Journal of Trace Elements in Medicine and Biology 36 (2016) 1–6

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Oleuropein ameliorates arsenic induced oxidative stress in mice

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ARTICLE INFO

Article history:
Received 25 January 2016
Received in revised form 26 February 2016
Accepted 9 March 2016

Keywords:
Arsenic toxicity
Oleuropein
Oxidative damage
MDA
NO
Protein carbonyl content

ABSTRACT

The objective of this study is to investigate the potential preventive effect of oleuropein in an experimental arsenic toxicity in mice. For this purpose, mice were exposed to 5 mg/kg/day sodium arsenite ($NaAsO_2$) in drinking water and treated with 30 mg/kg/day oleuropein for 15 days. At the end of the experiment, animals were sacrificed and selected organs were processed for biochemical and histopahtological investigations. Blood, liver, kidney and brain malondialdehyde (MDA) and nitric oxide (NO) levels were determined by colorimetric methods. Protein carbonyl content is measured by a commercial kit. Liver morphology and immunoreactivity for inducible NOS (iNOS) and endothelial NOS (eNOS) was evaluated microscopically. Level of NO was determined to decrease in blood and tissues whereas MDA increased in arsenic given mice. Tissue protein carbonyl content also increased in this group. Immunoreactivity for iNOS and eNOS was noted to increase with arsenic treatment. Oleuropein treatment had significant effects in normalizing the MDA and NO levels as well as protein carbonyl content. Immunohistochemical staining also showed reduction of the expression of iNOS and eNOS in liver. The results indicate that oleuropein ameliorates oxidative tissue damage by scavenging free radicals.

1. Introduction

Arsenic (As) is one of the most important toxic heavy metal ubiquitously found in natural environment. Arsenic can be found in water, soil and air inorganic forms as well as organic forms. The most common source of arsenic exposure is through consumption of contaminated drinking water [1,2]. As^{+3} (trivalent) and As^{+5} (pentavalent) are forms of inorganic arsenic mostly found in water. The trivalent form of arsenic is more toxic than the pentavalent form [3,4].

It has been known for a long time that people are prone to cancers, black foot diseases, diabetes mellitus (type II) [5,6], cardiovascular diseases [7], hypertension [8] and neurological diseases such as Alzheimer and Parkinson [9,10] exposed to high level of arsenic.

Arsenic causes toxicity in human and animal cells by development of oxidative stress [11,12]. Oxidative stress in arsenic toxicity is mediated by its oxidizing effect on metals such as iron (Fe) or reaction with antioxidants [13]. Arsenic also increases consumption of oxygen by the cells which increase reactive oxygen species (ROS) production and oxidative stress [14,15]. Indeed damage to proteins is more important than lipids in situation of oxidative stress [14].

Olive oil as a source of fat is one of the main nutrients in the conventional Mediterranean diet. The most bioactive component in olive oil is oleuropein. Oleuropein act as a free radical scavenger due to its strong antioxidant activity [16]. Most epidemiological studies revealed that in Mediterranean where the olive oil used abundantly widespread the incidence of certain cancers such as breast and colon and coronary heart disease is lower [17]. Because of the strong antioxidant capacity of oleuropein and the known mechanism of arsenic toxicity, it was aimed in this study to investigate the potential preventive effect of oleuropein in an experimental arsenic toxicity model by means of biochemistry and histopathology.

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http://dx.doi.org/10.1016/j.jtemb.2016.03.006
0946-672X/

2. Materials and methods

2.1. Experimental design

The ethics approval for research was confirmed by Kafkas University Animal Care and Use Committee (Registration Number: 2012-08). All procedures were conducted in accordance with the 'Guide for Care and Use of Laboratory Animals' published by the National Institutes of Health and the ethical guidelines of the International Association for the Study of Pain.

Male Swiss albino mice used in this experiment were purchased from Laboratory Animal Experimental Units of Ataturk University, Turkey. The mice were housed at 25 ± 2 °C, with an average humidity 50 ± 5% and with a lighting schedule of 12 h light and 12 h dark. Total of 40 male *swiss albino* mice weighing 26–32 g at 10–11 weeks of age were randomly divided into 4 equal groups, 10 mice each, and kept in cages. No attempt was made to normalize different groups by body weight. Animals had free access to a standard pellet diet and water *ad libitum*. No experimental treatment was performed on Control group (C). Mice in arsenic group (As) received 5 mg/kg/day sodium arsenite ($NaAsO_2$) (Sigma Chemical Company) in drinking water. Arsenic plus oleuropein group (As + OLE) received 5 mg/kg/day sodium arsenite plus 30 mg/kg/day oleuropein in drinking water, and oleuropein group (OLE) had only 30 mg/kg/day oleuropein. All experimental treatments lasted 15 days.

2.2. Biochemical analysis

At the end of the experiment blood samples were collected into heparin tubes from the hearts via cardiac puncture under ether anesthesia for MDA and NO measurements. The blood samples were centrifuged (3000*g*, 4 °C) for 10 min, and the plasma were obtained and then kept at −25 °C until the analyses were carried out. At necropsy liver, kidney, and brain tissues were collected to determine tissue MDA, NO and protein carbonyl content concentrations. For this purpose, the tissues were rinsed with ice-cold 0.9% NaCl. Then, 1 g of tissue was homogenized in phosphate buffer (pH 7.4) in an ice bath and the homogenates were centrifuged (5000*g*, 4 °C) for 15 min.

MDA as an end product of lipid peroxidation concentrations were measured by the method of Yoshoiko et al. [18] based on the reaction between thiobarbituric acid and MDA. The end products were read at 535 nm.

NO levels were determined according to the method described by Miranda et al. [19] in that nitrate is reduced to nitrite by $VaCl_3$, and then in acidic environment nitrite was reacted with sulphanilamide to produce colored diazonium compound, which was read at 540 nm.

Protein carbonyl content is measured by a commercial spectrophotometric assay kit (Cell Biolabs, INC). Bovine serum albumin (BSA) standards or protein samples which are derivate with dinitrophenylhydrazine (DNPH) first. Proteins are then trichloroacetic acid (TCA) precipitated and free DNPH is removed by washing the protein pellet. After dissolving the protein pellet in guanidine hydrochloride (GuHCl), the absorbance of protein-hydrozone is measured at 375 nm. MDA, NO and protein carbonyl content levels were determined by using a spectrophotometer (Epoch, Biotech, USA).

2.3. Histopathology and immunohistochemistry

Pieces of liver tissue samples collected at necropsy were fixed in 10% neutral buffered formalin solution and embedded in paraffin. Then, 5 μm thick sections were cut from the paraffin blocks, and were routinely processed for hematoxylin and eosin staining. The tissue sections were observed under a light microscope for evaluation of pathological changes.

iNOS and eNOS activities in liver tissues were assessed immunohistochemically. For this purpose, 4 μm thick sections were cut from the paraffin blocks, deparaffinized via conventional methods, and then washed in phosphate buffered saline (PBS, pH 7.4). Tissue endogenous peroxidase activity was blocked by 0.3% H_2O_2 for 20 min. Then, tissue antigen retrieval was accomplished by microwave treatment in 10 mM citrate buffer, pH 6.0 for 10 min. Following several PBS washes, the sections were incubated for 30 min at room temperature with non-immune goat serum (Zymed Laboratories Inc., Cat No: 85-9043A) to block non-specific antibody binding. The sections were treated with rabbit anti-iNOS antibody (Thermo Scientific, Cat No: RB-9242-PO) diluted 1:100 and rabbit anti-eNOS antibody (Thermo Scientific, Cat No: RB-9279-PO) diluted 1:50. Antibody incubations were performed in a humidified chamber at room temperature for 1 h. Biotinylated secondary antibody and then streptavidin peroxidase complex (Zymed Histostain- Plus Bulk Kit, cat. No. 85-9043) were applied on to the sections for 30 min each with several PBS washes between. Antibody binding was visualized by color development using 3,3-diaminobenzidine–H_2O_2. Finally, the sections were counterstained with Mayer's hematoxylin, washed with water, processed in an alcohol–xylene series, covered with a cover slip, and examined under a light microscope.

Table 1
Effects of oleuropein on MDA and NO levels (μmol/L) in blood. Values are expressed as mean ± SD. Values with different superscripts within the same column significantly differ from each other ($P < 0.01$).

	MDA	NO
Control	14.21 ± 0.24[a]	10.94 ± 0.25[a]
As	23.16 ± 0.88[b]	5.83 ± 0.63[b]
As + OLE	17.32 ± 0.63[c]	7.36 ± 0.32[c]
OLE	12.25 ± 0.57[d]	8.25 ± 0.74[d]

2.4. Statistical analysis

Statistical analyses of biochemical data were done by SPSS software (Windows version 20.0). The data were expressed as median (X) ± standard deviation (SD). Importance level of difference among the groups was determined by variance analysis (ANOVA) and Duncan multiple comparison test.

3. Results

3.1. Levels of MDA and NO

The results of blood MDA and NO levels were summarized in Table 1. The levels of MDA in blood were significantly higher in As group, compared to those of control group animals. Conversely blood MDA level in OLE group decreased compared to As and control groups. NO levels decreased significantly in mice treated $NaAsO_2$ compared to that of mice in control ($p < 0.01$).

Liver, kidney and brain MDA and NO levels were shown in Table 2. The kidney and brain tissue levels of MDA in As group were determined to be significantly higher than the control group ($p < 0.01$). Liver, kidney and brain NO levels were detected to be significantly lower in As group than the control. The brain MDA and NO levels in OLE group were noted to be significantly lower than the control ($p < 0.01$).

3.2. Protein carbonyl content

Protein carbonyl content levels are shown in Table 3. Treatment of arsenic significantly increased protein carbonyl content