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EFFECTS OF ORAL FERULAGO ANGULATE HYDROALCOHOLIC EXTRACT ON LIPID PEROXIDATION INDUCED BY ISCHEMIA / HYPOPERFUSION IN THE HIPPOCAMPUS RAT

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ABSTRACT

Cerebral ischemia leads to neuronal death of invulnerable sections of brain which is due to free radical production and oxidative damage. This study aimed to evaluate the effect of 14 days of oral administration of Ferulago angulate hydroalcoholic extract (FAE) (100, 200 and 400 mg/pkg) on brain oxidative stress indices after permanent bilateral common carotid artery occlusion or ischemia/hypoperfusion in male adult rats. A number of 35 rats were divided into test and control groups. To make animal model of permanent cerebral hypo perfusion/ischemia, carotid arteries were ligatured as upper and lower and cut bilaterally. Rat brain tissue was extracted to separate hippocampus and measure malondialdehyde. The results showed that ischemia/hypoperfusion increased brain oxidants such as lipid per oxidation (LPO) ($p<0.001$). Post ischemic FAE treatment significantly reduced LPO in hippocampus ($p<0.001$). FAE administration could remove or scavenge oxidants from brain tissue and improve its function. They could be possibly used as a way for treating this disease.

Keywords: Cerebral Ischemia, FAE, LPO, Hippocampus, Rat

INTRODUCTION

Global cerebral ischemia is a condition in which insufficient blood and oxygen reaches all or parts of the brain; therefore, normal functioning of neurons is disrupted **[1]**. Two-vessel occlusion model of ischemia is associated with common carotid artery ligation **[1]** and lowers blood pressure in rats to approximately 50 mmHg and blood flow by to only 15% at control levels. Permanent bilateral common carotid artery occlusion is a model for chronic cerebral hypoperfusion that is associated with neurodegenerative diseases **[2]**. In normal conditions there is a balance between production and elimination of free radicals; imbalance in this process leads to oxidative stress and incidence of several pathological changes at level of cellular macromolecules **[3]**.

A destructive aspect of oxidative stress is production of reactive oxygen species (ROS) which include free radicals and peroxides and their reduction leads to increase in longevity **[4]**. Disruptions of the oxidation state of toxic effects resulting from production of peroxides and free radicals causes damage to all cellular components, including proteins, lipids and DNA **[5-7]**. Lipid peroxidation refers to the oxidative damage of lipids. Removal of electrons from the lipids in cell membranes caused by free radicals leads to cell defects. Reactive oxygen species reduces highly unsaturated lipids and produces malondialdehyde (MDA) **[7]**. MDA organic compound with formula CH 2 (CHO) 2 is a reactive aldehyde that is presented as a final product of lipid peroxidation **[8]**. Ferulago angulata (FA) belonging to the family of Apiaceae (Umbllifererae) is a natural and native plant which grows in western part of Iran. Except Iran, it grows in other countries, including Turkey, Greece, Serbia and Macedonia **[9]**.

Ferulago angulata is an herb that contains phenolic compounds **[10]**. FA, also known as Chovir, is one of the indigenous plants used as Persian medicinal herbs **[11, 12]**. Ferulago angulata extract (FAE) is composed of ferulagone, β-hydroxy-13- epi-manoyl oxide, α-pinene, 2,5-dimethoxy-p-cymene, pcymene, methyl carvacrol, transchrysanthenyl acetate, γ- terpinene (Z)-β- ocimene, α-pinene, myrcene, (Z)-β-ocimene, terpinolene, 2,4,5 trimethylbenzaldehyde and α- phellandrene predominantly **[13]**. This plant has been used ethnically as sedutive, tonic, and remedy of digestive pains, aphrodisiac properties, chronic ulcers, snakebites and headache **[9, 12]**. FAE has antioxidant and anti-diabetic properties **[14]**. According to antioxidant

compounds extracted from Ferulago angulata, it was decided to investigate effects of Ferulago angulate hydroalcoholic extract on factors arising from oxidative stress such as lipid peroxidation in the hippocampal tissue in animal models of permanent global ischemia by bilaterally cutting arteries.

MATERIAL AND METHOD

Animals and Experimental Procedure: Thirty-five adult male Wistar rats (220±30g) were obtained from Central Animal House of Jundishapur University of Medical Sciences, Ahvaz, Iran. They were housed individually in standard cages and maintained in a temperature- controlled room (21 ± 2°C) on a 12/12-h light/dark cycle, humidity (of 50-55%) with food and water available ad libitum. The rats were randomly divided to five equal numbered groups of 7 in each: 1) control; 2) ischemic group submitted to occlusion of both common carotids arteries (ischemia); 3) ischemic animals receiving 14 days of FAE (100 mg/kg, orally) (ischemia+ FAE100); 4) ischemic animals receiving 14 days of FAE (200 mg/kg, orally) (ischemia+ FAE200); 5) ischemic animals receiving 14 days of FAE (400 mg/kg, orally) (ischemia+ FAE400).

Two-Vessel Occlusion Procedure

Cechetti's method, **[15]** with a little modification was used. In summary, the rats were anesthetized by ketamine/xylazine (50/5mg/kg, i.p). A neck ventral midline incision was made and the common carotid arteries were then exposed and gently separated from the vagus nerve. Carotids were occluded at a 1-week interval between the interventions, the right common carotid being the first to be assessed and the left one being occluded 1 week later **[15]**.

FAE Preparation

FA plants were collected from the western parts of Iran (Khozestan). Miss Ghayour from Department of Botany, Islamic Azad University, Izeh Branch, Khozestan, Iran identified the plant. The aerial parts of the plant were separated, air dried in shade for one week and milled to fine powder (electric mill, Panasonic Co. Japan). The powder was macerated in 75% ethanol for 72 h at room temperature. The ethanol extract was evaporated to remove ethanol and FAE was obtained as a lyophilized powder (yield 35%) **[16]**.

Brain Sample Collection and Biochemical Assays

At the end of experiments, the animals were decapitated and the hippocampus was removed quickly and rinsed with saline. The tissues were Immediately homogenized in cold KCl solution (1.5%) to obtain 10% homogenate suspension used for measuring

thiobarbituric acid reactive substances (TBARS) value, expressed as malondialdehyde equivalents (MDA) **[17] (Figure 1)**.

Data Analysis

The data were expressed as mean ± SEM. Significance level was determined by one-way ANOVA applying LSD's post-hoc test (SPSS, 18). A value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

In this study, in which effect of FAE was studied on antioxidant status and lipid peroxidation in animal models of ischemia, malondialdehyde (MDA) significantly increased in hippocampus ($p<0.001$) in the ischemic group compared with the control. The observations showed that malondialdehyde in hippocampus tissue in ischemia group treated with three doses (100, 200 and 400 mg/kg) of FAE significantly reduced ($p <0.001$) compared with the ischemia group **(Figure 2)**. Given that studies have shown that therapeutic effects of extract of many herbs have anti-oxidant compounds **[16]**, then the observed results were probably due to the existence of material in FA extract. ROS production and oxidative stress are known to cause disturbance in brain function in neurodegenerative diseases, including ischemia **[1]**.

Cerebral ischemia may trigger a signaling cascade, leading to production of free radicals, and oxidative stress depends on severity and duration of complications **[1]** and leads to the oxidation of lipids and proteins in the central nervous system, and which is followed by cell damage **[1]**. The difference between the amounts of ROS produced in any area of the brain might be related to oxygen consumption **[18]** and regions of the hippocampus are highly sensitive to oxygen deficiency **[19]**. Oxidative stress not only increases production of free radicals, but also reduces cellular antioxidant mechanisms **[8]**. Evaluation of oxidative stress is done using different indicators, one of which is the end products of lipid peroxidation (MDA). MDA with thiobarbituric acid (TBA) creates a red fluorescent derivative that is measured by spectrophotometrically **[8]**. Recent studies have focused on the role of antioxidants in the treatment of nervous system disorders and possibly ischemia, which is based on the assumption that if oxidative stress occurs when there is an imbalance between free radical production and antioxidant availability, administration of supplemental antioxidants may be used to sweep radicals and change disease development, progression or both. Recently, health food or nutritional

supplements have attracted much of public attention. Ferulago angulata is a traditional medicinal plants which is used in Iran for many purposes, particularly for gastrointestinal disorders and analgesia **[20]**.

The essential oil of aerial parts of this plant contains a variety of components with different therapeutic effects **[11]**. FAE was found to be selectively time-and dose-dependent inhibit proliferation of lymphoma and leukemic cells probably via an apoptosis-dependent pathway **[12]**. The essential oil of FA showed stronger antimicrobial activity **[14, 21]**. Khanahmadi (2006) reported that essential oil and extract of FA decreased peroxide value in oil; but, it was shown in, contrast that extract of FA gained better results **[22]**. Antioxidant activity of FAE was measured using two different tests of ferric thiocyanate method and thiobarbituric acid by Khanahmadi (2010) **[23]**. Their results indicated that methanol extracts of leaves, both male and female, and fruits of FA possessed antioxidant activity when tested using both methods **[23]**. Because of antioxidant activity of FAE that may be due to the presence of phenolic, especially polyphenolic compounds, the extracts have the potential to be used as a natural antioxidant agent in food and pharmaceutical industries **[10]**. There is a linear correlation between phenolic content and antioxidant activity of plant extracts and phenolic compounds have antioxidant activity and protect cells from oxidative reactions **[24]**. Powerful antioxidant activity is associated with free radical trapping molecules (hydroxyl-radical lipid molecules, iron and lipid peroxides) and delaying lipid oxidation **[25]**.

Functional disruptions after brain ischemia due to the damage caused to brain cells are a result of oxidant production. Therefore, FA with the ability of removing oxidant material from specific areas of the brain probably resulted in reduction of oxidative stress in ischemic hypoperfusion. Conclusion: The present results showed for the first time that different doses of FAE were able to reduce malondialdehyde as an indicator of lipid peroxidation in ischemic hypoperfusion model.

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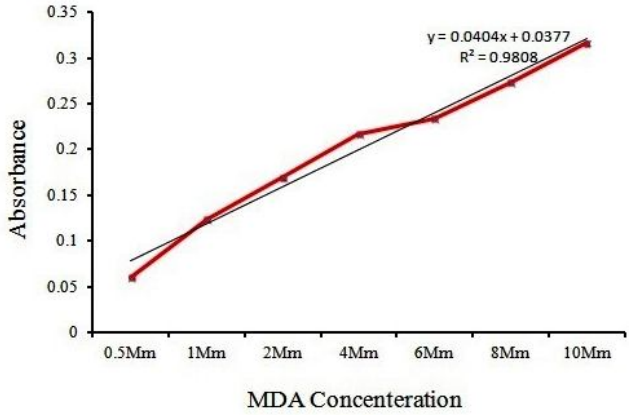

Figure 1: The Standard Curve of MDA was Constructed Over the Concentration Range of 0–10 µM

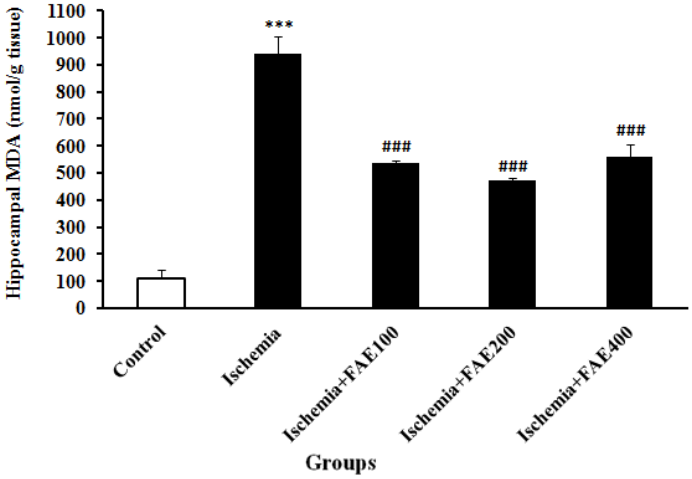

Figure 2: Mean ± SD of MDA Between Control Group, Ischemia and Ischemic Group Orally Receiving 100, 200 and 400 mg/kg FAE for 14 Days