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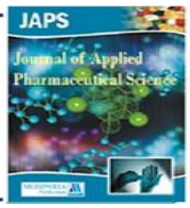

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Antioxidant and neuroprotective activities of essential oil, isolated from Chinese herb pairs of *Angelica sinensis* and *Sophora flavescens*

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ABSTRACT

The antioxidant and neuroprotective activities of essential oil (EO), isolated from Chinese herb pairs of *Angelica sinensis* and *Sophora flavescens* were studied in this paper. EO significantly improved the outcome in rats after cerebral ischemia and reperfusion in terms of neurobehavioral function. EO treatment decreased the levels of tumor necrosis factor alpha, interleukin 1 beta and oxidative stress. Our results had showed that EO was a potent neuroprotective medicine.

Keywords: Angelica sinensis; Sophora flavescens; Chinese herb pairs; essential oil.

INTRODUCTION

Many Chinese therapeutic herbs that are traditionally used in combination demonstrate significantly better pharmacological effects when used in the combination than when used alone. *Angelica sinensis* and *Sophora flavescens* constitute one traditional Chinese herb pairs (ASHP), which are the basic unit in traditional Chinese prescriptions and consist of two relatively standard single herbs. Although ASHP has been commonly used as a prescription for anti-inflammatory, free radical scavenging and antimicrobial activities for many years (Chiu *et al*., 2004, Kuroyanagi *et al*., 1999, Hwang *et al*., 2005), there is no research on mechanisms of its effects and chemical composition of ASHP. Volatile chemicals present in natural plants have been widely used in aroma therapy since ancient times, suggesting that they have some beneficial health effects in addition to their pleasant odor. There have been many reports on the volatile chemicals in plants because they inhibit oxidative damage and consequently prevent diseases, such as cancer (Huang *et al*., 1999), leukemia (Devi *et al*., 2000), aging (Butterfield *et al*., 1999), atherosclerosis (Steinbrecher *et al*., 1990) and rheumatoid arthritis (Jira *et al*., 1997). In this study, the essential oil of ASHP was studied for its antioxidant and neuroprotective activities as well as its chemical composition.

EXPERIMENTAL

Preparation of essential oil (EO)

Roots of *Angelica sinensis* and *Sophora flavescens* were collected from Gansu Province, China, in October 2009, and were identified by Professor Zhou. The voucher specimens (039-26 and 046-69) were deposited at the herbarium of Shandong University of Traditional Chinese Medicine. Fresh Roots of *Angelica sinensis* (100 g) and *Sophora flavescens* (100 g) were subjected to hydrodistillation using a modified clevenger-type apparatus for 3 h, dried over anhydrous sodium sulphate and transferred into amber-colored vials at 5°C for further work. Essential oil was stored in an airtight container prior to analysis by gas chromatography-mass spectrometry.

Experimental design

Wistar rats (2 months old and weighing 225 ± 25 g) were used in the study. This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals. Care was taken to minimize discomfort, distress, and pain to the animals. The animals were separated into five groups of ten rats each. The first group served as sham (SHAM). The second group was the ischemic group (MCAO). Group I and group II were treated orally by distilled water for 30 days respectively. Group III (EO-20), Group Ⅳ (EO -40) and Group Ⅴ (EO-80) were treated orally by EO (20, 40and 80 mg/kg/day respectively) for 30 days followed by MCAO induced cerebral ischemia. The right middle cerebral artery occlusion (MCAO) was performed using an intraluminal filament model and the method described by Longa *et al.* (Longa *et al.*, 1989). In sham rats, the ECA was surgically prepared for the insertion of the filament, but the filament was not inserted.

Neurobehavioral test

The sensorimotor integrity was conducted to assess the neurobehavior at 24 h after MCAO in rats (Lee *et al*., 2002）. Five categories of motor neurological findings were scored: 0, no observable deficit; 1, forelimb flexion; 2, forelimb flexion and decreased resistance to lateral push; 3, forelimb flexion, decreased resistance to lateral push and unilateral circling; 4, forelimb flexion, unable or difficult to ambulate.

Grip strength study

Grip strength in all the animals was measured for evaluation of neuromuscular strength, as described by Ali *et al.* (Ali *et al*., 2004）. The neuromuscular strength tests were carried out between 9:00 a.m. to 4:00 p.m. under standard laboratory conditions.

Estimation of inflammatory cells and inflammatory mediator estimations

Inflammatory processes not only have fundamental roles in the pathophysiology of cerebral ischaemia but also are considered to be a risk or trigger factor for human stroke (Lindsberg, *et al.*, 2003). Protein concentration of IL-1β and TNF-α were measured by the way introduced by Z. Cai (Cai *et al*., 2003) using a commercial ELISA kit (Shanghai Jinma Biological Technology, Inc., China) following the manufacture's instruction.

Estimation of oxidative stress

In serum, lactate dehydrogenase (LDH) was estimated using a method described by Lum *et al* (Lum G *et al*., 1974). Brains were used for the assay of glutathione (GSH) content, Lipid peroxidation (LPO), glutathione peroxidase (GPx) activity, glutathione reductase (GR) activity, catalase (CAT) activity, Na^+K^+ATPase activity and glutathione S transferase (GST) activity.

RESULTS AND DISCUSSION

Volatile chemicals identified

Yield of the volatile extract from ASHP was 0.83 ± 0.03% (w/w). The essential oil was yellow with a sharp odor. Seventy-six constituents in the essential oil of ASHP were identified corresponding to 96.23% of the total oil. The main constituents in the ASHP essential oil were ligustilide (24.58%), 2-ethyl-1-hexanol (3.25%) and geranyl acetone (2.1%) respectively.

Neurobehavioral test and grip strength study

The behavioral tasks adopted in this study were designed to assess impairments consistent with the known functional architecture of the rat brain. Twenty-four hours after MCAO in rats, neurological deficit scores were significantly reduced in EO-40 -treated rats and EO-80 -treated rats. The neurobehavior for the SHAM group was 0.9 (0.6-1.1), the MCAO group was 3.9 (2.6-6.3), the EO-20 group was 3.4 (2.7-4.8), the EO-40 group was 2.1 (0.6-4.2) and the EO-40 group was 1.2 (1.0-3.8). It is clear that the behavioral abnormality was significantly developed in the MCAO group as compared with the sham (**Figure 1**). In contrast, the EO-40 group and EO-80 significantly suppressed the development of behavioral abnormality as compared with the MCAO group (**Figure 1**).

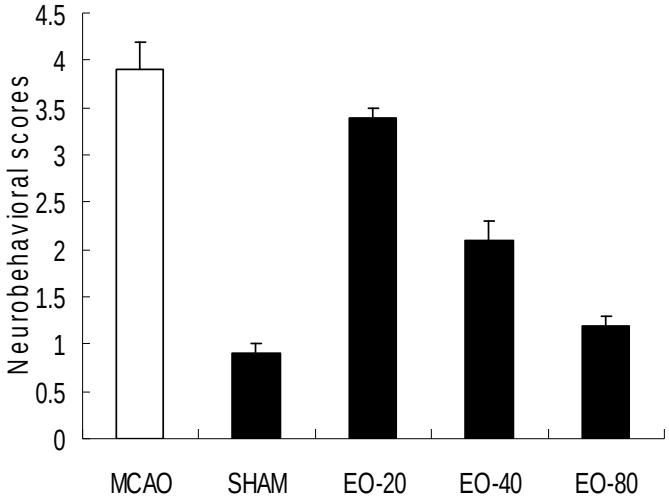

Fig. 1: Effect of EO on the Development of Behavioral Abnormalities after middle cerebral artery occlusion.
Values are shown as means ± SEM. *p < 0.05 vs. inflammatory group, **p <0.01 vs. inflammatory group

The grip strength in the SHAM group was found to be 0.960 ± 0.003 kg units. A significant decrease in the grip strength was observed in the MCAO group, as compared to the sham rats (P < 0.01). EO-40 and EO-80 treated rats showed a significant increase in grip strength, as compared to the MCAO group (P < 0.01) (**Table 1**).

Table. 1: Effect of EO on basal grip strength.

Different groups	Grip strength (Kg Units)	P value
MCAO group	0.587 ± 0.002 [b]	
SHAM group	0.960 ± 0.003 [a]	0.001
EO-20 group	0.661 ± 0.022[b]	0.438
EO-40 group	0.778 ± 0.015[a]	0.005
EO-80 group	0.890 ± 0.010[a]	0.003

Values are shown as means ± SEM. The different letters in the same column indicate a statistical difference (p<0.01 vs. MCAO group).

Estimation of inflammatory cells and inflammatory mediator estimations

Cytokines are upregulated in the brain in a variety of diseases, including ischaemic brain injury. Among these inflammatory mediators, IL-1β and TNF-α are of particular importance because they play a major role in coordinating mechanisms that command pro-inflammation. **Figure 2** shows that cerebral ischemia/reperfusion significantly increased protein concentration of IL-1β and TNF-α in the brain. EO-40 and EO-80 treatment decreased the level of IL-1β and TNF-α as compared to the MCAO group respectively. However the same result did not occur in the EO-20 treated group.

Estimation of oxidative stress

Increasing evidence has indicated that ischemia/reperfusion occurs due to oxidative stress that may potentiate ischemic injury (Traystman *et al*, 1991). In this study, a significant increase in the activity of LDH and LPO was observed in MCAO group, as compared to the SHAM group (P < 0.01, P<0.001 respectively); whereas, EO-40 and EO-80 treatment significantly (P < 0.05, P<0.01 respectively) resulted in decreased LDH and LPO levels when compared with MCAO group rats (Table 2). Concentrations of GSH were lower in MCAO group than those in SHAM group (Table 2). EO produced the increase in the level of GSH. The activity of endogenous antioxidant enzymes was decreased significantly (P < 0.01) in the MCAO group, as compared to the sham group, whereas in the EO-40 and EO-80 group, EO-treatment showed a significant (P<0.05–0.01) restoration in the level of various enzyme as compared with MCAO group (Table 2).

Table. 2: Effect of EO on LDH, GSH and LPO levels and the activity of various enzymes.

Different groups	LDH (IU/L)	GSH (nmol /mg pro.)	LPO (nmol /mg pro.)	GPx	GR	GST	CAT	Na^+K^+ATPase
SHAM	85.322± 2.660**	1.833± 0.016*	14.33 ± 0.56***	16.00±2.23**	36.56±2.56**	17.22±1.00**	7.23±0.33**	4.55±0.62*
MCAO	171.211 ± 3.711	1.112 ± 0.011	20.01 ± 1.41	7.89±0.33	21.11±2.23	9.07±1.11	4.66±0.10	2.22±0.23
EO-20	152.622 ± 4.121	1.401 ± 0.022*	19.82 ± 1.22	7.16±0.32	24.31±2.02	10.60±0.66	4.88±0.32	3.00 ±0.31
EO-40	122.603 ± 4.221*	1.500± 0.021*	17.00 ± 2.23*	13.22±1.33*	26.55±2.11**	15.10±1.12*	5.45±0.55*	4.30 ±0.31*
EO-80	91.566 ± 3.333**	1.500 ± 0.033*	14.30 ± 3.21*	14.11±1.12**	30.21±6.03**	17.66±2.33**	6.66±0.44*	4.00 ±0.20*

Values are shown as means ± SEM. *p < 0.05 vs. MCAO group, **p<0.01 vs. MCAO group, ***p<0.001 vs. MCAO group.

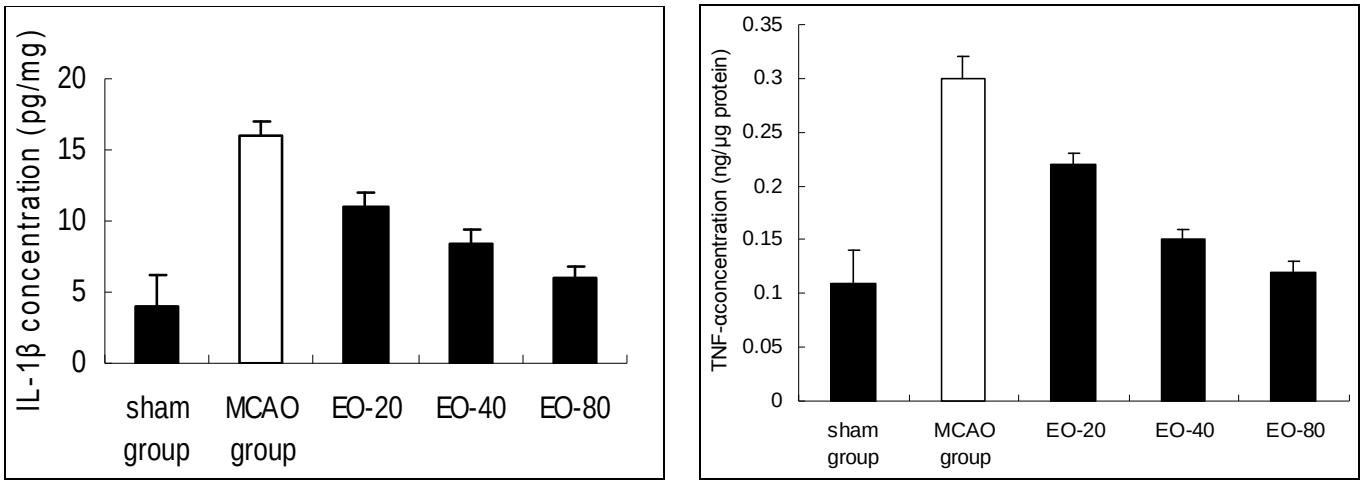

Fig. 2: Effect of EO on cytokines levels.
Values are shown as means ± SEM. *p < 0.05 vs. inflammatory group, **p <0.01 vs. inflammatory group

CONCLUSIONS

Here we showed supplementation of EO significantly boosted the defense mechanism against cerebral ischemia by increasing antioxidants activity related to lesion pathogenesis. Restoration of the antioxidant homeostasis in the brain after reperfusion may have helped the brain recover from ischemic injury.

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