesthesia before proceeding to a thoracotomy initiated at the abdominal region. Locating the heart, a syringe equipped with a flat-tipped needle was inserted into the left ventricle and an incision was made in the right atrium to allow for perfusion. Around 50 mL of PBS was perfused to clear the blood, monitored by the discoloration of the liver and limbs, indicating blood clearance. Subsequent to this, a 4% paraformaldehyde solution was perfused for fixation. The brain was then carefully extracted post opening the skull, further fixed in 4% paraformaldehyde and refrigerated at 4°C. After a 24 hrs fixation, the brain underwent dehydration through successive immersions in 10, 20 and 30% sucrose solutions, each for 24 hrs. Following this, the brain tissue was embedded in OCT compound and frozen at -80°C. Brain sections of 15 µm thickness were prepared using a cryostat and then stored at -20°C. Prior to immunostaining, sections were equilibrated to room temperature for 10 min and then fixed with a suitable fixative for another 10 min. After three washes with an immunostaining wash solution, antigen retrieval was conducted by incubating the sections in an antigen retrieval solution for 5 min, followed by additional washes. Blocking solution was applied to the sections for 1 hr before overnight incubation with specific fluorescent primary antibodies at 4°C. Following three washes, the sections were exposed to fluorescent secondary antibodies for 3 hrs at room temperature, washed again and briefly dipped in water before applying DAPI for nuclear staining. After a 5 min room temperature incubation, the sections were visualized and documented using a Leica TCS SP5 laser confocal microscope (Leica, Deerfield, Illinois, USA).

qPCR: Tissue was dissected and subjected to a precise protocol for total RNA isolation. This RNA was then reverse-transcribed into Complementary DNA (cDNA) using reverse transcriptase enzymes, forming the basis for subsequent Polymerase Chain Reaction (PCR) amplification. The real-time PCR process was conducted in a thermal cycler, involving repeated cycles of denaturation at 95°C for 30 sec, annealing at 53°C for 60 sec and extension at 72°C for 60 sec. The cycle threshold (Ct) value, indicative of the fluorescence intensity reaching a specific threshold, was measured to assess the initial quantity of the target gene. Relative expression of the target genes, such as IL-1β (Forward: 5'-TGCCACCTTTT GACAGTGATG-3'; Reverse: 5'-TGATGTGCTGCTGCGAGATT-3'), IL-6 (Forward: 5'-CCCCAATTTCCAATGCTCC-3'; Reverse: 5'-CGCACTAGGTTTGCCGAGTA-3') and TNF-α (Forward: 5'-GAT CGGTCCCCAAAGGGATG-3'; Reverse: 5'-CCACTTGGTGGTTT GTGAGTG-3'), was normalized to the housekeeping gene GAPDH (Forward primer: TGAGGCCGGTGCTGAGTATGT; Reverse primer: CAGTCTTCTGGGTGGCAGTGAT). Gene expression levels were quantified using the $2^{-\Delta\Delta CT}$ method, which calculates the relative amount of each target gene normalized to GAPDH and relative to a control sample.

Statistical analysis: Data are presented as Mean±SEM. Statistical analyses were conducted using SPSS software. The normality of data distribution was confirmed using the Kolmogorov-Smirnov test before applying one-way ANOVA. The *post hoc* analysis with Tukey's test was performed following ANOVA to ascertain differences between groups. Statistical significance was defined at a p-value of less than 0.05.

RESULTS

Diosgenin treatment does not alter BV2 cell viability and inhibits LPS-induced reduction in BV2 cell viability: The effect of Diosgenin on BV2 microglial cell viability was assessed using the CCK-8 assay after exposing the cells to various Diosgenin concentrations from 2.5 to 160 µM over 6 hrs. As shown in Fig. 1a, Diosgenin did not significantly impact the viability of BV2 cells within this concentration spectrum. Moreover, when BV2 cells were pretreated with Diosgenin for 3 hrs and subsequently exposed to LPS (10 µg/mL) for 6 hrs, significant effects were observed (Fig. 1b). The LPS exposure markedly decreased the viability of BV2 cells, whereas pretreatment with Diosgenin markedly alleviated this decrease in cell viability, evident at concentrations of 10, 20 and 40 µM Diosgenin. These results indicated that Diosgenin, at 10 to 40 µM, can protect against the reduced viability of cells under inflammatory stress, highlighting its prospective therapeutic benefit in scenarios of microglial cell activation.

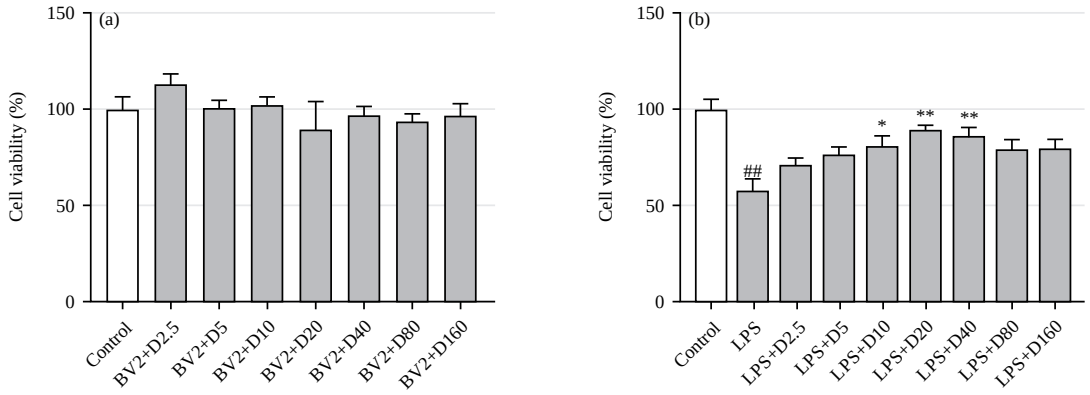

Fig. 1(a-b): Effects of Diosgenin on BV2 microglial cell viability and activation. This figure demonstrates how Diosgenin impacts BV2 microglial cells under two different scenarios (a) Exposure to Diosgenin alone and (b) Exposure to Diosgenin prior to LPS administration

Each scenario was evaluated across six separate experiments (n = 6). Statistical significance is indicated as follows: ##p<0.01 relative to the control group; *p<0.05 and **p<0.01 relative to the LPS-treated group in (b)

Diosgenin treatment inhibits LPS-induced increase in inflammatory factor levels in BV2: The effectiveness of Diosgenin in mitigating LPS-induced inflammation was assessed by quantifying the levels of the inflammatory cytokines IL-1β, IL-6 and TNF-α via PCR and ELISA. Figure 2a illustrated that LPS significantly upregulated IL-1β mRNA levels compared to controls, with Diosgenin at 40-80 μM concentrations substantially lowering IL-1β expression. In Fig. 2b, an increase in IL-6 mRNA levels was observed following LPS treatment, which was effectively reduced by Diosgenin. Additionally, Fig. 2c shows that TNF-α mRNA expression was significantly increased by LPS ($p<0.01$), but was reduced with Diosgenin pretreatment. At the protein level, Fig. 2d indicates that LPS elevated IL-1β protein levels significantly, which were decreased by Diosgenin at 40-160 μM. Figure 2e shows an increase in IL-6 protein levels following LPS exposure, reduced by Diosgenin. Lastly, Fig. 2f revealed that LPS enhanced TNF-α protein expression, which was diminished by Diosgenin. These results collectively demonstrated the anti-inflammatory effects of Diosgenin in BV2 cells challenged with LPS, effectively attenuating the expression of key inflammatory cytokines including IL-1β, IL-6 and TNF-α.

Diosgenin treatment inhibits LPS-induced apoptosis in BV2 cells: Current research utilized flow cytometry to assess the ability of Diosgenin to attenuate apoptosis. Cells underwent pretreatment with Diosgenin at concentrations ranging from 10 to 40 μM for 3 hrs before being subjected to LPS (10 μg/mL) for 24 hrs to prompt apoptosis. As shown in Fig. 3 that, LPS exposure increased the prevalence of late-stage apoptotic cells in comparison to the blank control. However, Diosgenin pretreatment markedly reduced the incidence of apoptosis, demonstrating its protective capacity against LPS-induced cellular mortality. These observations indicate the efficacy of Diosgenin in curtailing apoptosis within cells challenged with LPS.

Diosgenin treatment inhibits hippocampal inflammatory factor levels in mice: Compared to the normal control, the LPS-administered group exhibited significantly elevated mRNA levels of IL-1β, IL-6 and TNF-α. Administration of Diosgenin at a low dose notably diminished the hippocampal mRNA expression of IL-1β and TNF-α ($p<0.05$ for both), relative to the LPS-treated group. Additionally, medium-dose Diosgenin significantly lowered the expression levels of IL-1β, IL-6 and TNF-α in the hippocampus, highlighting a dose-responsive action (Fig. 4a-c). These results implied that Diosgenin effectively attenuates the production of pro-inflammatory cytokines in the central nervous system, thus demonstrating significant anti-inflammatory properties in the context of neuroinflammation.

Diosgenin treatment reduces complement C3 and C3aR expression in mice: As shown in Fig. 5a-b, C3/C3aR pathways was significantly activated in the LPS-treated mice compared to the normal controls. Notably, Diosgenin administration at various doses resulted in a significant decrease in the levels of C3 and C3aR within the LPS group. This reduction suggests the potent capability of Diosgenin to alleviate neuroinflammation by suppressing the activity of the C3/C3aR pathways.

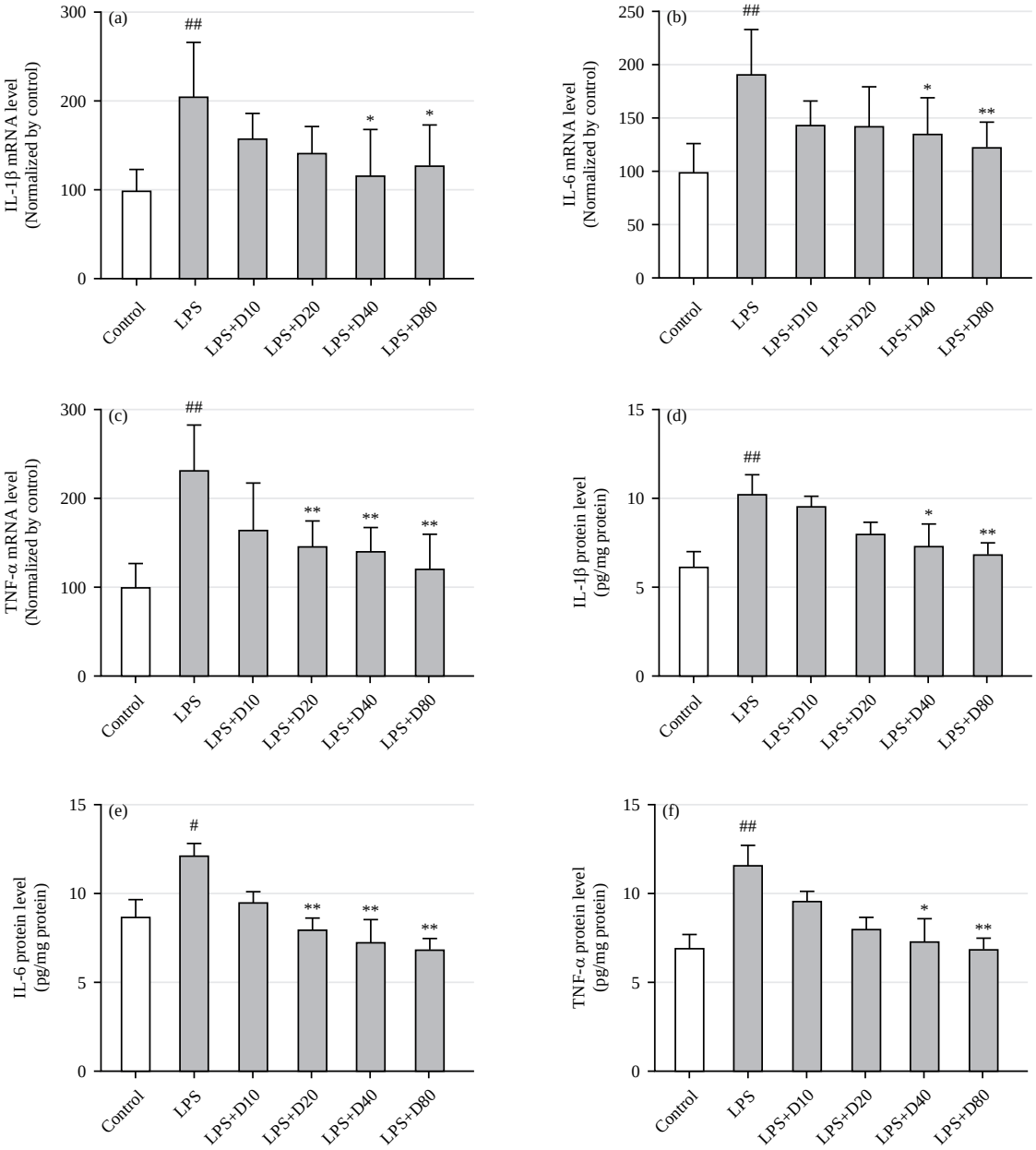

Fig. 2(a-f): Effects of Diosgenin on pro-inflammatory cytokine expression in LPS-stimulated BV2 cells. The levels of mRNA for (a) IL-1β, (b) IL-6, (c) TNF-α and protein levels for (d) IL-1β, (e) IL-6 and (f) TNF-α in BV2 cells treated with Diosgenin after LPS stimulation

Analysis was performed with six replicates (n = 6) for each assessed condition. Statistical significance is denoted as follows: $^{\#}p<0.05$ and $^{\#\#}p<0.01$ compared to the normal control group; $^{*}p<0.05$ and $^{**}p<0.01$ compared to the LPS-treated group

Diosgenin attenuates synaptic pruning of neurons by LPS-induced microglia activation: As shown in Fig. 6a-b, synaptic pruning by hippocampal microglia was significantly enhanced in the LPS-injected mice compared to the controls ($p<0.05$). However, Diosgenin pretreatment significantly curtailed this microglial activity at dosages of 20, 40 and 60 mg/kg ($p<0.05$ for each) when compared to the LPS group. This decline in synaptic pruning activity suggests a potential modulation of microglial activation towards a more quiescent state and supports the notion that Diosgenin could aid in the recovery of neuronal structure and function disrupted by excessive pruning.

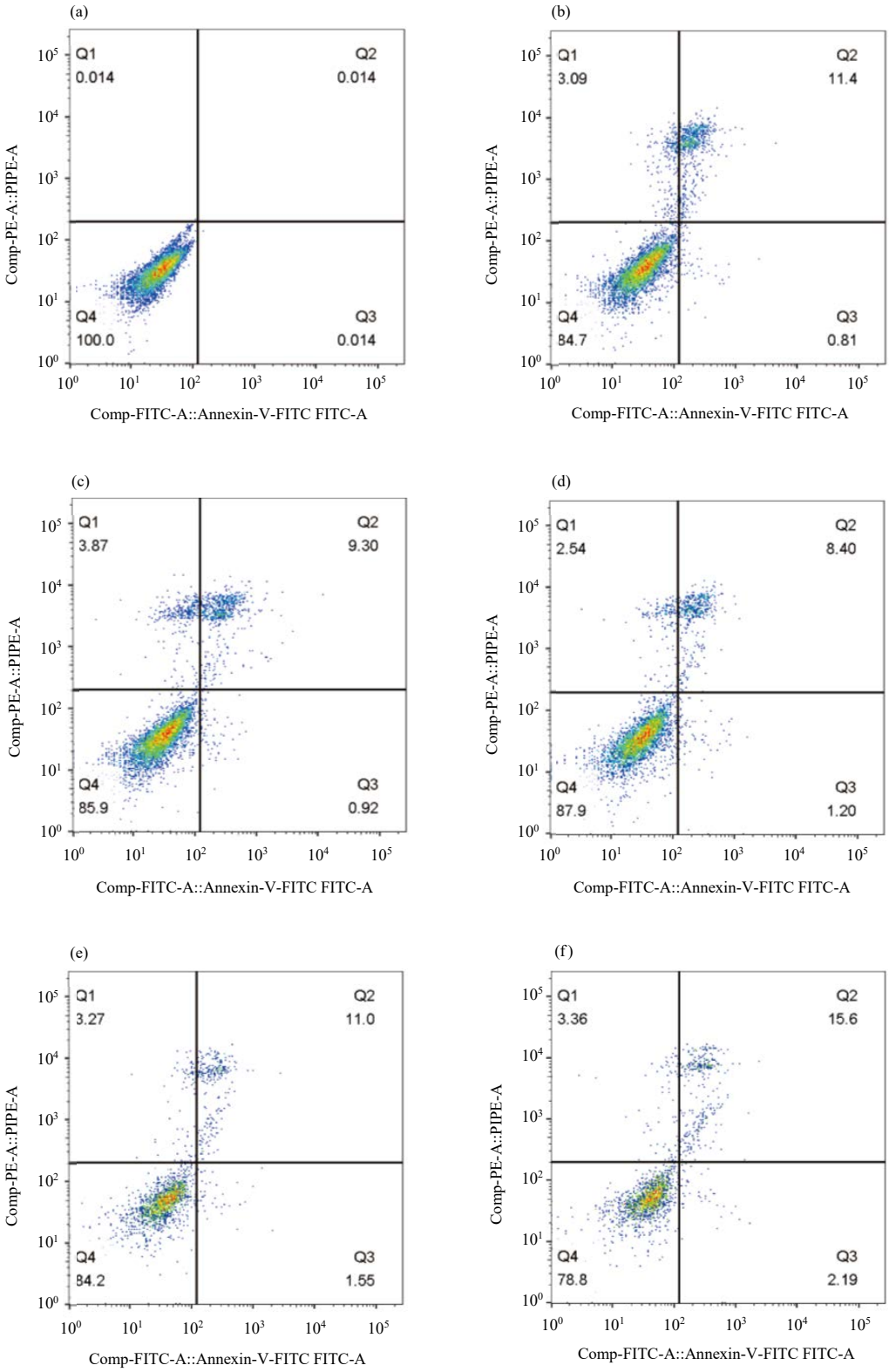

Fig. 3(a-f): Influence of Diosgenin on LPS-induced apoptosis in BV2 cells. The effect of Diosgenin on apoptosis in BV2 microglial cells subjected to LPS stimulation, showcasing data from six experimental conditions, (a) Blank control, (b) LPS alone and Diosgenin pretreatment at (c) 10 μM, (d) 20 μM, (e) 40 μM and (f) 80 μM

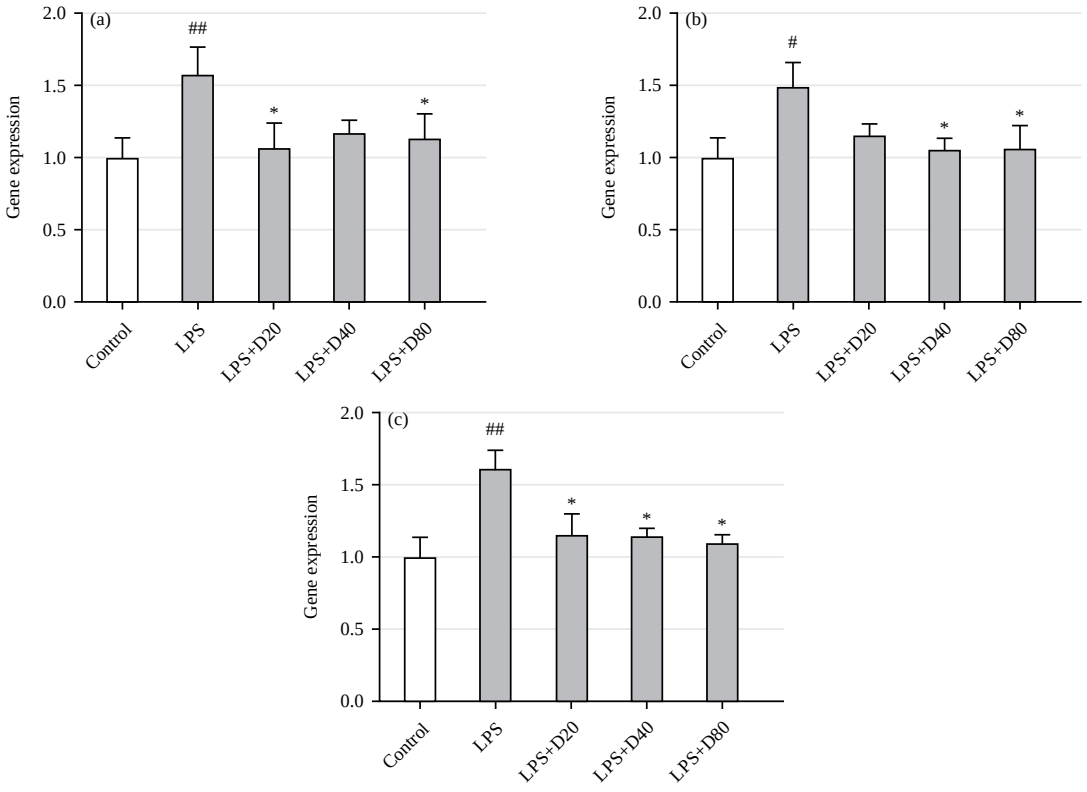

Fig. 4(a-c): Effects of Diosgenin on LPS-induced inflammatory cytokines in the mouse hippocampus. The effect of Diosgenin on the levels of major pro-inflammatory cytokines post-LPS treatment, (a) IL-1β, (b) IL-6 and (c) TNF-α

Results are from six sample per group (n = 6). Statistical significance is denoted by $^{\#}p<0.05$ and $^{\#\#}p<0.01$ compared to the normal control group and *p<0.05 compared to the LPS-injected group

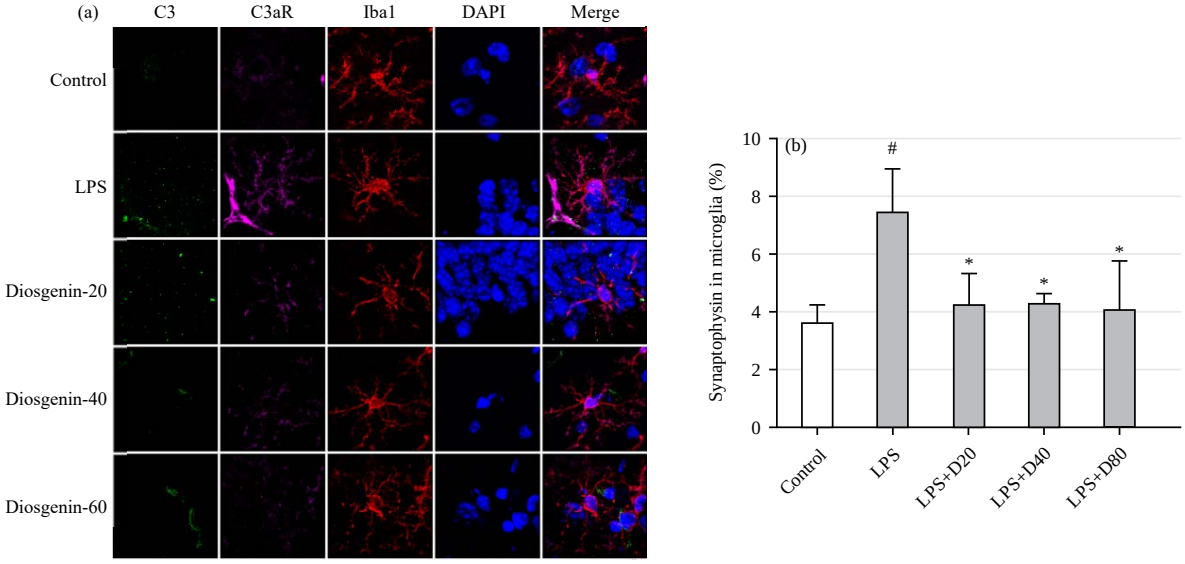

Fig. 5(a-b): Influence of Diosgenin on C3/C3aR signaling pathways induced by LPS in the mouse hippocampus, (a) Fluorescence microscopy images showing C3 in red, C3aR in green and cell nuclei in blue and (b) With a histogram quantifying C3 and C3aR expression levels

Results are from four sample per group (n = 4). Statistical significance is denoted by $^{\#}p<0.05$ against the normal control group and *p<0.05 against the LPS-injected group

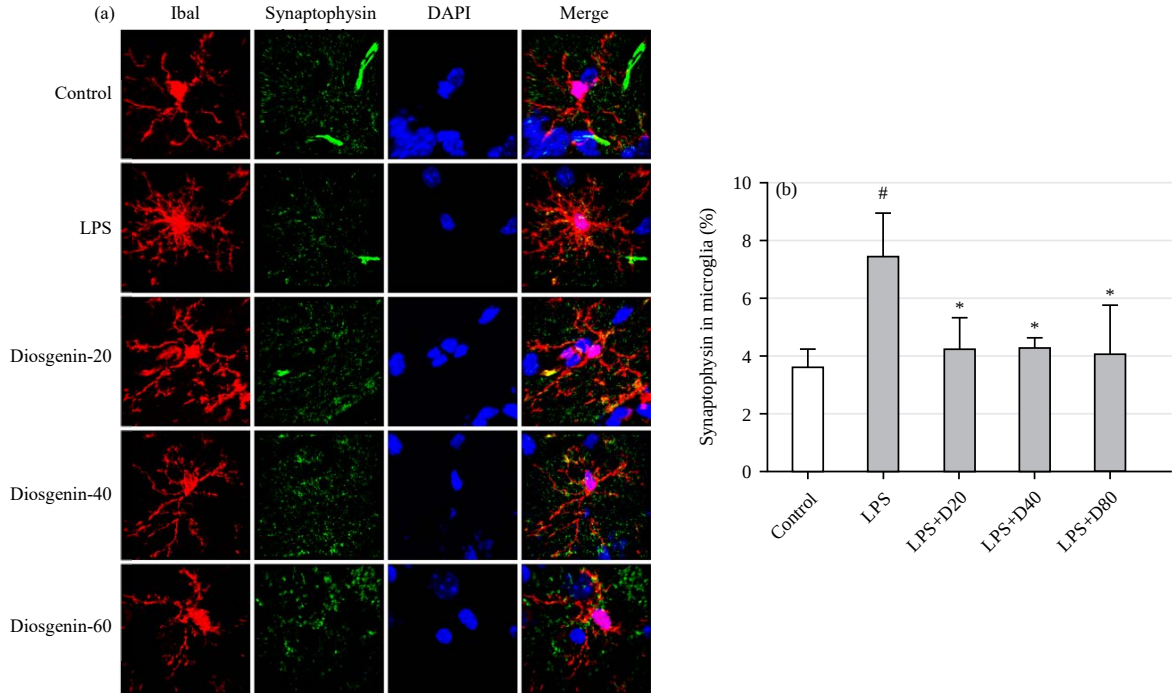

Fig. 6(a-b): Effects of Diosgenin on synaptic pruning induced by LPS in the mouse hippocampus, (a) Fluorescence microscopy images with microglia labeled in red, the presynaptic protein Synaptophysin in green and cell nuclei in blue and (b) Histogram quantitatively illustrates the level of synaptic pruning

Results are from four sample per group (n = 4). Statistical significance is denoted by $^{\#}p<0.05$ relative to the normal control group and $^{*}p<0.05$ relative to the LPS-injected group

DISCUSSION

This study has elucidated the protective role of Diosgenin against LPS-induced apoptosis in BV2 microglial cells and its capacity to modulate pro-inflammatory cytokines. A significant increase was observed in IL-1β, IL-6 and TNF-α levels in LPS-treated BV2 cells, which was substantially attenuated by Diosgenin pretreatment. This reduction suggested the protective effect of Diosgenin in preventing LPS-induced cellular stress. *In vivo*, Diosgenin significantly reduced inflammatory markers in the hippocampus post-LPS injection, indicating its anti-inflammatory efficacy. Furthermore, Diosgenin suppressed C3/C3aR pathways, crucial in neuroinflammation and inhibited excessive microglial synaptic pruning, a process vital to neuroinflammatory responses.

An increasing volume of research highlights the critical role of neuroinflammation in a range of neurological disorders, including Alzheimer's disease[12], Parkinson's disease[13], amyotrophic lateral sclerosis[14] experimental autoimmune encephalomyelitis[15] and depression[16]. Neuroinflammation serves as the cellular and molecular response of central nervous system to diverse stimuli like stress, infections or injuries, playing key roles in protection and healing. When the CNS encounters such stimuli, immune cells trigger an inflammatory reaction, engulfing cellular debris and toxins, thereby fulfilling protective and healing roles. Although neuroinflammation is protective under controlled scenarios, unchecked inflammatory responses can lead to cytokine overproduction, causing neurotoxicity and compromised neuronal function. Notably, depression patients, especially those prone to suicidal behavior, exhibit significantly elevated levels of inflammatory markers such as IL-1β, IL-6 and TNF-α, both genetically and proteomically[17]. This elevation suggests that these inflammatory mediators could act as biomarkers for depression and help gauge the effectiveness of antidepressant therapies[18]. The dualistic nature of neuroinflammation, as both a defense mechanism and a potential driver of neurodegenerative and psychiatric disorders, highlights its intricate influence on brain health and pathology.

The LPS, a major component of Gram-negative bacterial cell walls, serves as a potent endotoxin inciting substantial immune and inflammatory reactions in both peripheral and brain tissues[19]. Intraperitoneal LPS injections in mice induce neuroinflammatory behaviors, such as reduced exploratory activity linked to anhedonia and disrupt central nervous system functionality, impacting synaptic long-term potentiation[20]. The LPS acts as a pathogen-associated molecular pattern, binding to macrophage pattern recognition receptors and igniting inflammatory signaling cascades, leading to cytokine secretion[21]. Specifically, LPS binding to the TLR4 receptor on microglia initiates the TLR4/NF-κB signaling pathway, resulting in the release of large quantities of inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, thereby exacerbating neuroinflammatory responses. Current research showed that LPS exposure in BV2 microglial cells drastically decreases cell viability while increasing pro-inflammatory cytokine levels, emphasizing the role of LPS role in activating neuroinflammation. Conversely, Diosgenin pretreatment notably diminished these cytokines levels, highlighting its potential to reverse LPS-induced inflammatory responses in microglial cells.

Microglia, the primary immune cells of the Central Nervous System (CNS), are vital in maintaining homeostasis and immune regulation. They are key players in neurogenesis, phagocytosis and the clearance of dead neurons, acting as the brain macrophages to regulate neural development, ensure the integrity of neural networks and repair damage[22]. These cells are essential in modulating synaptic transmission and shaping neural circuitry[23].

Microglia are integral to brain development, ensuring the proper function of central nervous system through synaptic pruning, which removes seldom-used synaptic connections to enhance neurotransmission efficiency[24]. This process is partly regulated via a complement-dependent mechanism[25]. Activation of astrocytes leads to the production of complement components C1q and C3, which prompt microglia to prune synapses, potentially diminishing synaptic density and disrupting normal neuronal function. Additionally, evidence indicates that complement C4a can intensify synaptic phagocytosis by microglia, resulting in excessive pruning[26]. Thus, microglial activation, especially under neuroinflammatory conditions, can deteriorate synaptic architecture and function, potentially triggering neurological disorders[27]. Current findings revealed that LPS activates hippocampal microglia, leading to heightened synaptic pruning, whereas Diosgenin alleviates this over-pruning by inhibiting the C3/C3aR signaling pathway, thereby preserving neuronal functionality.

CONCLUSION

Diosgenin effectively countered the reduction in BV2 microglial cell activity, inflammatory responses and apoptosis triggered by LPS. Additionally, it alleviated LPS-induced neuroinflammation in mice, primarily through reducing microglial-mediated synaptic pruning, thereby enhancing neuronal function. These outcomes demonstrate the anti-neuroinflammatory capabilities of Diosgenin both in cellular and animal models, highlighting its potential therapeutic value for treating neuroinflammation-associated disorders.

SIGNIFICANCE STATEMENT

This study aimed to investigate the potential of Diosgenin to mitigate neuroinflammation and its associated synaptic alterations. Our key findings show that Diosgenin significantly reduces the secretion of proinflammatory cytokines, diminishes apoptosis in microglial cells and prevents synaptic pruning in the hippocampus caused by LPS-induced neuroinflammation. These findings are crucial as they highlight the therapeutic potential of Diosgenin to preserve synaptic integrity and improve outcomes in neuroinflammatory disorders. Future research should explore the long-term effects of Diosgenin on cognitive function and its efficacy across different models of neuroinflammation to fully establish its role in clinical applications.

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