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Effect of ellagic acid on oxidative stress duo to brain ischemia/hypoperfusion in male rat

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ABSTRACT

Background & Aim: Free radicals are produced in ischemic processes. Nerve damage caused by free radicals may play a role in neurological diseases and antioxidants are protective activity. Ellagic acid is a polyphenol compound with antioxidant properties which is found in fruits like pomegranate, blackberry, and all types of mulberry**.** This study aimed to evaluate the effect of 14 days of oral administration of ellagic acid (50 mg/kg) on brain oxidative stress indices after permanent bilateral common carotid artery occlusion or ischemia/hypoperfusion in male adult rats.

Experimental: A number of 30 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, and thiol (-Sh) groups.

Results & Discussion: Our results indicated that malondialdehyde and thiol in ischemia group has significantly increased ($p < 0.001$) than to control group. In addition, the rate of malondialdehyde and thiol in ischemia group receiving the ellagic acid has significantly decreased ($p < 0.001$)**,** than to ischemia group**.**

Recommended applications/industries: Ellagic acid possibly with powerful antioxidant properties, can improve the effect of ischemia such as production of free radicals.

1. Introduction

Ischemia means reducing cerebral blood flow (CBF) which damages the whole or part of brain (Rafiei Rad *et al*., 2011). 2-VO (Two Vessel Occlusion) model is an experimental model for chronic brain hypoperfusion, in which permanent occlusion of both common carotid arteries and their cutting occur (in rats) (Accardo-Palumbo *et al.,* 2010). In normal conditions, there is a balance between production and elimination of free radicals and an imbalance in these processes leads to oxidative stress and incidence of pathological changes in multiple cellular macromolecules (Farkas *et al.,* 2007). As a result, oxidative stress can alter neurotransmission, neuronal function and whole brain activity (Rammal *et al.,* 2007). One of factors causing oxidative stress is reactive oxygen species (ROS) which is generated

within brain tissue during ischemia, and plays a role in the development of cerebral damages (Gil *et al.*, 2000). ROS are highly reactive and attack lipids, proteins, and nucleic acids, which eventuates in tissue injury and cell death (Gil *et al.*, 2000). Interestingly, oxidative stress state was recently linked to other behavioral disorders, such as aggressive behavior and depression, and also to deterioration of short-term spatial memory (Bouayed *et al.*, 2009a; Bouayed *et al.*, 2009b), highlighting that oxidative stress disturbances could be implicated in the pathophysiology of conditions that are more specific for the nervous system impairment (Bouayed *et al.*, 2009b).

In recent years, it has been well shown that consuming fruits and vegetables containing natural antioxidants prevents many diseases such as cardiac complications and even different cancers (Casanova *et al.*, 2008). Ellagic acid (8, 7, 3, 2- tetra-hydroxy-chromno (3, 4, 5-CDA) chromno-10, 5-dion with molecular weight of 302 g/mole) is a bioactive compound with many pharmaceutical and industrial applications (Ascacio-Valdes *et al.*, 2010). This molecule has different properties such as antioxidant ones (Ascacio-Valdes *et al.*, 2010). The studies conducted on ellagic acid in cancer cells have shown apoptosis induction, cellular death and stopping continual growth of tumor (Ito *et al.*, 1999). Results of studies suggest that ellagic acid provides effects of useful neural protection against oxidative damage in diabetic rats (Uzar *et al.*, 2012).

In this research, effects of ellagic acid on oxidative stress in ischemia hypoperfusion model were investigated.

2. Materials and Methods

2.1. Animals and experimental groups

The present research was done using adult male Wistar rats reproduced in Reproduction and Care Center, Animal House of Ahwaz University of Medical Sciences, in weight range of 200-250 g. The animals were being kept inside standard cages in quartet groups in Animal Care Center of Islamic Azad University, Izeh Branch, Khuzestan, Iran. They were kept in standard conditions at 20±2 °C and 12-h light-dark cycle (lighting started at 7 a.m.) with enough access to compressed food of Dam Pars Tehran Company, Chavdaneh Company, Shahreza, Isfahan, and purified tap water of Izeh City. To facilitate the work and adapt to peripheral conditions and the tester, the animals were petted for some minutes on a daily basis in advance. They were randomly classified into the following groups:

1. Healthy group without ischemic induction and medicine intake (control);
2. Ischemic hypoperfusion group without medicine intake (ischemia);
3. Ischemic hypoperfusion group receiving 50 mg/kg of ellagic acid every day for 14 days using intragasteric administration or gavage (ischemia + 50 mg/kg ellagic acid) (Girish *et al.*, 2013; Rafieirad *et al.*, 2014a)

2.2. Brain ischemia/hypoperfusion Surgery Method

After completely anesthetizing the animals using Ketamin / xylazine (intra protaneal injection of 10.90 mg / kg of body weight), left and right common carotid arteries were opened by longitudinal incision of skin below their neck and pushing muscles aside. After making two knots using surgical suture silk on top and at bottom of each artery, middle of both knots was cut by a pair of scissors. Then, places of the surgery were sutured and then the animals were treated to recover from the surgery. After the animals became conscious after one week, a similar surgery was performed on the other side (Rafieirad *et al.*, 2014). Afterward, the animals of both groups were treated for two weeks with one of different doses of ellagic acid using gavage method.

2.3. Preparation of ellagic acid

Ellagic acid from Sigma-Aldrich (Steinheim, Germany) was solved in normal saline. It was administered orally by gavage for 14 days (Rafieirad *et al.*, 2014a).

2.4. 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) (Sarkaki *et al.*, 2013; Rafieirad *et al.*, 2014b) (Fig 1.).

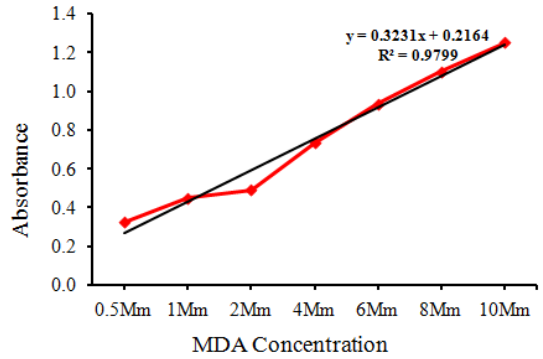

Fig 1. The Standard Curve of MDA was constructed Over the Concentration Range of 0–10 µM

2.6. Statistical analysis

Data of this research were analyzed using one way ANOVA by $SPSS_{ver\ 20.0}$ software. Means were comprised LSD test methods with $p < 0.05$ and then they presented as mean ± SEM.

3. Results and discussion

In this study, in which effect of ellagic acid was studied on antioxidant status such as lipid peroxidation and thiol group (-Sh) in animal models of ischemia, malondialdehyde (MDA) significantly increased in hippocampus ($p \leq 0.001$) in the ischemic group compared with the control. The observations showed that malondialdehyde in hippocampus tissue in ischemia group treated with (50 mg/kg) of ellagic acid significantly reduced ($p \leq 0.001$) compared with the ischemia group (Fig 2.). On the other thiol in ischemia group has significantly increased ($p \leq 0.001$) than in control group and rate of thiol in ischemia group receiving ellagic acid has significantly decreased ($p \leq 0.001$) (Fig 3.).

As can be seen in figure our results indicated that ellagic acid could reduce oxidative stress factors that caused by ischemia and these favorable effects were significant. Cerebral ischemia caused increase of Reaction Oxygen Species (ROS) and finally activation of the pathways leading to cellular death in vulnerable areas of brain (Puyal *et al.*, 2013). Neurons have the highest sensitivity to ischemia in CA1 area of hippocampus (Eichenbaum, 2004). Previous researches have shown that obstruction of both common carotid arteries for 30 min caused extensive cellular death in CA1 area of hippocampus so that significant reduction was found in mean number of CA1 area in ischemic group compared with other groups and significant changes were found in behavioral tests of the animals (Hazelton *et al.*, 2010). Oxidative stress not only increases production of free radicals, but also reduces cellular antioxidant mechanisms.

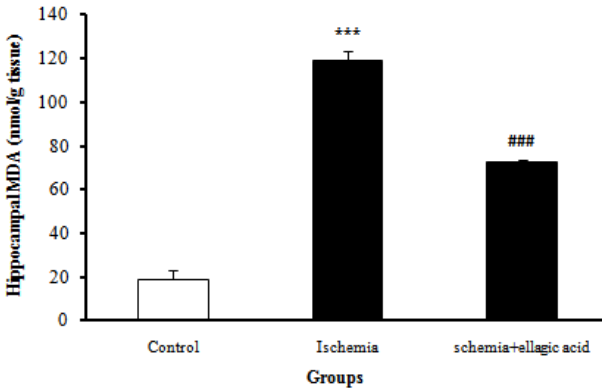

Fig 2. Mean ± SD of MDA between Control Group, Ischemia and Ischemic Group orally receiving 50 mg/kg ellagic acid for 14 Days.

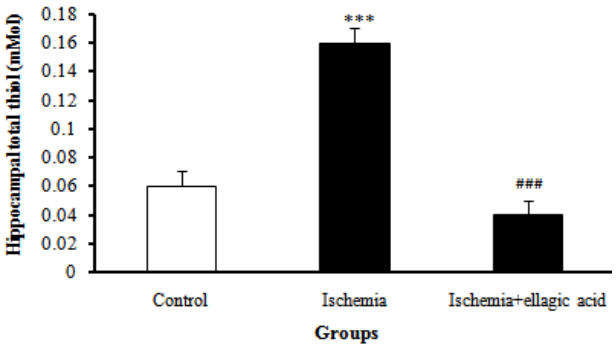

Fig 3. Mean ± SD of total thiol between Control Group, Ischemia and Ischemic Group orally receiving 50 mg/kg ellagic acid for 14 Days

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 (Acikgoz *et al.*, 1998). Philip et al. (2003) found that after 40 minutes of ischemia, the level of total protein S - thiolation significantly increased in kidney tissue and remained significantly higher than control after 5 min

reperfusion. Low molecular weight thiols such as glutathione can act as a protection against the irreversible loss of biological activity of macromolecules. Glutathione is synthesized in two reactions, gamma-glutamyl cysteine synthetase by successive ATP-dependent (γ-GCS), the rate-limiting enzyme, glutathione synthetase (Eaton *et al.*, 2003). GSH concentrations is inhibited by a feedback mechanism of γ-GCS activity (Lu, 2000). Results have shown that increasing glutathione levels in old mice significantly improved by grape seed extract, as to justify that flavonoids in grape seed extract produces the rate-limiting enzyme in the synthesis of glutathione (gamma-glutamyl cysteine synthetase) (Balu *et al.*, 2005). 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 (Motamedi *et al.*, 2010). Ellagic acid is one of the most important compounds available in pomegranate and phenol structure and nature of this compound causes its strong antioxidant activity (Sopala & Danysz, 2001).

Results a study by Spencer et al. (2011) indicated that ellagic acid prevented oxidative damage of DNA due to copper transporters and catecholamine; as a result, protective role of ellagic acid has been suggested for preventing ROS**,** lipid peroxidation and breakage of DNA string (Spencer *et al.*, 2011). Recent studies have investigated ability of cytotoxic activity and antigrowth factor of ellagic acid in human cells, lung cancer, colorectal cancer, breast cancer and prostate cancer and shown that 1 to 100 M/lit inhibits expansion of cell cancers. In addition, it has been demonstrated that application of ellagic acid probably leads to apoptosis in cancer cells by inhibiting the factors that cause metastasis (Spencer *et al.*, 2011). The ellagic acid has been recognized as antimicrobial and antiviral factor and it has been also mentioned that ellagic acid is able to inhibit growth of pathogens in humans; this action is probably performed by being coupled with protein in wall of bacteria (Huetz *et al.*, 2005). In addition, ellagic acid has some inflammatory effects and causes reduced oxidative stress (Chao *et al.*, 2009). According to the results of this study, confirm previous reports functional disorders after brain ischemia that is caused by damage on brain cells is due to oxidant production.

4. Conclusion

Therefore, ellagic acid with antioxidant ability to remove oxidant substances from specific and important areas of the brain can be this compensatory effect on oxidative stress in animal model of hypoperfusion ischemia.

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