Ameliorative Effects of Arctigenin From *Arctium Lappa L* Against LPS-induced Neuroinflammation and Cognitive Impairments in Mice

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Abstract

Arctigenin is a phenylpropanoid dibenzylbutyrolactone lignan in *Arctium lappa* L, which has been widely applied as a traditional Chinese herbal medicine for treating inflammation. In the present study, we explored the neuroprotective effect and the potential mechanisms of arctigenin against LPS-evoked neuroinflammation, neurodegeneration, and memory impairments in the mice hippocampus. Daily administration of arctigenin (50 mg/kg per day, i.g.) for 21 days revealed noticeable improvements in spatial learning and memory deficits after exposure to LPS treatment. Arctigenin prevented LPS-induced neuronal/synaptic injury and inhibited the increases in A β generation and the levels of amyloid precursor protein (APP) and β -site amyloid precursor protein cleavage enzyme 1 (BACE1). Moreover, arctigenin treatment also suppressed glial activation and reduced the production of proinflammatory cytokines. In LPS-treated BV-2 microglial cells and mice, activation of the TLR4 mediated NF- κ B signaling pathway was significantly suppressed by arctigenin administration. Mechanistically, arctigenin reduced the LPS-induced interaction of adiponectin receptor 1 (AdipoR1) with TLR4 and its coreceptor CD14 and inhibited the TLR4-mediated downstream inflammatory response. The outcomes of the current study indicate that arctigenin mitigates LPS-induced apoptotic neurodegeneration, amyloidogenesis and neuroinflammation as well as cognitive impairments, and suggest that arctigenin may be a potential therapeutic candidate for neuroinflammation/neurodegeneration-related diseases.

Introduction

Neuroinflammation is associated with several neurodegenerative diseases (Glass et al. 2010), including Alzheimer's disease (AD) (Heneka et al. 2015), Parkinson's disease (PD) (Hirsch et al. 2012), amyotrophic lateral sclerosis (ALS) (Lewis et al. 2012), and Huntington's disease (Möller 2010). Under neuroinflammatory conditions, extensively activated microglia promote neuronal damage by releasing various inflammatory mediators. Meanwhile, extracellular aggregates such as A β enhance glial activation and trigger a vicious circuit of chronic neuroinflammation (Jucker and Walker 2013). Therefore, constant neuroinflammation and progressive neuronal injury accelerate brain damage, which consequently gives rise to behavioral abnormalities (Morales et al. 2014). Lipopolysaccharide (LPS, endotoxin) is a membrane component of gram-negative bacteria, that triggers the immune response. LPS injection induces inflammation by upregulating various inflammatory mediators and proinflammatory cytokines (Erickson and Banks 2011; Parajuli et al. 2012). LPS primarily binds to TLR4 and the coreceptor CD14, which are expressed in microglia, astrocytes, and macrophages in the brain (Marinelli et al. 2015). Once the interaction between LPS and the TLR4/CD14 complex occurs, the TLR4 mediated signaling cascade is launched and activates the NF- κ B signaling pathway, leading to neuroinflammation and neurodegeneration (Gárate et al. 2014). Numerous studies have indicated that systemic administration of LPS can induce neuroinflammation in the mouse brain and provoke cognitive impairment (Qin et al. 2007). Activation of the NF- κ B pathway as a result of LPS administration increases A β generation by upregulating β -secretase 1 (BACE1) transcription activity (Bourne et al. 2007). Several reports have

confirmed that LPS treatment increases A β generation and accumulation, induces neuroinflammation, and impairs synaptic function and learning abilities (Liu et al. 2017b).

It is well-documented that anti-inflammatory compounds ameliorate LPS-induced neuroinflammation and improve cognitive impairment (Lee et al. 2012, 2013; Liu et al. 2017b). Arctigenin, a phenylpropanoid dibenzylbutyrolactone lignan, is responsible for most of the bioactive effects of *Arctium lappa* L and has been applied as a traditional Chinese herbal to treat inflammatory diseases (Park et al. 2007). Arctigenin has also been shown to suppress LPS-induced inflammatory responses and exert anti-inflammatory effects in vivo (Shi et al. 2015; Zhang et al. 2015), and in vitro (Lee et al. 2010; Hyam et al. 2013). It is well-known that arctigenin is able to cross the blood–brain barrier and has been applied for treating encephalitis in traditional medicine (Saxena and Dhole 2008; Zhang et al. 2014). Moreover, arctigenin exerts its neuroprotective effects in stab wound injury models (Song et al. 2016), mPTP-induced PD models (Li et al. 2014), A β -induced cognitive impairment models (Qi et al. 2016), and ischemic stroke models (Zhang et al. 2017). Recently, adiponectin (Jenke et al. 2013) or adiponectin receptor 1 (AdipoR1) agonists (Badshah et al. 2016), which bind to its receptor, were shown to intervene in TLR4/CD14 signaling and prevent LPS-elicited inflammatory responses. Arctigenin has been reported to possess potential AdipoR1 agonist properties (Sun et al. 2013; Ng and Chan 2017). Based on the above findings, we hypothesized that arctigenin would interact with the TLR4/CD14 complex and then suppress downstream inflammatory signal transduction. In the present study, intraperitoneal injection of LPS induced chronic inflammation, exerted deleterious neurobiological effects, and triggered cognitive deficits. This model was employed to evaluate the neuroprotective effects of arctigenin against LPS-induced neuroinflammation and the underlying mechanisms.

Materials And Methods

Animals

C57BL/6 mice weighing 25–30 g were kept in an air-conditioned room set at $23 \pm 1^\circ\text{C}$ with 50% humidity and maintained on a standard 12-12 light/dark cycle with food available *ad libitum*. This study design and all animal procedure were approved by the Animal Experiment Committee of the Henan University of Science and Technology, China.

Group and Treatment

Forty-eight male mice were randomly divided into the following groups ($n = 12$, per group): control group, LPS group, LPS+Arctigenin group, Arctigenin group. Mice were orally administered Arc ($50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$; i.g.) or its solvent for consecutive 21 days. On the 3rd week, 2 h after administration of Arc, LPS ($250 \mu\text{g}/\text{kg}$) was injected intraperitoneally for 7 consecutive days. Arctigenin (Bellancom Chemistry, USA) was of high purity ($> 99\%$) as determined by HPLC analysis, and was dissolved in 0.5% carboxymethylcellulose sodium (CMC) in PBS followed by daily intragastric (i.g.) administration. LPS (*Escherichia coli* serotype 0111:B4, Sigma-Aldrich, St. Louis, MO, USA) was diluted in saline and

intraperitoneally administered 2h after drug administration. To determine the optimum dosage, a preliminary study was performed with three different dosages of arctigenin (25, 50, 100 mg·kg⁻¹·d⁻¹; i.g.). Our results showed that both high dose (50 mg·kg⁻¹·d⁻¹; i.g.) and higher dose (100 mg·kg⁻¹·d⁻¹; i.g.) arctigenin administration markedly attenuated LPS-induced learning and memory, as assessed by the Morris water maze (MWM) in mice. However, no significant difference was observed between the two doses of arctigenin administration (data not shown). Therefore, the dose of arctigenin (50 mg·kg⁻¹·d⁻¹; i.g.) was selected as the optimum dosage for further studies.

Morris Water Maze

The Morris water maze was employed to assess spatial learning and memory ability with minor modifications (Laczó et al. 2009; Wang et al. 2017a). Briefly, the circular apparatus (100 cm diameter) was filled with white nontoxic paint and water (22 ± 1 °C), and an invisible platform (10 cm diameter) was submerged 1 cm beneath the surface of the water. During the place navigation test, each mouse was trained to escape and climb onto the hidden platform (4 trials per day × 5 days). In each trial, each mouse was given a maximum time of 60 s to find and reach the platform. If the mouse failed to find the platform within 60 s, it was guided toward it and kept there for 15 s. The probe trial was carried out on the 6th day. The mice were allowed to swim freely for 60 s (the platform was withdrawn), and the velocity and the path length and the time spent in the target quadrant were recorded. All the behavioural parameters were tracked, recorded, and analyzed by SMART system (Panlab, Barcelona, Spain).

Tissue preparation.

Following behavioral tests, mice received transcardial perfusion with 0.9% saline and 4% paraformaldehyde. Mouse brains were collected and postfixed with 4% paraformaldehyde followed by 30% w/v sucrose in 0.1 M phosphate buffer (pH 7.4). Brain tissues were cut into serial 20 µm-thick coronal sections in coronal planes. Sections were stored at -20°C until immunostaining was performed.

Nissl staining

Following staining in 0.1% cresyl violet solution for 5 min, brain slices were rinsed with deionized water twice and dehydrated with an ascending series of ethanol. After clearing in xylene, slides were mounted on glass coverslips. Images were obtained using a microscope, and healthy neurons containing Nissl substance were quantified from 4 randomly selected visual fields of each section (4-5 representative sections per animal) using Image-Pro Plus 6.0 software.

Immunohistochemistry

Following blocking in 10% goat serum for 1 h, brain slides were incubated with anti-Iba1 and anti-GFAP at 4 °C overnight. After washing three times in PBS, sections were incubated with biotinylated secondary antibodies for 1 h at room temperature. Then, they were colored and visualized by a 3, 3'-diaminobenzidine kit (DAB). Images were captured using a Nikon Eclipse Ti Microscope. The integral

optical density of immunopositive cells was assessed from 4 randomly selected visual fields of each section (4-5 representative sections per animal) and quantified using Image-Pro Plus 6.0 software (Wang et al. 2018).

Immunofluorescence staining

Immunofluorescence staining was carried out on brain and cell slices as previously described (Wang et al. 2017b, 2019). Following blocking in 10% goat serum at room temperature for 1 h, the slides were incubated with the primary antibodies: Synapsin-1 (1:200; Cell Signaling Technology), PSD95 (1:300; Millipore), A β (1:200; Cell Signaling Technology), and NF- κ B p65 (1:200; Cell Signaling Technology) at 4°C overnight. The slides were subsequently incubated with Alexa Fluor 488- or 594-conjugated secondary antibodies at room temperature for 1 h. Immunofluorescence images were obtained using a ZEISS LSM 800 confocal microscope (Carl Zeiss, Germany) and processed using ZEN software.

Cell culture and drug treatment

Immortalized murine microglial (BV-2) cells were maintained in DMEM supplemented with 10% FBS and antibiotics. BV-2 microglial cells were grown to 70–80% confluence and pretreated for 1 h in the absence or presence of arctigenin (12.5, 25, 50, or 100 μ M) followed by stimulation with LPS (1 μ g/ml) for 24 h. After the indicated drug treatment, CCK-8 (Beyotime Institute of Biotechnology, Jiangsu, China) was employed to examine cell viability by changes in the absorbance at 450 nm. Following treatment, the cellular protein was obtained for further analyses.

AdipoR1 siRNA transfection

siRNA targeting AdipoR1 and a nontargeting control siRNA were designed and synthesized (Genepharma, Shanghai, China). Transfection was carried out as described in the manufacturer's instructions using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Following transfection with AdipoR1 siRNA for 48 h, BV-2 cells were pretreated with arctigenin (50 μ M) before additional LPS incubation for 24 h. Finally, the cellular protein was acquired for further analyses.

Assay of proinflammatory cytokines levels

Protein from cellular lysate or brain homogenate was centrifuged at 14,000 $\times$ g for 30 min at 4 °C, and the obtained supernatants were sampled in triplicate to examine proinflammatory cytokine levels including TNF- α , IL-1 β , and IL-6 using commercial ELISA kits (R&D Systems and Invitrogen).

Western blot analysis

Equal amounts of soluble protein from cellular lysate or hippocampal homogenate were electrophoretically separated using 10%-15% SDS-PAGE gel and transferred onto a nitrocellulose membrane. After blocking, membranes were incubated with primary antibodies specific for TNF- α , cleaved caspase-3, BACE1, p-I κ B- α , I κ B- α , p-NF- κ B p65, and NF- κ B p65 (Cell Signaling Technology);

cleaved PARP-1, APP, Iba-1, GFAP, AdipoR1, TLR4, and CD14 (Abcam) at 4°C overnight. The membranes were probed with HRP-conjugated secondary antibodies, and immunoreactive images were exhibited using a chemiluminescent detection system and collected on Bio-Rad Chemidoc Imaging System, and the intensities of the protein bands were determined using Image-Pro Plus 6.0 software.

Immunoprecipitation (IP)

Aliquots of the cellular lysates were incubated with anti-TLR4 antibody (Santa Cruz Biotechnology) at 4 °C overnight on a rotating device. Protein A/G Plus-Agarose beads (Santa Cruz Biotechnology) were added to the cell lysate for 4 h at 4 °C on rotation, and the beads were washed three times with ice-cold buffer. The washed beads were resuspended in SDS sample buffer and denatured for 5 min at 100 °C. Following centrifugation, the supernatants were subjected to the western blot analysis.

Statistical analysis

Values were presented as the mean $\pm$ SEM. All statistical procedures were performed using SPSS version 23.0. For the Morris water maze task, escape latency during the place navigation test was analyzed using two-way repeated measures ANOVA. The data that were not included in repeated measures were analyzed with one-way ANOVA followed by the Bonferroni post-hoc test for multiple comparisons. Differences were considered significant at a p value < 0.05 .

Results

Arctigenin rescued learning-associated deficits in LPS-treated mice

To evaluate the effects of arctigenin on spatial learning and memory abilities, a Morris water maze test was conducted. During the place navigation test, two-way ANOVA indicated a marked group difference in escape latency ($F(3, 44) = 9.56, p < 0.01$). No significant interaction was observed between group and training day ($F(12, 156) = 1.06, p > 0.05$). The LPS-treated mice exhibited increased escape latencies relative to the levels observed in the control mice, whereas arctigenin administration resulted in a noticeable increase in escape latencies relative to that observed in the LPS group. These results suggested that the impaired spatial learning abilities induced by LPS were rescued by arctigenin administration (Fig. 1a). In the subsequent probe test, the time spent in the target quadrant was significantly decreased in LPS mice compared to the controls; however, arctigenin administration increased the preference for the target quadrant (Fig. 1b and 1c). No statistically significant difference was found in the velocity (Fig. 1d) or the total path length (Fig. 1e) between the four groups. In addition, we employed a novel object recognition test to assess short-term recognition memory. The results showed that the impaired recognition memory deficit, manifested by a notable decrease in the discrimination index, in LPS-treated mice, was attenuated after arctigenin administration (Fig. 2). Collectively, the data revealed that arctigenin administration can alleviate memory deficits in a mouse model of LPS challenge.

Arctigenin reversed LPS-mediated neuronal apoptosis and neurodegeneration in the hippocampus of mice

Nissl staining was employed to evaluate structural atrophy and neuron loss. Our results demonstrated that the numbers of neurons in the hippocampal CA1, CA3, and DG regions were significantly lower following LPS injection than in the control group. However, arctigenin treatment markedly prevented the loss of Nissl bodies and mitigated neuronal injury elicited by LPS in the hippocampus (Fig. 3a). Furthermore, arctigenin also markedly downregulated LPS-mediated increase in cleaved caspase-3 and cleaved PARP-1 expression (Fig. 3b).

Arctigenin alleviated the LPS-induced reduction in synaptic density in the hippocampal CA1 region of mice

To examine the effects of arctigenin against LPS-induced synaptic degeneration, immunofluorescence staining with the presynaptic marker synapsin-1 and the postsynaptic marker PSD95 was conducted. Synaptic density measured by the number of synapsin-1 and PSD95 dual-positive puncta was markedly reduced in the hippocampal CA1 subfield of LPS-treated mice compared to the control. However, LPS plus arctigenin treated mice displayed a distinct elevation in synaptic density in the hippocampal CA1 area (Fig. 4a). Additionally, arctigenin elevated the decreased expression of synapsin-1 and PSD95 mediated by LPS (Fig. 4b).

Arctigenin treatment prevented amyloidogenesis in the hippocampus of LPS-treated mice

Amyloidogenesis is known to contribute to the pathogenesis of AD. Emerging evidence indicates that LPS administration in mice promotes A β generation and accumulation, induces synaptic dysfunction, and results in neuronal injury and cognitive impairment (Wang et al. 2019). To investigate whether arctigenin suppressed A β production and accumulation in the brains of LPS-treated mice, immunofluorescence staining of A β was performed. As illustrated in Fig. 5a, the LPS-injected mice exhibited significantly increased A β accumulation in the hippocampus, while pretreatment with arctigenin significantly reduced A β accumulation in the hippocampus. In addition, arctigenin substantially downregulated the LPS-evoked increase in the levels of APP and BACE1, thereby lowering A β generation and accumulation (Fig. 5b).

Arctigenin inhibited glial activation in the hippocampus of LPS-treated mice

To investigate the inhibitory effects of arctigenin on the activation of astrocytes and microglia, we performed immunohistochemistry (IHC) with antibodies against the marker Iba-1 (marker for microglia) and the marker GFAP (marker for astrocyte). Compared with the controls, the LPS-treated mice exhibited significant elevations of Iba-1 and GFAP immunoreactivities in the hippocampal CA1, CA3, and DG subfields, whereas arctigenin significantly attenuated LPS-evoked glial overactivation in these regions (Fig. 6a and 6b). Consistent with the IHC staining results, the upregulation of Iba-1 and GFAP was also inhibited by arctigenin (Fig. 6c).

Arctigenin mitigated elevated protein expression of the TLR4/NF- κ B signaling pathway in the hippocampus of LPS-treated mice

TLR4 is activated by LPS and triggers a signaling cascade, which results in early phase activation of NF- κ B p65 and results in the production of proinflammatory cytokines. Western blot results showed that the expression of TLR4, CD14, p-I κ B/I κ B, and p-NF- κ B p65/NF- κ B p65 were markedly increased in the hippocampus of LPS-treated mice compared to control mice. However, activation of the TLR4/NF- κ B pathway was attenuated by arctigenin administration (Fig. 7a). Moreover, arctigenin substantially reduced the LPS-evoked elevated levels of proinflammatory cytokines including TNF- α , IL-1 β , and IL-6 in mice (Fig. 7b).

Anti-inflammatory mechanism of arctigenin against LPS-evoked neuroinflammation in BV-2 microglial cells.

Our results demonstrated that BV-2 cells treated with 12.5, 25, or 50 μ M arctigenin did not affect the viability of BV-2 cells (Fig. 8a). However, 100 μ M arctigenin slightly lowered cell viability. Furthermore, the results indicated that 12.5, 25, or 50 μ M arctigenin did not affect the viability of LPS-stimulated microglial cells (data not shown). To examine the effects of arctigenin on the neuroinflammatory process in LPS-treated BV-2 cells, ELISA was employed to assess the levels of proinflammatory cytokines. As shown in Fig. 8b, the levels of TNF- α , IL-6, and IL-1 β were dramatically upregulated after LPS stimulation, whereas arctigenin significantly decreased proinflammatory cytokines in a concentration-dependent manner. Based on the above results, a dose of 50 μ M arctigenin was selected for subsequent experiments. TLR4 is triggered by LPS, resulting in the activation of its downstream effector NF- κ B p65, which subsequently induces the production of proinflammatory cytokines. To investigate the anti-inflammatory mechanisms of arctigenin, the protein expression levels of p-I κ B- α and p-NF- κ B p65 were also determined by western blotting. Arctigenin effectively prevented the LPS-evoked increase in p-I κ B- α and p-NF- κ B p65 (Fig. 8c), thereby inhibiting the translocation of NF- κ B p65 to the nucleus (Fig. 8d and 8e) in BV-2 microglial cells.

AdipoR1 has been demonstrated to interact with and stabilize the TLR4/CD14 complex, which facilitates TLR4 mediated signal transduction and inflammatory responses. Consistent with these effects, our results showed an interaction of AdipoR1 with TLR4 under basic culture conditions. Furthermore, LPS stimulation enhanced the interaction. Arctigenin significantly suppressed the increased interaction of AdipoR1 with TLR4 and decreased the stability of the TLR4/CD14 complex (Fig. 8f). Adiponectin or AdipoR1 agonists binding to its receptor caused dissociation of the ligand-receptor complex from TLR4/CD14, resulting in the inhibition of downstream signal transduction. Arctigenin has been reported to possess potential AdipoR1 agonist properties. AdipoR1 knockdown by siRNA was employed to elucidate the role of AdipoR1 in arctigenin against LPS-induced neuroinflammation. As illustrated in Fig. 8g, AdipoR1 siRNA or pretreatment with arctigenin lowered NF- κ B phosphorylation and TNF- α expression after LPS stimulation *in vitro*.

Discussion

The findings of this research demonstrated that arctigenin administration alleviated hippocampus-related learning and memory performance following systemic LPS challenge. The protective effects of arctigenin on memory deficits in mice may be ascribed to inhibition of neuroinflammation and its associated amyloidogenesis and hippocampal mitigation of neuronal/synaptic injury. Furthermore, this study suggested that arctigenin would suppresses the TLR4 signaling pathway by interfering with the stability of the TLR4-CD14 receptor complex and preventing neuroinflammation-elicited neurodegeneration.

Activation of the TLR4 receptor has been considered as an essential event during the irritation of inflammatory responses. LPS specifically recognizes TLR4 and binds to TLR4-CD14 complexes on glial cells in the CNS, and triggers immune responses (Gorina et al. 2011; Zanoni et al. 2011, p. 14; Park and Lee 2013). This receptor complex induces nuclear factor NF- κ B p65 activation and releases inflammatory mediators, such as iNOS, COX-2, and TNF- α (Kawai and Akira 2010; Parajuli et al. 2012). NF- κ B p65, as transcription factors and has been a central link in the process of inflammatory responses (Gasparini and Feldmann 2012). It has been documented that, even at the primary level, LPS is capable of binding TLR4-CD14 complexes and inducing NF- κ B p65 signal activation and subsequent cellular events (Chow et al. 1999; Lakhani and Bogue 2003). Consistently, our study also observed that exposure to LPS stimulation induced TLR4/NF- κ B p65 pathway activation and triggered inflammatory responses *in vivo* and *in vitro*, evidenced by the apparent augmentation in protein expression in the TLR4/ NF- κ B p65 pathway and the levels of proinflammatory cytokines. Notably, recent studies have demonstrated that AdipoR1 stabilizes the TLR4-CD14 complex and facilitates LPS-induced inflammation (Jenke et al. 2013). Following ligand binding, AdipoR1 dissociates from the TLR4/CD14 complex, thereby suppressing downstream signal transduction after exposure to LPS stimulation (Badshah et al. 2016). Adiponectin prevents the interaction of its receptor AdipoR1 with the TLR4/CD14 complex and destabilizes the complex, thereby inhibiting downstream inflammatory processes. The potential property of arctigenin as an AdipoR1 agonist led us to hypothesize that arctigenin would suppress LPS-evoked inflammation in a similar way as adiponectin. In line with this hypothesis, arctigenin could reduce the interaction of AdipoR1 with TLR4 and the stability of the TLR4/CD14 complex, resulting in substantial inhibition of the TLR4 downstream inflammatory response. Arctigenin, as an AdipoR1 agonist, suppressed LPS-induced activation of TLR4 by interacting with AdipoR1 and prevented downstream neuroinflammatory signals.

Numerous studies have indicated that chronic neuroinflammation is a crucial driver during the neuropathogenesis of AD (Millington et al. 2014; Calsolaro and Edison 2016). Glial activation and augmented neuroinflammatory responses were also observed in AD patients (Wyss-Coray 2006). A β aggregates, an essential neuropathological hallmark in AD brains, are surrounded by inflammatory mediators and acute-phase reactant proteins (Akiyama 2000). Several studies confirmed that LPS-elicited glial activation and synaptic abnormalities were closely correlated with amyloidogenesis in rodents (Sheng 2003; Deng et al. 2014; Liu et al. 2017a). Consistent with previous reports, our findings demonstrated that LPS stimulation induced TLR4/NF- κ B p65 mediated neuroinflammation that upregulated the expression of BACE-1 and APP and subsequently increased A β levels. However, arctigenin, significantly downregulated BACE1 and APP expression and reduced the production and

accumulation of A β , which might, in part, benefit from the inhibitory effects of arctigenin on TLR4/NF- κ B signaling activation.

Systemic administration of LPS has been shown to trigger the mitochondrial apoptotic pathway and promote apoptotic neurodegeneration (Hattori et al. 2010; Santhanasabapathy and Sudhandiran 2015). Activation of TLR4 signaling induces the JNK signaling cascade following LPS administration, resulting in the activation of the mitochondrial apoptotic pathway and neurodegeneration (Tournier et al. 2000; Putcha et al. 2003). In line with these observations, we also found that systemic LPS administration markedly enhanced the expression levels of critical apoptotic markers, including the cleaved forms of caspase-3 and PARP-1, while arctigenin attenuated LPS-induced apoptosis by reducing the expression levels of cleaved caspase-3 and PARP-1. Moreover, histopathological analysis revealed that LPS-treated mice exhibited substantial neuronal injury in the hippocampus, as manifested by the marked loss of Nissl substance and healthy neuronal cells (Choi et al. 2009; Chen et al. 2018). Arctigenin administration reversed LPS-evoked neuronal apoptosis and neurodegeneration in mice.

In conclusion, our results demonstrated the protective effects of arctigenin against systemic LPS-induced cognitive deficits by multiple pathological processes, including neuroinflammation, amyloidogenesis, and neuronal/synaptic injury. Our findings indicate that arctigenin may be a potential treatment for neuroinflammation and its associated cognitive decline.

Abbreviations

AD, Alzheimer's disease; AdipoR1, Adiponectin receptor 1; ALS, Amyotrophic lateral sclerosis; APP, Amyloid precursor protein; BACE1, β -site amyloid precursor protein cleavage enzymes 1; COX-2, Cyclooxygenase-2; CMC Carboxymethylcellulose sodium; iNOS, Inducible nitric oxidesynthase; LPS, Lipopolysaccharide; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; TNF- α , Tumor necrosis factor- α .

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Quan Yuan, Yiran Wu , Gang Wang, Xiang Zhou, Xiaohui Dong, Zihan Lou and Sanqiang Li. The first draft of the manuscript was written by Dongmei Wang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Animal Experiment Committee of the Henan University of Science and Technology, China.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

The authors affirm that human research participants provided informed consent for publication of the images.

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Figures

Figure 1

Arctigenin improved spatial learning and memory impairment in LPS-treated mice. (a) Escape latency during the place navigation tests, (b) representative movement tracks during the training phase (5th day) and the probe test (6th day), (c) the duration in the target quadrant, (d) the velocity, and (e) the path length in the probe trial were measured. Values were expressed as mean $\pm$ SEM ($n = 12$ per group, ** $p < 0.01$ vs. Control mice; # $p < 0.05$, ## $p < 0.01$ vs. LPS mice)

Figure 2

Arctigenin attenuated recognition memory deficits in LPS-treated mice. (a) Schematic representation of the NOR task protocol. (b) Recognition index in the NOR task. Values were expressed as mean $\pm$ SEM ($n = 12$ per group, ** $p < 0.01$ vs. Control mice; # $p < 0.05$, ## $p < 0.01$ vs. LPS mice).

Figure 3

Arctigenin reversed LPS-mediated neuronal apoptosis and neurodegeneration in the hippocampus of mice. (a) Nissl staining and normal neuronal counts in the hippocampal CA1, CA3, and DG subfields (scale bars = 100 μ m). (b) Western blot analysis of caspase-3 and PARP-1 in the hippocampus. Values were expressed as mean $\pm$ SEM (n = 3-4 per group, * p <0.05, ** p <0.01 vs. Control mice; # p <0.05, ## p <0.01 vs. LPS mice)

Figure 4

Arctigenin alleviated the LPS-induced reduction in synaptic density in the hippocampal CA1 region of mice. (a) Representative immunostaining images of synapsin-1 (green) and PSD95 (red) in the hippocampal CA1 region (scale bars = 50 μ m), and quantitative analysis of the numbers of synaptic puncta. Insert indicates higher magnification of synapsin puncta. (b) Western blot analysis of synapsin-1 and PSD95 in the hippocampus. Values were expressed as mean $\pm$ SEM (n = 3-4 per group, * p <0.05, ** p <0.01 vs. Control mice; # p <0.05, ## p <0.01 vs. LPS mice)

Figure 5

Arctigenin treatment prevented LPS-induced amyloid genesis in the hippocampus of LPS-treated mice. (a) Immunofluorescence staining of A β in the CA1 area of the hippocampus (scale bars = 50 μ m). (b) Western blot analysis of APP and BACE1 in the hippocampus. Values were expressed as mean $\pm$ SEM (n = 3 per group, ** p <0.01 vs. Control mice; ## p <0.01 vs. LPS mice)

Figure 6

Arctigenin inhibited glial activation in the hippocampus of LPS-treated mice. (a) Immunohistochemical images of microglia and astrocytes (scale bars = 50 μ m). (b) Quantitative analysis of Iba-1- and GFAP-positive cells in the hippocampal CA1, CA3 and DG subfields. Inserts indicate higher magnification of Iba1- positive or GFAP-positive cells from the CA1 and CA3 subfields. (c) Western blot analysis of Iba-1 and GFAP in the hippocampus. Values were expressed as mean $\pm$ SEM (n = 3 per group, ** p <0.01 vs. Control mice; ## p <0.01 vs. LPS mice)

Figure 7

Arctigenin mitigated elevated protein expression of the TLR4/ NF- κ B p65 signaling pathway in the hippocampus of LPS-treated mice. (a) The expression of the inflammatory pathway of TLR4, CD14, p-I κ B, and p-NF- κ B p65 was detected by western blotting in the mouse brain. (b) Levels of TNF- α , IL-1 β , and IL-6 in mouse brain were detected by ELISA. Values were expressed as mean $\pm$ SEM (n = 3-4 per group, ** p <0.01 vs. Control mice; ## p <0.01 vs. LPS mice)

Figure 8

Anti-inflammatory mechanism of arctigenin against LPS-evoked neuroinflammation in BV-2 microglial cells. (a) BV-2 microglial cell viability was determined by CCK-8 assay after treatment with different concentrations of arctigenin for 24 h. (b) The proinflammatory levels were examined by ELISA in 1 μ g/ml LPS-treated BV-2 microglial cells for 24 h in the absence or presence of different concentrations of arctigenin pretreatment. ** p <0.01 vs. Control group; # p <0.05, ## p <0.01 vs. LPS group. (c) The expression levels of p-I κ B- α and p-NF- κ B p65 were examined by western blotting. (d) Immunofluorescence staining of NF- κ B p65 (scale bars = 20 μ m). (e) the levels of nuclear NF- κ B p65 and cytosolic NF- κ B p65 were detected by western blotting in BV-2 microglial cells treated with LPS (1 μ g/ml) in the absence or presence of arctigenin (50 μ M). ** p <0.01 vs. Control group; # p <0.05, ## p <0.01 vs. LPS group. (f) The interaction of AdipoR1 with TLR4 and its coreceptor CD14 was analyzed by immunoprecipitation-western blotting in BV-2 microglial cells treated with LPS (1 μ g/ml) in the absence or presence of arctigenin. ** p <0.01 vs. Control group; # p <0.05, ## p <0.01 vs. LPS group. After incubation with AdipoR1 siRNA for 48 h, BV-2 microglial cells were administered 50 μ M arctigenin for 2 h followed by LPS (1 μ g/ml) for an additional 24 h. (g) The expressions of p-NF- κ B p65 and TNF- α were examined by western blotting. ** p < 0.01 vs. Control group, ## p <0.01 vs. LPS group, && p < 0.01 vs. LPS + arctigenin group. Values were expressed as mean $\pm$ SEM (n = 5)