Original Article
Astragalus polysaccharide inhibits lipopolysaccharide-induced depressive-like behaviors and inflammatory response through regulating NF-κB and MAPK signaling pathways in rats

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Abstract: Astragalus polysaccharide (APS) is a natural compound extracted from astragalus membranaceus which has an antidepressant activity. Initially APS was studied for its immunomodulatory potential, and later was found to exhibit multiple pharmacological effects, including anti-inflammatory activity. More recently APS was shown to attenuate the lipopolysaccharide (LPS) induced neuroinflammation and improve the learning and memory ability of rats. The major objectives of this study were to investigate whether APS would exhibit antidepressant effects in an animal model of depression induced by LPS, and whether this effect might be associated with regulating nuclear factor-κappaB (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways. Three groups of Wistar rats were injected LPS (i.p.), two groups of which were pretreated with APS (200 mg or 400 mg, i.p.). Behaviors were evaluated by forced swim test, saccharin preference test and open field test. Levels of NF-κB p65, phospho-NF-κB p65, phospho-IκBα, ERK1/2, JNK, p38 MAPK, phospho-ERK1/2, phospho-JNK and phospho-p38 MAPK in hippocampus and hypothalamus were measured to assess the activities of NF-κB and MAPK signaling pathways. In addition, levels of TNF-α, IL-1β and IL-6 (both protein and mRNA levels) in hippocampus and hypothalamus were determined. Results showed LPS induced depressive behaviors, as well as activated the NF-κB and MAPK signaling pathways in rats. APS treatment dose-dependently alleviated depressive-like symptoms and inhibited the activation of NF-κB and MAPK signaling pathways induced by LPS. The data indicate an antidepressant-like activity of APS in a LPS-induced animal model of depression possibly via inhibition of NF-κB and MAPK signaling pathways.

Keywords: Astragalus polysaccharide, depression, inflammation, lipopolysaccharide, NF-κB, MAPK

Introduction

Major depressive disorder (MDD) is a recurrent and incapacitating mood disorder being related to high mortality and morbidity, affecting about 15% of the population worldwide [1, 2]. It is generally accepted that the pathogenesis of MDD is complex and has not been completely elucidated yet, which is far more beyond the typical “monoamine hypothesis”. Based on the complexity of the pathogenesis, novel therapeutic strategies targeting at specific pathogenesis are required.

It has been proved that inflammatory mediators can interact with pathophysiological pathways relevant to mood regulation, such as neurotransmitter metabolism, neuroendocrine function, synaptic plasticity and regional brain activity. To date, more and more studies revealed the association between the immune system activation and MDD [3-5]. Depressive patients and animal models of depression have been found to exhibit increased levels of inflammatory biomarkers in the periphery and the brain, for example TNF-α, IL-1 and IL-6 [6-8]. Furthermore, data from laboratory animals
showed that administration of IFN-α or TNF-α could induce depressive behaviors in rodents [9-11]. These data confirmed the role of inflammatory response in the pathophysiology of depression.

Regarding the signaling pathways through which the stimuli induce inflammatory response and depressive behaviors, there is a growing body of research that has begun to clarify the roles of NF-κB and MAPK signaling pathways [12, 13]. Extracellular signal-regulated kinases (ERK) 1/2 and p38 MAPK have been found to influence both the expression and the activity of the membrane reuptake pumps for both dopamine and serotonin which are neurotransmitters playing crucial roles in depression. MAPK signaling pathway has also been well demonstrated to mediate the LPS-induced neuroinflammation [14, 15] which is associated with depression. Besides, studies showed IFN-α and TNF-α induction was accompanied by p38 MAPK activation [16]. NF-κB signaling pathway is another one which promotes inflammation. Many studies have proved the activation of the NF-κB signaling pathway in depressive patients and animal models of depression [17, 18]. The activation of the NF-κB signaling pathway can lead to the overproduction of inflammatory cytokines, such as TNF-α, IL-1 and IL-6. The NF-κB activation also influences neurotransmitters, neurogenesis and the glucocorticoid receptor signaling which are involved in depression. Therefore, NF-κB is regarded as a critical mediator of stress-induced and LPS-induced depressive behaviors [18, 19]. Based on the crucial role of NF-κB and MAPK in depression, suppressing the activation of these inflammation associated signaling pathway has been tested as new therapeutic targets for depression [19-21].

Many natural compounds possessing anti-inflammatory activities have been tested to treat depression. For example, resveratrol, salidroside and EGb761 respectively attenuated the depressive-like behaviors induced LPS in rodents [22-24]; Apigenin exhibited antidepressant effect in the chronic mild stress-induced rat model of depression [25]. Herbal drug *Astragalus membranaceus* has a long history of medical use for various diseases. Astragalus injection, a crude extract from *Astragalus membranaceus*, has been reported to attenuate the depressive-like behaviors induced by chronic stress in rats [26]. APS, a natural compound, is one of the active pharmacological constituents of *Astragalus membranaceus*. APS has been found to have multiple pharmacological actions, including anti-inflammatory activity, and has been used to treat inflammation associated diseases such as asthma, diabetic nephropathy and liver injury in preclinical and clinical trials. Our laboratory also has tested its renoprotective, anti-inflammatory and neuroprotective effects in our previous studies [27-29]. A recent study found APS attenuated LPS-induced neuroinflammation by regulating NF-κB signaling [30]. It is also has been proved that APS could regulate the p38 MAPK signaling pathway [31, 32]. In addition, a study reported APS improved the learning and memory ability of the rats [33]. Based on the antidepressant activity of *Astragalus membranaceus* as well as the anti-inflammatory and neuroprotective effects of APS, we suppose that APS might have an antidepressant effects in inflammation induced depression. In this study, we induced depressive behaviors in rats by LPS injection and treated the rats with APS to assess its antidepressant effect. Moreover, we also investigated the effects of APS on NF-κB and MAPK signaling pathways to explore the possible mechanisms by which APS exerted its antidepressant effect in the LPS-induced depression model.

**Material and methods**

**Animals**

Wistar rats (200-220 g upon arrival) were obtained from the Laboratory Animal Center of Shandong University (Jinan, China). Animals were subjected to a 1-week acclimatization period upon the arrival and all efforts were made to minimize the number of animals used. Throughout the study, the rats were provided food and water *ad libitum* with the exception of behavioral tests. The animal room was maintained at 23-26°C on a 12-h light/dark cycle. Behavioral testing and drug injections occurred during the light phase of the cycle. All studies conformed to the requirements of the National Institutes of Health Guide for the Care and Use of Laboratory Animals, as approved by the Committee of Animal Care and Use in our university.

**APS pretreatment and LPS administration**

After the acclimation period, rats were randomly assigned to control group, LPS group, APS-
Astragalus polysaccharide attenuates depressive-like behaviors

200 group and APS-400 group (n=10 each). LPS (dissolved in saline at a concentration of 200 µg/ml) (Sigma Chemical Company, St Louis, MO, USA.) was intraperitoneally injected to rats in LPS group, APS-200 group and APS-400 group at a dose of 200 µg/kg, which could induce depressive-like behaviors in rats according to previous reports [34, 35]. Prior to the LPS injection, rats were pretreated with two doses of APS (APS-200 group: 200 mg/day; APS-400 group: 400 mg/day) or saline (control group and LPS group) for 5 days, and the treatments were further given for another 2 days post LPS administration.

**Forced swim test**

The forced swim test consisted of two exposures to a tank (46 cm tall × 20 cm in diameter) filled with water (24-26°C; 35 cm depth) in which rats could swim. The first exposure was a training phase, during which rats were placed in the water for 15 min. The test phase lasting for 5 min was carried out 24 h after the training phase. The second test phase (conducted 24 h post LPS administration) was videotaped and the immobility time in the test was scored by experienced observers that were blind to the experimental conditions. Immobility was defined as: the rat remained floating in the water without struggling and only made movement necessary to keep its head above the water.

**Exploratory activity in the open-field test**

Exploratory activity in the open-field test was conducted after the forced swim test. The apparatus was a wooden box (100 cm × 100 cm × 40 cm) which had 25 same squares outlined by white lines on the floor of the arena. The rats individually underwent the test. During the test, the rat was placed into the center of the arena and left free to explore the arena for 5 min and the exploratory activity of the rat was video-recorded. Rearrings and crossings of the rats were scored by experienced observers that were blind to the experimental conditions. The area was wiped clean with a 75% alcohol solution after each trial.

**Saccharin preference test**

Rats were trained for optimal saccharin preference (0.5% solution) before testing. After 20 hr’s deprivation of water, each rat was given two bottles, one containing tap water and the other with a 0.5% saccharin solution for 12 h in their individual home cages. The amount of liquid consumed from each bottle was measured and the saccharin preference was calculated as a percentage of total liquid (saccharin solution intake to saccharin solution plus water consumed).

**NF-κB and MAPK signaling activity**

After the behavioral tests, left hippocampus and hypothalamus were immediately removed from the brain and homogenized. The supernatant of the homogenates was collected after centrifugation at 3000 rpm for 10 min. NF-κB p65, phospho-NF-κB p65, phospho-IκBα, ERK1/2, JNK, p38 MAPK, phospho-ERK1/2, phospho-JNK and phospho-p38 MAPK in hippocampal and hypothalamic tissue homogenate were determined using enzyme-linked immunosorbent assay (ELISA) kits, and the procedures were performed in accordance with the kit instructions (Bangyi Biotechnology Co. Ltd, Shanghai, China; Huamei Biotechnology Co. Ltd, Wuhan, China; R & D Systems, Inc., Minneapolis, MN, USA; Cell Signaling Technology, Beverly, Massachusetts, USA).

**TNF-α, IL-1β and IL-6**

Concentrations of inflammatory cytokines TNF-α, IL-1β and IL-6 in hippocampal and hypothalamic homogenates were assessed using radio-immunoassay kits strictly following the manufacturer’s instructions (Beijing North Institute of Biological Technology Company, China).

**mRNA expression of TNF-α, IL-1β and IL-6**

Total tissue RNA was extracted from the hippocampus and hypothalamus samples with TRizol reagent (TaKaRa Bio, Dalian, China), according to the manufacturer’s recommended procedures. The cDNA was synthesized from 2 µg total RNA. The cDNA was amplified through quantitative real-time reverse transcription PCR analysis to determine the expression of IL TNF-α, IL-1β and IL-6. The sequences of primers were: β-actin, (forward) 5’-GCCAGAGTGATGAGTGGAG-3’ and (reverse) 5’-ACGCAGCCTCAAGTAA CAGTCC-3’; IL-1β, (forward) 5’-GCTAGGGAGGAGCTTGGTCGAG-3’, and (reverse) 5’-AGGCAGGAAGAACCATGGTTCGAG-3’; IL-6, (forward) 5’-GCTAGAGGCAG GGCCCCTTGTCGAG-3’, and (reverse) 5’-AGG CAGGAGGAAAAACACAGCTTGGTTCGAG-3’; TNF-α, (forward) 5’-GCCACCAGCCTTCTTCTGTC-3’, and
Astragalus polysaccharide attenuates depressive-like behaviors

(reverse) 5'-GCTACGGGCTTGTCACTCG-3'. The amplification reactions were carried out as the following: 1 min at 95°C, 35 cycles at 95°C for 10 seconds, 60°C for 30 seconds, and 72°C for 20 seconds. The mRNA expression levels of the target genes were normalized to those of β-actin.

Statistical analyses
All values were expressed as the mean ± SD. All analyses were conducted using the SPSS 13.0. The statistical significance of differences among groups was assessed with one-way ANOVA followed by the Tukey-Kramer multiple comparison. P<0.05 was considered to be statistically significant.

Results
APS had no significant effects on the locomotor activity of the rats

(Figure 1) In the open field test, rats in the LPS group showed a significant decrease in numbers of rearings and crossings when compared to the control group (respectively P<0.05), while APS (both 200 mg and 400 mg) treated rats displayed similar levels of rearings and crossings to the LPS group (respectively P>0.05).

APS reduced immobility time in forced swim test

(Figure 2) During the forced swim test, rats in the LPS group showed a significant increase in immobility time when compared to the control group (P<0.05). APS (both 200 mg and 400 mg) treated rats displayed much shorter immobility time than the LPS group (respectively P<0.05), and the APS-400 group displayed even shorter immobility time than the APS-200 group (P<0.05).

APS increased the saccharin preference

(Figure 2) In the saccharin preference test, rats in the LPS group showed a significant decrease in saccharin preference when compared to the control group (P<0.05). APS (both 200 mg and 400 mg) treated rats displayed much higher saccharin preference than the LPS group (respectively P<0.05), and the APS-400 group displayed higher saccharin preference than the APS-200 group (P<0.05).

APS inhibited the NF-κB signaling activity

(Tables 1 and 2) Rats in the LPS group had much higher levels of NF-κB p65, phospho-NF-κB p65 and phospho-IκBα in hippocampal and hypothalamic tissue homogenate than the control group (respectively P<0.05), indicating LPS injection activated the NF-κB signaling. APS (both 200 mg and 400 mg) treated rats displayed much lower levels of NF-κB p65, phospho-NF-κB p65 and phospho-IκBα than the LPS group (respectively P<0.05), and the APS-400 group had even lower levels of these parameters than the APS-200 group (respectively P<0.05).

APS inhibited the MAPK signaling activity

(Tables 3 and 4) LPS injection significantly increased levels of ERK1/2, JNK, p38 MAPK, phospho-ERK1/2, phospho-JNK and phospho-p38 MAPK in hippocampal and hypothalamic tissue homogenate in the LPS group if compared to the control group (respectively P<0.05). APS significantly reversed the alterations of these parameters induced by LPS injection in the APS-200 group and the APS-400 group if compared to the LPS group (respectively P<0.05). Moreover, the APS-400 group displayed lower levels of these parameters than the APS-200 group (respectively P<0.05).

APS decreased levels of inflammatory cytokines TNF-α, IL-1β and IL-6

(Tables 5 and 6) Rats in the LPS group showed much higher levels of inflammatory cytokines
Astragalus polysaccharide attenuates depressive-like behaviors

Astragalus membranaceus has a long history of medical use in traditional Chinese medicine. It has been widely used as diuretic, antiperspirant, antihypertensive, anti-inflammatory, hypoglycemic, tonic and anti-depressant treatments. Saponins, polysaccharides and flavonoids are the active pharmacological constituents of Astragalus membranaceus. Among the constituents, APS has been most widely studied. Initially APS was studied for its immunomodulatory potential, and later was found to exhibit multiple pharmacological effects, including anti-inflammatory activity. It has been tested to treat autoimmune diseases, diabetes, cardiovascular diseases, tumor, cell damage, and so on, and has exhibited potent therapeutical effect [27-32]. Recently, anti-inflammatory and neuroprotective effects were reported [30, 33]. However, whether it has an antidepressant activity has not been investigated. Considering the anti-inflammatory effects of APS, in the current study, we designed to investigate the effects of APS on a LPS induced animal model of depression, an inflammation associated depression model.

Figure 2. Effects of APS on the depressive behaviors of the rats. The depressive behaviors are evaluated by saccharin preference test and forced swim test. A: The saccharin preference was calculated as a percentage of total liquid (saccharin solution intake/saccharin solution plus water consumed). B: Immobility was defined as: the rat remained floating in the water without struggling and only made movement necessary to keep its head above the water. The immobility time in the FST was measured. The results are expressed as means ± SD. *P<0.05, versus control; ▼P<0.05 versus LPS, ▲P<0.05 versus APS-200.

Table 1. Effects of APS on the hippocampal NF-κB signaling activity of the rats

<table>
<thead>
<tr>
<th></th>
<th>NF-κB p65 (pg/ml)</th>
<th>phospho-NF-κB p65 (pg/ml)</th>
<th>phospho-IκBα (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>48.09±4.75</td>
<td>12.36±1.48</td>
<td>13.14±0.95</td>
</tr>
<tr>
<td>LPS</td>
<td>103.14±4.03a</td>
<td>55.26±7.01a</td>
<td>38.72±4.08a</td>
</tr>
<tr>
<td>APS-200</td>
<td>84.52±6.38b</td>
<td>40.33±5.17b</td>
<td>28.30±2.54b</td>
</tr>
<tr>
<td>APS-400</td>
<td>65.11±5.96bc</td>
<td>26.01±3.83bc</td>
<td>16.44±1.50bc</td>
</tr>
</tbody>
</table>

The NF-κB signaling activity is evaluated by measuring the levels of NF-κB p65, phospho-NF-κB p65, phospho-IκBα in hippocampal tissue homogenate. The results are expressed as means ± SD. *P<0.05 versus control; ▼P<0.05 versus LPS, ▲P<0.05 versus APS-200.

Table 2. Effects of APS on the hypothalamic NF-κB signaling activity of the rats

<table>
<thead>
<tr>
<th></th>
<th>NF-κB p65 (pg/ml)</th>
<th>phospho-NF-κB p65 (pg/ml)</th>
<th>phospho-IκBα (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>42.50±4.08</td>
<td>11.20±1.14</td>
<td>12.05±1.40</td>
</tr>
<tr>
<td>LPS</td>
<td>78.25±6.21a</td>
<td>36.05±5.73a</td>
<td>29.14±4.01a</td>
</tr>
<tr>
<td>APS-200</td>
<td>64.70±4.53b</td>
<td>26.00±3.55b</td>
<td>22.86±2.17b</td>
</tr>
<tr>
<td>APS-400</td>
<td>53.82±4.51bc</td>
<td>19.22±2.10bc</td>
<td>17.19±2.05bc</td>
</tr>
</tbody>
</table>

The NF-κB signaling activity is evaluated by measuring the levels of NF-κB p65, phospho-NF-κB p65, phospho-IκBα in hypothalamic tissue homogenate. The results are expressed as means ± SD. *P<0.05 versus control; ▼P<0.05 versus LPS, ▲P<0.05 versus APS-200.
Astragalus polysaccharide attenuates depressive-like behaviors

Table 3. Effects of APS on the hippocampal MAPK signaling activity of the rats

<table>
<thead>
<tr>
<th>Group</th>
<th>ERK1/2 (pg/ml) ± SD</th>
<th>JNK (pg/ml) ± SD</th>
<th>p38 (pg/ml) ± SD</th>
<th>phospho-ERK1/2 (pg/ml) ± SD</th>
<th>phospho-JNK (pg/ml) ± SD</th>
<th>phospho-p38 (pg/ml) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25.12±4.35</td>
<td>33.92±3.40</td>
<td>43.07±3.67</td>
<td>7.08±1.25</td>
<td>10.15±1.46</td>
<td>12.27±2.12</td>
</tr>
<tr>
<td>LPS</td>
<td>57.19±4.11</td>
<td>69.27±5.38</td>
<td>91.25±8.28</td>
<td>28.37±4.88</td>
<td>38.07±4.11</td>
<td>46.82±6.40</td>
</tr>
<tr>
<td>APS-200</td>
<td>45.55±3.02</td>
<td>55.08±4.72</td>
<td>75.22±5.07</td>
<td>19.13±3.08</td>
<td>24.75±3.40</td>
<td>28.18±3.44</td>
</tr>
<tr>
<td>APS-400</td>
<td>36.06±3.79</td>
<td>46.99±4.51</td>
<td>61.70±5.33</td>
<td>13.01±2.10</td>
<td>16.51±2.53</td>
<td>19.50±2.29</td>
</tr>
</tbody>
</table>

The NF-κB signaling activity is evaluated by measuring the levels of ERK1/2, JNK, p38 MAPK, phospho-ERK1/2, phospho-JNK and phospho-p38 MAPK in hippocampal tissue homogenate. The results are expressed as means ± SD. *P<0.05 versus control; †P<0.05 versus LPS, ‡P<0.05 versus APS-200.

Table 4. Effects of APS on the hypothalamic MAPK signaling activity of the rats

<table>
<thead>
<tr>
<th>Group</th>
<th>ERK1/2 (pg/ml) ± SD</th>
<th>JNK (pg/ml) ± SD</th>
<th>p38 (pg/ml) ± SD</th>
<th>phospho-ERK1/2 (pg/ml) ± SD</th>
<th>phospho-JNK (pg/ml) ± SD</th>
<th>phospho-p38 (pg/ml) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36.09±5.63</td>
<td>40.95±5.85</td>
<td>55.09±7.38</td>
<td>8.89±1.33</td>
<td>11.70±1.88</td>
<td>14.47±2.11</td>
</tr>
<tr>
<td>LPS</td>
<td>76.01±6.40</td>
<td>91.66±7.23</td>
<td>124.53±10.92</td>
<td>40.36±6.25</td>
<td>43.01±5.53</td>
<td>60.05±8.51</td>
</tr>
<tr>
<td>APS-200</td>
<td>60.77±4.08</td>
<td>73.54±6.20</td>
<td>97.80±9.08</td>
<td>26.17±3.54</td>
<td>31.29±4.20</td>
<td>41.35±5.49</td>
</tr>
<tr>
<td>APS-400</td>
<td>48.38±5.21</td>
<td>58.14±4.97</td>
<td>80.72±7.11</td>
<td>18.70±2.21</td>
<td>22.80±3.66</td>
<td>25.70±4.11</td>
</tr>
</tbody>
</table>

The NF-κB signaling activity is evaluated by measuring the levels of ERK1/2, JNK, p38 MAPK, phospho-ERK1/2, phospho-JNK and phospho-p38 MAPK in hypothalamic tissue homogenate. The results are expressed as means ± SD. *P<0.05 versus control; †P<0.05 versus LPS, ‡P<0.05 versus APS-200.

Table 5. Effects of APS on the protein and mRNA expressions of inflammatory cytokines in hippocampus

<table>
<thead>
<tr>
<th>Group</th>
<th>TNF-α (pg/ml) ± SD</th>
<th>IL-1β (pg/ml) ± SD</th>
<th>IL-6 (pg/ml) ± SD</th>
<th>TNF-α mRNA</th>
<th>IL-1β mRNA</th>
<th>IL-6 mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>55.07±4.17</td>
<td>63.16±5.90</td>
<td>45.30±6.55</td>
<td>1.00±0.12</td>
<td>1.00±0.15</td>
<td>1.00±0.13</td>
</tr>
<tr>
<td>LPS</td>
<td>162.59±13.22</td>
<td>180.15±20.05</td>
<td>119.48±14.36</td>
<td>2.49±0.30</td>
<td>2.82±0.39</td>
<td>2.27±0.19</td>
</tr>
<tr>
<td>APS-200</td>
<td>115.28±12.99</td>
<td>136.62±15.44</td>
<td>88.12±12.80</td>
<td>1.99±0.14</td>
<td>2.04±0.18</td>
<td>1.86±0.15</td>
</tr>
<tr>
<td>APS-400</td>
<td>82.11±10.25</td>
<td>97.27±14.31</td>
<td>65.83±7.60</td>
<td>1.55±0.11</td>
<td>1.63±0.17</td>
<td>1.50±0.11</td>
</tr>
</tbody>
</table>

Levels of TNF-α, IL-1β and IL-6 in the hippocampal tissue homogenate were measured; the mRNA levels of the target genes were normalized to those of β-actin. The results are expressed as means ± SD. *P<0.05 versus control; †P<0.05 versus LPS, ‡P<0.05 versus APS-200.

Table 6. Effects of APS on the protein and mRNA expressions of inflammatory cytokines in hypothalamus

<table>
<thead>
<tr>
<th>Group</th>
<th>TNF-α (pg/ml) ± SD</th>
<th>IL-1β (pg/ml) ± SD</th>
<th>IL-6 (pg/ml) ± SD</th>
<th>TNF-α mRNA</th>
<th>IL-1β mRNA</th>
<th>IL-6 mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>46.87±8.55</td>
<td>56.00±6.21</td>
<td>43.92±5.87</td>
<td>1.00±0.09</td>
<td>1.00±0.12</td>
<td>1.00±0.14</td>
</tr>
<tr>
<td>LPS</td>
<td>123.05±14.36</td>
<td>138.65±16.27</td>
<td>101.08±14.69</td>
<td>2.10±0.20</td>
<td>2.76±0.29</td>
<td>2.05±0.16</td>
</tr>
<tr>
<td>APS-200</td>
<td>91.89±10.22</td>
<td>101.13±13.41</td>
<td>77.44±7.01</td>
<td>1.70±0.11</td>
<td>2.11±0.15</td>
<td>1.77±0.13</td>
</tr>
<tr>
<td>APS-400</td>
<td>65.24±8.67</td>
<td>76.77±8.20</td>
<td>59.10±5.89</td>
<td>1.46±0.08</td>
<td>1.72±0.12</td>
<td>1.41±0.11</td>
</tr>
</tbody>
</table>

Levels of TNF-α, IL-1β and IL-6 in the hypothalamic tissue homogenate were measured; the mRNA levels of the target genes were normalized to those of β-actin. The results are expressed as means ± SD. *P<0.05 versus control; †P<0.05 versus LPS, ‡P<0.05 versus APS-200.

We administrated LPS (i.p.) to the Wistar rat at dose of 200 µg/kg body weight, which is a dose that has been reported to successfully depressive-like behaviors in rats [34, 35]. Similar to the previous reports [22, 24, 36], we found LPS significantly increased the immobility time of the rats in the forced swim test which is a behavioral test widely used to evaluate the depressive state of rodents. Immobility time represents the severity of hopelessness which is a typical symptom of depression. Interestingly, rats with APS treatment (200 mg and 400 mg)
displayed much shorter immobility time than the LPS group, and the effect was dose-dependent. In addition, anhedonia, another common symptom of depression, was assessed by saccharin preference test. In line with the forced swim test, APS also dose-dependently improved the anhedonia induced by LPS. These findings indicated the dose-dependent antidepressant activity of APS in the LPS induced depression model. However, according to previous reports, LPS may elicit sickness behavior in animals which is a usual response to infection characterized by endocrine, autonomic, and behavioral changes triggered by the activation of the peripheral innate immune system [38]. As is known, sickness behavior may influence the performance of rodent animals in other behavioral tests, resulting in false results. In order to exclude the influence of sickness on other behavioral tests, sickness behavior was accessed in open field test. No differences in locomotor activity were observed between the LPS treated animals and LPS+APS treated animals. The result indicated that the attenuation of the behavioral performance in forced swim test was specific to the antidepressant effects of APS without influence from locomotor activity. All the behavioral tests confirmed the antidepressant activity of APS in LPS induced rat model of depression.

The evidence from preclinical and clinical studies supports that depression is usually accompanied by the activation of the inflammatory-response system [3-8]. And overproduction of inflammatory cytokines plays a role in the pathophysiology of depressive disorders. The cytokines has been found to influence neurogenesis and the HPA axis activity, which are both associated with depression. In preclinical studies, administration of IFN-α or TNF-α has been tested to establish animal model of depression [9-11, 16, 38]. And suppressing the over-production of these cytokines has exhibited a potent antidepressant effect [23, 39]. LPS can activate TLR-4 and induce systemic inflammation and neuroinflammation. After LPS administration, overproduction of IL-1, IFN-α and TNF-α in the peripheral tissue and brain has been found in rodents. In the current study, we analyzed the expression of IL-1β, IL-6 and TNF-α in hippocampus and hypothalamus which play a crucial role in mode regulation. Consistently with previous reports [18, 19, 40], we also found the rats in LPS group had much higher levels of inflammatory cytokines TNF-α, IL-1β and IL-6 (both protein and mRNA levels) than the control animals. Yet, APS effectively inhibited the over-expression of these cytokines (both protein and mRNA levels). Thus, the effects of APS on these cytokines may explain the positive results of APS treatment in the behavioral tests. Besides our findings, some other natural products, such as resveratrol, salidroside, EGB761, apigenin, curcumin, also exhibited anti-inflammatory and antidepressant effects in animal model of depression induced by LPS, stress and other means [22-25, 41].

Inhibition of the NF-κB and MAPK pathways has been proposed to be a major mechanism underlying the attenuation of LPS-induced inflammatory cytokine production. Activation of NF-κB signaling is involved in LPS induced inflammation response. LPS combines with TLR-4 and triggers a cascade of signaling reactions, ultimately leading to the degradation of transcription inhibition factor-κB (IκB) by phosphorylation. The phosphorylation of IκB activates NF-κB, and the activated NF-κB p65 sub-unit (phosphorylated NF-κB p65) is translocated to the nucleus where it promotes the transcription of target genes, such as TNF-α, IL-1β and IL-6 genes. Consistently, we observed much high levels of NF-κB p65, phospho-NF-κB p65 and phospho-IκBα in hippocampus and hypothalamus in the LPS treated rats in this study. And this was in line with the increases in levels of TNF-α, IL-1β and IL-6 (both protein and mRNA levels) LPS treated rats. In agreement with our results, other studies also demonstrated the activation of NF-κB signaling in the LPS induced animal model of depression [21, 42]. However, in this study APS (200 mg and 400 mg) inhibited the NF-κB signaling activation in a dose dependent manner, which was in line with the attenuation of inflammatory cytokine production by APS mentioned above. Similarly to our findings, there is other evidence that inhibition of NF-κB signaling contributes to the improvement of depressive-like behaviors [22, 42]. MAPK signaling, which includes JNK, ERK1/2 and p38 MAPK, is another pathway that plays an important role in inflammatory responses and is involved in pathophysiology of depression. Studies have proved that MAPK signaling is activated at least in inflammation associated depression and the managements suppressing the over-activation of the signaling manifested.
Astragalus polysaccharide attenuates depressive-like behaviors

antidepressant effects [16, 38]. In line with the studies that reported peripheral administration of LPS triggered inflammation and activated MAPK signaling [43, 44], we found much higher levels of ERK1/2, JNK, p38 MAPK, phospho-ERK1/2, phospho-JNK and phospho-p38 MAPK in hippocampus and hypothalamus of the LPS treated rats. But rats with APS treatment displayed much lower levels of these MAPKs. All the findings suggested that APS inhibited the activation of NF-κB and MAPK signaling pathways which could mediate the inflammatory cascade elicited by LPS in rats.

Conclusions

In summary, we observed for the first time that APS exhibited antidepressant effects in an animal model of depression induced by a single administration of LPS, and the antidepressant action of APS was paralleled by significant inhibition of TNF-α, IL-1β and IL-6 expression, as well as the activity of NF-κB and MAPK signaling pathways in hippocampus and hypothalamus of the Wistar rats. The findings suggest a therapeutic potential of APS in at least inflammation associated depression. However, the potential effects of APS on the other aspects of neuroinflammation, the neurogenesis, neurotransmitter and the HPA axis activity which are associated with depression and on other types of depression models needs further study in the future work.

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Disclosure of conflict of interest

None.

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