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Research Paper

Potential Anti-inflammatory Activity of Brown Propolis Against Brain Ischemia Damage in Mice

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ABSTRACT

Background: Inflammation plays a major part in brain ischemia. Propolis is a polyphenol-rich hive product with a set of pharmaceutical properties.

Objectives: This research aims to investigate the impact of water extracts of brown propolis (WEPs) on stroke outcomes and inflammatory responses in a rat model of permanent middle cerebral artery occlusion (MCAO).

Materials & Methods: This experimental study was conducted in Rafsanjan, Iran, in 2017. WEPs were experimentally prepared from two regions in Iran. Gas chromatography–mass spectrometry and Folin–Ciocalteu assays were used to determine chemical portrayal and the total polyphenol content, respectively. A total of 66 male adult mice were divided randomly into the surgical sham, control (vehicle-treated), and four WEPs-treated animal groups. WEPs-treated groups received doses of 100 and 200 (mg/kg, IP) four times, and their behavioral tests, brain edema, infarct volume, and tumor necrosis factor-alpha (TNF-α) level were evaluated.

Results: The samples were not significantly different in terms of the concentration of the total polyphenol content. Compared to the control, WEPs led to a substantial decrease in the TNF-α level ($P<0.05$) as well as a subsequent reduction in the brain edema and infarct volume ($P<0.001$) in all treatment groups. Furthermore, there was a significant improvement in neurological deficits and sensory-motor impairments level ($P<0.05$).

Conclusion: According to the study findings, WEPs reduce brain ischemia damage, perhaps by exerting a neuroprotective effect on stroke-induced neuroinflammatory responses.

Keywords: Brain ischemia, Neuroprotection, Neuroinflammatory diseases, Polyphenols

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Highlights

- Administration of water extracts of brown propolis (WEPs) is neuroprotective by reducing the level of pro-inflammatory factor, i.e. TNF-α.
- WEPs led to a reduction in infarct volume and brain edema compared to the control.

Introduction

Stroke is one of the most common causes of death and morbidity worldwide [1, 2]. Brain ischemia injury, resulting from a type of restricted perfusion, has some complications, including brain edema, infarction, neuroinflammation, as well as neurological damage [3]. Cell death is accelerated in the penumbra due to several pathophysiological mechanisms, including inflammation, apoptosis, peri-infarct excitotoxicity, depolarization, and oxidative stress [2, 3]. Now, neuroprotective agents are considered in treating stroke, in addition to thrombolytic therapy [4].

In ischemic brain injury pathogenesis, called neuroinflammation, the inflammatory response has played an important role [5]. According to clinical and experimental animal studies, the detrimental implications of stroke result from neuroinflammation [2, 5]. Within the first hours after stroke, levels of pro-inflammatory interveners, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 (IL-1) rise, resulting in the exacerbation of ischemic complications [6]. In developing inflammatory reactions in brain tissue, TNF-α has been very effective [6, 7]. Both experimental and clinical studies indicate a positive association between the TNF-α level and the extent of ischemic injury [7]. Thus, TNF-α-targeted therapies could be a novel neuroprotective approach to treating cerebral ischemia [7].

Propolis is a resinous compound rich in polyphenols, provided when secretions of honeybees are mixed with resinous sap obtained from different plant sprouts and bark [8-10]. Since old times, propolis has been used as a traditional and alternative treatment by humans for its biological features [8, 10, 11], including antimicrobial effects [12], anti-apoptotic [13] anti-inflammatory [14], antioxidant [15, 16] and neuroprotective [17, 18]. According to the reports, the propolis compounds and characteristics depend on the vegetation, area, and time of collection [11, 16]. It has been reported that propolis produced in Brazil and Turkey reduces post-ischemic neuroinflammation and neuronal damage [15, 18, 19]. In addition, many biological impacts of propolis have been ascribed to its phenolic compounds [12, 20]. These compartments have shown anti-inflammatory and antioxidative properties [12, 21]. The useful role of phenolic compounds has been reported in preventing and treating some neurodegenerative diseases [22]. Accordingly, the current research aims to evaluate the phenolic content and chemical features of water extract of brown propolis (WEPs) in two locations in Iran, in addition to assessing its impact on stroke consequences and inflammatory response in a rat model of permanent middle cerebral artery occlusion (MCAO).

Materials and Methods

In this study, 2,3,5-triphenyl tetrazolium chloride (TTC) was provided from Merck KGOA (Darmstadt, Germany). Moreover, the TNF-α ELISA kit was obtained from Crystal Day Biotech Co., Ltd. (Shanghai, China). The researchers also provided diethyl ether, ketamine hydrochloride, formaldehyde, and xylazine from Sigma-Aldrich.

Gas chromatography-mass spectrometry

To perform gas chromatography-mass spectrometry (GC-MS) analysis, Agilent Gas Chromatograph 6890 and Agilent 5973N mass spectrometer, with 0.25 mm id, 30 m long, and WEPs X5 capillary column film thickness of 0.25 mm, were both used. A temperature range of 50°C-300°C was set at a rate of 3°C/min. Helium with a 1 mL/min flow rate was defined as the carrier gas. In the split/splitless mode, the injection was conducted at 220°C, with an ionization voltage of 70 eV. Reconnaissance was performed using ChemStation software. The Kovats retention index and ChemStation software were employed to determine the chemical characterization in the following manner:

Kovats retention index (I), total carbon atoms in the smaller n-alkane, total carbon atoms in the larger n-alkane, and the adjusted retention time.

Preparing water extracts of brown propolis (WEPs)

Brown propolis samples were obtained from Hezar Masjed mountains, Khorasan Razavi Province, Iran, and beehives in the Lalehzar area, Kerman Province, Iran. Afterward, they were transferred to Soren Tech Toos, Inc. Ltd. (Mashhad City, Iran). Under a company-specific protocol, the samples were extracted, and total polyphenol content was measured using the modified Folin–Ciocalteu colorimetric test. The propolis was extracted with WEPs. The total phenolic content is shown in mg/g [23].

Study animals

In the current research, 66 male adult Balb/C mice (30–35 g) were employed and were kept on a light/dark 12-h cycle, and food and water were provided for them. Six groups were provided, and the mice were randomly divided into groups (11 in each group, 6 for the neurological test, brain edema, and the infarct volume, as well as 5 for the TNF-α level) after being acclimatized. Accordingly, group 1 was the sham (craniotomy with no MCAO), group 2 was the control (ischemia-exposed and vehicle-treated), as well as WEPs-treated groups (4 groups). All animal measures were confirmed by the Ethics Committee of Rafsanjan University of Medical Sciences Research (IR.RUMS.REC.1399.203). They were also consistent with the National Institute of Health guidelines and national laws on using and caring for laboratory animals.

Experimental protocol

The WEPs were obtained from diverse regions of Iran (Khorasan Razavi: KhWEP, and Kerman: KeWEP). At 48, 24, and 1 h before as well as 4 h after inducing focal brain ischemia, the KhWEP was administered intraperitoneally with the doses of 100 and 200 mg/kg (group 3: KhWEP100; group 4: KhWEP200) and the KeWEP with similar doses (group 5: KeWEP100; group 6: KeWEP200). The extract doses were suggested according to the researchers' early findings and the study conducted before [16, 17].

Model of MCAO Establishment

Xylazine (8 mg/kg) and ketamine (80 mg/kg) were injected intraperitoneally to anesthetize the mice. By employing a rectal probe, the body's core temperature was checked and reserved at 37°C throughout anesthesia. The right middle cerebral artery (MCA) was permanently occluded to induce MCAO, as described in previous research [16, 24]. In short, a minor skin incision was created between the external auditory meatus and the lateral part of the orbit. Later on, the connective tissue and muscles were pulled fully aside. Next, the dura mater was removed by drilling a minor hole (approximately 1 mm in diameter) over the MCA proximal part; besides, a thermal coagulator was used to permanently cauterize the MCA. Finally, the researchers substituted the connective tissue and the temporal muscle and sutured the skin. The mice were returned to their cages after recovering in a heated cage. Except for the cauterization MCA, a similar method was performed for the surgical sham group.

The brain edema and infarct volume measurement

As earlier reported, brain edema and infarct volume were measured [3, 16]. Diethyl ether was used to anesthetize the animals, and 48 h after MCAO, they were decapitated. Next, the brains were separated and divided into 5 coronal slices with a thickness of 1 mm using the brain matrix. At 37°C for 30 min, 2% TTC was used to stain the slices. They were then immersed in a solution of 10% phosphate-buffered formalin overnight. The infarcted tissue was not stained (white), but the normal one was stained (red). Next, ImageJ software (NIH Image, Version 1.61 Bethesda, Maryland, USA) and a color flatbed scanner (Scan jet 5370C, Hewlett-Packard, Palo Alto, CA, USA) were used to determine and analyze the volume of the infarct. To calculate the overall infarct size, all slices of the infarct zones were added and multiplied by the brain slice thickness (1 mm) to achieve the infarct volume. The researchers computed the volume of the corrected infarct to compensate for the brain edema effect with the volume of the modified infarct=the measured infarct area×[1-(ipsilateral hemisphere area–the contralateral hemisphere area)/the contralateral hemisphere)].

Next, the formula of edema=(the left hemisphere volume –the right hemisphere volume)/the right hemisphere volume was used to determine brain edema. In addition, brain edema and infarct volume were reported in percentages.

TNF-α level quantification

The animals were beheaded about 48 h after MCAO. Their brains were quickly separated, and the slices were cut in half. The right halves were weighed and homogenized in the buffer (PBS, pH 7.4) on an ice-cold pad and then centrifuged at 4°C for 20 min at 6000 RPM. Next, each sample supernatant was collected and preserved at -80°C up to the analysis. The commercially available kit (crystal day biotech, Shanghai, China) was utilized to measure the

Table 1. Effects of WEPs on neurological deficits measured by the Bederson scoring system following the MCAO at 4 and 48 h

Groups	Time	
	After 4 h	After 48 h
Sham	0(0-1)	0(0-0)
Control	2(1.75-3.5)¶	2(1-3)¶
KhWEP100	1(0.75-1)**	0(0-1.25)*
KeWEP100	1(0-1)*	0.5(0-1)*
KhWEP200	1(0–1.25)*	0.5(0-1)*
KeWEP200	1(0-1.25)*	1(0–1.25)*

Data are presented as the median, 25th, and 75th percentiles (percentiles in parentheses).

P<0.01 vs the sham group, **P<0.01, and *P<0.05 vs the control group.

Abbreviations: WEPs: Water extract of brown propolis; KhWEP: WEP of Khorasan Razavi Province; KeWEP: WEP of Kerman Province.

TNF-α level immune-enzymatically (ELISA), according to the manufacturer's guidelines. In brief, a particular antibody was coated in wells against every antigen (TNF-α), and the antigen coverage was performed between primary and secondary HRP(horseradish peroxidase)-coated antibodies. Next, an ELISA reader at 450 nm with a correction wavelength of 630 nm was used to evaluate color progress within 10 minutes. In addition, tissue TNF-α concentrations were expressed as a nanogram (ng) of the antigen per milligram (mg) of the protein.

Behavioral tests

For 2 days before the stroke, the sensorimotor function of the mice was tested on sticky tape (adhesive). Next, the animals were tested 4 and 48 h after the stroke and before MCAO. The touching duration was confirmed and averaged in 3 trials until removing the adhesive labels from the stroke-impacted contralateral forepaw [25]. Through the modified Bederson 6-point scoring system, neurological deficiencies were verified and defined at 4 and 48 h following MCA occlusion [26]. In this regard, a non-observable deficit was defined by score 0, forelimb flexion by score 1, forelimb flexion and reduced resistance to lateral push by score 2, unidirectional circling by score 3, unidirectional circling and decreasing levels of consciousness by score 4, and score 5 implied death. In addition, a blinded experiment was performed for behavioral tests.

Statistical analysis

SPSS software version 20 was used for Statistical analysis. Data were presented as Mean±SEM. The two-way ANOVA was used to compare behavioral tests, the infarct volume, brain edema, and TNF-α level by SPSS software, version 20. Furthermore, to determine individual differences, the Tukey test was applied. The Kruskal-Wallis test was used for comparing neurological deficits due to the non-normality of the data. P≤0.05 were regarded as significant.

Results

The total polyphenols content, as well as the GC-MS analysis of WEPs for KhWEP and KeWEP, showed that the overall polyphenols content was 40 and 45 mg/g, respectively. The polyphenols types also varied slightly from one sample to the other. For KhWEP and KeWEP, GC-MS chromatograms are shown in Figure 1. The main observed flavonoids in the KhWEP extract were derivatives of pinobanksin and 3,7- dihydroxy-5-methoxyflavanone. The main flavonoids in the KeWEP, in contrast, were naringenin and quercetin. However, the two samples had the same phenolic acid ester and cinnamic acid derivatives.

WEPs effect on the TNF-α level

Forty-eight hours after the stroke, the anti-neuroinflammatory activity of the WEPs was evaluated by quantifying TNF-α in the brain tissue. Compared to the sham group, a significant increase was observed in the level of TNF in the control group (P<0.01). Compared to the control group, although KeWEP reduced the TNF level in KeWEP-treated groups (P<0.05), its reduction was greater in the KhWEP-treated groups (P<0.01) (Figure 2).

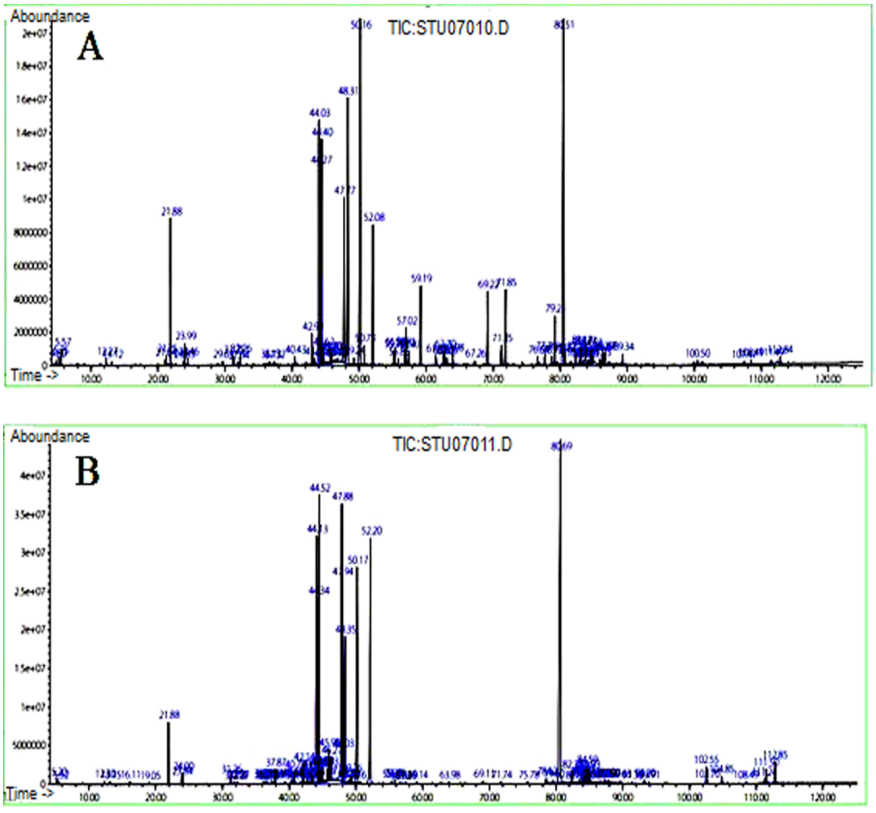

Figure 1. Water extract gas chromatography-mass spectrometry (GC-MS) of brown propolis

A: Khorasan Razavi Province (KhWEP) and B: Kerman Province (KeWEP), Iran

Effect on the Infarct volume and brain edema

TTC-stained brain sections quantified infarct volumes. There was no infarction in the group of the sham. As against the control group, doses of 100 and 200 mg/kg from both regions reduced the volume of infarct significantly (P<0.001) (Figure 3). According to Figure 4, compared to the sham group, although the control group's brain edema sharply increased (P<0.001), it decreased by WEPs treatment significantly as against the control (P<0.001).

WEPs' effect on behavioral function

Compared to the sham, the ischemia induction significantly increased the duration of touching until removing the labels off the control group stroke-impacted contralateral forepaws (P<0.01) (Figure 5). A 100-200 mg/kg-dose treatment of KhWEP at 4 and 48 h (P<0.05), as well as 100 mg/kg of KeWEP at 48 h (P<0.01) significantly decreased the average time taken for the duration of touching until removing the adhesive tape from forepaws after MCAO (Figure 5). Table 1 shows that based on Bederson scoring system, in contrast to the control, at 4 and 48 h after the stroke, 100-200 mg/kg-dose WEPs samples reduced neurological deficits substantially (P<0.05 for the entire treated groups excluding KhWEP100 at 4 h P<0.01).

Discussion

One major clinical purpose of neuroscience study is to use neuroprotective agents to reduce brain ischemia injury [4]. Propolis is a natural compound rich in polyphenols broadly used in traditional and alternative medicine [9, 12]. The results revealed that after permanent MCAO in the samples, WEPs treatment from two locations in Iran (KeWEP and KhWEP) provided neuroprotection. Administrating WEPs was neuroprotective, proven by the reduction in the level of pro-inflammatory factor, TNF-α, and this beneficial effect was associated with a decrease in brain edema, volume of infarct, and an improvement in neurological experiments.

It has become known that inflammatory response is a vital pathological stroke process throughout the acute phase, in particular [27, 28]. Thus, during the first stroke phase, the inhibition of inflammation could lead to a striking neuroprotective tactic [28, 29]. Variations in the propolis chemical properties in various regions indicate its diverse biological activities. However, several studies have reported that the polyphenol content is responsible for its

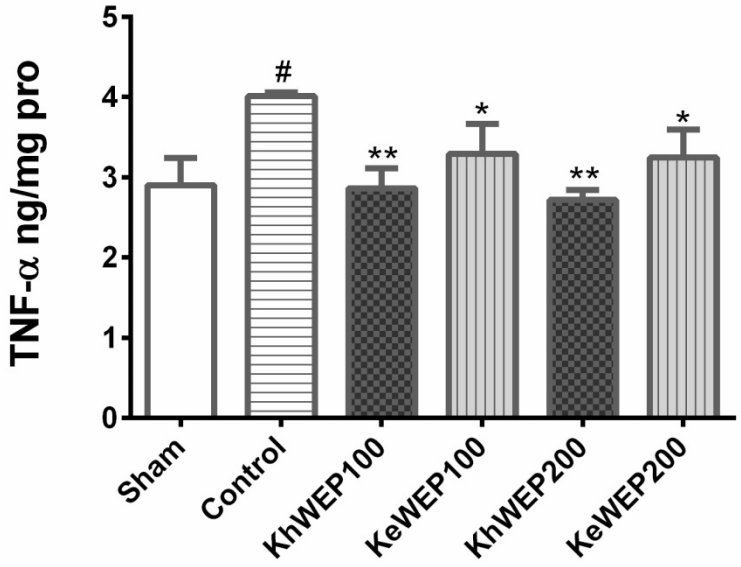

Figure 2. Effects of WEPs on the TNF-α level (ng/mg protein) in brain tissue at 48 h following stroke

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Data are presented as Mean±SEM. #P<0.01 vs the sham group, **P<0.01 and *P<0.05 vs the control group.

Abbreviations: WEPs: Water extracts of brown propolis; KhWEP: WEP of Khorasan Razavi Province; KeWEP: WEP of Kerman Province.

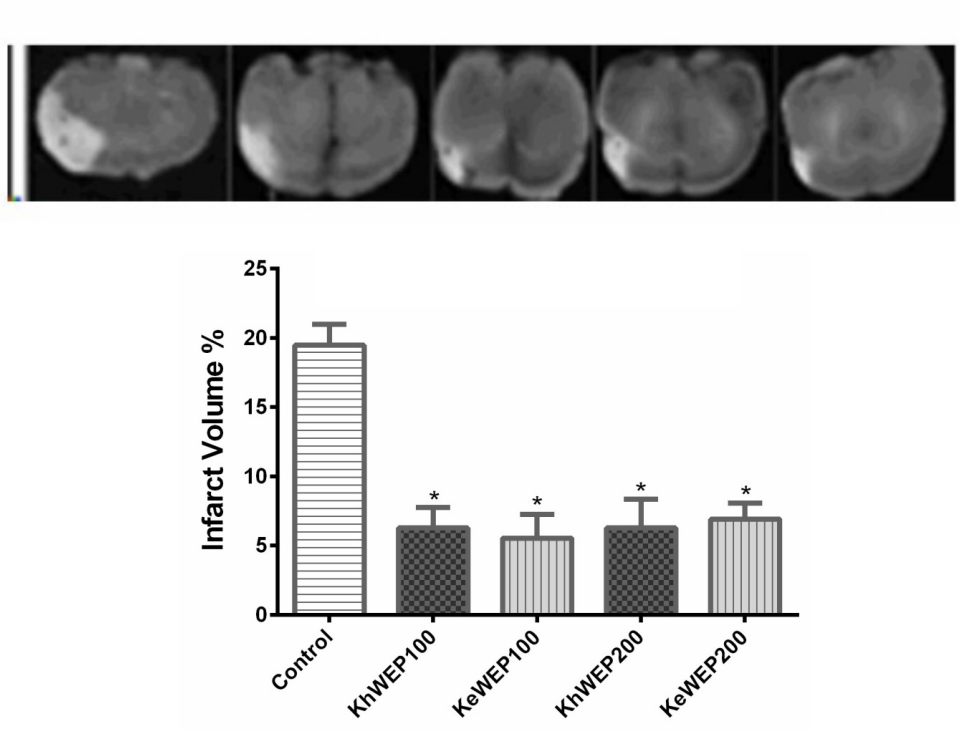

Figure 3. WEPs effects on the infarct volume (%)

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The infarct volume was defined by calculating TTC-stained brain sections (the upper panel) gained at 48 h following stroke (the lower panel). Data are presented as Mean±SEM. *P<0.001 vs the control group.

Abbreviations: WEPs: Water extracts of brown propolis; KhWEP: WEP of Khorasan Razavi Province; KeWEP: WEP of Kerman Province.

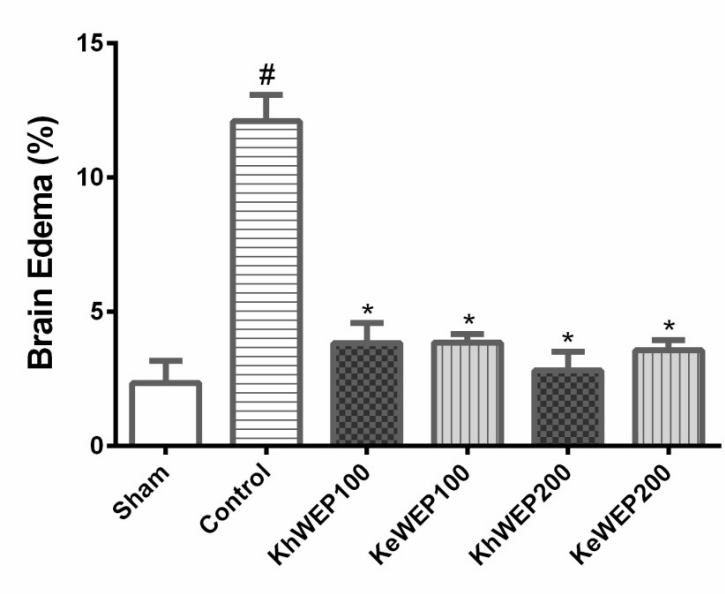

Figure 4. Effects of WEPs on brain edema (%) at 48 h following stroke

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Data are presented as Mean±SEM.

#P<0.001 vs the sham group, *P<0.001 vs the control group.

Abbreviations: WEPs: Water extracts of brown propolis; KhWEP: WEP of Khorasan Razavi Province; KeWEP: WEP of Kerman Province.

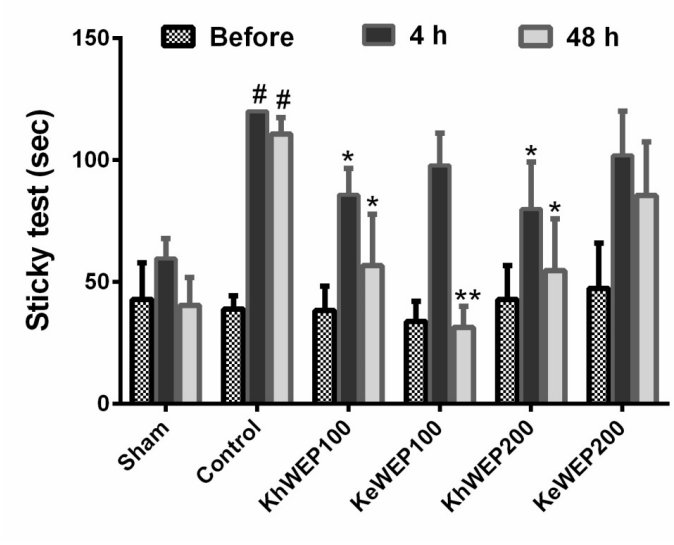

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Figure 5. Effects of WEPs on sensorimotor performance, assessed by the sticky tape test at 4 and 48 h following stroke data are presented as Mean±SEM.

#P<0.01 vs the sham group at the same time, **P<0.01 and *P<0.05 vs the control group at the same time.

Abbreviations: WEPs: Water extracts of brown propolis; KhWEP: WEP of Khorasan Razavi Province; KeWEP: WEP of Kerman Province.

physical activities: synergistic interactions among propolis alignments are required as the necessary factor in these activities [8, 23, 30]. Owing to its compound diversity, particularly flavonoids, this substance has a high anti-inflammatory capacity [14, 22]. This substance is non-toxic, and research studies have indicated that 1400 mg/kg of body weight per day could not exert toxic impacts on rats [31]. Because of its anti-inflammatory and protective features, propolis seems to be a potential defensive agent with natural therapeutic effects in ischemic brain injury [19, 31].

Our findings revealed that the TNF-α level, brain edema, and infarct volume increased after the induction of permanent MCAO. Cytokines are key in increasing the local inflammatory response and infarct extension]5, 32]. It has been demonstrated that TNF-α is expressed in ischemic neurons after permanent MCAO, being concerned with early stroke-induced inflammation mechanisms [32]. Patients with brain ischemia have also exhibited high TNF-α levels in cerebrospinal fluid and the serum over the first 24 h [7]. The TNF-α level is an indicator of the activation of macrophages, microglia, and other inflammatory reactions, as well as a stroke outcome predictor [6, 33, 34]. TNF-α is a powerful pro-inflammatory cytokine produced immediately after the stroke [6]. This cytokine participates in a wide range of events during cerebral ischemia, such as the stimulation of acute-phase protein synthesis, increased vascular permeability of the blood-brain barrier, expression of endothelial adhesion molecules and other pro-inflammatory cytokines, recruitment of inflammatory cells, as well as generation of inducible NOS (iNOS) and cyclooxygenase-2 [6, 32]. These events are vital in improving the secondary inflammatory reaction (neuroinflammation), resulting in stroke complications, particularly brain edema [35]. According to Fukuda et al., in several brain diseases, neuroinflammation is a prevalent pathological incident typically followed by brain edema, consistent with the present study findings [29]. In addition, a positive association has been established between the TNF-α level and the infarct volume in human and animal studies [7, 32]. It has been observed that the TNF-α-neutralizing antibody diminishes brain edema and the infarct volume after permanent or transient focal cerebral ischemia [32, 35]. In this research, we investigated whether the WEPs' neuroprotective impacts were related to the neuroinflammatory response. The assessment of pro-inflammatory mediator TNF-α demonstrated that the WEPs at 200 and 100 mg/kg in both regions could decrease the TNF-α level, thereby reducing brain edema and the infarct volume. Swamy et al. showed the propolis neuroprotective impact in Malaysia by reducing the number of cytokines, including TNF-α concentrations involved in neuroinflammation [36]. Wu et al. showed that Brazilian green propolis could inhibit *in vitro* hypoxia-induced neuroinflammatory reactions in microglia [19].

To the author's knowledge, this is the first research that evaluated the Iranian WEPs' impact on detrimental complications of stroke by inhibiting neuroinflammation in the permanent model of MCAO. The neuroprotective effects in the WEPs-treated groups could have been due to propolis phenolic compounds' anti-inflammatory activity [22]. Sala et al. (2003) reported that pinocembrin, i.e. a phenolic compound in the KhWEP, displayed anti-inflammatory properties in the sheep's red blood cell-induced hypersensitivity reaction [37]. In addition, research shows that naringenin and quercetin, i.e. phenolic compounds in the KeWEP, block the production of inflammatory mediators to show the anti-inflammatory capability *in vitro* [38].

In the present research, the mice treated with both doses and samples of WEPs showed significantly less brain edema than the control group. WEPs treatment was also neuroprotective as it reduced the infarct volume. In addition, administering KhWEP and KeWEP at doses of 200 and 100 mg/kg decreased the infarct volume considerably by approximately 64%-73%. In the same vein, Shimazawa et al. showed that green propolis from Brazil could decrease the infarct volume statistically [17]. Our previous studies also showed that Iranian brown propolis significantly reduced the volume of ischemia and brain edema in a rat model of permanent MCAO [9, 16].

Often, patients surviving a stroke suffer sequelae, including neurological deficits [39]. The sticky tape test and the Bederson scoring system are the most common methods for evaluating behavioral deficits after stroke [40]. Thiyagarajan et al. showed that tests of functional improvement, regardless of the infarct size, could assess neuroprotective agent effects [41]. This research showed that both indices were impaired following the induction of stroke. In addition, treatment with WEPs improved behavioral function. The neurological performance improvement in functional tests once medications are received could be because of the inhibition of WEPs' neuroinflammatory and neuroprotective effects. However, further research must be conducted to identify this compound's probable impact on functional recovery.

Conclusion

The current research showed the possible neuroprotective effects of Iranian brown propolis on stroke-induced neuroinflammatory reactions. The protective effects could include attenuating the TNF-α-dependent pathway in an

animal model of MCAO. This property might be useful in preventing and treating ischemia-induced function deficits.

Ethical Considerations

Compliance with ethical guidelines

The study procedures were consistent with the ethical guidelines of the Declaration of Helsinki 2013. The animal measures were confirmed by Rafsanjan University of Medical Sciences Research Ethics Committee, Iran (Code: IR.RUMS.REC.1399.203).

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Authors contributions

Conceptualization, funding acquisition, resources, and writing the original draft: All authors; Methodology, supervision, and investigation: Mohammad Allahtavakoli, Zahra Kamiab, Gholamreza Bazmandegan, and Mohammad Taher Boroushaki; Writing, review, and editing: Zahra Kamiab, Gholamreza Bazmandegan, Mohammad Yassin Zamanian.

Conflict of interest

The authors declared no conflict of interest.

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References

[1] Vakilian A, Bazmandegan G, Saeedi Nezhad M, Asadollahi Z, Moghadam Ahmadi A. Association of HbA1c levels with extent and functional status of ischemic and hemorrhagic stroke. J Kermanshah Univ Med Sci. 2018; 25(4):307-17. [Link]

[2] Hakimizadeh E, Shamsizadeh A, Roohbakhsh A, Kazemi Arababadi M, Hajizadeh MR, Shariati M, et al. TRPV1 receptor-mediated expression of toll-like receptors 2 and 4 following permanent middle cerebral artery occlusion in rats. Iran J Basic Med Sci. 2017; 20:863-9. [DOI:10.22038/IJBMS.2017.9107]

[3] Allahtavakoli M, Amin F, Esmaeeli-Nadimi A, Shamsizadeh A, Kazemi-Arababadi M, Kennedy D. Ascorbic acid reduces the adverse effects of delayed administration of tissue plasminogen activator in a rat stroke model. Basic Clin Pharmacol Toxicol. 2015; 117(5):335-9. [DOI:10.1111/bcpt.12413] [PMID]

[4] Wang C, Pei A, Chen J, Yu H, Sun ML, Liu CF, et al. A natural coumarin derivative esculetin offers neuroprotection on cerebral ischemia/reperfusion injury in mice. J Neurochem. 2012; 121(6):1007-13. [DOI:10.1111/j.1471-4159.2012.07744.x] [PMID]

[5] Lakhan SE, Kirchgessner A, Hofer M. Inflammatory mechanisms in ischemic stroke: Therapeutic approaches. J Transl Med. 2009; 7:97. [DOI:10.1186/1479-5876-7-97] [PMID] [PMCID]

[6] Buttini M, Appel K, Sauter A, Gebicke-Haerter PJ, Boddeke HW. Expression of tumor necrosis factor alpha after focal cerebral ischaemia in the rat. Neuroscience. 1996; 71(1):1-16. [DOI:10.1016/0306-4522(95)00414-9] [PMID]

[7] Zaremba J, Skrobanski P, Losy J. Tumor necrosis factor-alpha is increased in the cerebrospinal fluid and serum of ischaemic stroke patients and correlates with the volume of evolving brain infarct. Biomed Pharmacother. 2001; 55(5):258-63. [DOI:10.1016/S0753-3322(01)00058-0] [PMID]

[8] Bankova V, De Castro S, Marcucci M. Propolis: Recent advances in chemistry and plant origin. Apidologie. 2000; 31(3):3-15. [DOI:10.1051/apido:2000102]

[9] Bazmandegan G, Shamsizadeh A, FathiNajafi M, Assadollahi Z, Allahtavakoli M, Kamiab Z, et al. Iranian brown propolis possesses neuroprotective effect against ischemic neuronal damage in mice. J Herbmed Pharmacol. 2020; 9:121-9. [DOI:10.34172/jhp.2020.16]

[10] Liu Y, Wu Z, Zhu A, Nakanishi H, Ni J, Zhong X, et al. Brazilian green propolis improves cognitive functions and modulates systemic cytokines in elderly people living at high altitude. J Neurol Sci. 2017; 381 Supplement:678-9. [DOI:10.1016/j.jns.2017.08.1911]

[11] Popova M, Bankova V, Naydensky C, Tsvetkova I, Kujumgiev A. Comparative study of the biological activity of propolis from different geographic origin: A statistical approach. Maced Pharm Bull. 2004; 50:9-14. [Link]

[12] Silva JC, Rodrigues S, Feas X, Estevinho LM. Antimicrobial activity, phenolic profile and role in the inflammation of propolis. Food Chem Toxicol. 2012; 50(5):1790-5. [DOI:10.1016/j.fct.2012.02.097] [PMID]

[13] Liu R, Gao M, Yang ZH, Du GH. Pinocembrin protects rat brain against oxidation and apoptosis induced by ischemia-reperfusion both in vivo and in vitro. Brain Res. 2008; 1216:104-15. [DOI:10.1016/j.brainres.2008.03.049] [PMID]

[14] De Almeida E, Menezes H. Anti-inflammatory activity of propolis extracts: A review. J Venom Anim Toxin. 2002; 8(2):191-212. [DOI:10.1590/S0104-79302002000200002]

[15] Kakoolaki S, Talas ZS, Cakir O, Ciftci O, Ozdemir I. Role of propolis on oxidative stress in fish brain. Basic Clin Neurosci. 2013; 4(2):153-8. [PMID] [PMCID]

[16] Bazmandegan G, Boroushaki MT, Shamsizadeh A, Ayoobi F, Hakimizadeh E, Allahtavakoli M. Brown propolis attenuates cerebral ischemia-induced oxidative damage via affecting antioxidant enzyme system in mice. Biomed Pharma-

cother. 2017; 85:503-10. [DOI:10.1016/j.biopha.2016.11.057] [PMID]

[17] Shimazawa M, Chikamatsu S, Morimoto N, Mishima S, Nagai H, Hara H. Neuroprotection by Brazilian green propolis against in vitro and in vivo ischemic neuronal damage. Evid Based Complement Alternat Med. 2005; 2(2):201-7. [DOI:10.1093/ecam/neh078] [PMID] [PMCID]

[18] Wu Z, Liu Y, Zhu A, Wu S, Nakanishi H. Brazilian green propolis suppresses microglia-mediated neuroinflammation by inhibiting NF-kB activation. J Neurol Sci. 2017; 381:678. [DOI:10.1016/j.jns.2017.08.1910]

[19] Wu Z, Zhu A, Takayama F, Okada R, Liu Y, Harada Y, et al. Brazilian green propolis suppresses the hypoxia-induced neuroinflammatory responses by inhibiting NF-kappaB activation in microglia. Oxid Med Cell Longev. 2013; 2013:906-16. [DOI:10.1155/2013/906726] [PMID] [PMCID]

[20] Turan I, Deger O, Aliyazicioğlu Y, Demir S, Kilinç K, Sumer A. Effects of Turkish propolis on expression of hOGG-1 and NEIL-1. Turk J Med Sci. 2015; 45:804-11. [DOI:10.3906/sag-1406-98] [PMID]

[21] Paduch R, Woźniak A, Niedziela P, Rejdak R. Assessment of eyebright (euphrasia officinalis l.) extract activity in relation to human corneal cells using in vitro tests. Balkan Med J. 2014; 31(1):29-36. [DOI:10.5152/balkanmedj.2014.8377] [PMID] [PMCID]

[22] Frautschy SA, Hu W, Kim P, Miller SA, Chu T, Harris-White ME, et al. Phenolic anti-inflammatory antioxidant reversal of abeta-induced cognitive deficits and neuropathology. Neurobiol Aging. 2001; 22(6):993-1005. [DOI:10.1016/S0197-4580(01)00300-1] [PMID]

[23] Najafi MF, Vahedy F, Seyyedin M, Jomehzadeh HR, Bozary K. Effect of the water extracts of propolis on stimulation and inhibition of different cells. Cytotechnology. 2007; 54(1):49-56. [DOI:10.1007/s10616-007-9067-2] [PMID] [PMCID]

[24] Vakili A. [Mini-review for methods of induction experimental ischemia stroke: Type of model, evaluation ischemic damage and neurological deficit (Persian)]. Koomesh. 2006; 8(1):1-10. [Link]

[25] Li X, Blizzard KK, Zeng Z, DeVries AC, Hurn PD, McCullough LD. Chronic behavioral testing after focal ischemia in the mouse: Functional recovery and the effects of gender. Exp Neurol. 2004; 187(1):94-104. [DOI:10.1016/j.expneurol.2004.01.004] [PMID]

[26] Bederson JB, Pitts LH, Tsuji M, Nishimura MC, Davis RL, Bartkowski H. Rat middle cerebral artery occlusion: Evaluation of the model and development of a neurologic examination. Stroke. 1986; 17(3):472-6. [DOI:10.1161/01.STR.17.3.472] [PMID]

[27] Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: An integrated view. Trends Neuroscie. 1999; 22(9):391-7. [DOI:10.1016/S0166-2236(99)01401-0] [PMID]

[28] Muir KW, Tyrrell P, Sattar N, Warburton E. Inflammation and ischaemic stroke. Curr Opin Neurol. 2007; 20:334-42. [DOI:10.1097/WCO.0b013e32813ba151] [PMID]

[29] Fukuda AM, Badaut J. Aquaporin 4: A player in cerebral edema and neuroinflammation. J Neuroinflammation. 2012; 9:279-84. [DOI:10.1186/1742-2094-9-279] [PMID] [PMCID]

[30] Afrouzan H, Tahmasbi GH, Bankova V, Bigdeli M. [Comparison of quantity and quality of propolis produced by gymnosperms and angiosperms plants in northeast of Tehran, Iran (Persian)]. Vet Res Biol Products. 2007; 20(4):156-62. [Link]

[31] Victorino FR, Franco SL, Estivalet Svidzinski TI, Avila-Campos MJ, Nakamura Cuman RK, Hidalgo MM, et al. Pharmacological evaluation of propolis solutions for endodontic use. Pharm Biol. 2007; 45(9):721-7. [DOI:10.1080/13880200701575411]

[32] Nawashiro H, Martin D, Hallenbeck JM. Inhibition of tumor necrosis factor and amelioration of brain infarction in mice. J Cereb Blood Flow Metab. 1997; 17:229-32. [DOI:10.1097/00004647-199702000-00013] [PMID]

[33] Zhang F, Li N, Jiang L, Chen L, Huang M. Neuroprotective effects of (−)-Epigallocatechin-3-Gallate against focal cerebral ischemia/reperfusion injury in rats through attenuation of inflammation. Neurochem Res. 2015; 40:1691-8. [DOI:10.1007/s11064-015-1647-5] [PMID]

[34] Dong XQ, Du Q, Yu WH, Zhang ZY, Zhu Q, Che ZH, et al. Anti-inflammatory effects of oxymatrine through inhibition of nuclear factor-kappa b and mitogen-activated protein kinase activation in lipopolysaccharide-induced BV2 microglia cells. Iran J Pharm Res. 2013; 12(1):165-74. [PMID] [PMCID]

[35] Hosomi N, Ban CR, Naya T, Takahashi T, Guo P, Song XY, et al. Tumor necrosis factor-alpha neutralization reduced cerebral edema through inhibition of matrix metalloproteinase production after transient focal cerebral ischemia. J Cereb Blood Flow Metab. 2005; 25(8):959-67. [DOI:10.1038/sj.jcbfm.9600086] [PMID]

[36] Swamy M, Suhaili D, Sirajudeen KN, Mustapha Z, Govindasamy C. Propolis ameliorates tumor nerosis factor-alpha, nitric oxide levels, caspase-3 and nitric oxide synthase activities in kainic acid mediated excitotoxicity in rat brain. Afr J Tradit Complement Altern Med. 2014; 11(5):48-53. [DOI:10.4314/ajtcam.v11i5.8] [PMID] [PMCID]

[37] Sala A, Recio MC, Schinella GR, Manez S, Giner RM, Cerda-Nicolas M, et al. Assessment of the anti-inflammatory activity and free radical scavenger activity of tiliroside. Eur J Pharmacol. 2003; 461(1):53-61. [DOI:10.1016/S0014-2999(02)02953-9] [PMID]

[38] Hämäläinen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E. Anti-inflammatory effects of flavonoids: Genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-κB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-κB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. Mediators Inflamm. 2007; 5:38-45. [DOI:10.1155/2007/45673] [PMID] [PMCID]

[39] Tahamtan M, Allahtavakoli M, Abbasnejad M, Roohbakhsh A, Taghipour Z, Taghavi M, et al. Exercise preconditioning improves behavioral functions following transient cerebral ischemia induced by 4-vessel occlusion (4-VO) in rats. Arch Iran Med. 2013; 16(12):697-704. [PMID]

[40] Balkaya M, Krober JM, Rex A, Endres M. Assessing post-stroke behavior in mouse models of focal ischemia. J Cereb Blood Flow Metab. 2013; 33(3):330-8. [DOI:10.1038/jcbfm.2012.185] [PMID] [PMCID]

[41] Thiyagarajan M, Fernández JA, Lane SM, Griffin JH, Zlokovic BV. Activated protein C promotes neovascularization and neurogenesis in postischemic brain via protease-activated receptor 1. J Neurosci. 2008; 28(48):12788-97. [DOI:10.1523/JNEUROSCI.3485-08.2008] [PMID] [PMCID]