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The possible neuroprotective effect of ellagic acid on sodium arsenate-induced neurotoxicity in rats

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ABSTRACT

Objective: Arsenic is a well-known environmental contaminant, causing toxicity in different organs. The aim of this study was to investigate the possible neuroprotective effect of ellagic acid (EA) on arsenic-induced neurotoxicity in rats.

Design: Animals were divided into five groups. The first group received normal saline (2 mL/kg) for 21 days as control group. Group 2 was orally treated with sodium arsenite (SA, 10 mg/kg) for 21 days. Groups 3 and 4 were orally treated with SA (10 mg/kg) for 7 days prior to EA (10 and 30 mg/kg respectively) treatment and continued up to 21 days simultaneously with SA administration. Group 5 was orally treated with EA (30 mg/kg) for 14 days. Passive avoidance test and rotarod test were done to evaluate the behavioral changes following SA and/or EA treatment. Different biochemical, histological and molecular biomarkers were assessed in the brain tissue.

Results: Our data showed that SA significantly elevated brain tissue arsenic levels and malondialdehyde, nitric oxide, protein carbonylation, tumor necrosis factor-alpha, and interlukein-1β production. A decrease in the total antioxidant capacity, reduced glutathione content and glutathione peroxidase activity occurred in the brain of rats exposed to SA. SA-treated rats showed a significant impairment in long-term-memory, motor coordination and equilibrium. These results were supported by histopathological observations of the brain. Results revealed that administration of EA (30 mg/kg) reversed all neural markers alternation and ameliorated behavioral and histopathological changes induced by SA.

Conclusion: EA can effectively protect brain tissue against SA-induced neurotoxicity via its antioxidant and anti-inflammatory effects.

1. Introduction

Nowadays, industrialization of societies and expansion of factories has dramatically increased the amount of contaminants and environmental pollutions. Arsenic is one of the most important worldwide environmental toxicants, which is found in air, water and soil. Arsenic is mainly distributed in the environment through using pesticides and herbicide, burning coal and treated wood, mining wastes, smelting metal and glass [27,44]. The main exposure to arsenic is occupational and the most frequent reason of poisoning is the use of contaminated water. Long term exposure to arsenic and its metabolites can cause skin and lung cancer, neurological effects, hypertension and cardiovascular diseases [9,42,51]. Since arsenic can cross the blood brain barrier (BBB), it accumulates in the brain and causes neurobehavioral abnormalities. The neurotoxicity of arsenic includes symmetrical peripheral neuropathy, decreased muscle strength, paralysis, sleep disturbances, delirium, confusion, disorientation, severe agitation, paranoid ideation, emotional liability, blurred vision, loss of hearing and taste as well as impairment of superior neurological functions such as learning, recent memory and concentration [21,27].

Although the exact mechanism of arsenic neurotoxicity is not clear, the evidence suggests that arsenic induces ROS-mediated oxidative

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stress along with impaired mitochondrial functions. Furthermore, some studies have shown that arsenic induces cell oxidative damage through elevating malondialdehyde (MDA) level and reducing superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activity as well as reduced glutathione (GSH) content [24,32,41,54].

Antioxidant compounds have been shown to protect tissues from arsenic-induced oxidative damage [46]. Ellagic acid (EA) (C14H6O8) is a phenolic component found in various flowering plants including grapes, raspberries, blackberries, strawberries and walnuts [13,53]. Recent studies have shown antioxidant, anti-mutagen and anti-carcinogen effects of EA. The antioxidant effects of EA have been found to be mediated by its metal chelating capacity, free radical scavenging ability and induction of cellular antioxidant enzymes activity [3,28,52]. EA has also been shown to protect cells from DNA damage caused by free radicals [10,52]. Moreover, EA has anti-inflammatory effects and could prevent cognitive, learning and memory impairment following traumatic brain injury (TBI). The anti-inflammatory effects of EA have been suggested to be related to its modulatory effect on IL-1β and IL-6 production [13]. Furthermore, EA can improve Alzheimer's disease associated dementia through reducing oxidative and inflammatory cell damage and enhancing antioxidant content [19]. Current work was therefore carried out to investigate the possible protective effect of EA on brain toxicity induced by sodium arsenite (SA).

2. Materials and methods

2.1. Chemicals

Sodium arsenite (SA, $NaAsO_2$), Ellagic acid (EA) and all other chemicals and reagents were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA) and Merck Company (Darmstadt, Germany).

2.2. Animals

Adult male Wistar albino rats weighing 180–200 g were used for the study. The animals were obtained from animal house of Ahvaz Jundishapur University of Medical Sciences. Animals were housed seven per cage. They were held in a room with temperature of 22 ± 2 °C (room temperature) at 12-h light: 12-h dark cycle with free access to food and water. The investigation was performed according to the Animal Ethics Committee Guidelines for the use of experimental animals (Ethic code: IR.AJUMS.REC.1395.641) and all rats used in the experiment received merciful care.

2.3. Experimental protocol

Animals were randomly divided in to five groups, each group consist of seven rats. Group 1 received normal saline (2 mL/kg) for 21 days as control group. Group 2 received SA 10 mg/kg/day, p.o. for 21 days. Groups 3 and 4 received SA for 7 days prior to EA (10 and 30 mg/kg/day, respectively) treatment and continued up to 21 days simultaneously with SA administration (14 days). Group 5 received EA (30 mg/kg/day, p.o.) for 14 consecutive days. All administrations were orally by gavage. On the 22nd day after the first treatment, the behavioral tests were done and then samples were collected. Animals were habituated to the research lab for 30 min before starting the test.

2.4. Passive avoidance test

A passive avoidance test was performed to evaluate the effects of SA and/or EA on the long-term memory in rats. The experiments were carried out using a step-through type passive avoidance apparatus (Ugo Basile model 7551, Comerio, Italy), consisted of equal sized light and dark compartments (22 × 21 × 22 cm), and separated by a guillotine door. A 40 W lamp was fixed 30 cm above the floor in the center of the light compartment. The floor was made from stainless steel and connected to a shock stimulator. Single electrical shocks (0.5 mA, 75 V, 50 Hz) were delivered to the grid floor of the dark compartment by a stimulator. The experiments consisted of training and test sessions. In the training session, each rat was placed in the illuminated compartment, and allowed 1 min for habituation. The guillotine door was opened and closed immediately when the animal entered the dark compartment, then an electric shock was delivered through the grid floor. In the test session, each animal was again placed in the illuminated compartment, 24 h after the training session. The step-through latency to enter the dark compartment was measured in both sessions. The cut-off time was 600 s training session [2,34].

2.5. Rotarod test

The motor performance was performed using the rotarod equipment (ROTA-ROD, Borj-Sanaat, Iran. Model: M.T6800) as previously described [36]. The unit consists of a rotating spindle, a power source for turning the spindle and grids beneath the rotating roller where the rat can fall without injury. All animals were pre-trained on the rotarod apparatus in order to reach a stable performance. The training consisted of three separate trials on 1 day, with at least 20 min of rest between trials, under an accelerating protocol starting at 4 rpm and reaching 40 rpm in 5 min and the latency to fall was recorded.

2.6. Sample collection

After the behavioral tests, the animals were sacrificed by decapitation, brain tissues were isolated and then washed with saline quickly. For histological studies, a part of cortex was fixed in 10% phosphate buffered formalin. For biochemical estimations, the remained part of brain was homogenized (1/10 w/v) in ice-cold Tris-HCl buffer (0.1 M, pH 7.4).

2.7. Assay the arsenic concentration in the brain tissue

The flameless atomic absorption spectrophotometric technique [1,38] was used for the determination of arsenic concentration. The tissue was digested with a mixture of nitric acid, sulfuric acid and perchloric acid (3:1:1). Values are expressed in μg of arsenic per g tissue.

2.8. Assay for redox status in tissue homogenates

Activity of GPx in the brain was determined by ZellBio. Lipid peroxidation was determined by the method described by Buege and Aust, which estimates the malondialdehyde (MDA) formation [6]. Levels of GSH were detected by the method described by Ellman [11].

2.9. NO assay

The levels of nitric oxide (NO) in brain tissues were determined indirectly using the Griess reaction for evaluation of nitrite and nitrate concentrations [18]. Brain tissue was homogenized in 0.1 M sodium phosphate buffer pH 7.4 in 1:10 (w/v) and centrifuged at 1000*g* for 10 min. Deproteinization of the sample was carried out by mixing 40 μl of 30% (w/v) ZnSO4 with 800 mL of sample using vortex. Then, the solution was allowed to stand for 10 min. The tubes were centrifuged at 4000 *g* for 10 min. For nitrate estimation, 2.5–3 g cadmium granules were added in every tube and the samples were kept for 2 h at room temperature. Then, equal volumes of sample and Griess reagent (5% phosphoric acid containing 0.1% NEDD, 1% sulphanilamide) were mixed and incubated in dark for 10 min at room temperature. The absorbance of the reaction mixture was measured at 540 nm on a UV–visible Spectrophotometer (UV-160A, Shimadzu, Japan). A standard curve was generated with sodium nitrite (NaNO2) in concentrations