Protective Effects of Hydroalcoholic Extract of *Nasturtium officinale* on Rat Blood Cells Exposed to Arsenic

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ABSTRACT

Background: Arsenic is one of the most toxic metalloids. Anemia and leukopenia are common results of poisoning with arsenic, which may happen due to a direct hemolytic or cytotoxic effect on blood cells. The aim of this study was to examine the effects of hydroalcoholic extract of *Nasturtium officinale* on blood cells and antioxidant enzymes in rats exposed to sodium (meta)arsenite.

Methods: 32 Male Sprague Dawley rats were randomly divided into four groups; Group I (normal healthy rats), Group II (treated with 5.5mg/kg of body weight of $NaAsO_2$), Group III (treated with 500mg/kg of body weight of hydro-alcoholic extract of *N. officinale*), and Group IV (treated with group II and III supplementations). Blood samples were collected and red blood cell, white blood cell, hematocrit, hemoglobin, platelet, total protein and albumin levels and total antioxidant capacity were measured. Data was analyzed with Mann-Whitney U test.

Results: WBC, RBC and Hct were decreased in the rats exposed to $NaAsO_2$ ($p<0.05$). A significant increase was seen in RBC and Hct after treatment with the plant extract ($p<0.05$). There was no significant decrease in serum albumin and total protein in the groups exposed to $NaAsO_2$ compared to the group I, but $NaAsO_2$ decreased the total antioxidant capacity, significantly.

Conclusion: The *Nasturtium officinale* extract have protective effect on arsenic-induced damage of blood cells.

Keywords: Arsenic; Blood Cells; *Nasturtium officinale*; Oxidative Stress.

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INTRODUCTION

Arsenic is one of the most toxic metalloids which is found in soil, water and air as a potential human carcinogen [1]. Many studies have confirmed the production of free radicals during arsenic metabolism in the cell [1-4]. It has been reported that exposure to sodium arsenite decreased the antioxidant enzymes, e.g. superoxid dismutase (SOD), catalase (CAT) and glutathion peroxidase (GPx) activity, significantly [5].

Anemia and leukopenia are common results of poisoning with arsenic which may happen due to a direct hemolytic or cytotoxic effect on blood cells [6]. Trivalant arsenics are potent inhibitors of thioredoxin reductase (a NADPH-dependent flavoenzyme), so they inhibit the cellular responses to oxidative stress [7]. Defense system against free radical-induced oxidative stress includes enzymatic antioxidants (superoxide dismutase, catalase and gluthation peroxidase) and non-enzymatic antioxidants (vitamin C, vitamin E, carotenoids and flavonoids) [8].

In recent years, attentions have been paid toward medicinal plants. Phenolic compounds, particularly flavonoids, have antioxidant

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activities [9, 10]. *Nasturtium officinale* (watercress) from Brassicacea family is a perennial plant that thrives in cold water and is found in streams and contains vitamins A, C, E, gluconasturtine and some minerals, e.g. iron and phosphorus [11, 12]. Watercress is used as a home remedy for hypertension, cardiovascular disease and hyperglycemia [13].

The aim of this study was to examine the effects of hydroalcoholic extract of *Nasturtium officinale* on blood cells and antioxidant enzymes in rats exposed to sodium (meta)arsenite ($NaAsO_2$).

MATERIALS AND METHODS

Plant material and extraction

Aerial parts of *N. officinale* were gathered from Kaleybar City, East Azerbaijan Province, Iran, and are identified (NO. 711Tbz-fph) by an expert faculty of Tabriz University of Medical Sciences, Iran (Dr. Nazemiyeh). The samples were air-dried and powdered and were kept in a closed container at 8°C until extraction. 700g of the powder was extracted three times with the mixture of ethanol/water (4:1) at the room temperature overnight. The solvent was completely removed by a rotary evaporator (Heidolph; Germany) at 50°C. The final residue of the extract was used for tests.

Animals and experimental groups

32 Male Sprague Dawley rats weighing 180-200g (Iran; Central Animal House of Tabriz Medical School) were housed at 22±2°C and 60±5% relative humidity with a 12h light/dark cycle. They had access to water and normal diet *ad libitum*. The experimental protocol was approved by the medical ethics committee, and all animals received human care in compliance with the guidelines of Tabriz University of Medical Sciences. After the adaptation period (7 days), the rats were randomly divided into four groups (n=8); Group I (normal healthy rats), Group II (treated with 5.5mg/kg of body weight of $NaAsO_2$), Group III (treated with 500mg/kg of body weight of hydro-alcoholic extract of *N. officinale*), and Group IV (treated with group II and III supplementations). The groups were treated every day for 28 days with their supplementations and after the day 28, blood samples were collected.

Assessments

Hematological parameters: Whole blood samples were collected using EDTA as anticoagulants and were analyzed automatically with an Auto Hematology MINDRAY (BC-2800) for for red blood cell (RBC), white blood cells (WBC), hematocrit (Hct), hemoglobin (Hb), platelet (Plt).

Biochemical analysis: Total Antioxidant Capacity (TAC) was measured using Randox TAC status test (United Kingdum; Randox Laboratories Ltd.) in serum. ABTS (2,2′-Azino-di-[3-ethylbenzthiazoline sulphate]) was incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the ABTS radical cation that has a relatively stable blue-green color at 600nm. Antioxidants suppress this color production to a degree which is propotional to their concentration. Serum albumin and total protein were measured by an autoanalyzer (Abbott ALCYON^{TM300}; USA) and enzymatic Kits (Pars Azmoon; Iran).

Statistical analysis

Data was inserted into SPSS 16 software and was analyzed with Mann-Whitney U test to compare the groups at the significance level $p<0.05$.

RESULTS

WBC, RBC and Hct were decreased in the rats exposed to $NaAsO_2$, significantly ($p<0.05$). A significant increase was seen in RBC and Hct after treatment with the plant extract ($p<0.05$). There was no significant decrease in serum albumin and total protein in the groups exposed to $NaAsO_2$ compared to the group I, but $NaAsO_2$ decreased the total antioxidant capacity, significantly (Table 1).

Table 1. The average of hematological and biochemical parameters analysis of all 4 groups (Alb: Albumin; TP: Total Protein; TAC: Total Antioxidant Capacity; WBC: White Blood Cells; RBC: Red Blood Cells; Hb: Heamoglobin; Hct: Hematocrit; Plt: Platelet).

Parameters	Group I	Group I	Group III	Group IV
Hematological				
WBC (10^9/l)	13.80±2.10	10.45±2.40*	13.00±3.20	11.18± 1.30
RBC (10^{12}/l)	8.38±0.30	7.11±0.60*	7.80±0.50	8.20±0.30**
Hb (g/dl)	14.17±0.50	13.54±2.00	14.15±0.50	13.60±1.20
Hct (%)	44.90±1.50	39.04±2.90*	43.53±1.40	42.44±2.80**
Plt (10^9/l)	564.60±59.70	678.70±77.90*	519.60±95.00	663.30±45.90
Biochemical				
Alb (g/dl)	2.91±0.10	2.90±0.1	3.08±0.20*	3.39±0.20**
TP (g/dl)	7.84±0.40	7.80±0.6	8.35 ±0.40*	8.87±0.70**
TAC (mmol/dl)	0.89±0.13	0.67±0.23*	1.07±0.33**	1.15±0.37**

*p<0.05 compared to group I; **p<0.05 compared to group II

DISCUSSION

WBC, RBC and Hct were decreased in the rats exposed to sodium arsenite. The reduction of these hematological parameters might be due to the effect of arsenic on heme synthesis by inhibition of Delta-aminolevulinic acid dehydratase (ALAD), detoxification mechanism of arsenic (methylation of arsenic and depletion of gluthation), inhibition of pyruvate dehydrogenase and reduction of ATP and oxidative damage to blood cells.

Our results suggest that hydro-alcoholic extract of *N. officinale* may improve some hematological parameters. Many studies have reported that arsenic decreased WBC, RBC and hemoglobin [14-17]. Bone marrow depression in humans has been reported with high dose of arsenic [18]. 95-99% of the arsenic is located in erythrocytes after absorption, bound to the globin of hemoglobin and transported to the other parts of the body [19]. Rats differ from most mammalian species by accumulating dimethyl arsenic in erythrocytes probably by binding to a cysteine component of hemoglobin [20, 21].

Anemia and leukopenia may be due to hemolytic or cytotoxic effect of arsenic on the blood cells. The mechanism of hemolysis is depletion of intracellular GSH (gluthation) resulting in oxidation of sulfhydryl groups in the hemoglobin from ferrous to ferric in mice and rats [18, 22]. Erythrocytes may be susceptible to the oxidative damage due to the presence of heme iron, polyunsaturated fatty acids (PUFA) and oxygen, which may initiate the reactions that induce oxidative changes in RBC [23, 24]. Inhibition of pyruvate dehydrogenase (by binding of arsenic to dihydrolipoic acid, a pyruvate dehydrogenase cofactor, and inhibiting the conversion of pyruvate to acetyl coenzyme-A) or reduction of succinyl coenzyme-A decrease the production of ATP and may damage the cell slowly [18, 25, 26]. Antioxidant enzymes activity was decreased in rats exposed to sodium arsenite [27]. So sodium arsenite-induced oxidative stress and reduction of antioxidant enzymes activity and inhibition of heme synthesis may lead to the damage of blood cell and reduction of RBC, WBC and Hct in the present study.

Treatment of rats with polyphenols might have ameliorated the hematological system of rats exposed to lead [28]. Watercress may be able to improve antioxidant enzymes activity and some of hematological parameters due to the phenolic compounds or other compounds, e.g. isothiocyanates or beta-carotene.

Consumption of the plant extract with- (group IV) and without sodium arsenite (group III) showed a significant increase in serum levels of albumin, total protein and total antioxidant capacity. Tanju & Madhuri have reported that sodium arsenite decrease total protein and albumin in serum [29]. Increasing of serum protein and albumin has been reported with

consumption of ascorbic acid and plant antioxidants [29, 30]. Albumin plays an important role in transportation of some compounds in blood, so increasing of albumin in serum may help transporting of plant extract and sodium arsenite in blood. As these proteins are produced in the liver, any changes in the concentration of serum proteins and albumin indicate a change in the normal function of liver [30]. Plant extract has been reported to decrease the lipid peroxidation and aminotransferase activity and increase the status of antioxidant enzymes in liver [27]. So, increasing the total protein of serum is thought to be associated with hepato-protective effects of plant extract and improving of proteins synthesis in liver or immune responses [31]. Asadi *et al.* have indicated that watercress enhances globulin levels and improves the immune system [31]. Increasing of the total antioxidant capacity might be due to the effects of substances such as vitamin C, vitamin E, carotenoieds (e.g. β-carotene) or superoxide scavenging activity (e.g. quercetin) of flavonoids in the plant extract.

Further studies are needed to determine the possible mechanisms of action and specific bioactive compounds in *Nasturtium officinale*. It is recommended that long-term animal studies on depending use of other oxidants, the dose and route of arsenic exposure to evaluate the effects of the extract be carried out.

CONCLUSION

The *Nasturtium officinale* extract have protective effect on arsenic-induced damage of blood cells and can improve hematological parameters and total antioxidant capacity.

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