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Hemp (*Cannabis sativa* L.) Seed Phenylpropionamides Composition and Effects on Memory Dysfunction and Biomarkers of Neuroinflammation Induced by Lipopolysaccharide in Mice

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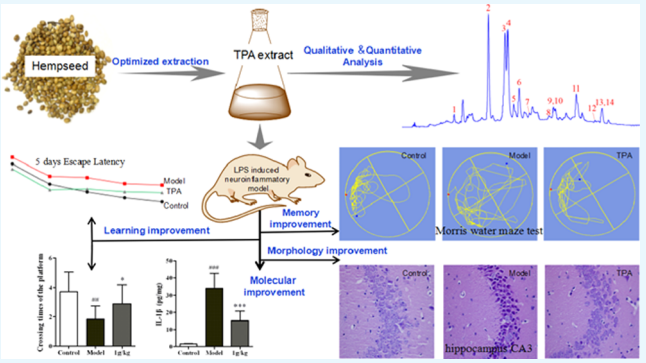
Supporting Information

ABSTRACT: Hempseed has achieved a growing popularity in human nutrition, particularly regarding essential amino acids and fatty acids. The multiple positive attributes of hempseed have led to the further study of its constituents. In this study, hempseed extract containing phenylpropionamides (TPA) was obtained and its chemical profile and content were obtained using high-performance liquid chromatography technology based on previous study. The anti-neuroinflammatory effect of TPA extract was evaluated using a lipopolysaccharide (LPS)-induced mouse model. Fourteen phenylpropionamides (TPA) were identified in the obtained extract with a total content of 233.52 ± 2.50 μg/mg extract. In mice, TPA prevented the learning and spatial memory damage induced by LPS. Increased brain levels of IL-1β, IL-6, and TNF-α in the LPS-induced mice were reduced by TPA treatment. Furthermore, TPA attenuated LPS-induced hippocampal neuronal damage in mice. This study demonstrates the nutraceutical potential of hempseed from a neuroprotective perspective.



1. INTRODUCTION

Cannabis sativa L. (Cannabaceae family) has been an important source of food, fiber, and traditional medicine since historical times.[1] There are two varieties that could be distinguished by the content of Δ^9-tetrahydrocannabinol (THC), *C. sativa* subsp. *indica* (marijuana or hashish) and *C. sativa* subsp. *sativa* (industrial hemp). The hemp has a low content of THC, less than 0.2% on a dry basis as legal limit for the cultivation.[2,3]

Hempseed has been used in different way in human nutrition, including hempseed oil,[1,4] milled hempseed as a source of vegetable protein, dietary fiber,[5] and incorporation into food preparations (snack bars, bread, cookies, yogurt, etc).[6] It is an excellent source of nutrients, especially due to its unsaturated fatty acids and essential amino acids, which are rich and in correct ratio for human dietary demands.[4,7] Moreover, hempseed exert many positive effects, including alleviating constipation,[8] providing cardiovascular health benefits,[9] immunomodulation, and ameliorating dermatological diseases[4] and gastrointestinal diseases.[9] Hempseed extract showed antimutagenic effects,[10] antioxidant and anti-ageing effects,[11,12] and could improve chemical drugs induced learning and memory impairment in mice.[13,14] It worthy noting that the ethyl acetate extract showed prominent effect among extracts prepared by different solvents (petroleum ether, ethyl acetate, *n*-butanol, and aqueous extracts).[13] Thus, hempseed should be further investigated not only regarding amino acids or fatty acids but also other bioactive constituents.[10,15]

Besides amino acids and fatty acids, hempseed is rich in lignanamides[10,16] such as cannabisin A, B, C, etc., caffeoyltyramine-like compounds, and other polyphenols.[10,17] Because the common part of these constituents is the phenylpropionamide moiety, to simplify, we refer to such constituents as phenylpropionamides in the text. In our previous study on hempseed, more than 30 phenylpropionamides were isolated.[16,17] We further established a lipopolysaccharide (LPS)-stimulated BV2 microglia cells model to explore the anti-neuroinflammatory activity of these compounds. The results showed that most lignanamides had good anti-neuroinflammatory activity by inhibiting the NF-κB signaling pathway.[15] Neuroinflammation is a critical pathological hallmark in the development of Alzheimer's disease and other neurodegenerative diseases, and high expression of inflammatory mediators in the brain is associated with Aβ deposition and neurofibrillary tangles.[18] Lipopolysaccharide (LPS), a critical component in the outer membrane of Gram-negative bacteria, has been used as a stimulator of microglial cells and in animal models to trigger systemic and central inflammatory responses.[19] Recent studies have shown that the expression

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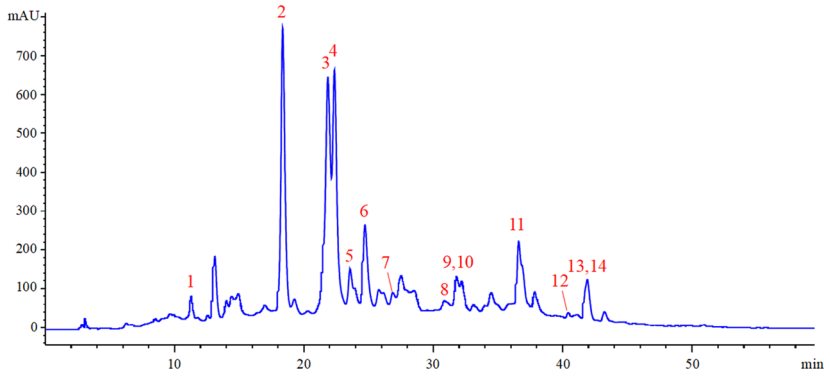

Figure 1. HPLC chromatogram of hempseed extract.

Table 1. Compounds Identified in TPA by HPLC and UV Data and Compared with Standard Compounds

peak no.	identity	RT (min)	UV λ_{max} (nm)	molecular weight	molecular formula	references
1	*N-trans*-caffeoyloctopamine	11.3	220, 290, 320	315	$C_{17}H_{17}NO_5$	17
2	*N-trans*-caffeoyltyramine	18.4	220, 290, 320	299	$C_{17}H_{17}NO_4$	27
3	cannabisin A	21.8	255	594	$C_{34}H_{30}N_2O_8$	27
4	cannabisin B	22.3	220, 245, 280, 340	596	$C_{34}H_{32}N_2O_8$	25
5	*N-trans*-coumaroyltyramine	23.6	220, 310	283	$C_{17}H_{17}NO_3$	17
6	*N-trans*-feryroyltyramine	24.7	220, 290, 320	313	$C_{18}H_{19}NO_4$	17
7	cannabisin C	26.9	225, 245, 285, 335	610	$C_{35}H_{34}N_2O_8$	27
8	cannabisin D	30.9	222, 245, 285, 335	624	$C_{36}H_{36}N_2O_8$	25
9	cannabisin E	31.8	221, 281, 315	642	$C_{36}H_{38}N_2O_9$	16
10	3,3′-demethyl-grossamide	32.2	225, 285, 300, 320	596	$C_{34}H_{32}N_2O_8$	16
11	cannabisin M	36.6	220, 285, 315	596	$C_{34}H_{32}N_2O_8$	16
12	isocannabisin N[a]	40.4	225, 281, 311	610	$C_{35}H_{34}N_2O_8$	16
13	cannabisin F	41.8	221, 285, 320	624	$C_{36}H_{36}N_2O_8$	26
14	grossamide	41.9	221, 285, 300, 320	624	$C_{36}H_{36}N_2O_8$	16

[a]Compound **12** is (2,3-*trans*)-3-(3-hydroxy-5-methoxyphenyl)-*N*-(4-hydroxyphenethyl)-7-{(*E*)-3-[(4-hydroxy phenethyl)amino]-3-oxoprop-1-enyl}-2,3-dihydro-benzo[*b*][1,4]dioxine-2-carboxamide; to simplify, this is referred to as isocannabisin N.

Table 2. Peak Area and Content of TPA

compounds	peak area	μg/mL	peak area	μg/mL	peak area	μg/mL
1	190.1	4.02	221.8	4.57	209.3	4.35
2	2190.1	39.03	2277.2	40.55	2335.4	41.57
3	3024.1	117.13	3108.5	120.34	3128.2	121.08
4	2472.4	96.20	2307.7	89.94	2099.0	82.02
5	939.0	17.13	971.4	17.69	876.6	16.03
6	1372.9	24.72	1377.3	24.80	1317.1	23.74
7	419.3	18.28	408.1	17.85	365.2	16.22
8	376.5	16.65	356.7	15.90	454.3	19.60
9, 10	870.6	15.93	891.3	16.29	854.2	15.64
11	917.8	16.75	882.7	16.14	932.3	17.01
12	56.6	1.68	55.5	1.66	58.9	1.72
13, 14	584.1	11.60	585.3	11.63	632.3	12.45
TPA (μg/mL)		379.12		377.36		371.44
TPA (μg/mg)		235.48		234.38		230.71

of Aβ, progressive neurodegeneration, loss of learning and memory, and the release of neuroinflammatory mediators have been detected in LPS-induced animals.[20,21]

The aim of this study was to investigate the anti-neuroinflammatory effect of hempseed extract containing mainly phenylpropionamides. The phenylpropionamide fraction was enriched from hempseed and the chemical profile of the extract was obtained using high-performance liquid chromatography (HPLC) technology based on our previous phytochemical study of hempseed. The total phenylpropanamides (TPA) content and representative constituents were measured. The anti-neuroinflammatory effects of the TPA extract were evaluated using an LPS-induced mouse model. This study demonstrates the nutraceutical potential of hempseed from a new perspective.

2. RESULTS

2.1. Phytochemical Analysis of the TPA Extract of Hempseed. To obtain the TPA extract of hempseed, the dried hempseed was defatted with petroleum ether and

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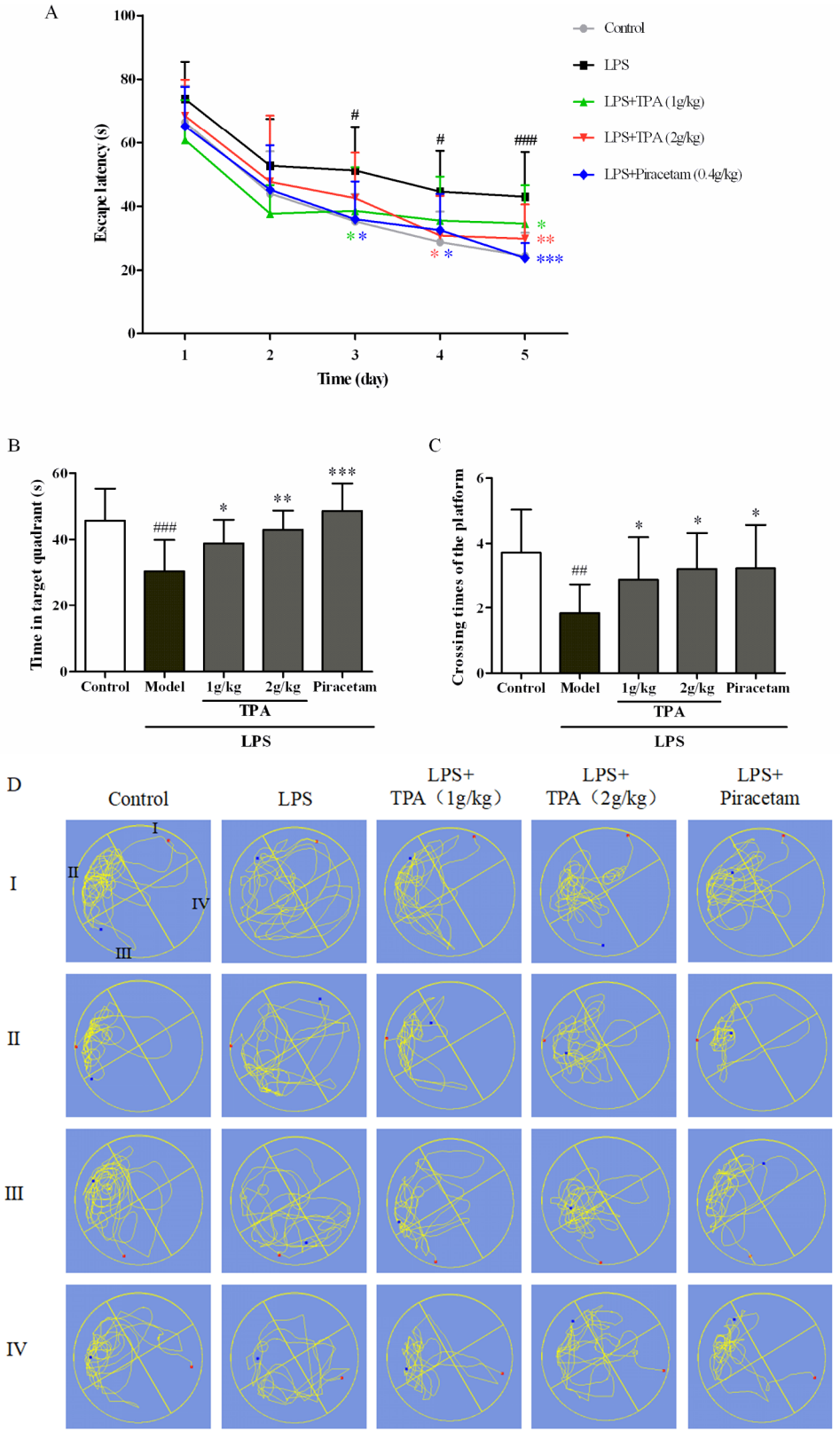

Figure 2. Effects of TPA on behavioral parameters of LPS-induced mice in the Morris water maze test (n = 12). (A) Escape latency during five consecutive days test. (B) Time spent in the quadrant of the platform. (C) Crossing times of the former platform location. (D) Motion trails of the mice. (Data are expressed as the mean ± standard deviation (SD), $^{###}p < 0.001$, $^{##}p < 0.01$, $^{#}p < 0.05$ vs control; $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ vs LPS model.)

extracted with 70% EtOH. The crude extract was subjected to an D101 macroporous adsorption resin column using H_2O, 75%, and 95% EtOH successively as eluents. Finally, the 75% EtOH fraction was collected and concentrated. HPLC technology was used to study the chemical composition of the TPA extract. Figure 1 shows the HPLC chromatogram of

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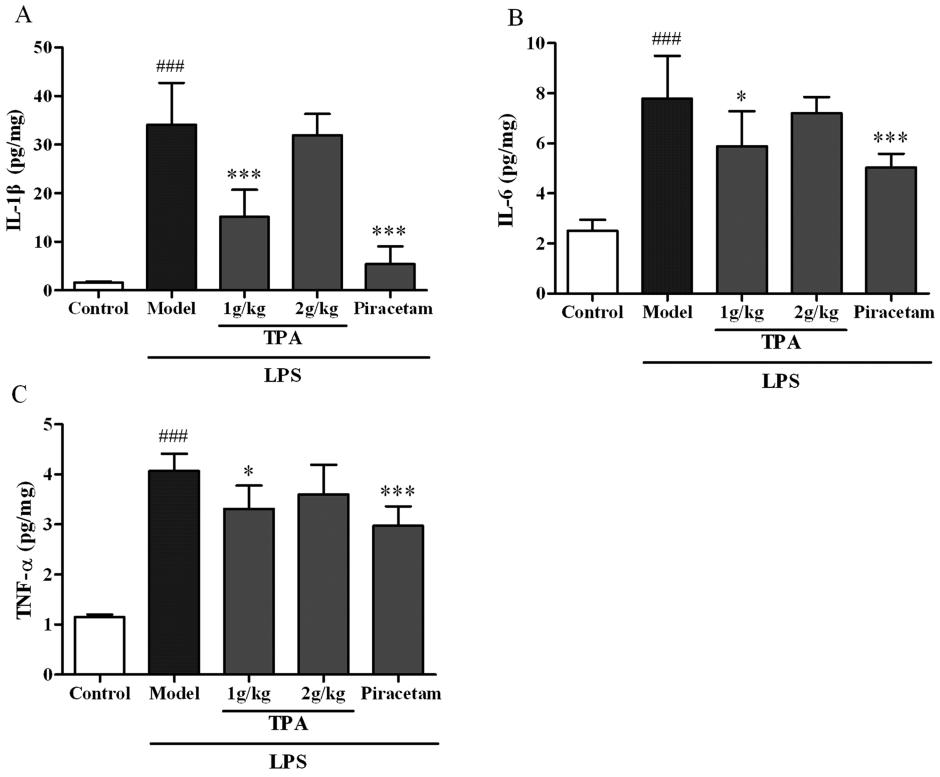

Figure 3. Effects of TPA on brain levels of pro-inflammatory cytokines in LPS-induced mice ($n = 6$): (A) IL-1β level, (B) IL-6 level, and (C) TNF-α level. (Data are expressed as the mean ± SD, $^{###}p < 0.001$ vs control; $***p < 0.001$, $*p < 0.05$ vs LPS model.)

the TPA extract. By combining the data from HPLC, including the retention times, the UV data on the peaks, and co-elution with previously isolated authentic samples (Figures S1−S3, Table S1 in the Supporting Information), 14 phenylpropionamides were identified (Table 1, see their structures in Figure S4).

As shown in Figure 1, most compounds in the hempseed extract were identified. Therefore, the content of TPA in the extract was quantified by summing up the contents of the identified 14 compounds. Because the UV spectra of compounds **3**, **4**, **7**, and **8** were similar to that of cannabisin A (CA) and those of the others were similar to that of *N-trans*-caffeoyltyramine (NC), we quantified the two kinds of compounds using CA and NC as reference compounds, respectively. The standard curves of CA and NC are given in Figure S5. The content of the 14 compounds was calculated based on their measured peak area. As shown in Table 2, after summing up, the content of TPA was 233.52 ± 2.50 μg/mg extract.

2.2. TPA Extract Attenuates the Cognitive Deficit Induced by LPS. Morris water maze (MWM) test was used to test the spatial learning and memory ability of mice. As shown in Figure 2A, during the five training days, the escape latency of all the groups declined progressively. This suggests that with continuous learning, the learning ability of each group increased. However, compared with the other groups, the LPS model mice spent a longer period finding the platform. Especially, on the third to fifth days, the escape latency of the model mice was significantly longer than that of the control mice ($p < 0.05$, $p < 0.05$, and $p < 0.001$, respectively). These results show that the LPS-treated mice had significant cognitive impairment. Moreover, piracetam (0.4 g/kg)-treated and TPA (1 and 2 g/kg)-treated mice showed a significant shortened escape latency compared with the model mice on the fifth day ($p < 0.001$, $p < 0.05$, and $p < 0.01$, respectively). In the probe trial, the platform was removed. As shown in Figure 2B−D, the model mice spent less time ($p < 0.001$) in the target quadrant and crossed to the platform fewer times ($p < 0.01$) compared with the control group. Compared with the model group, piracetam-treated and TPA (1 and 2 g/kg)-treated mice showed significant increase in both the time spent in the quadrant of the platform and the number of crossing counts (both $p < 0.01$). These results demonstrate that TPA treatment significantly improved the memory loss induced by LPS treatment in mice.

2.3. TPA Extract Reduces Brain Levels of Inflammatory Cytokines in LPS-Induced Mice. Figure 3 shows the effects of TPA extract on the brain levels of inflammatory cytokines (IL-1β, IL-6, and TNF-α) induced by LPS in mice. Enzyme-linked immunosorbent assay (ELISA) analysis indicated that exposure to LPS significantly increased the expression of IL-1β, IL-6, and TNF-α in the brain compared with the control group (all $p < 0.001$). However, pretreatment with TPA (1 g/kg) and piracetam significantly inhibited the LPS-induced increase in IL-1β (both $p < 0.001$, Figure 3A), IL-6 ($p < 0.05$ and $p < 0.001$, respectively, Figure 3B), and TNF-α ($p < 0.05$ and $p < 0.001$, respectively, Figure 3C) levels in the brain in comparison to model mice, whereas TPA (2 g/kg) had no significant effect compared with the model group. The results show that a low dose of TPA (1 g/kg) and piracetam inhibited the overexpression of inflammatory cytokines in the brain in an LPS-induced neuroinflammation model, but a high dose of TPA may induce toxicity and counteract its role in inhibiting inflammatory cytokines production.

2.4. Protective Capacity of TPA Extract on Hippocampal Neurons. The hippocampus plays an essential role in

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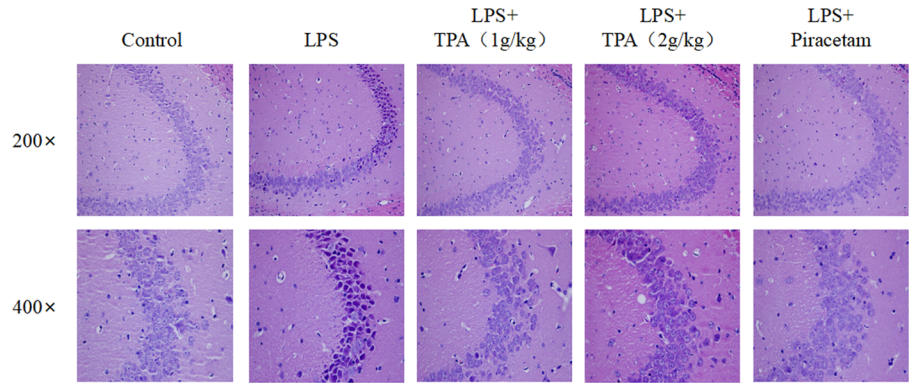

Figure 4. Effects of TPA on LPS-induced neuronal damage in hippocampal CA3 region indicated by HE staining.

spatial learning and memory function. As shown in Figure 4, hematoxylin−eosin (HE) staining indicated that the intraperitoneal injection of LPS (250 μg/kg/day) for 7 days caused neuronal damage. In the LPS model group, this was observed as dark-stained and deformed neurons, clearly identified in the hippocampal CA3 region. However, there were no evident neuronal morphological alterations in the TPA groups and the piracetam group, indicating that TPA and piracetam can alleviate LPS-induced neuronal damage.

3. DISCUSSION

The chemical constituents of hempseed are complex and diverse, including fatty acids and their esters,[4] phenylpropionamides,[16,17] cannabinoids,[22] steroids and terpenoids,[4] flavonoids and their glycosides,[23] alkaloids,[24] and proteins. Except fatty acids and protein, phenylpropionamides are the most studied and well-reported. Compounds of this class mainly include *N-trans*-caffeoyltyramine, *N-trans*-feryroyltyramine, and their various structural dimers called lignanamides.[16,17,25−27] According to reports in the literature, more than 20 kinds of phenylpropionamides have been isolated from hempseed.[11,26] Our previous study on hempseed further increased the varieties of phenylpropionamides.[16,17] Some of them showed good antioxidant and anti-neuroinflammatory activities.[16,17] Their bioactivities and benefits must be considered when evaluating hempseed as a functional food. Presently, studies in this field are not enough. Cai et al.[28] determined the anti-aging effect of hempseed oil, protein, and liganamide on old mice and found that all of them showed obvious effect. Lin et al.[13] investigated the effect of hempseed extracts prepared by different solvents (petroleum ether, ethyl acetate, *n*-butanol and aqueous) on mice with experimental Alzheimer's disease and found that hempseed extracts conspicuously alleviated learning and memory ability, as well as improved brain tissue pathological changes in experimental dementia mice, whereas the ethyl acetate extract demonstrates a more prominent improvement than other extracts. According to our experience,[16] the ethyl acetate extract contains mainly liganamide and other phenylpropionamides. This study obtained a hempseed extract containing mainly phenylpropionamides and described its chemical profile based on our phytochemical study on hempseed.[16,17] Fourteen phenylpropionamides were identified from the extract and the total content was obtained. Moreover, the neuroprotective effect of the TPA extract was evaluated using an LPS-induced neuroinflammatory model in mice.

The results showed that TPA treatment significantly improved the learning and memory of LPS-induced neuroinflammatory mice, and significantly improved their cognitive function. Low-dose TPA significantly reduced the expression of IL-1β, IL-6, and TNF-α in the brains of LPS-induced mice. From the observation of the morphology of hippocampal neurons in mice, it was found that TPA prevented damage to nerve cells in the hippocampal CA3 region induced by LPS, indicating that TPA has neuroprotective effects. However, as we can see in the experiment, the dose-dependent relation of TPA regarding the expression of IL-1β, IL-6, and TNF-α in the brains of LPS-induced mice was not as expected, suggesting that the safe and effective dose for TPA is less than 2 g/kg; this should be researched in more detail. According to the reports in literature,[20,21] most studies use Swiss or ICR mice for modeling. Here, we have demonstrated that Kunming mice are also applicable to the LPS model. However, further studies are necessary to evaluate the anti-neuroinflammatory function of hempseed, including the use different mouse models and measuring more biomarkers.

Presently, fourteen phenylpropionamides have been identified from the extract, including caffeoyloctopamine, caffeoyltyramine, coumaroyltyramine, feryroyltyramine, cannabisins (A−F, M, N), grossamide, and demethyl-grossamide. In our previous cell-level screening test, most of them showed anti-neuroinflammatory activity in LPS-induced BV2 microglia cell, especially caffeoyltyramine, cannabisin F, G, M, and grossamide.[17] Actually, some other phenylpropionamides of low content also showed good anti-neuroinflammatory activity,[17] but they could not be identified and measured online from the extract because of their low content and lack of standard substances. We selected grossamide as a representative compound to study its anti-neuroinflammatory mechanism and found it able to significantly inhibit the LPS-mediated secretion of pro-inflammatory mediators, reduce the phosphorylation levels of NF-κB subunit p65 in a concentration-dependent manner, and suppress the translocation of NF-κB p65 into the nucleus, suggesting that grossamide could intervene the NF-κB signaling pathway.[15] Further study still need to be done to elucidate the anti-neuroinflammatory contribution of hempseed constituents.

Overall, our previous study found that the phenylpropanamide compounds in hempseed have significant anti-neuroinflammatory activities at the cellular level. In this study, we established a mouse model of neuroinflammation induced by LPS and found that hempseed extract rich in phenylpropionamides possesses effective anti-neuroinflammatory

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activity. This neuroprotective function of hempseed makes it worth noting as a functional food.

4. MATERIALS AND METHODS

4.1. Plant Material and Extract Preparation. Hemp (*C. sativa* L.) seed material was collected in Bama county, Guangxi province of China, in October 2016 and identified by Professor Lan Xiang, Department of Pharmacognosy, Shandong University. Voucher specimens (201610-1) have been deposited in Dr Fan's laboratory at Shandong University, China.

The dried and crushed hempseed (3 kg) was first defatted with petroleum ether (25 L) under ultrasound for 1 h three times and then extracted with 70% aqueous ethanol (EtOH) under reflux (three times, 25 L × 2 h). The ethanol solution was subsequently concentrated to 500 mL by evaporating in a vacuum. This solution was suspended in distilled water and subjected to a D101 macroporous adsorption resin column using H_2O, 75%, and 95% EtOH successively to obtain the 75% EtOH fraction (41.2 g, 1.37%, extract of hempseed).

4.2. Phytochemical Identification and Quantitation of TPA in Hempseed Extract. *4.2.1. HPLC–DAD Qualitative Analysis.* The 75% EtOH fraction was dissolved in methanol as the sample solution and analyzed by HPLC with a diode array detector (DAD) detector. Identification of the sample solution was performed on an Agilent 1200 series HPLC system (Agilent Technologies) equipped with a G-1315D DAD detector. The samples were separated through an Eclipse XDB-C18 column (4.6 × 250 mm^2, 5 μm; Agilent) using water (A)–acetonitrile (B) as the mobile phase, and detected under 280 nm at 25 °C. The gradient elution for the mobile phase was set as follows: 10% B at the beginning, 20% at 5 min, 30% at 25 min, 45% at 45 min, and 75% at 55 min. The flow rate was 0.8 mL/min and the injection volume was 20 μL.

The compounds were identified based on a comparison with the retention times and UV spectral characteristics and co-elution with authentic samples previously isolated and identified by NMR analysis in our laboratory.[16]

4.2.2. HPLC–DAD Quantitative Analysis. To a weighed quantity containing 16.1 mg of hempseed extract, 10 mL of methanol was added as the sample solution; this was repeated three times. The sample solution was analyzed according to the above-mentioned HPLC–DAD chromatographic conditions and the peak area was recorded. A standard curve was prepared using cannabisin A (CA) and *N-trans*-caffeoyltyramine (NC) as standard compounds, and the total content of the identified compounds was calculated.

4.3. Animals. Male Kunming mice (31.1 ± 2.2 g, 8 weeks old) were obtained from Shandong University Animal Services (Shandong, China). The mice were housed under standard laboratory conditions of temperature (24 ± 1 °C), humidity (50 ± 5%), and light (12 h light/dark cycle). The animals were allowed free access to food and water. All the procedures were approved by the Shandong University Animal Care and Use Committee and carried out in compliance with the National Institutes of Health guidelines for the care and use of laboratory animals (Publication No. 85-23, revised 1985).

4.4. Drugs and Reagents. Lipopolysaccharides (LPS) from *Escherichia coli* (055:B5), piracetam, and radio immunoprecipitation assay (RIPA) lysis buffer were purchased from Solarbio (Beijing, China). LPS was dissolved in 0.9% saline solution, and all the other drugs were dissolved in distilled water. TNF-α, IL-1β, and IL-6 enzyme-linked immunosorbent assay (ELISA) kits were purchased from Boster (Wuhan, China).

4.5. Experimental Groups and Treatment. Sixty male Kunming mice were randomly divided into five groups (n = 12/group), including control (1–3 weeks, 0.9% saline, 10 mL/kg/day), model (third week, 0.9% saline, 10 mL/kg/day), TPA (1–3 week, 1, 2 g/kg/day), and piracetam (2–3 week, 0.4 g/kg/day) treatment groups. Piracetam was used as a positive control due to its therapeutic effect on cognition in aging and dementia, as well as its ability to improve mitochondrial function to ameliorate the detrimental effects of Aβ on brain function.[29,30]

After 2 weeks of administration, the model, TPA, and piracetam groups were injected intraperitoneally with LPS (250 μg/kg/day) on day 15, 30 min after treatment with TPA (1, 2 g/kg/day) and piracetam (0.4 g/kg/day). Intraperitoneal injection of LPS continued for an additional week. One hour after the LPS administration on day 16, memory function was assessed using the Morris water maze (MWM) test for 6 consecutive days (for the time line of drug treatment and experimental schedule, see Figure S6).

4.6. Morris Water Maze Test. The Morris water maze (MWM) test was used to evaluate the spatial learning and memory of the mice.[31] The equipment consists of a black circular pool (120 cm in diameter, 50 cm in height), platform, and recording system. The pool was divided into four quadrants, and an invisible transparent platform (9 cm in diameter) was placed at the center of the second quadrant and submerged 1 cm below the water surface. A black edible pigment was added to the water, so the water was opaque. The water temperature was maintained at 22–24 °C. This behavioral task included hidden-platform training (spatial learning) and probe trial (spatial memory) sessions.

In the hidden-platform training session, the mice were allowed four daily trials in the presence of the platform for five subsequent days. In this session, the mice were placed in the pool facing the wall in one of the four quadrants. When the mouse located the platform, the time required to find the hidden escape platform (escape latency) was recorded and the mouse was allowed to stay on the platform for 15 s. If the mouse did not locate the platform within 90 s, it was placed on the platform for 15 s to familiarize it and the escape latency was considered to be 90 s. On day 6, the probe trial was performed. In this session, the platform was removed. The time spent in the target quadrant and the number of times that the mouse crossed the removed platform was recorded and analyzed during 90 s.

4.7. Tissue Processing. After the MWM test, the animals (n = 6/group) were decapitated and the brain was rapidly moved on ice. Thereafter, each mouse brain was weighed, homogenized in RIPA lysis buffer (with 1:100 PMSF, a protease inhibitor) to a concentration of 100 mg/mL, and centrifuged at 10 000 rpm at 4 °C for 15 min. The supernatants were collected and stored at −80 °C for ELISA.

In addition, another cohort (n = 6/group) of mice was anesthetized with 5% chloral hydrate and transcardially perfused with a 0.9% saline solution, followed by 4% paraformaldehyde (pH 7.4). The brains were removed from the skull and then fixed in the same 4% paraformaldehyde solution at 4 °C.

4.8. Detection of the Levels of TNF-α, IL-1β, and IL-6 by ELISA. The levels of TNF-α, IL-1β, and IL-6 in the mice

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brains were assessed using ELISA kits (Boster, China) according to the manufacturer's instructions. Absorbance was read at 450 nm on a microplate spectrophotometer (BioTek). All the results are expressed as pg/mg.

4.9. Hippocampal Morphology Examination. To observe the histological changes in the hippocampus in the LPS-induced mice, the brain tissues were fixed in 4% paraformaldehyde solution at 4 °C for 24 h. The brain specimens were paraffin-embedded and coronal sections were cut (4 μm thick) before mounting on silane-coated slides. The coronal sections were processed for HE staining according to a previously described method.[32] The images were taken using an optical microscope (Olympus BX53F, Tokyo, Japan) at magnifications of 200× and 400×, respectively.

4.10. Statistical Analysis. All the data are expressed as mean ± standard deviation (SD). Statistical analysis was done by one-way analysis of variance followed by the Student–Newman–Keuls test using GraphPad Prism version 5.0 (GraphPad software, La Jolla, CA). *P* values less than 0.05 were considered statistically significant.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02250.

HPLC chromatogram of standard compounds and their mixture; UV spectrum of each standard compound; the chemical structures of the identified compounds; the standard curves of NC and CA and the concentration and peak area of NC and CA; the time line of drug treatment and experimental schedule (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CA, cannabisin A; ELISA, enzyme-linked immunosorbent assays; HE, hematoxylin–eosin staining; HPLC, high-performance liquid chromatography; LPS, lipopolysaccharide; MWM, Morris water maze; NC, *N-trans*-caffeoyltyramine; TPA, total phenylpropanamides

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