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Neuroprotective Effects of Oral Ellagic Acid on Locomotor Activity and Anxiety-Induced by Ischemia/Hypoperfusion in Rat

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ABSTRACT

Ischemia-associated depression and anxiety may occur due to brain damage caused by oxidative stress. A number of reports indicate that treatment with herbal plant extracts with antioxidant properties could lead to a significant reduction in Ischemic complications. The aim of this study was to evaluate the behaviors of ischemic rats through animal models of depression -the forced swim test (FST) - and anxiety and exploratory behavior–the open field to determine the efficiency of ellagic acid as antioxidant. In this study, 21 male wistar rats (250 ± 20 g) were used. Animals were randomly divided into three groups with 7 in each: 1) Control; 2) Ischemic; 3) Ischemic received ellagic acid for 14 days. In order to create ischemia/hypoperfusion, carotid arteries were ligatured and cut bilaterally. Ischemic rats showed a significant increase in anxiety or decrease approach and reduction locomotor activity compared to control group and were more immobile during the forced-swimming test. 14-days administration of ellagic acid significantly improved the immobilization and anxiety-induced ischemia. In conclusion, ellagic acid exhibits therapeutic potential for anxiety and depression, which is most likely related at least in part to its antioxidative and free radical scavenging actions.

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INTRODUCTION

Permanent occlusion of bilateral common carotid artery is a model for chronic cerebral hypoperfusion/ Ischemia which is associated with neurodegenerative diseases [1]. 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 [2]. As a result, oxidative stress can alter neurotransmission, neuronal function and whole brain activity [3]. One of the 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 [4]. ROS are highly reactive and attack lipids, proteins, and nucleic acids, which eventuates in tissue injury and cell death [4]. 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 [3,5,6], highlighting that oxidative stress disturbances could be implicated in the pathophysiology of conditions that are more specific for the nervous system impairment [6]. Hall et al. initially described the open field test for the study of emotionality in rat [7,8]. Research in recent years has seen considerable interests in exploring non-traditional pathophysiological mechanisms involved in oxidative stress, such as those involved in anxiety disorders[8]. Ellagic acid (2, 3, 7, 8-tetrahydroxy [2] benzopyrano(5, 4, 3,-cde) [2] benzopran-5, 10-dione) is a naturally occurring polyphenolic compound which has been reported to be involved in a wide range of pharmacological activities such as such as antioxidant [10], anticancer [11], antiallergic [12], antimalarial [13], antiwrinkle [14], antiglycative and anti-inflammatory activities.[15-17] Furthermore, it exhibits antioxidative properties both *in vivo* and *in vitro* [17]. Ellagic acid shows antidepressant-like activity in unstressed mice probably by interaction through adrenergic and serotonergic systems [15]. In this study, the effect of ellagic acid on anxiety and depression in animal models of global ischemia permanently with common carotid artery occlusion was examined.

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MATERIALS AND METHODS

Animals:

Thirty healthy adult female albino rats of Wistar strain (250 ± 20 g, 3–4 months) obtained from Ahvaz Jundishapur University of Medical Sciences (AJUMS) Laboratory Animal Centre. Animals were housed in standard cages under controlled room temperature (20 ± 2 °C), humidity (55%–60%) and light exposure conditions 12:12 h light–dark cycle (light on at 07:00 am). All experiments were carried out during the light phase of the cycle (8:00 am to 6:00 pm). Access to food and water were ad libitum except during the experiments. Animal handling and experimental procedures were performed under observance of the University and Institutional legislation, controlled by the Local Ethics Committee for the Purpose of Control and Supervision of Experiments on Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used. Prior to the onset of behavioral testing, all rats were handled for 5 days (5 minutes daily). Animals were divided randomly into three groups consisting of 7 animals in each. Group 1: Control. Group 2: untreated ischemic group with occlusion of bilateral common carotid arteries, Group 3: Ischemic rats received 50 mg/kg, ellagig acid for 14 days.

hypoperfusion/ Ischemia procedure:

We used Cechetti's method (2010) with little modification [18]. Briefly, rats were anaesthetized with ketamine/xylazine (50/5 mg/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 with one week intervals between interventions, the right common carotid being the first to be assessed and the left one being occluded one week later.

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

Open field:

The apparatus made of wood, had a white floor of 72 × 72 cm divided into 16 squares of 18 × 18 cm. the walls, 36 cm high, were also painted white. A central square (18 cm x 18 cm) was drawn in the middle of the open field [19]. The central square is used because some mouse strains have high locomotor activity and cross the lines of the test chamber many times during a test session. Also, the central square has sufficient space surrounding it to give meaning to the central location as being distinct from the outer locations [19]. Each rat was placed in the center of open field and the following variables were recorded after the latest administration of ellagic acid for 5 min: number of outer squares crossed (outer locomotion) as Locomotor Activity; number of inner squares crossed (inner locomotion) as Anxiety or Approach/Avoidance. A high frequency/duration of these behaviors indicates high exploratory behavior and low anxiety levels. And Total number of fecal boli (defecation). The whole area was cleaned between two tests [20].

Forced swimming test (FST):

One day after open field test, animals were adapted to the test room for 1 h before the beginning of the FST experiment. During the FST, the animals were placed in a glass cylindrical tank with 60 cm height and 38 cm width, which was filled with water (23°C) to the depth of 40 cm. Animals, were placed individually inside the water cylinder. The time of floating (immobility) during the FST was recorded for 5 min in each session [21]. Rats were considered immobile when they floated in the water.

Statistics:

Data are expressed as mean±SEM. Significance 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 DUSCUSSION

Results:

Factor analysis of open field behavior generally yields 3 factors that were described by Ramos et al. (1997) known as anxiety or approach/avoidance (locomotion in center squares); locomotor activity (lines crosses in outer squares) and defecation scores [20]. Accordingly, our findings showed a significant increase in anxiety or decrease in approach and reduction of locomotor activity in ischemic rats compared to control group at $p < 0.01$ and $p < 0.001$, respectively (figure 1 and 2). A 14-day administration of ellagic acid in ischemic rats led to reduced anxiety and exploratory behavior and increase their physical activity compared with untreated ischemic that were significant ($p < 0.05$) (figure 1 and 2). Defecation (total number of fecal boli) is often used as a

measure of anxiety. In this study, number of stools significantly increased in ischemia group ($p < 0.01$) compared to a control group, while on ischemic group receiving ellagic acid there was a significant reduction in the number of feces (figure 3). Another study was conducted in the forced swimming test. This test is an animal test for depression. In these conditions, the animals were swimming in the water to maintain its stability. After a while, the animals off their hands and feet movements of activity remained open, and stay afloat that conventionally called the immobilization. Ischemia in rats increased immobility time in the forced swim test ($p < 0.01$) compared with controls. Whereas ellagic acid administered to ischemic rats could reduce the time significantly ($p < 0.001$) in comparison with ischemia group that did not receive ellagic acid (figure 4).

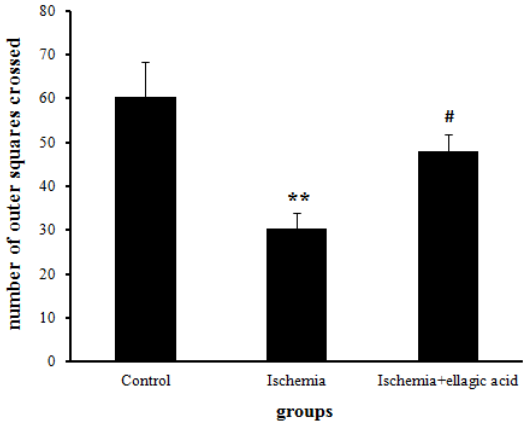

Fig. 1: Mean ± SD of number of outer squares crossed between control group, Ischemia and Ischemic group receiving 50mg/kg ellagic acid for 14 days, orally

* Significant compared to the control, # Significant compared to the Ischemia (One-way ANOVA-Post Hoc LSD test, n=7, $^{\#}P<0.05$, $^{**}P<0.01$)

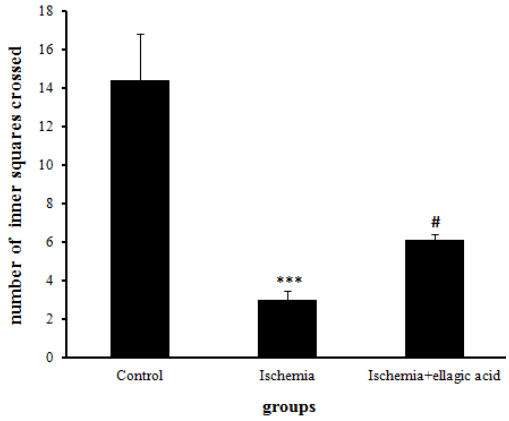

Fig. 2: Mean ± SD of number of inner squares crossed between control group, Ischemia and Ischemic group receiving 50mg/kg ellagic acid for 14 days, orally.

* Significant compared to the control, # Significant compared to the Ischemia (One-way ANOVA-Post Hoc LSD test, n=7, $^{\#}P<0.05$, $^{***}P<0.001$)

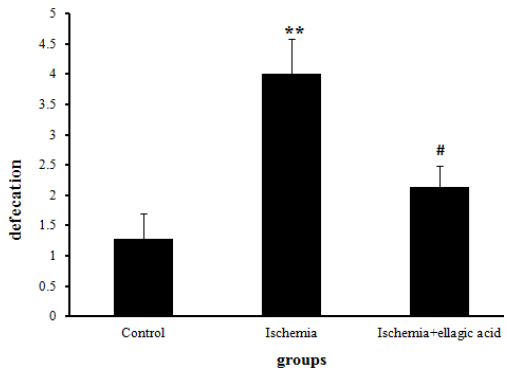

Fig. 3: Mean ± SD of defecation between control groups, Ischemia and Ischemic group receiving 50mg/kg ellagic acid for 14 days, orally.

* Significant compared to the control, # Significant compared to the Ischemia (One-way ANOVA-Post Hoc LSD test, n=7, $^{\#}P<0.05$, $^{**}P<0.01$)

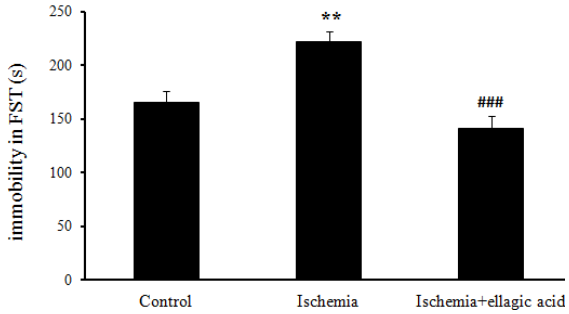

Fig. 4: Mean ± SD of immobility in FST (s) between control groups, Ischemia and Ischemic group receiving 50 mg/kg ellagic acid for 14 days, orally

* Significant compared to the control and # Significant compared to the Ischemia (One-way ANOVA-Post Hoc LSD test, n=7, $^{\#\#\#}P<0.001$, $^{**}P<0.01$).

Discussion:

According to our findings, hypoperfusion/ischemia has showed anxiety and depression in rats. In addition, we found that 14 days oral administration of ellagic acid can improve both the anxiety and depression in ischemic rats. Neuropsychiatric sequelae of stroke include a wide range of emotional and cognitive disturbances and have important clinical implications for functional outcome and rehabilitation [22, 23]. Depression and other poststroke emotional and behavior disorders include anxiety disorder, mania, bipolar disorder, insomnia, apathy, and pathologic crying [23, 24]. Oxidative stress is known to cause brain dysfunction in neurodegenerative diseases, including ischemia [25]. Cerebral ischemia may trigger a signaling cascade that, depending on the severity and duration of symptoms, will lead to free radicals and oxidative stress [26]. Kuloglu et al (2002) have established a relationship between oxidative stress and anxiety disorder. Anxiety of rodents is detected from specific behavioral model tests, among which the elevated plus maze, the light/dark choice test, open field test and hold board test are most employed [6, 26]. These behavioral tests are also sensitive to pharmacological agents with anxiolytic or anxiogenic properties, causing a decrease or an increase in the anxiety-related behavior of animals, respectively [6]. At physiologic conditions, antioxidants play a crucial role in maintaining redox homeostasis by maintaining the level of ROS at physiological doses necessary for optimal cellular functioning [6]. Yasunari *et al*. (2006) found a significant relationship between trait anxiety and ROS formation in monocytes of hypertensive individuals [28]. In addition, Berry *et al*. (2007) showed that a deletion of the p66Shc longevity gene in mice, which results in lower levels of oxidative stress and an extended life span, decreased anxiety-related behavior [29]. Thus, the excess of ROS is neutralized by antioxidants avoiding the oxidation of cellular components and consequently their damage. The principal source of exogenous antioxidants is our diet. However, diets relatively deficient in antioxidants may favor oxidative stress. Vitamin E, vitamin C, carotenoids, zinc, selenium, and polyphenols (e.g. phenolic acids and flavonoids) constitute the principal dietary antioxidants existing in food [6]. Our findings in 2012 showed that the antioxidant effect of grape seed extract on oxidative stress-induced ischemia and improve cognitive functions in rats with chronic hypo perfusion [25]. The highest levels of ellagic acid are found including blackberries, cranberries, pecans, pomegranates, raspberries, strawberries, walnuts, wolfberry and grapes [30]. Ellagic acid has antiproliferative and antioxidant properties in a number of *in vitro* and small-animal models [30-32]. As other polyphenol antioxidants, ellagic acid has a chemo protective effect in cellular models by reducing oxidative stress [30]. Currently, there is increasing evidence that the advantageous effects of antioxidants on health are not only attributed to their antioxidant properties. This is due to the fact that antioxidants can also act e.g. as signaling molecules or as chemo preventive agents by displaying other activities such as anti-inflammatory activity [6]. In conclusion, functional disorders after brain ischemia are mainly due to the damage caused by oxidant production in brain cells. Therefore, the ability of ellagic acids to remove oxidant from brain leads to the recovery and reduces oxidative stress in a model of ischemic hypo perfusion.

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