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Research Article

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Caffeic Acid Ameliorates Cerebral Ischemic Damage via Altering Lipid Peroxidation, Antioxidant, and Bio-metal Levels

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Abstract: Caffeic acid is one of the bioactive compounds and is reported to exert multi-beneficial functions, especially regarding anti-oxidation and anti-inflammation. Ischemic stroke is one of the leading causes of death in the older population. The bio-metals magnesium (Mg), zinc (Zn) and antioxidant enzymes of superoxide dismutase (SOD) and catalase (CAT) are essential substance for brain to maintain normal physiological functions. In the present study, we explore whether caffeic acid, a constituent, is related to prevention against cerebral ischemic damage caused by one hour of middle cerebral artery (MCA) occlusion and whether its protective mechanism is correlated with altering the level of bio-metal, antioxidant enzyme, and reactive oxygen species (ROS)-induced lipid peroxidation. Significantly, experimental results showed that caffeic acid not only attenuates ROS-mediated lipid peroxidation status, as represented by a decreased MDA concentration, but also enhances antioxidant activity of SOD, CAT, and the bio-metal concentration of Mg and Zn in the ischemic brain cortex. Altogether, it is of note that caffeic acid can potentially ameliorate cerebral ischemic injury.

Keywords: Caffeic acid, cerebral ischemia, bio-metal, antioxidant, lipid peroxidation

1. Introduction

Caffeic acid is a bioactive compound classified as a hydroxycinnamic acid and an organic component composed of the functional group of polyphenol [1]. Also, the caffeic acid constituent is widely distributed in fruits and plants such as blueberries, apples, blue eucalyptus bark, barley, freshwater ferns, and mushrooms [2-5]. In addition, caffeic acid exists not only in brewed coffee and red wine beverages but also in Chinese herbal medicines from the mint family, such as sage, thymoquinone, ceylon cinnamon, and star anise [6-8]. Experimental studies have suggested that caffeic acid exerts anti-inflammatory and antioxidant activities on living organisms [9, 10]. Furthermore, toxicological investigation has evidenced that caffeic acid is superior to other antioxidants because it can decrease 95% of cellular oxidative stress and toxicity caused by Aspergillus and aflatoxin [11]. Specifically, it is worth noting that cytological research has evidenced that caffeic acid has no cytotoxicity to normal cells [11]. Similarly, neuroscience research has shown that caffeic acid markedly attenuates oxidative stress, thus reducing the severity of neurological disorders, including atherosclerosis and cardiovascular diseases [10-11]. Ischemic stroke is the second leading cause of death in the United States [12]. In addition, ischemic events may generate numerous reactive

oxygen species (ROS), such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals. These ROS spontaneously attack the polyunsaturated fatty acid (PUFA) component in cells via lipid peroxidation processes. Under these circumstances, oxidative stress and injury occur [12].

Recently, bio-metals have received more attention due to their positive biological effects [13]. Among them, magnesium (Mg) and zinc (Zn) achieve more attraction due to their anti-inflammatory and innate antioxidant efficacies [14-15]. Proper Mg and Zn levels are helpful for cells to reduce ROS-induced oxidative brain damage [14-17]. The preceding in vivo experiment has indicated that Zn can reduce lipid peroxidation effects [14]. On the contrary, another animal study has suggested that decreased Zn level is responsible for cerebral vascular disease such as stroke [12, 14].

It is known that superoxide dismutase (SOD) and catalase (CAT) are two important antioxidant enzymes to protect from ROS attack in the situations of ischemic stroke and coronary artery disease [12, 18]. Therefore, decreasing both enzyme activities represents increased oxidative stress and oxidative devastation. This study aimed to elucidate whether caffeic acid can attenuate cerebral ischemia-induced oxidative injury and whether its protective mechanism is related to the modulations of the levels of bio-metals, antioxidant activity, and lipid peroxidation.

2. Materials and Methods

Animal grouping, cerebral ischemic surgery, and brain cortex harvest

Forty male Sprague-Dawley rats, weighing from 200-250 g, were enrolled in this research. All rats were housed under controlled conditions in the animal room (22 ± 2°C, 50 ± 20% relative humidity, 12-h light-dark cycle). The experimental rats were randomly divided into four groups of 10 each as below: control (rats were treated with physiological saline once in a day for 10 days); ligation (rats were treated with physiological saline once in a day for 10 days before ligation of right middle cerebral artery (RMCA) for 1 hour); caffeic acid (rats were intraperitoneally injected with caffeic acid at the dosage of 100 mg/Kg once in a day for consecutive 10 days); and prevention (rats were pretreated with caffeic acid at the dosage of 100 mg/Kg once in a day for 10 days followed by ligation of the RMCA for 1 hour). All rats were sacrificed on day 11 and the cerebral cortex was harvested for further analysis. Experimentally, all animal-used protocols listed and mentioned above have been approved in advance by the Institutional Animal Care and Use Committee (IACUC) of Central Taiwan University of Science and Technology.

Measurement of the malondialdehyde (MDA) concentration in brain cortex

The malondialdehyde (MDA) concentration, an end-product of lipid peroxidation, was measured to evaluate the intensity of ROS-mediated oxidative brain injury. Firstly, 0.2 g of the right brain cortex tissue was pipetted into Pyrex tube with 4.8 ml of the cold H3PO4 solution (1% w/v) and adding 1 ml of the TBA reagent followed by boiling at 100 ℃ for 1 hour. Secondly, 4 ml of the butanol was added followed by centrifuging at 1600 g for 5 minutes. The supernatant was collected and the MDA level was measured by spectrophotometry (U-1900, Hitachi, Japan) at the wavelength of 532 nm.

Bio-metal concentration analysis in brain cortex

Experimentally, 0.2g of the cerebral cortex tissues was taken to analyze the bio-metal concentration of Mg and Zn. The standard solutions of both bio-metals were dissolved in the concentration of 0.1 mol/L nitric acid solution purchased from Merck, Germany. The Mg and Zn levels in the cerebral cortex were measured via SavantAA Z graphite furnace atomic absorption spectrophotometer (GBC Scientific Equipment Pty Ltd., Melbourne, Australia) and longitudinal Zeeman Effect background correction system throughout the experiment.

Detection of antioxidant enzyme activity in brain cortex

The antioxidant activity of SOD was measured according to the commercial kit purchased from Cayman Chemical Company; USA. The assay principle is that xanthine oxidase reacts with the hypoxanthine to produce superoxide radicals (O2-). Superoxide radicals interacted with tetrazolium salts and the SOD activity was measured using a spectrophotometer (Thermo Scientific Multiskan Spectrum; USA). On the other hand, CAT activity was measured using a commercial catalase reagent kit (Cayman Chemical Company; USA). In short, the principle is to utilize the reaction of methanol with hydrogen, and hydrogen peroxide (H2O2) generates formaldehyde under the catalysis of

CAT. Finally, the chromogen of 4-amino-3-hydrazino-5-mercapto-1, 2, 4-triazole was reacted with formaldehyde and CAT and measured by spectrophotometer obtained from the Thermo Scientific Multiskan Spectrum; USA.

Statistical analysis for all analytical parameters

All the obtained data were expressed as mean ± S.D. The experimental results were analyzed by statistical method of Kruskal-Wallis one-way analysis of variance (ANOVA). If the analyzed values showed significant differences among groups, each group was compared using the Fisher's Least Significant Difference (FLSD) test. The significant differences were considered significantly at a P-value of less than 0.05. a: $P < 0.05$, vs. control group; b: $P < 0.05$, vs. ligation subject.

3. Results

The results of the study showed that caffeic acid significantly reduced the lipid peroxidation status in the prevention group as compared to the ligation subject (Figure 1)

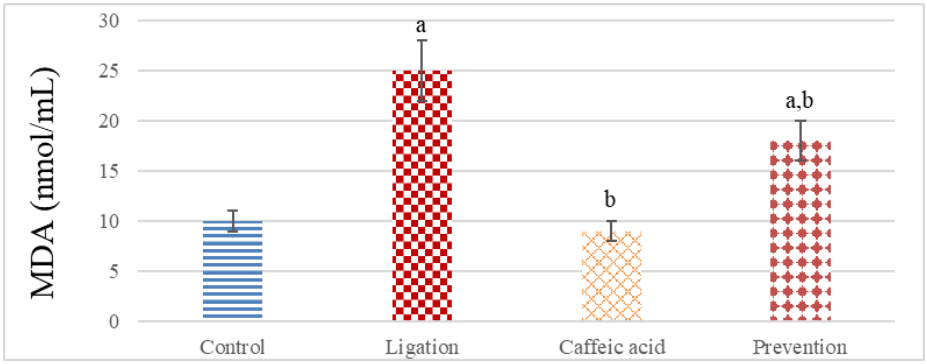

Figure 1: Profiles of the MDA level in the brain cortex. Data were expressed as mean ± S.D.

(N=40). The statistical method of One-way ANOVA followed by Least Significant Difference test was used. a: Significant difference ($p < 0.05$) from the control subjects. b: Significant difference ($p < 0.05$) from the ligation group.

The antioxidant activity of SOD was markedly enhanced in the prevention group as compared to the ligation subject as showed in Figure 2.

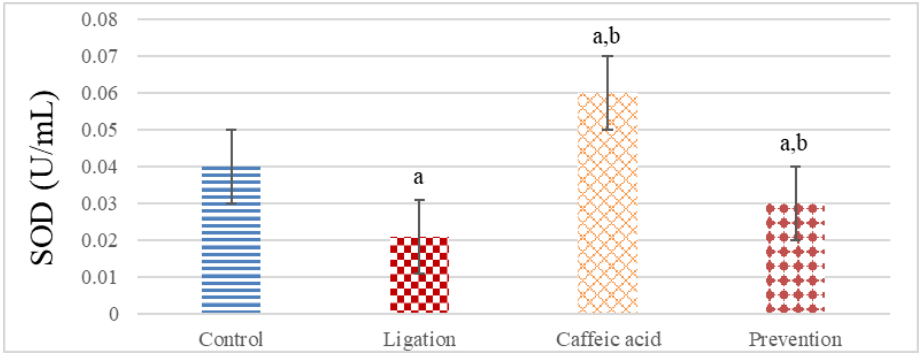

Figure 2: Profiles of the SOD activity in the brain cortex. Data were expressed as mean ± S.D.

(N=40). The statistical method of One-way ANOVA followed by Least Significant Difference test was used. a: Significant difference ($p < 0.05$) from the control subjects. b: Significant difference ($p < 0.05$) from the ligation group.

The antioxidant activity of CAT was obviously elevated in the prevention group as compared to the ligation subject (Figure 3).

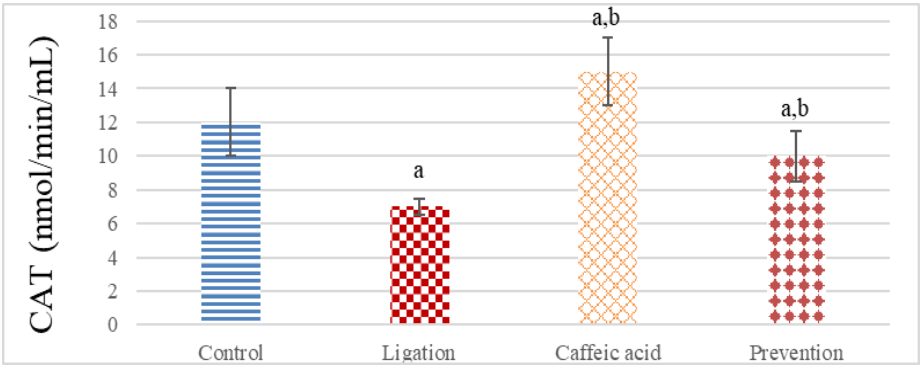

Figure 3: Profiles of the CAT activity in the brain cortex. Data were expressed as mean ± S.D.

test was used. a: Significant difference ($p < 0.05$) from the control subjects. b: Significant difference ($p < 0.05$) from the ligation group.

The bio-metal concentration of Mg in the prevention group was prominently higher than that of the ligation group as listed in Figure 4.

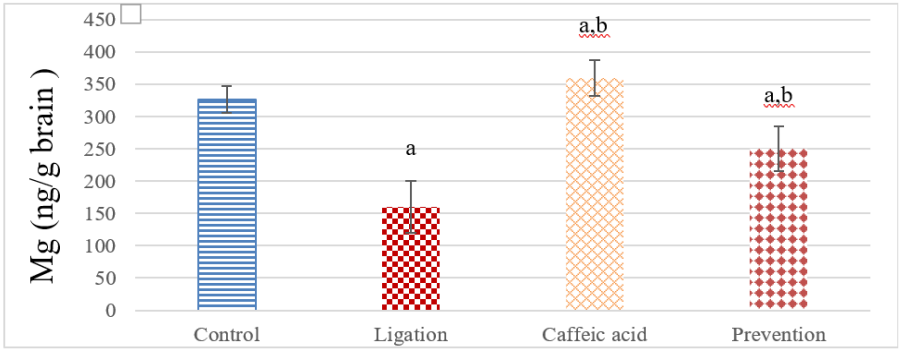

Figure 4: Profiles of the Mg concentration in the brain cortex. Data were expressed as

mean ± S.D. (N=40). The statistical method of One-way ANOVA followed by Least Significant

Difference test was used. a: Significant difference ($p < 0.05$) from the control subjects. b: Significant difference ($p < 0.05$) from the ligation group.

The bio-metal concentration of Zn in the prevention group was significantly higher than that of the ligation group as listed in Figure 5.

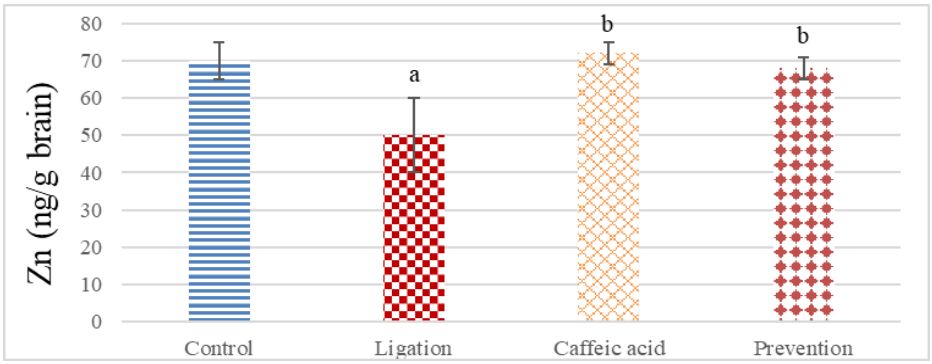

Figure 5: Profiles of the Zn concentration in the brain cortex. Data were expressed as

mean ± S.D. (N=40). The statistical method of One-way ANOVA followed by Least Significant Difference test was used. a: Significant difference ($p < 0.05$) from the control subjects. b: Significant difference ($p < 0.05$) from the ligation group.

4. Discussion

Our experimental findings suggest that caffeic acid is not only related to decreasing the ischemic stroke-induced lipid peroxidation but also is correlated with increasing the bio-metal concentration of Mg, Zn as well as the antioxidant activity of SOD and CAT in the ischemic cerebral cortex. Studies have demonstrated that caffeic acid is one of the polyphenol constituents that widely exist in a variety of plants and food sources such as apples, coffee, blueberries, and cider [1]. Meanwhile, caffeic acid possesses multiple beneficial characteristics in anti-oxidation and anti-inflammation [1, 11]. Inflammation is mainly post-stroke damage followed by results in further cell death after ischemic stroke. The caffeic acid has indeed gained remarkable attention recently for its contribution to powerful neuroprotection against a variety of neurological disorders, such as cardiovascular diseases, stroke, and atherosclerosis [2-4, 11]. Cerebral ischemia is the leading cause of disability and mortality and increases with age globally [12]. Ischemic stroke may generate a large amount of toxic ROS. It is also recognized that brain tissue is susceptible to reactive oxygen species (ROS) attack. These deleterious ROS can spontaneously oxidize the constituent of the polyunsaturated fatty acid (PUFA) in the brain cells, thus initiating further lipid peroxidation effects and producing a carcinogenetic product, so-called malondialdehyde (MDA) [12, 17]. Given this fact, increased oxidative stress may eventually result in further cellular injury. The antioxidant property of caffeic acid is indeed reported to be correlated with the structure of the two hydroxyl groups on its aromatic ring [2, 3, 6-7]. Also, this chemical structure is useful to eliminate free radicals and prevent ROS formation. Experimentally, our present result follows the preceding study, evidencing that pretreating rats with caffeic acid before ischemic damage significantly decreases ROS-mediated lipid peroxidation [9].

Oxidative stress occurs during ischemic injury when there is an imbalance between ROS production and the capacity of antioxidant enzymes to eliminate ROS. In normal situations, maintaining the balance between the antioxidant system and ROS levels in the brain is crucial for protecting brain tissue from ROS attack [12, 17]. Antioxidant enzymes can inhibit or attenuate the adverse effects triggered by ROS to protect the cells from oxidative injury. Once an imbalance is observed between elevated free radical-generating and decreased radical-scavenging, oxidative stress occurs [18, 19]. Under these circumstances, cellular integrity is damaged due to the harmful lipid peroxidation pathway, thus, further oxidative cellular injury occurs. In the resent study, the antioxidant SOD can convert toxic superoxide radical into the hydrogen peroxide and the CAT is capable of detoxifying hydrogen peroxide into non-toxic water and oxygen [19, 20]. In the current study, the SOD and CAT activities were significantly increased in the prevention group as compared to the ischemic subject. Our previous studies have proposed that cerebral ischemia may result in a decreased SOD and CAT activities, and our present finding is in agreement with the previous result [20-21]. As stated, the caffeic acid has recently gained remarkable attention based on its beneficial characteristics of anti-oxidation and anti-inflammation to provide a strong neuroprotection [1, 7, 9]. Importantly, our current finding indicates that pretreating rats with caffeic acid before ischemic injury significantly increases both the SOD and CAT activities. Also, it is consequently suggested here that caffeic acid possesses the ability to enhance antioxidant activities and, crucially, this positive effect, at least in part, is useful for the ischemic brain to obviously ameliorate ROS-induced oxidative stress and further injury in the ischemic brain.

Bio-metal magnesium (Mg) and zinc (Zn) are necessary for brain tissues to maintain normal physiological functions [12-15]. Previous research has proposed that Mg and Zn have innate anti-inflammatory and antioxidant abilities [12, 13]. The in vivo study indicates that Mg possesses profound beneficial effects, such as reducing ROS-induced lipid peroxidation, maintaining cell membrane integrity, and attenuating the inflammatory impact [17]. Former research showed that pesticide paraquat-induced oxidative lung injury can be markedly improved by treating rats with magnesium isoglycyrrhizinate [16]. In addition, clinical research reveals that intravenous administration of stroke patients with magnesium sulfate significantly attenuates cerebral injury [13]. Similarly, an in vivo study demonstrates that supplementation of fetal mice with magnesium sulfate not only can mitigate the MDA

concentration via attenuating lipid peroxidation effects but also can reduce the inflammatory effect [15]. Furthermore, animal studies indicated that restriction of the Mg intake in rats significantly enhances oxidative toxicity in organs [12-13]. On the other hand, experimental study suggests that essential bio-metal Zn can attenuate ROS-induced biological damage due to its powerful anti-inflammatory and antioxidant abilities [12, 14]. Conversely, reduction of the Zn level in living organisms is closely responsible for an increased oxidative injury [12]. In addition, a study proposes that depletion of Zn concentration is related to enhancing the inflammatory effect and together with an enhanced lipid peroxidation in rats [14]. Similarly, our previous experiment indicates the fact that a reduction in Zn concentration is responsible for the etiology of ischemic stroke in an animal model [12]. Altogether, our current data suggest that cerebral ischemic lesions may decline Mg and Zn levels, but specifically, pretreating rats with caffeic acid before the ischemic event markedly enhances Mg and Zn concentration in the ischemic cerebral cortex. Based on our experimental evidence, it is reasonable to conclude that caffeic acid is capable of increasing the Mg and Zn concentrations. In this regard, increased Mg and Zn levels may exert their innate anti-inflammatory and antioxidant capacities to attenuate cerebral ischemia-induced ROS. Given this fact, further ROS-mediated oxidative cerebral cortex injury is significantly ameliorated.

5. Conclusion

Taken all experimental findings together, we demonstrate that the protective effect of caffeic acid on the ischemic cerebral cortex is via the following mechanisms including (1) declining ROS-mediated lipid peroxidation effect as represented by an attenuated malondialdehyde (MDA) concentration; (2) enhancing the antioxidant activity of superoxide dismutase and catalase; and (3) increasing the magnesium and zinc bio-metal concentrations.

References

[1]. Boerjan, W., Ralph, J., & Baucher, M. (2003). Lignin biosynthesis. Annual review of plant biology, 54, 519–546.

[2]. Santos, S. A., Freire, C. S., Domingues, M. R., Silvestre, A. J., & Pascoal Neto, C. (2011). Characterization of phenolic components in polar extracts of Eucalyptus globulus Labill. bark by high-performance liquid chromatography-mass spectrometry. Journal of agricultural and food chemistry, 59(17), 9386–9393.

[3]. Pearson, Jarryd & Lee, Samiuela & Suresh, Harsha & Low, Mitchell & Nang, Marnilar & Singh, Swastika & Lamin, Franklin & Kazzem, Magdy & Sullivan, Shaun & Khoo, Cheang. (2014). The Liquid Chromatographic Determination of Chlorogenic and Caffeic Acids in Xu Duan (Dipsacus asperoides) Raw Herb. ISRN Analytical Chemistry. 2014. 1-6. 10.1155/2014/968314.

[4]. Choudhary, M. I., Naheed, N., Abbaskhan, A., Musharraf, S. G., Siddiqui, H., & Atta-Ur-Rahman (2008). Phenolic and other constituents of fresh water fern Salvinia molesta. Phytochemistry, 69(4), 1018–1023.

[5]. Lee, Y. S., Kang, Y. H., Jung, J. Y., Lee, S., Ohuchi, K., Shin, K. H., Kang, I. J., Park, J. H., Shin, H. K., & Lim, S. S. (2008). Protein glycation inhibitors from the fruiting body of Phellinus linteus. Biological & pharmaceutical bulletin, 31(10), 1968–1972.

[6]. Mattila, P., & Kumpulainen, J. (2002). Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. Journal of agricultural and food chemistry, 50(13), 3660–3667.

[7]. Neveu, V., Perez-Jiménez, J., Vos, F., Crespy, V., du Chaffaut, L., Mennen, L., Knox, C., Eisner, R., Cruz, J., Wishart, D., & Scalbert, A. (2010). Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. Database : the journal of biological databases and curation, 2010, bap024.

[8]. Caffeic acid. (1993). IARC monographs on the evaluation of carcinogenic risks to humans, 56, 115–134.

[9]. Rajendra Prasad, N., Karthikeyan, A., Karthikeyan, S., & Reddy, B. V. (2011). Inhibitory effect of caffeic acid on cancer cell proliferation by oxidative mechanism in human HT-1080 fibrosarcoma cell line. Molecular and cellular biochemistry, 349(1-2), 11–19.

[10]. Olthof, M. R., Hollman, P. C., & Katan, M. B. (2001). Chlorogenic acid and caffeic acid are absorbed in humans. The Journal of nutrition, 131(1), 66–71.

[11]. Basu Mallik, S., Mudgal, J., Nampoothiri, M., Hall, S., Dukie, S. A., Grant, G., Rao, C. M., & Arora, D. (2016). Caffeic acid attenuates lipopolysaccharide-induced sickness behaviour and neuroinflammation in mice. Neuroscience letters, 632, 218–223.

[12]. Fang, K. M., Cheng, F. C., Huang, Y. L., Chung, S. Y., Jian, Z. Y., & Lin, M. C. (2013). Trace element, antioxidant activity, and lipid peroxidation levels in brain cortex of gerbils after cerebral ischemic injury. Biological trace element research, 152(1), 66–74.

[13]. Afshari, D., Moradian, N., & Rezaei, M. (2013). Evaluation of the intravenous magnesium sulfate effect in clinical improvement of patients with acute ischemic stroke. Clinical neurology and neurosurgery, 115(4), 400–404.

[14]. Powell S. R. (2000). The antioxidant properties of zinc. The Journal of nutrition, 130(5S Suppl), 1447S–54S.

[15]. Burd, I., Breen, K., Friedman, A., Chai, J., & Elovitz, M. A. (2010). Magnesium sulfate reduces inflammation-associated brain injury in fetal mice. American journal of obstetrics and gynecology, 202(3), 292.e1–292.e2929.

[16]. Minakata, K., Suzuki, O., Saito, S., & Harada, N. (1998). Dietary Mg and/or K restriction enhances paraquat toxicity in rats. Archives of toxicology, 72(7), 450–453.

[17]. Lin, M. C., Huang, Y. L., Liu, H. W., Yang, D. Y., Lee, C. P., Yang, L. L., & Cheng, F. C. (2004). On-line microdialysis-graphite furnace atomic absorption spectrometry in the determination of brain magnesium levels in gerbils subjected to cerebral ischemia/reperfusion. Journal of the American College of Nutrition, 23(5), 561S–565S.

[18]. Saboori, S., Koohdani, F., Nematipour, E., Yousefi Rad, E., Saboor-Yaraghi, A. A., Javanbakht, M. H., Eshraghian, M. R., Ramezani, A., & Djalali, M. (2016). Beneficial effects of omega-3 and vitamin E coadministration on gene expression of SIRT1 and PGC1α and serum antioxidant enzymes in patients with coronary artery disease. Nutrition, metabolism, and cardiovascular diseases : NMCD, 26(6), 489–494.

[19]. Buettner G. R. (2011). Superoxide dismutase in redox biology: the roles of superoxide and hydrogen peroxide. Anti-cancer agents in medicinal chemistry, 11(4), 341–346.

[20]. Xu, D., Hu, M. J., Wang, Y. Q., & Cui, Y. L. (2019). Antioxidant Activities of Quercetin and Its Complexes for Medicinal Application. Molecules (Basel, Switzerland), 24(6), 1123.

[21]. Wang, D., Yuan, X., Liu, T., Liu, L., Hu, Y., Wang, Z., & Zheng, Q. (2012). Neuroprotective activity of lavender oil on transient focal cerebral ischemia in mice. Molecules (Basel, Switzerland), 17(8), 9803–9817.