Sinapic Acid Attenuates the Neuroinflammatory Response by targeting AKT and MAPK in LPS-activated microglial models

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Abstract

Sinapic acid (SA) is a phenolic acid that is widely found in fruits and vegetables, which has various bioactivities, such as antidiabetic, anti-inflammatory, anticancer and anxiolytic functions. Overactivated microglial cells play a major role in the development of neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. The objective of this study was to investigate the effect and mechanism of sinapic acid in microglia-derived neuroinflammatory models. Our results demonstrated that SA inhibited production of the nitric oxide (NO) pro-inflammatory factor, induced nitric oxide synthase (iNOS), induced interleukin (IL)-6 and enhanced the release of IL-10 in a dose-dependent manner. Subsequently, a mechanistic study revealed that SA remarkably attenuated the phosphorylation of AKT and MAPK cascades in LPS-induced microglia. Consistently, administration of SA regulated the production of inflammation-related cytokines and inhibited the phosphorylation of MAPK cascades and Akt in the mouse cerebral cortex. These results suggested that SA may offer a possible treatment for anti-inflammatory activity by targeting the AKT/MAPK signaling pathway.

1. Introduction

Sinapic acid (SA) is a naturally occurring phenolic acid that is present in various edible, functional and pharmaceutical plants, including lemon, orange and various Brassica vegetables, and it is common in the human diet(Hameed et al., 2016; Nguyen et al., 2021). Importantly, SA has various bioactivities, such as antidiabetic, anti-inflammatory, anticancer and anxiolytic functions (Bin Jardan et al., 2020; Chen, 2016; Huang et al., 2020; Lee, 2018). SA has been reported to attenuate IL-1β secretion and suppress the NLRP3 inflammasome in macrophages and in a mouse model(Lee et al., 2021). Doxorubicin-induced inflammation is mediated by sinapic acid via downregulation of NF-κB in rat heart muscle(Bin Jardan et al., 2020). In addition, sinapic acid derivatives in broccoli sprouts also downregulate the release of proinflammatory cytokines (TNF-α and IL-6) and increase the production of IL-10 from LPS-stimulated human peripheral blood mononuclear cells(Olszewska et al., 2020). Interestingly, one study has also reported that SA does not show anti-inflammatory activity when it is isolated from the roots of Polygala arillata (Xiang et al., 2019). However, for the treatment of other diseases, SA has been demonstrated to improve cognitive impairment in a scopolamine-induced mouse model and attenuate proinflammatory cytokine secretion in a dementia rat model (Verma et al., 2020).

Neuroinflammation has long been recognized as a key pathophysiological process that is involved in various neurodegenerative diseases, such as Alzheimer’s disease and Parkinson’s disease. Microglial cells are innate immune cells that maintain the health condition of the central neuron system by removing injurious stimuli or repairing damaged cells at the beginning of neuroinflammation (Perry et al., 2010). Lasting pathological stimulation induces microglial cells to be overactivated, releasing a large number of proinflammatory factors and cytokines, which initiate the neuroinflammatory response and damage brain tissue (Saha & Pahan, 2006; Zhao et al., 2021). Recently, many studies have indicated that microglia-derived neuroinflammation plays an indicative role in the pathogenesis of neurodegenerative diseases (Krause & Muller, 2010; Streit et al., 2004). Therefore, inhibiting the overactivation of microglia

may be useful to reduce or slow the progression of neuroinflammation and neurodegenerative conditions, which will be beneficial for neurodegenerative disease therapy. During the neuroinflammatory process, cells of the immune system, including microglia, are activated through the recognition of a pathogen endotoxin by Toll-like receptors (TLRs) and then propagate antigen-induced signal transduction pathways to subsequently activate other downstream proteins, such as mitogen-activated protein kinases (MAPKs) and protein kinase B (AKT) (Chen et al., 2019). MAPK cascades, including c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK) and p38, are central intracellular mediators that are activated in response to lipopolysaccharide stimulation, which is an endotoxin of the outer membrane of gram-negative bacteria. AKT, a serine/threonine kinase, is also considered one of the most important effector kinases downstream of PI3K and the core of the PI3K/AKT signaling pathway(Tang et al., 2018). It has been demonstrated that these signaling pathways are involved in neuroinflammation by regulating the expression of inflammation-related cytokines and enzyme complexes. Thus, targeting inflammation-related signaling pathways may be a beneficial way to slow the progression of neuroinflammation and neurodegenerative conditions.

Therefore, SA may have the potential to impede the key cycle of cytokine secretion, inflammation and neurodamage, thereby providing a new therapeutically beneficial option for neuroinflammation. However, it remains unclear whether SA has an anti-inflammatory effect on LPS-induced microglial neuroinflammation. Thus, it is necessary not only to investigate whether SA has an anti-inflammatory effect in the CNS but also to clarify the potential underlying mechanisms.

2. Material And Methods

2.1 Chemicals and reagent.

Antibodies (iNOS (2982), COX-2 (12282), phospho-Akt (9271), ERK (9102), phosphor-ERK (9101), JNK (9252), phosphor-JNK (9251), p38 (9212) and phosphor-p38 (9211) primary antibodies as well as an anti-rabbit immunoglobulin G (IgG) secondary antibody) were purchased from Cell Signaling Technology (Boston, MA, USA). Fetal bovine serum (FBS) was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Penicillin/streptomycin (P/S) and Dulbecco's modified Eagle's medium (DMEM/F12) were purchased from Gibco (Grand Island, NY, USA). LPS originating from Escherichia coli O55:B5 and sinapic acid (purity > 98%) were obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2 Cell culture.

The BV-2 mouse microglial cell line was obtained from Prof. Lee Sung Jung's research team at the School of Dentistry, Seoul National University and was cultured in Dulbecco's modified Eagle medium (DMEM; Gibco, Invitrogen, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Invitrogen, Grand Island, NY, USA) and 1% penicillin/streptomycin (P/S; Gibco, Invitrogen, Grand Island, NY, USA) at 37°C under a humidified atmosphere containing 5% CO_2.

2.3 Cell Viability Assay.

Cell viability was measured by a colorimetric assay to detect cell metabolic ability according to a previously reported protocol (Zhao et al., 2019). BV-2 microglial cells were cultured in DMEM/F12 medium (Gibco) in 96-well plates (1×10^4 cells/well) for 24 hours. Cells were then treated for 12 hours with various concentrations of sinapic acid and compounds in the presence or absence of 100 ng/ml LPS. After incubation for 12 hours, 10 µl of EZ-Cytox reagent (Daeil Lab Co., Ltd., Seoul, Republic of Korea) was added to each well followed by incubation for 30 min at 37°C. The absorbance of each reaction product was then measured using a microplate reader (Infinite 1000, Tecan Trading AG, Switzerland) at a wavelength of 450 nm. The results are presented as a percentage of the MTT absorbance of the control cells.

2.4 Measurement of Nitric Oxide.

Cells were grown in 6-well plates (5×10^5 cells/well) for 24 hours. Cells were then pretreated with 25, 50 and 100 µg/ml WIN-1001X for 1 hour and then treated with 100 ng/ml LPS for 12 hours. The medium was collected and centrifuged at 13,000 rpm to remove dead cells. The supernatant medium was collected and mixed with an equal volume (50 µl) of Griess reagents (1% sulfanilamide and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride in 2.5% H_3PO_4) for 10 min followed by incubation at room temperature. The nitric oxide concentration was measured by a sodium nitrite standard curve at 540 nm using a microplate reader (Multiskan SkyHigh Plate Reader, Thermo Fisher Scientific).

2.5 Animal experiments.

Male ICR mice (aged 8 weeks and weighing 26 ± 2 g) were purchased from Orient Bio, Inc., Seongnam, Republic of Korea. Animals were kept in the housing facility for one week to adapt to the environment before the experiment and were maintained at 24 ± 1°C under a 16/8 h light/dark cycle with food and water provided *ad libitum*. Five groups of animals were maintained in different cages with 6 mice per cage. The normal control group and LPS-injected only group were administered PBS orally. The positive control group was administered dexamethasone (2.5 mg/kg/day) orally. The other two groups were administered sinapic acid (dissolved in PBS) orally (10 and 20 mg/kg/day). The oral administration continued for 8 days. Three days before sampling the LPS-injected group, the positive control group and the two sinapic acid-administered groups were i.p. (intraperitoneal) injected with LPS (2.5 mg/kg/day) after 30 min of oral gavage treatment. Animals were anesthetized by diethyl ether and sacrificed after the last LPS stimulation for 3 h. Brain tissue samples were collected and stored at -80°C until further analysis.

2.6 Total Protein Isolation from BV-2 Cells.

Total protein was isolated according to a previous study (Zhao et al., 2019). Briefly, cells were washed 3 times with ice-cold PBS and centrifuged at 13,000 rpm for 5 min to remove dead cells. After discarding the supernatant, the collected cells were lysed with lysis buffer (Cell Signaling Technology, Boston, MA, USA), containing protease inhibitor cocktail (PIC, Roche, Penzberg, Germany) and phenylmethylsulfonyl fluoride (PMSF), for 20 min on ice. The lysates were centrifuged (13,000 rpm) for 20 min. Protein

quantification was conducted with the Bradford reagent (Bio–Rad, Hercules, CA, USA), and the BSA standard (0 – 20 mg/ml) was prepared as a standard curve. Western blot samples were prepared with lysate and an equal volume of 2× NuPAGE LDS sample buffer (Thermo Fisher Scientific, Inc., Lafayette, CA, USA) with 10% 2-mercaptoethanol. After denaturation at 99°C, the samples were stored at -80°C until further analysis.

2.7 Total Protein Isolation from the Mouse Brain.

For the preparation of mouse brain samples as described previously(Zhao et al., 2019), the mouse cerebral cortex was collected, stored at -80°C and then homogenized with PRO-PREP protein extraction buffer (iNtRON, Seongnam-si, Republic of Korea) supplemented with 1× PIC set III (Sigma–Aldrich, St. Louis, MO, USA). The homogenates were then centrifuged (13,000 rpm) for 20 min at 4°C. Protein quantification assays were performed as described for total protein samples.

2.8 Western blot Analysis.

Total protein samples (20 μg) were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS – PAGE) with 10% acrylamide/bis gels and electrotransferred to Immobilon-P polyvinylidene difluoride (PVDF) membranes (Merck KGaA, Darmstadt, Germany). The membranes were then blocked with 5% BSA in Tris-buffered saline with Tween (TBST) and incubated overnight at 4°C with specific primary antibodies (1:1000). After washing with TBST, the membranes were incubated with secondary HRP-conjugated IgG (1:2000) at room temperature for 1 h and visualized using SuperSignal™ West Femto Chemiluminescent Substrate (Thermo Fisher Scientific). Band signal densitometry analysis was performed with the LAS4000 system (Fujifilm, Tokyo, Japan), and the intensities of the proteins were quantified by Multi Gauge V3.0 software (Fujifilm, Japan).

2.9 Statistics.

Data are expressed as the mean ± SEM of each independent replication. For comparison of three or more replications, data were analyzed by one-way analysis of variance (ANOVA) with Dunnett's post-hoc test. A value of $P < 0.05$ was considered statistically significant. Statistical tests were performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1 Effect of sinapic acid on cell viability, NO and iNOS/COX-2 production in LPS-stimulated BV-2 cells.

To elucidate the potential protective effect of sinapic acid on BV-2 cells, an MTT assay was performed. As shown in Fig. 1A, the viability of BV-2 cells was not strongly affected in the presence of sinapic acid, and cytotoxicity was slightly increased when the concentration of sinapic acid was higher than 20 μM (Fig. 1A). The anti-inflammatory effects of sinapic acid were also evaluated in terms of the production of NO as well as the expression of iNOS and COX-2. As depicted in Fig. 1B, treatment with LPS significantly stimulated the production of NO in the supernatant of the BV-2 cell culture medium, and the expression of

iNOS was significantly decreased in the sinapic acid-treated group compared to the LPS groups (Fig. 1C). In addition, there was no significant reduction in COX-2 expression after exposure to sinapic acid and LPS (Fig. 1D). Therefore, these results indicated that sinapic acid participates in the anti-inflammatory response in microglial cells.

3.2 Effect of sinapic acid on the production of inflammatory cytokines in LPS-stimulated microglial cells.

To determine the effects of sinapic acid on the release of proinflammatory and anti-inflammatory cytokines, the secretion of IL-6 and IL-10 was detected after LPS exposure. As shown in Fig. 2A, pretreatment of LPS-stimulated BV-2 microglia with sinapic acid (5, 10 and 20 μM) facilitated a reduction in IL-6 levels in the cells. Moreover, sinapic acid increased the production of IL-10 compared to cells induced by LPS (Fig. 2B). Thus, these results demonstrated that sinapic acid pretreatment significantly suppresses the LPS-induced inflammatory response by regulating the secretion of inflammation-related cytokines.

3.3 Sinapic acid suppresses MAPK phosphorylation in LPS-stimulated BV-2 microglial cells.

The MAPK signaling pathway is involved in the regulation of numerous inflammatory responses, including LPS-induced microglial activation. To investigate the participation of sinapic acid in the molecular mechanism of the LPS-induced inflammatory response, JNK, p38 and ERK protein phosphorylation was detected in LPS-treated BV-2 cells. As shown in Fig. 3A-C, LPS treatment resulted in a significant increase in the phosphorylation of JNK, p38 and ERK in BV-2 cells. However, sinapic acid significantly attenuated the LPS-stimulated phosphorylation levels of JNK, p38 and ERK. These data suggested that sinapic acid may attenuate proinflammatory factors by inhibiting the MAPK signaling cascade.

3.4 Sinapic acid suppresses AKT phosphorylation in LPS-stimulated BV-2 microglial cells.

As illustrated in Fig. 3D, sinapic acid significantly decreased the phosphorylation of AKT compared to that in the LPS group, indicating that sinapic acid may induce the activation of the AKT signaling pathway. To further determine whether sinapic acid suppresses the AKT signaling pathway, pretreatment with an Akt1/2 kinase inhibitor (A6730) and AKT enhancer (SC79) also ameliorated the increase in LPS-induced NO secretion, further suggesting that the AKT signaling pathway may mediate the LPS-induced secretion of proinflammatory factors in BV-2 cells. Moreover, the AKT inhibitor reduced not only NO production but also the expression levels of iNOS, while cotreatment with sinapic acid and the inhibitor further suppressed NO production. The SC79 AKT enhancer had a significant effect on the phosphorylation levels of AKT (Fig. 4A). However, cotreatment with SC79 and sinapic acid rescued this trend and attenuated the phosphorylation levels of AKT. Therefore, these results indicated that sinapic acid may protect microglia against the LPS-induced inflammatory response by inhibiting AKT activation.

3.5 Sinapic acid suppresses IL-6 and enhances IL-10 secretion in LPS-induced mouse serum.

Because systemic LPS injection modulates inflammatory cytokine production in the serum and brain, the effect of sinapic acid on the inflammatory response in an LPS-stimulated mouse model was investigated. As shown in Fig. 5A, systemic LPS injection significantly increased IL-6 levels in the serum, but this effect was reversed by sinapic acid administration. Moreover, sinapic acid treatment induced a further increase in IL-10 in the serum of LPS-treated mice, revealing that the anti-inflammatory response is activated by sinapic acid (Fig. 5B). These data suggested that sinapic acid systemically regulates the LPS-mediated inflammatory response.

3.6 Sinapic acid suppresses the MAPK and AKT signaling pathways in the LPS-induced mouse cerebral cortex.

The *in vitro* data revealed that sinapic acid mediated LPS-treated microglial activation by attenuating the AKT and MAPK pathways. Thus, we further investigated whether sinapic acid is involved in these inflammation-related signaling pathways in LPS-injected mouse brains. Western blot results indicated that LPS treatment increased the expression of p-JNK, p-P38, p-ERK and p-AKT. In contrast, sinapic acid administration significantly reduced AKT and MAPK phosphorylation in cerebral cortex tissue compared to the LPS group (Fig. 6).

4. Discussion

This study investigated the effects of sinapic acid on inflammatory mediator secretion through the Akt/MAPK signaling pathway and its underlying mechanism in BV-2 microglia macrophages and *in vivo* mouse model exposed to LPS-induced inflammatory conditions. Our results revealed that sinapic acid treatment suppressed inflammatory response by modulating the phosphorylation of Akt, leading to the suppression of MAPK casedes phosphorylation under LPS-induced inflammatory challenge. Additionally, sinapic acid administration also suppressed the Akt/MAPK activation and the LPS-induced inflammatory response *in vivo*.

Chronic inflammation is a central process involved in many metabolic disorders, including neurodegenerative (Alzheimer and Parkinson diseases) and autoimmune diseases(Marginǎ et al., 2020). Dietary components have the ability to influence the immune response through the modulation of gut bacteria metabolism or impacting cells through the brain-blood barrier(Galland, 2010; Leigh & Morris, 2020). Thus, the constituents of daily diets, including vegetables and crops, have been focused on in recent studies. Sinapic acid is an interesting and widely present hydroxycinnamic acid, especially in the *Brassicaceae* family(Nguyen et al., 2021). In recent years, SA and some of its derivatives (sinapine and sinapoyl esters) have been studied due their various biological activities, including antioxidant and anti-inflammatory activities(Chen, 2016). In the present study, we found that LPS-induced excessive proinflammatory cytokine expression, such as intercellular NO and iNOS production, was inhibited by SA in LPS-treated microglia. Consistently, sinapic acid protected the mice against an acute inflammatory response and decreased proinflammatory cytokines, such as IL-6, after LPS administration. Moreover, SA

potently suppressed the activation of Akt and MAPK signaling in LPS-treated microglia. These findings revealed that sinapic acid has the potential to attenuate neuroinflammation.

Neuroinflammation is a complicated process derived from the initial inflammatory responses regulated by inflammatory mediators, including nitric oxide (NO) (Aktan, 2004). Nitric oxide eliminates microorganisms and has a protective effect on specific tissues, but it also induces cytotoxicity in these tissues at high concentrations (Maksoud et al., 2019). Lipopolysaccharide (LPS) induces systemic inflammation by boosting immune system cells and expanding the secretion of inflammatory mediators by regulating microglial activation (Calabrese et al., 2007; Xue et al., 2018). The production of NO is regulated by the nitric oxide synthase enzyme family, in which iNOS is mainly involved (Singh et al., 2019). Increased levels of NO have also been reported to connect neuroinflammation with brain dysfunctions and diseases, such as learning and memory impairments(Picón-Pagès et al., 2019). The present study demonstrated that SA treatment inhibits NO production and downregulates iNOS protein expression in BV-2 microglial cells, thereby suggesting the potential protective effect of SA against LPS-induced inflammatory stimuli.

The production of LPS-induced iNOS in microglial cells is modulated by MAPKs (Chen et al., 2019; Rai et al., 2019; Zhang & Liu, 2002). MAPKs are protein kinases that regulate important cellular processes in immune responses(Zhang & Liu, 2002), and several studies have suggested that MAPKs constitute a key step in the process of neuroinflammation and may be a potential target for pharmacological treatment (Kim & Choi, 2015; Rai et al., 2019). In the present study, we found reduced p-ERK, p–JNK and p-p38 in response to SA treatment, which suggested suppression of MAPK phosphorylation during LPS-induced upregulated inflammation. The MAPK pathway is activated by TLR4/MyD88 and modulates the expression of inflammatory mediators, including iNOS, IL-6 and IL-10(Arthur & Ley, 2013). Huang *et al.* reported that SA inhibits IL-1β-induced inflammation via blockade of IL-1β-induced MAPK signaling activation in rat chondrocytes (Huang et al., 2018). The present study found that LPS activates the MAPK signaling pathway and that treatment with SA significantly moderates the phosphorylation of JNK, ERK and p38.

Akt is a serine kinase that plays the essential role in the PI3K/Akt signaling pathway. Akt is activated during all aspects of the development of neuroinflammation. Previous studies have revealed that the PI3K/Akt signaling pathway alters LPS-induced COX-2 and iNOS expression in BV-2 cells (Zhao et al., 2021). Activated Akt releases NF-κB to translocate to the nucleus, which triggers stimulation of inflammatory target genes and promotes the proinflammatory response (Zhu et al., 2021). In addition, other studies have demonstrated that treatment with SA attenuates the NF-κB signaling pathway both *in vivo* and *in vitro* (Bin Jardan et al., 2020; Verma et al., 2020). However, the relationship between Akt phosphorylation and SA requires further exploration. In the present study, western blot analysis indicated that the Akt activation induced by LPS was attenuated by SA in a dose-dependent manner in BV-2 microglia. For further confirmation, an Akt kinase inhibitor (A6730) and Akt agonist (SC79) were applied to investigate the relationship between the Akt signaling pathway and SA. These data suggested that the

Akt signaling pathway plays a major role in the anti-inflammatory activity of SA *in vitro*, contributing to further understanding of the mechanism of SA bioactivity in the anti-inflammation model.

An LPS-injected *in vivo* mouse model was also used to explore the effect of SA. After LPS administration, a systematic inflammatory response generates and releases proinflammatory cytokines. As proinflammatory cytokines are generated, glial activation from the brain shifts to counter the impacts of attacking pathogens by upregulating IL-10(Becher et al., 2017). The release of proinflammatory cytokines and their expression in the mouse cerebral tissue demonstrated that anti-inflammatory activity can be detected under both LPS-induced systemic inflammatory and neuroinflammatory conditions. SA also consistently inhibited the phosphorylation of LPS-stimulated Akt and MAPK in the mouse cerebral cortex, indicating that SA represses the inflammatory response of microglia by inhibiting Akt/MAPK signaling pathways.

In conclusion, our investigation revealed that SA is a potent suppressor of the neuroinflammatory pathway. Overall, both *in vivo* and *in vitro* findings showed that SA may protect against LPS-induced inflammation via the MAPK and Akt signaling pathways, thus acting as an active compound. Therefore, these results indicated that SA may be a potential therapeutic candidate against neurodegenerative diseases related to inflammation.

Declarations

COMPETING INTERESTS DECLARATIONS.

I declare that the authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

ETHICAL APPROVAL

All the animal protocols used in current study was approved and in accordance with the Korea Institute of Science and Technology Animal Care Committee guidelines.

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AUTHOR CONTRIBUTIONS

Tianqi Huang designed the experiments, performed the experiments, analyzed data and wrote the paper; Dong Zhao designed the experiments, analyzed the data and revised the paper; Sangbin Lee revised the

paper; Hyunok Yang and Gyochang Keum supervised the manuscript. All authors contributed to the manuscript.

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Figures

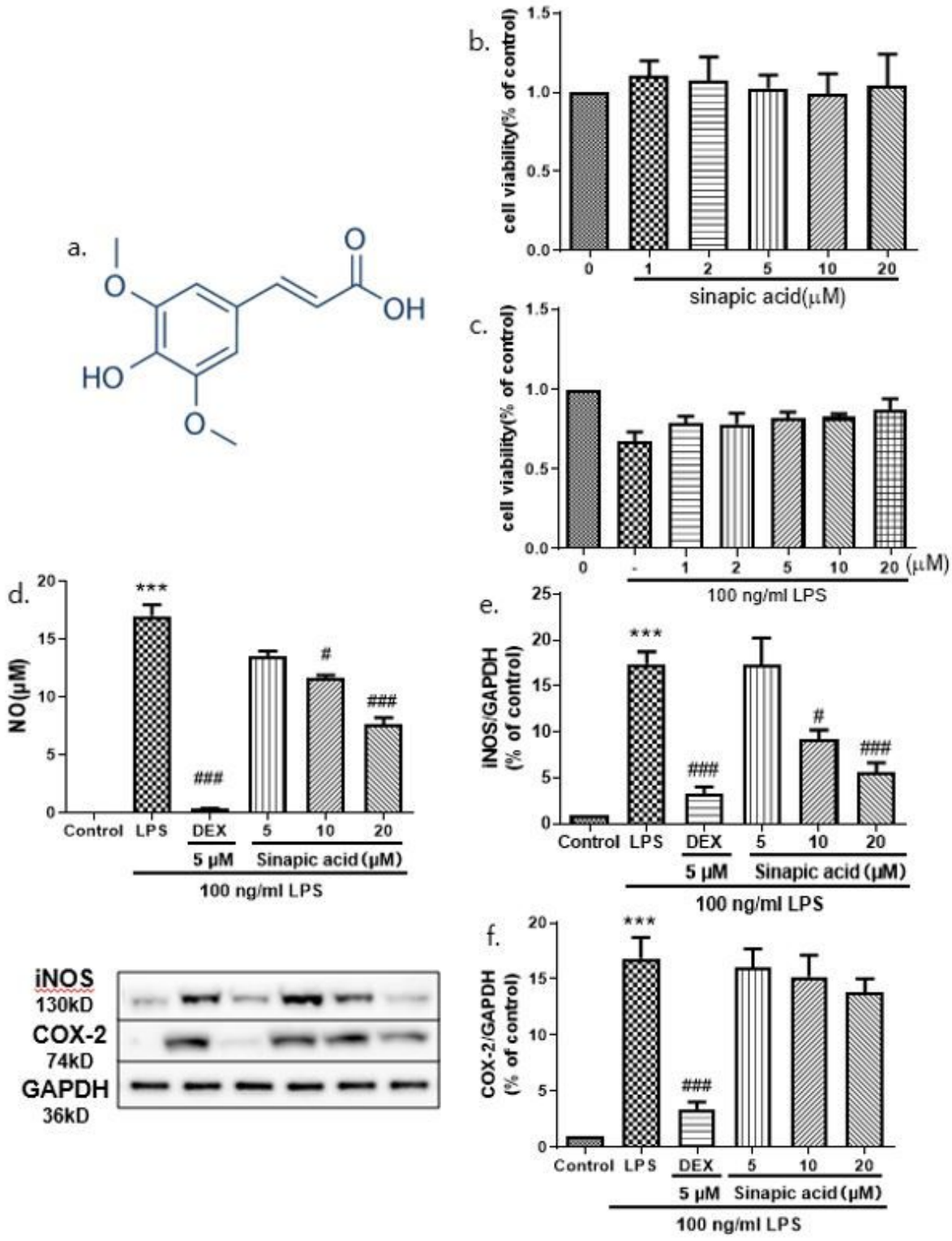

Figure 1

Effect of the sinapic acid on nitric oxide (NO) and iNOS/COX-2 production in LPS-stimulated BV-2 cells. Chemical structure of sinapic acid (A). MTT assay was measured to perform the cell viability with LPS (B) or without LPS (C). NO levels in the cell supernatant were measured by Griess reagent colorimetric reaction (D). Respective Western blots presenting the expression level of the iNOS (E) & COX-2(F). The Control added with 0.025% DMSO solvent for 12 h. Experimental results were presented as

mean (±SEM, n = 3). *** P < 0.001, the significant difference compared with the control. Compared with LPS group, #: p < 0.05, ###: p < 0.001.

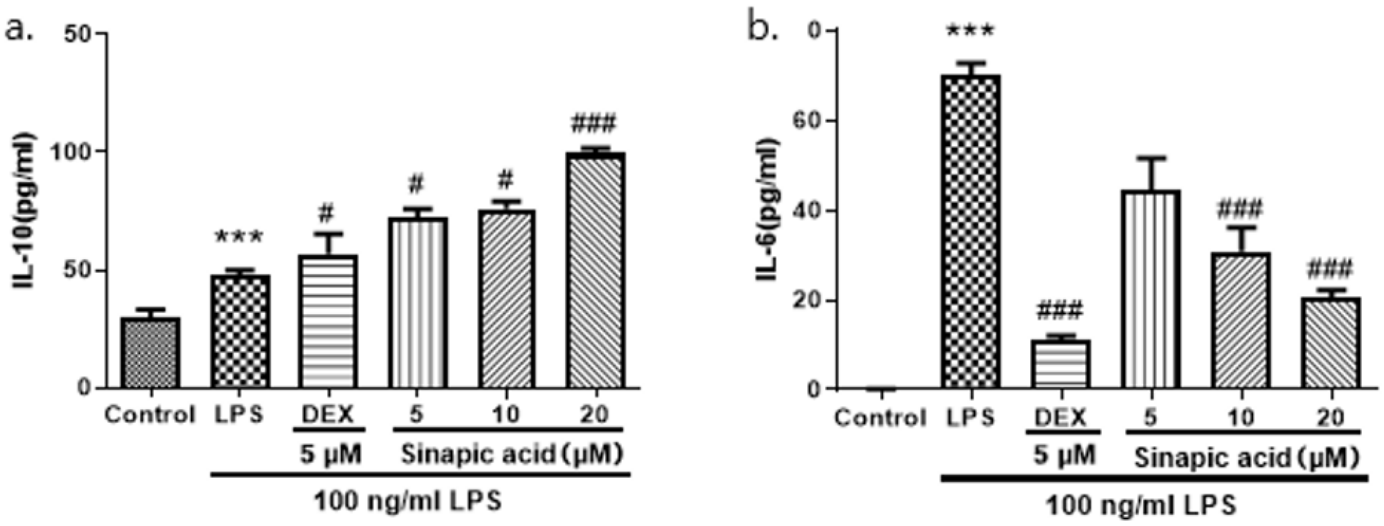

Figure 2

Effect of sinapic acid on the production of inflammatory cytokine production in LPS-stimulated microglial cells. Cells (5×105 cells/well) were cultured in 6-well culture dishes at 37 °C in presence of 5% CO_2. BV-2 Cells were pretreated with the indicated concentrations (5, 10 and 20 μM) of sinapic acid for 1 h and then stimulated with 1 μg/mL LPS for 24 h. The Elisa were performed to measure IL-6(A) & Il-10(B) secretions as the manufacture protocol. Data are detected as mean (±SEM, n = 3). *** P < 0.001, the significant difference compared with the control. Compared with LPS group, #: p < 0.05, ###: p < 0.001.

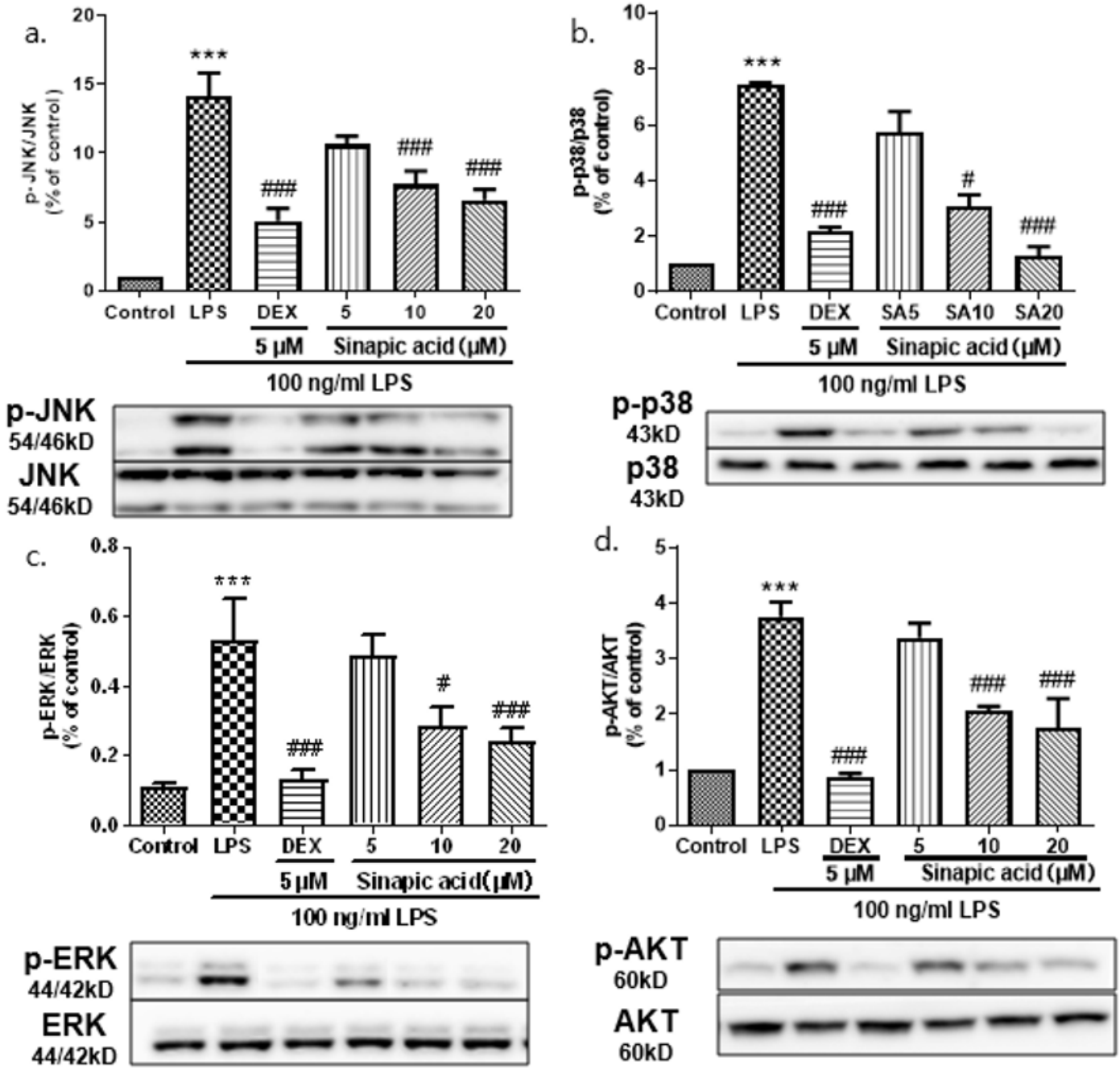

Figure 3

Sinapic acid suppressed MAPK phosphorylation in LPS-stimulated BV-2 Microglia cell. The phosphorylation of JNK(A), ERK(B), and p38 MAP kinases(C) as well as on AKT(D) were measured by western blot. Data are detected as mean (±SEM, n = 3). *** $P < 0.001$, the significant difference compared with the control. Compared with LPS group, **#: $p < 0.05$, ###: $p < 0.001$.**

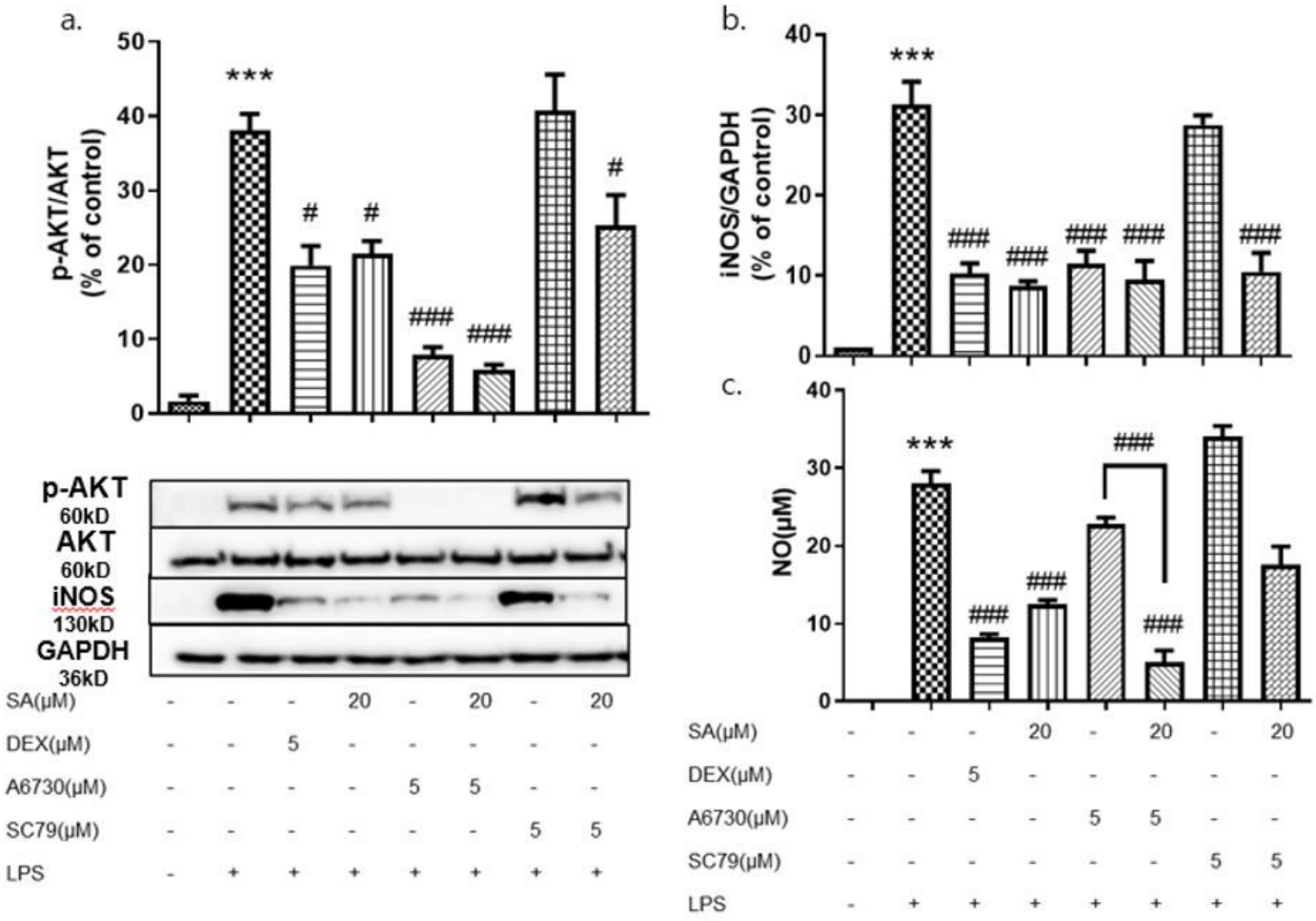

Figure 4

Sinapic acid suppressed AKT phosphorylation in LPS-stimulated BV-2 Microglia cell. AKT(A), iNOS(B) expression and NO production(C) was detected after co-treatment with the specific inhibitor Akt1/2 kinase inhibitor and specific enhancer SC79. Data are detected as mean (±SEM, n = 3). *** $P < 0.001$, the significant difference compared with the control. Compared with LPS group, #: $p < 0.05$, ###: $p < 0.001$.

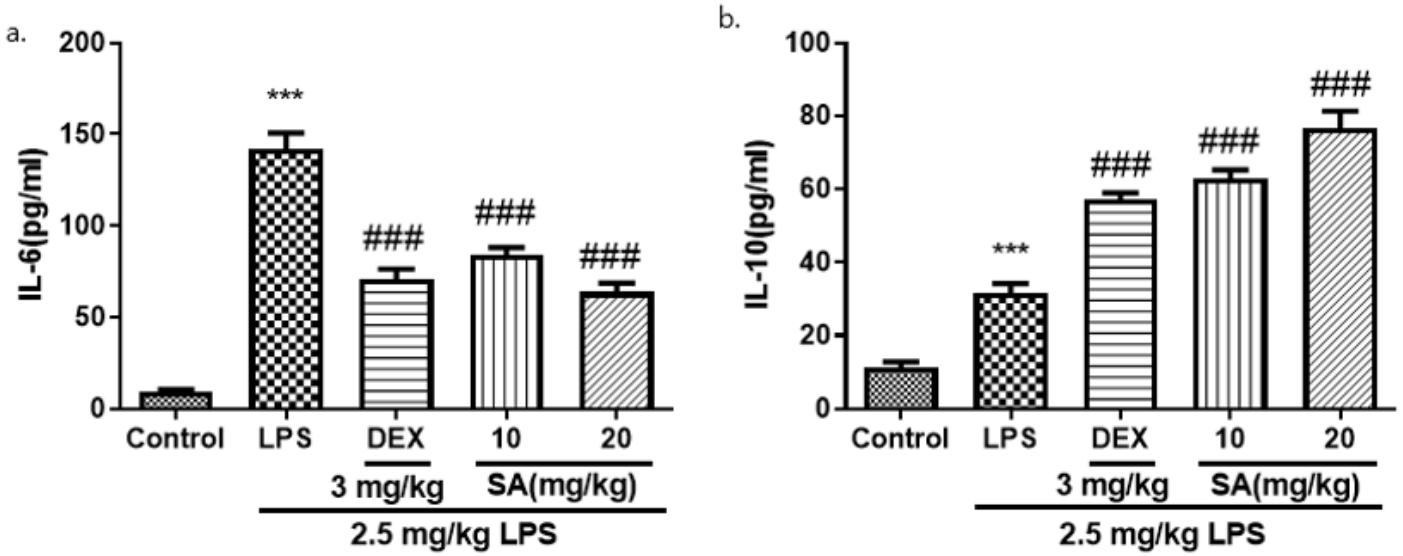

Figure 5

Sinapic acid suppressed IL-6 and enhanced IL-10 secretion in LPS-induced mouse serum. IL-6 (A,) and IL-10 (B) levels in the mouse serum were detected by ELISA. Data are detected as mean (±SEM, n = 5). *** $P < 0.001$, the significant difference compared with the control. Compared with LPS group, #: $p < 0.05$, ###: $p < 0.001$.

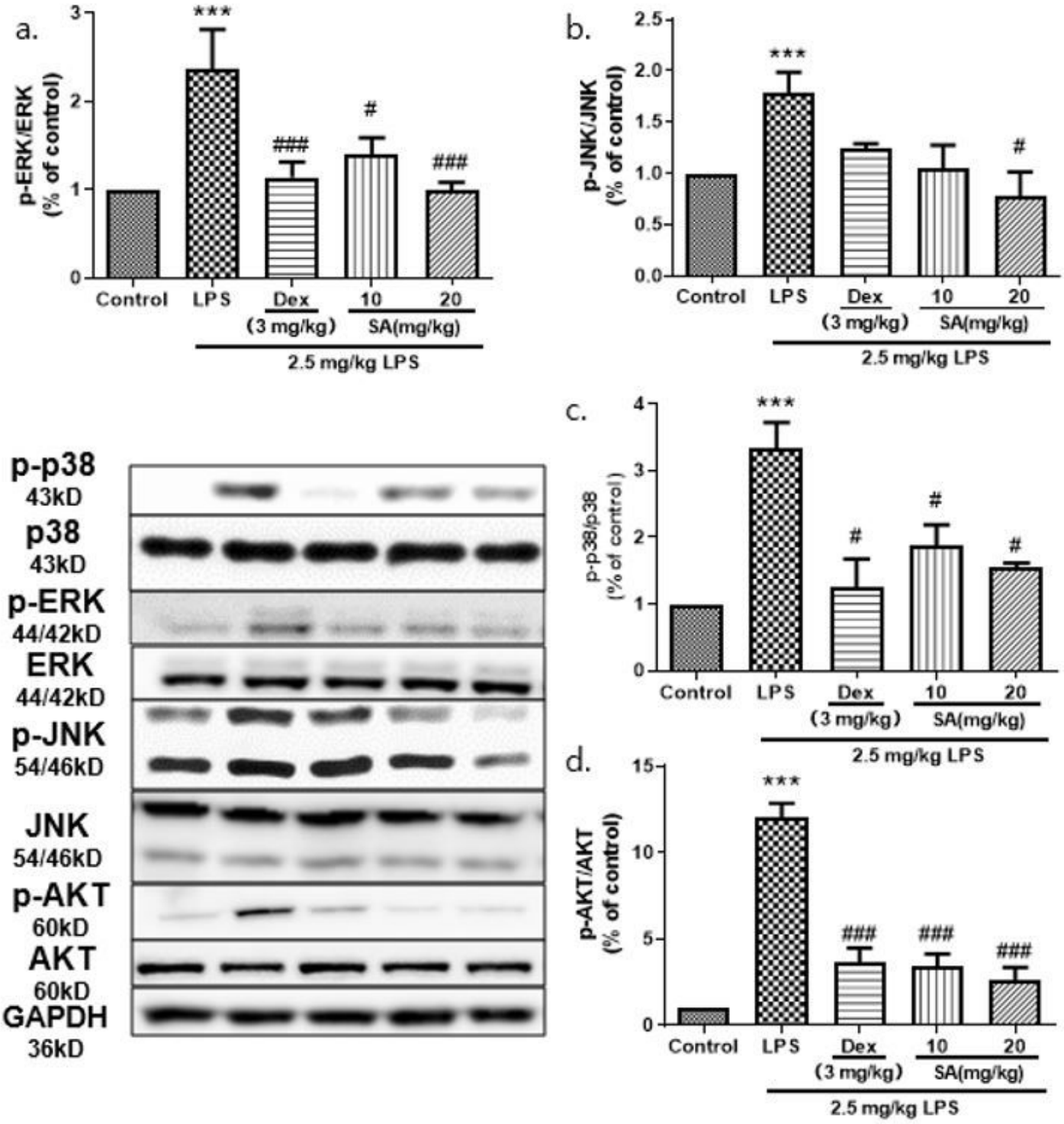

Figure 6

Sinapic acid supressed MAPK & AKT signaling pathway in LPS-induced mouse cerebral cortex. Protein level of p-ERK(A), p-JNK(B), p-p38(C), p-AKT(D) in mouse cerebral cortex were detected. Three independent experiments were performed. Data are detected as mean (±SEM, n = 5). *** $P < 0.001$, the significant difference compared with the control. Compared with LPS group, #: $p < 0.05$, ###: $p < 0.001$.