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Inhibitory effect of concomitant administration of Zataria multiflora Boiss. against oxidative damage-induced by sub-acute exposure to arsenic in rats

Farzad Nasrpour[1], Fariba Sharififar[2], Marzieh Barfe[1], Mohammad Mehdipour[3], Somayyeh Karami-Mohajeri[1,3]*

[1] Pharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran
[2] Herbal and Traditional Medicines Research Center, Kerman University of Medical Sciences, Kerman, Iran
[3] Department of Pharmacology & Toxicology, School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran

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ABSTRACT

To evaluate the protective effect of *Zataria multiflora boiss.* (Zm) extract against arsenic-induced oxidative damage in rats. Rats were orally treated with various doses of Zm (200, 400, and 600 mg/kg) and sodium arsenite (5.5 mg/ kg), alone or in combination, once daily for 30 consecutive days. Twenty-four hours after the last dose, rats were euthanized, and biochemical studies were conducted on their blood samples. Sub-acute exposure to the sub-lethal dose of arsenic markedly altered the blood levels of several biomarkers associated with oxidative stress. Treatment with Zm significantly inhibited the elevation of lipid peroxidation and protein carbonylation and the depletion in total antioxidant capacity in plasma. In addition, Zm effectively increased the total antioxidant capacity of plasma in a dose-dependent manner in control and arsenic-treated groups. The results reveal that Zm as an antioxidative medicinal plant reduces oxidative damages induced by arsenic in the doses much lower than the lethal dose (2-4 gr/kg). Since Zm is a safe herbal drug routinely used as condiment, it can be used as a good supplement for reducing toxicity of low dose of arsenic in long-term exposure. Further studies on human environmentally exposed to arsenic through drinking water and food are proposed to find out effective dose in human.

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Keywords
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Corresponding to:
Somayyeh Karami-Mohajeri,
Department of Toxicology and Pharmacology, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, 7616911319, Iran

Email:
s_karami@kmu.ac.ir and;
somayyehkarami@gmail.com

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INTRODUCTION

Industrialization of societies caused a myriad of environmental pollutions problem [1]. One of the environmental pollutants, which has become a global problem, is heavy metals including arsenic. Water and food resources are contaminated with arsenic through natural resources and extensive use of metal in various industries, including ceramics, dyeing, and medical and therapeutic research [2, 3]. Exposure to arsenic usually occurs through contaminated drinking-water and food [4-7]. The most serious problems including vascular disorders, neuropathy, and skin lesions tend to occur in long-term exposure to arsenic [8-11]. It has been shown that replacement of phosphorus by arsenic and binding of arsenic to thiol and sulfidryl groups affect the normal activity of cells [12, 13]. Sodium arsenite has also been shown to increase the production of hemeoxygenase as an indicator of oxidative stress [13, 14]. Arsenic can directly participate in production of reactive oxygen species, including hydrogen peroxide and superoxide anions [15].

Free radicals play an important role in development of many physiological and pathological processes including DNA damage, mutations, cell death, and aging [16]. In pathological conditions, excessive production of free radicals shifts balance between the oxidants and antioxidants in favor of oxidants and induces oxidative damage of important macromolecules leading to cell damage [17]. Enzymatic and non-enzymatic antioxidants react with free radicals and inhibit their harmful effects on cellular processes. [18, 19]. Over the years, herbal antioxidants has gained popularity as a potential therapeutic strategy to prevent oxidative damage caused by medications, environmental pollutants, and many diseases such as diabetes and cardiovascular problems [20].

Zataria multiflora Boiss. (Zm) belonging to the Lamiaceae family grows naturally in Iran, Pakistan and Afghanistan [21]. This plant with the vernacular name of Avishan-e-Shirazi (Shirazian thyme) in Iran is a valuable medicinal and condimental plant with therapeutic and pharmacological effects such as anti-bacterial, anti-fungal, anti-protozoa, anti-spasmodic, anti-inflammatory, and antioxidant [21]. This plant is used traditionally to treat some digestive problems such as dyspepsia, irritable bowel syndrome and bloating, bronchitis, and influenza [22-24]. Phytochemical studies of Zm showed the presence of flavonoid antioxidants such as lutein, non-flovenoid antioxidants such as rosemaric acid, and antioxidant with terpenoid structure such as thymol, carvacrol and Methyl Carvacrol [25, 26]. The plant also contains tryptophan, trionin, isoleucine, leucine, lysine, methionine and cystine amino acids and a small amount of vitamins [27].

The present study was aimed to evaluate antioxidant effect of concomitant administration of hydroalcoholic extract of Zm against sub-acute exposure to sub-lethal dose of arsenic by measuring the extent of oxidative damages of lipids and proteins as well as total antioxidant capacity in plasma.

MATERIAL AND METHODS

Preparation of plant specimen and extraction

Aerial parts of Zm were collected from mountainous regions of Kerman province and were used after confirmation by botanists (Herbarium number: Kf_1241). The amount of 250 g of the plant was weighted, grinded and passed through a sieve of 30 mesh. Methanol extract of Zm were prepared by warm maceration method in 80% methanol for 72 hours. The extract was concentrated under vacuum conditions and finally oven dried at 40-50 °C and stored at -20 °C. Prior to administration, dry extracts were weighed and dissolved in 5% tween (9.5 ml normal saline and 0.5 ml tween) to prepare different concentration of extract.

Experimental animals

In this study, male rats weighing 200-250 g were kept in the animal house of the Faculty of Pharmacy, Kerman University of Medical Sciences under a standard laboratory diet, without restriction on water and food. The conditions of the room were 12 hours light-dark cycles and 25 °C, and relative humidity of 25-30%. Before the experiment, the rats underwent adaptation for 7 days to eliminate the stress and adapt the animals to new conditions.

Animal treatment

Forty eight rats were randomly divided into 8 groups of 6 and treated for 30 days:

Control: Oral normal saline, daily

As: Oral sodium arsenite (5.5 mg/kg, equivalent to 13% of LD_{50}), daily

Zm 200: Oral Zm extract (200 mg/kg), daily

Zm 400: Oral Zm extract (400 mg/kg), daily

Zm 600: Oral Zm extract (600 mg/kg), daily

As + Zm 200: Oral sodium arsenite (5.5 mg/kg) + Zm extract (200 mg/kg), daily

As + Zm 400: Oral sodium arsenite (5.5 mg/kg) + Zm extract (400 mg/kg), daily

As + Zm 600: Oral sodium arsenite (5.5 mg/kg) + Zm extract (600 mg/kg), daily

The Zm extract was gavged 1 hour before administration of arsenic and 24 hours after the end of the treatment, the rats were anesthetized with ketamine and xylazine and blood samples were collected to measure oxidative stress biomarkers. All procedure approved by ethical committee of Kerman University of Medical Sciences (Approval no.: IR.KMU.REC.1393.50) and in accordance with the National Institutes of Health guidelines on animal care.

Measurement of total antioxidant capacity of plasma

Plasma total antioxidant capacity was performed according to ferric reducing antioxidant power (FRAP) method based on conversion of ferric tripyridyltriazine into ferrous tripyridyltriazine in acidic condition [28]. Briefly, 5 µL of plasma sample was added to 295 µL of FRAP reagent and incubated for 10 minutes at 37 °C, and then absorbance was read at 593 nm using BioTek spectrophotometer (Winooski, VT, USA) against blank solution. The change in absorbance using the standard curve of ferrous sulfate was expressed as ferrous equivalent.

Measurement of lipid peroxidation

Lipid peroxidation was evaluated by measuring thiobarbituric acid (TBA) reactive substances (TBARS) in plasma to determine malondialdehyde (MDA), a lipid peroxidation end product [29]. The plasma sample was mixed with 2 volumes of TBA reagent (15% trichloroacetic acid, 0.5% TBA, 2.5 N HCl) and heated in boiling water bath for 20 minutes. Optical density of pink colored complex of TBA-MDA was measured at 532 nm against blank. The MDA concentration of sample was calculated using the molar extinction coefficient of $1.56\times10^5\ M^{-1}\ cm^{-1}$.

Measurement of protein carbonylation

Protein carbonylation level in plasma was assayed using a method based on production of a yellow complex of 2,4dinitrophenylhydrazine (DNPH) with carbonyl groups in oxidized proteins [30]. Briefly, 50 µL of the plasma sample, 200

µl of distilled water, and 25 µl of TCA 20% were mixed thoroughly and centrifuged at 3000 g for 10 minutes. Then, 250 µl of 2 N HCL and 10 mM DNPH (2% w/v in 2 N HCl) were added to, respectively, the blank and the test tubes and kept at 37 ºC for 50 minutes. Proteins were then precipitated by adding 1ml of 100 % TCA and the pellets were washed three times with 1 ml of ethanol: ethyl acetate (1:1) solution and then dissolved in 600 µl of guanidine hydrochloride solution 6 M. the absorbance was read at 370 nm and carbonyl group level was calculated using the molar extinction coefficient of $2.2\times10^4\ M^{-1}\ cm^{-1}$.

RESULTS

Characteristics of ZM extract

The extract of Zm was dry, brittle and dark green with a bitter aromatic taste. The extraction yields from the aerial parts of Zm were 14.24% (w/w).

Result of total antioxidant capacity of plasma

The *in vivo* measurement of FRAP values indicated a significant ($p< 0.01$) decrease in the total antioxidant capacity of plasma in the arsenic-treated group compared to the other groups. Zm at the doses higher than 200 mg/kg increased total antioxidant capacity of plasma in both healthy and arsenic-treated rats in a dose-dependent manner (Fig. 1).

Result of lipid peroxidation

As shown in Figure 1, the lipid peroxidation levels in plasma were higher in the arsenic -exposed rats than the control group (2.1±0.25 *vs* 1.2±0.06 nmol/mg protein, $p<0.001$). On the contrary, the treatment with Zm caused a marked reduction in the plasma TBARS level at all three doses. The lipid peroxidation values did not change in healthy rats received different concentration of Zm (Fig. 2).

Result of protein carbonylation

The protein carbonylation values of plasma in the arsenic-treated group was increased compared to the control group but not significantly (0.35±0.04 *vs* 0.44±0.05 nmol/mg protein). Zm at all three concentration reduced protein carbonyl values in arsenic-treated rats. Zm at the concentration of 400 and 600 mg/kg caused a significant reduction in the

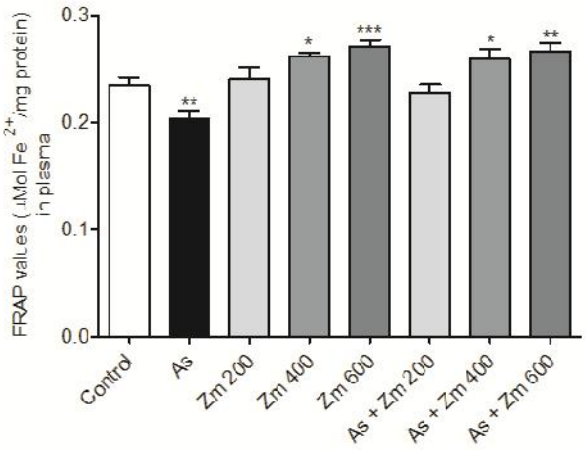

Figure 1. Total antioxidant capacity in control group, arsenic-treated group (As, 5.5 mg/kg), groups received *Zataria multiflora* extract at doses of 200, 400, and 600 mg/kg (Zm 200, Zm 400, and Zm 600), and arsenic-treated groups received *Zataria multiflora* extract at doses of 200, 400, and 600 mg/kg (As + Zm 200, As + Zm 400, and As + Zm 600). Each bar represented as mean ± Standard deviation of six measurements per group, data were analysed by one-way analysis of variance (ANOVA). *p<0.05, **p<0.01, *** p< 0.001

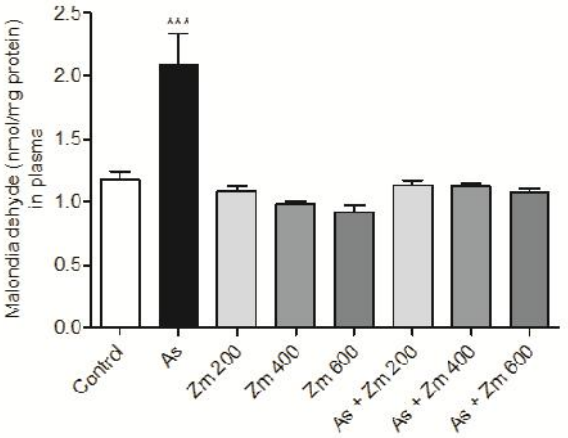

Figure 2. Concentration of malondialdehyde in control group, arsenic-treated group (As, 5.5 mg/kg), groups received *Zataria multiflora* extract at doses of 200, 400, and 600 mg/kg (Zm 200, Zm 400, and Zm 600), and arsenic-treated groups received *Zataria multiflora* extract at doses of 200, 400, and 600 mg/kg (As + Zm 200, As + Zm 400, and As + Zm 600). Each bar represented as mean ± Standard deviation of six measurements per group, data were analysed by one-way analysis of variance (ANOVA). *** p< 0.001

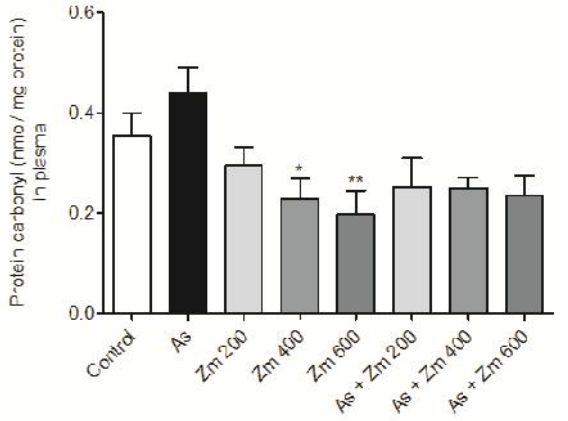

Figure 3. Protein carbonylation in plasma samples of control group, arsenic-treated group (As, 5.5 mg/kg), groups received *Zataria multiflora* extract at doses of 200, 400, and 600 mg/kg (Zm 200, Zm 400, and Zm 600), and arsenic-treated groups received *Zataria multiflora* extract at doses of 200, 400, and 600 mg/kg (As + Zm 200, As + Zm 400, and As + Zm 600). Each bar represented as mean ± Standard deviation of six measurements per group, data were analysed by one-way analysis of variance (ANOVA). *p<0.05, **p<0.01

protein carbonyl levels compared to the control group (Fig. 3).

DISCUSSION

Exposure of rats to the sub-lethal dose of sodium arsenite for 4 weeks resulted in an increase in the oxidation of plasma lipids and proteins with a decrease in the total antioxidant capacity of plasma, as reported before [28, 31-33]. The oxidative damages induced by arsenic have been attributed to disturbing the proxidant and antioxidant balance through thiols oxidation and covalent binding of arsenic to thiol groups which mitigate generation of free radicals [34]. Arsenic leads to excessive production of oxygen species and causes disorders in endogenous antioxidants [35]. Excessive production of free oxygen species also directly affects oxidative damage of membrane and cellular proteins, enzymes, and nucleic acids and increases lipid peroxidatin, protein carbonylation, and DNA fragmentation [36-38]. In the present study, administration of Zm at the doses of 200, 400, 600 mg/kg in rats exposed to sodium arsenite normalized the plasma lipid peroxidation and protein carbonylation, indicating the possible interception of arsenic-induced radical generation by administration of Zm. Previously, it was shown that Zm has beneficial effects on reduction of oxidative damages-induced by many chemicals and drugs [39-42][43-45]. It was also observed that the *in vivo* antioxidant effect of Zm increased dose-dependently, whereas reduction in oxidative damage of lipids and proteins was not significantly dose-dependent which may decrease by increasing the duration of treatment with Zm. However, the doses of ZM which used in this study were 6-12% of LD_{50} and much lower than the lethal dose of maceration extract of Zm [46].

CONCLUSION

Considering various application area of arsenic for industrial purposes and its resulting risks to the environment and human health, it has to be planned to restrict the use of arsenic by replacement with safer chemicals and to find out therapeutics strategies for toxicity outcomes of arsenic exposure by administration of natural products such as Zm as a dietary supplement.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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