Effects of sodium arsenite on the some laboratory signs and therapeutic role of thymoquinone in the rats

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Abstract. – OBJECTIVE: Serious health problems in humans are caused by arsenic (As) exposure, which is wide spread in the environment. Sodium arsenite (SAs), capable of inducing macromolecular damage is evaluated for its damaging effect in the blood vessels, liver and kidneys of Wistar rats. This study was undertaken to investigate the ameliorative effects of thymoquinone on SAs-induced oxidative and inflammatory damages in the serum of male Wistar rats.

MATERIALS AND METHODS: Wistar Albino rats divided into three groups of nine rats each were administered to controls saline (10 mg/kg), SAs (10 mg/kg), and SAs plus thymoquinone (10 mg/kg/day) for two weeks orally. Biochemical tests were analyzed by a otoanalyzer; nitric oxide levels specthrophometrically, and cytokines were measured by ELISA method in the rat serum samples.

RESULTS: Inflammatory cytokines and some biochemical variables were found to be increased in the SAs group compared to control group. On the other hand, thymoquinone supressed these laboratory signs, which are thought to be the characteristic signs of SAs toxicity, most probably by its ameliorative effects including anti-inflammatory and antioxidant properties.

CONCLUSIONS: From the results obtained, thymoquinone mitigates SAs-induced adverse effects in the serum of rats, which suggest that it may attenuate inflammation implicated in endotelial dysfunction.

Key Words:
Sodium arsenite, Inflammatory cytokines, Thymoquinone, Oxidative stress, Rats.

Introduction

Epidemiologic studies demonstrated that long-term exposure to inorganic As through ingestion and inhalation is associated with neurotoxicity, skin lesions, diabetes mellitus, cardiovascular diseases and cancers in human[1]. In some newly released publications, it is reported that As also has deleterious effects on the immune system, and its some chronic effects might be related to immunotoxic properties[1-3]. There is a strong body of evidence linking As-induced oxidative stress and endothelial inflammation which is a hallmark of atherosclerosis[4]. Animal and *in vitro* studies both suggest that oxidative stress may be a mechanism of As toxicity[5]. Interleukin-6 (IL-6), a proinflammatory cytokine, and monocyte chemoattractant protein-1 (MCP-1; also known as CCL2 chemokine), were induced in atherosclerotic lesions and plasma of As-exposed ApoE-/- mice[6], circulating lymphocytes of As-exposed human subjects[7] and As-treated vascular smooth muscle cells[8]. Like this, macrophage migration inhibitory factor (MIF), a pleiotropic cytokine, has been found to be associated with inflammation and immune responses[9]. Thymoquinone (TQ) has been known as an functional phytochemical isolated from *Nigella sativa* seeds. Therefore, it has been examined for its anti-inflammatory, antioxidant, and anticancer activities in experimental setups *in vitro* and *in vivo*[10]. Moreover, it has been observed that thymoquinone (Figure 1) could act as a superoxide radical (also a general free radival) scavenger, as

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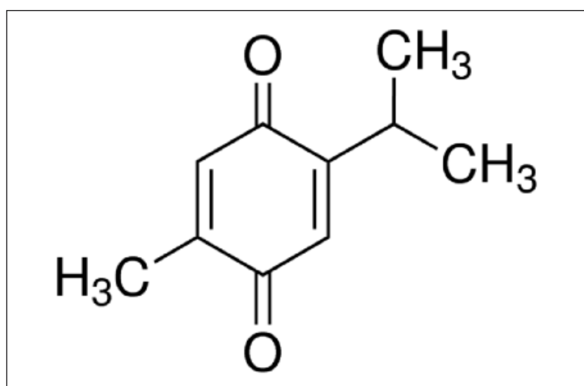

Figure 1. The chemical structure of thymoquinone.

well as conserving the activity of several antioxidant enzymes[11]. In the present study, it was aimed to investigate deleterious effects of SAs, and a possible therapeutic effects of thymoquinone treatment on the biochemical (inflammatory and oxidative) markers in the serum samples of As-induced rats.

Materials and Methods

Twenty-seven adult male Wistar Albino rats, weighting 250-300 grams were included in this study. Rats were kept at optimal conditions (room temperature between 22-25°C and humidity was kept between 65% and 70%) for 15 days in standard rat cages and free access standart pellet feed and water *ad libitum*, and 12-h light/dark cycle was maintained. All procedures involving animals were approved by the Institutional Committe for Animal Care. The animals were randomly selected and were assigned to the experiment groups as follows: control group, n:9, SAs, n:9, SAs plus TQ, n:9. Each group were administered intragastrically with saline (10 ml/kg), SAs (10 mg/kg), and SAs + TQ (10 mg/kg) for two weeks. Sodium arsenite and thymoquinone were purchased from Sigma-Aldrich Chemical (Deisenhofen, Germany). TQ was dissolved in corn oil, both SAs (soluble in water) and TQ solutions were prepared freshly daily.

At the end of the experiment rats were sacrificed under the ketamine/xylazine anesthesia; blood samples were obtained. They were kept on hold for half an hour and then centrifuged at 3000 rpm for 15 min at 4°C to separate serum which was stored at -20°C for the different biochemical measurements. The serum levels of MIF (catalog no: CSB-E07293r), and MCP-1 (catalog no: CSB-E07429r), (Cusabio Biotech, Wuhan, China) and IL-6 (catalog no: BMS625), (eBioscience, Vienna, Austria) were quantitatively detected by commercial enzyme-linked immunosorbent assay (ELISA) kits by using a plate reader. The following analytes were measured on a Cobas c501 (Roche Diagnostics, GmbH, Mannheim, Germany), using proprietary reagents; albumin, total protein, alanine amino transferase (ALT), aspartate amino transferase (AST), urea, creatinine, uric acid, triglyceride, total cholesterol, and HDL-cholesterol. There are some difficulties to detect NO levels in biological specimens. Therefore, nitrite and nitrate levels were measured to estimate the NO production in our experimental setup. Serum nitrite and nitrate levels were estimated by using a method based on the Griess reaction[12].

Statistical Analysis

Data were analyzed by using Statistical Package for Social Sciences (SPPSS) version 15.0 computing program (SPSS Inc., Chicago, IL, USA). Differences in measured parameters among the three groups were analyzed by a Kruskal-Wallis test, and Mann-Whitney U test were used to analyze the variance among groups if appropriate. Results were expressed as mean±standard deviation of means for the parameters; if $p < 0.05$ were considered as statistically significant.

Results

After going through the procedure stated in the methodology the following results were obtained. As shown in Table I, significant increases ($p < 0.01$) in SAs group rats in the activities of the liver enzymes; ALT, AST were observed in comparison to the control rats. The mean concentrations of serum creatinine, triglyceride, total cholesterol, IL-6, MCP-I and MIF were significantly increased and serum albumin concentration was significantly decreased in SAs group in comparison to the control group ($p < 0.01$). There were no significant differences between control group and SAs group in according to the mean serum total protein, urea, uric acid, NO and HDL-cholesterol concentrations. Compared to the serum concentrations for the SAs group, serum concentrations of ALT, AST, creatinine, triglyceride, total cholesterol, IL-6, MCP-1 and MIF were significantly lower and albumin concentration was significantly higher in SAs + TQ group ($p < 0.001$). There were no sig-

Table I. Results of biochemical parameters in the control, SAs-induced, and SAs-induced plus TQ-treated group serum samples (results were expressed mean ± standard deviation).

	SAs + TQ (n:9)	SAs (n:9)	Control (n:9)
Albumin (g/dl)	3.91 ± 0.17#	3.57 ± 0.13*	4.01 ± 0.26
Total protein (g/dl)	5.59 ± 0.31 ns	5.71 ± 0.37 ns	5.52 ± 0.30
ALT (U/L)	22.22 ± 2.5#	31.1 ± 2.8*	21.1 ± 1.9
AST (U/L)	115.1 ± 16.8#	150.8 ± 18.7*	99.8 ± 5.7
Urea (mg/dl)	42.8 ± 3.9 ns	47 ± 4.2ns	43.5 ± 3.8
Creatinine (mg/dl)	0.29 ± 0.02#	0.39 ± 0.04*	0.26 ± 0.03
Uric acid (mg/dl)	1.04 ± 0.28 ns	1.29 ± 0.43 ns	0.87 ± 0.17
Triglyceride (mg/dl)	54.6 ± 11#	75.4 ± 13*	53.8 ± 13
Total Cholesterol (mg/dl)	57.4 ± 5.1#	64.8 ± 7.7*	53.9 ± 6
HDL-Cholesterol (mg/dl)	35 ± 5.0 ns	37 ± 4.5 ns	32 ± 5.4
Nitric oxide (μmol/L)	61.7 ± 8.1 ns	70.6 ± 8.1 ns	67.4 ± 12.7
IL-6 (pg/ml)	14.6 ± 1.85#	26.7 ± 3.2*	12.8 ± 1.1
MCP-1 (pg/ml)	5.57 ± 0.8#	6.65 ± 0.6*	5.29 ± 0.7
MIF (pg/ml)	55.5 ± 7.7#	74.3 ± 10*	50.7 ± 6.5

*Significant compared to control group $p < 0.001$; ns Not significant; #Significant compared to SAs group $p < 0.001$.

nificant differences between SAs + TQ group and control group for serum albumin, ALT, creatinine, triglyceride, total cholesterol, IL-6, MCP-1 and MIF concentrations (Figure 2). Thymoquinone treatment resulted in marked decrease in serum levels of ALT, AST, creatinine, triglyceride, total cholesterol, IL-6, MCP-1 and MIF and increase in serum level of albumin. Serum levels of total protein, urea, uric acid, HDL cholesterol and NO were not different between the SAs and SAs + TQ group.

Discussion

Arsenic is an environmental and industrial pollutant affects almost every organ system in human and experimental animals. Chronic exposure to As may affects various internal organs in humans. Liver is one of the important target organs. Abnormal liver functions as manifested by clinical increases of several liver enzymes in blood including ALT and AST also are associated with chronic As exposure[1,2]. Similar to our study, the treatment with SAs exhibited a significant increase in hepatic and renal biochemical parameters (ALT, AST, total protein, cholesterol, urea and creatinine) in serum[13]. Furthermore, exposure of SAs significantly increased ALT and AST activities and leucocyte count in the rat blood[14]. However, curiously the co-administration of green tea extract[13] and Se[15] decreased the concentration of biochemical parameters such as ALT and AST activities, and improved the antioxidant status as well as in the SAs-induced rats. Otherwise, the kidney is also known as an important target organ for As toxicity and is critical for As biotransformation and elimination[16]. Blood test results obtained from the present study suggested that both liver and kidney were damaged in the SA-treated rats. Arsenic trioxide (As_2O_3) leads a broad range of organ-specific diseases and cancers, and promotes vascular remodeling, portal fibrosis and hypertension in the human liver[17]. On the other hand, although As is known to cause cancers of lung, skin and kidney, trivalent As_2O_3 has been recently recognized as one of the most effective novel anticancer agent for the treatment of acute promyelocytic leukemia[18,19]. These paradoxical effects of As may be dose-dependent, associated with its distinctive metabolism, or related with its direct or indirect effects on different cellular pathways which may result in altered cellular functions[19]. It should be noted that most laboratory animals appear to be substantially less susceptible to As than humans. It has been reported that chronic oral exposure to inorganic As (0.05-0.1 mg/kg/d) causes hematological toxicity in humans but not in rats exposed to As at doses of 0.72-2.8 mg/kg/d[2]. Caciari et al[20] demonstrated that chronic exposure to low-dose As causes decrease in red blood cells, and provokes an inflammatory response. It is reported[2] that chronic As exposure has many effects on the vascular system including thickening of blood vessels and occlution.

It has been reported previously that As exposure is related to increased incidence of auto-im-

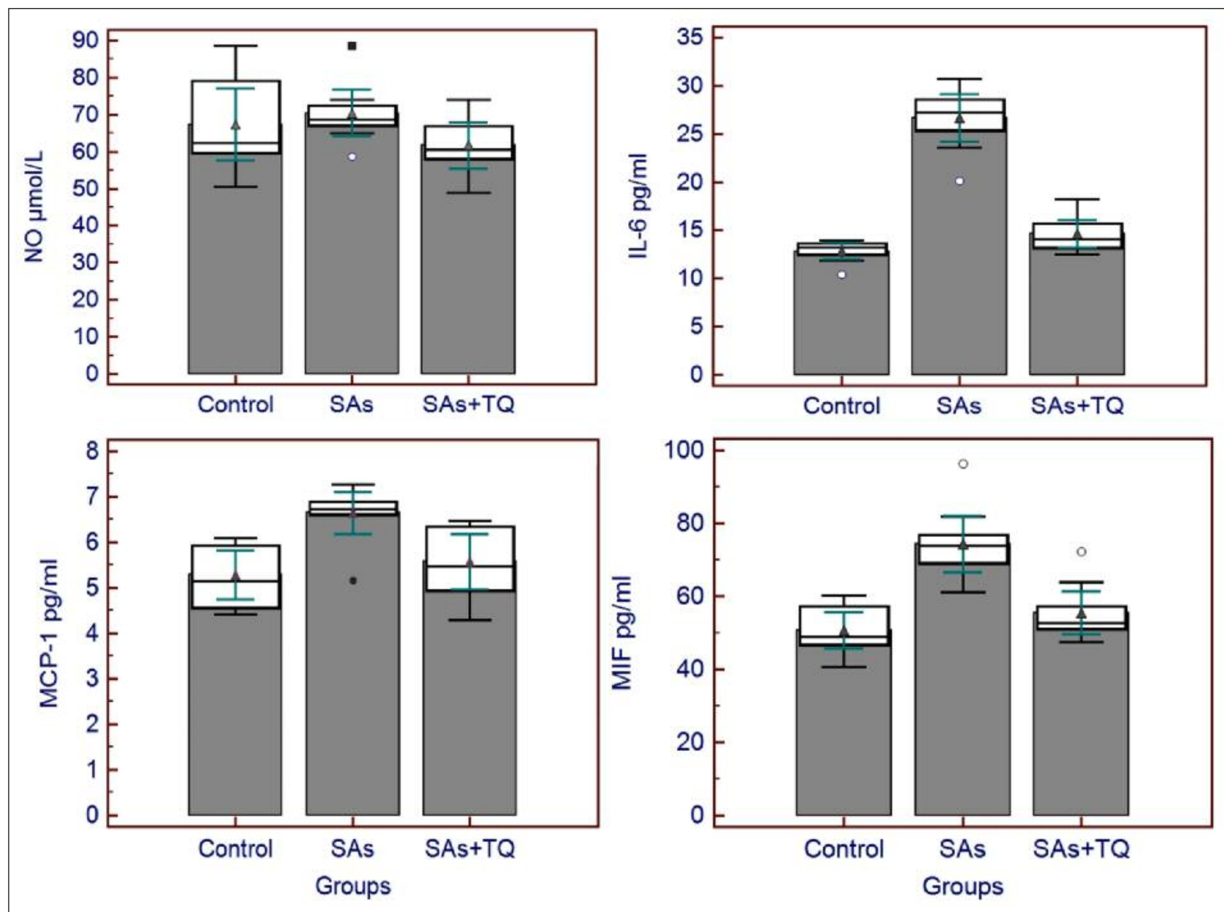

Figure 2. Comparison of the effects of SAs and TQ on the levels NO, IL-6, MCP-1 and MIF in the control and experimental groups.

mune diseases such as diabetes mellitus and development of atherosclerosis[21]. Moreover, inflammation is a key component of the generation of atherosclerotic lesions, and increased inflammatory molecule expression after As exposure has been reported[7]. Mechanistic evidence suggests that As exposure increases the production of ROS and influences inflammatory responses and endothelial nitric oxide (NO) homeostasis[22]). Banerjee et al[23] found that chronic As exposure induces cell rounding, a subsequent loss of cell adhesion capacity and F-actin expression. Additionally, As exposure reduces the NO production, and accompanied with impaired macrophage function in the subject with skin lesions. In another study, results indicated that superoxide readical production were increased in a time-dependent manner in blood monocyte-derived macrophages treated with As_2O_3 for 72 h *in vitro*[24]. Also, they found that As_2O_3 induced a prominent activation of NADPH oxidase, most probably by the stimulation of a Rho-kinase/p38-kinase pathway, and which have a potential to contribute to some of the harmful effects of iAs on macrophage phenotype. Das et al[1] demonstrated that circulating levels of IL-6, IL-8 and MCP-1 as indicators of cardiovascular disease associated with As exposure (although significantly higher IL-6 and IL-8, MCP-1 was not significant in the exposed group). Moreover, the significant increases in the laboratory results like ALT, AST and anti-nuclear antibody worsened the situation by generating autoimmune markers as observed in significant rise in serum IL-6 and IL-8 levels, which finally caused liver injury and increased As-associated cardiovascular risk. In this study, although NO levels were higher in As group compared to control group, the difference was not significant in the study. Whereas, as shown in the Table I and Figure 2, the levels of IL-6, MCP-1 and MIF levels were higher in the As group compared to control group significant-

ly. In a recent study[25], novel evidence that genetic variants related to As metabolism may play an important role in As-induced subclinical atherosclerosis. Also, both MCP-1 and MIF-1 are suggested to be involved in the development of atherosclerosis (26). In addition to OS effects in the development of atherosclerosis, As administration increased total cholesterol level as well as the reduced HDL cholesterol level in the serum of mice[27]. In this study, the serum triglyceride and total cholesterol levels significantly increased with As administration, and their enhanced levels reduced with TQ treatment.

Thymoquinone have long been ingested to treat a broad range of diseases, including inflammation and cancer. It has anti-inflammatory effects blocking the synthesis of leukotrienes, and the effect of TQ in remediating oxidative damage and inflammation of tissues have been cited in several reports[11,28]. Antioxidant potential of TQ is associated with scavenging ability against ROS (superoxide and hydroxyl radicals, hydrogen peroxide, and peroxynitrate) through modulation of hepatic and extra hepatic antioxidant enzymes[29]. Interestingly, TQ ameliorated hepatotoxicity of carbon tetrachloride as detected by the significant decline of the elevated levels of serum enzymes and significant increase of the hepatic GSH content[30]. Fouad et al[31] explored protective effects of TQ in the rats exposed to testicular injury induced by SAs (10 mg/kg/day, orally, for two days). TQ treatment decreased significantly high testicular malondialdehyde and NO levels, conversely increased low testicular GSH in the ASs-induced rats. In addition, SAs showed a marked increase in iNOS immunoreactivity in the cytoplasm of the cells of seminiferous tubules; but TQ an obvious decrease in iNOS immunostaining. Elsherbiny and El-Elsherbiny[32] reported that TQ attenuated renal oxidative stress, inflammation and reversed redox imbalance. According to their studies, it is not surprising that treatment with TQ reduced levels of inflammation and increased levels of anti-inflammatory cytokines such as IL-6 and TNF-α in renal tissues. Similar, in a study[33]), it is found that TQ reduced lipid peroxidation, NO and IL-6 levels in the kidney of diabetic rats. It is reported[34] that TQ dose-dependently reduced the expression of TNF-α and the intrinsic activity of MCP-1 promoter in pancreatic ductal adenocarcinoma cells. Additionally, it is concluded that TQ attenuates hypercholesterolemic atherosclerosis and its effect is associated with a decrease in serum lipids and oxidative stress[35]. Taken together these results, it is understood that the TQ have anti-inflammatory and antioxidant effects. Our study results also showed its reduced IL-6, MCP-1, MIF and NO levels in the serum of SAs-induced rats.

Conclusions

Causes of tissue damage are represented by disintegrating metabolic responsiveness and regulations, vitiating antioxidant systems, decomposing immune competent cells, and finally inducing DNA damages. Therefore, it may be concluded that supplementation of TQ significantly protects deleterious effects from SAs-induced toxicity by reducing inflammatory and oxidative damages, as indicated by levels of serum biomarkers.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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