Life Sciences 217 (2019) 91–100

Contents lists available at ScienceDirect

Life Sciences

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

Ameliorative effect of gallic acid on sodium arsenite-induced spleno-, cardio- and hemato-toxicity in rats

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ARTICLE INFO

Keywords:
Arsenic toxicity
Gallic acid
Reactive oxygen species
Antioxidant
Histopathology
Hematological parameters

ABSTRACT

Aim: Arsenic is an important toxic chemical affecting millions of people around the world. Exposure to inorganic arsenic results in various health problems including skin lesions, hypertension, hematological disturbance, cardiovascular disease, spleen enlargement and cancer. Gallic acid (GA) is an important phenolic compound possessing various pharmacological properties including anti-inflammatory, antioxidant and free radical scavenging activities. The present study investigated effects of GA against sodium arsenite (SA)-induced spleno-, cardio- and hemato-toxicity.

Main methods: Thirty-five adult male Wistar rats were randomly divided into five groups; group I received normal saline (2 ml/kg/day, p.o.) for 21 days, group II received SA (10 mg/kg/day, p.o.) for 14 days, group III and IV were treated with GA (10 and 30 mg/kg/day, respectively) for 7 days prior to receive SA and treatment was continued up to 21 days in parallel with SA administration, group V received GA (30 mg/kg/day, p.o.) for 21 days. The level of MDA, NO· and glutathione (GSH) and the activity of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase were measured in heart and spleen tissues. Creatine kinase-MB (CK-MB) activity and hematological and histopathological parameters were also assessed.

Key findings: GA significantly decreased SA-induced elevation of MDA and NO· levels and reduction of GSH level and GPx and SOD activity in heart and spleen tissues. Furthermore, GA improved SA-induced alteration in hematological and histopathological parameters and reduced SA-induced elevation of serum CK-MB activity.

Significance: Our results suggest that GA inhibits SA-induced spleno-, cardio- and hemato-toxicity through reducing oxidative stress.

1. Introduction

Arsenic is one of the most important toxic chemicals affecting about 150 million people around the world. Arsenic occurs in both organic and inorganic forms in the environment, which inorganic compounds (arsenite, As^{+3} or arsenate, As^{+5}) are highly toxic. Arsenite is reported to be more toxic compared to arsenate [1]. The use of contaminated drinking-water is the main source of human exposure to arsenic. Furthermore, exposure to arsenic may occur via ingestion of contaminated food, dermal contacts, and inhalation of arsenic-containing air [2,3]. Chronic exposure to toxic inorganic arsenic leads to various health problems including skin lesions, hypertension, hematological disturbance, cardiovascular disease, neurological disease, reproductive disorders, liver and kidney diseases, diabetes, spleen enlargement and cancer of skin and internal organs [4–6]. Exposure to arsenic contributes to myocardial injury, cardiac arrhythmias and cardiomyopathy [7,8]; this cardiac toxicity induced by arsenic has been reported to be associated with the reduction of antioxidant capacity [9]. Arsenic has also been reported to alter hematological parameters and increase sensitivity of people to infections [10,11]. Due to the role of spleen in the cellular immune function [12], arsenic is suggested to promote spleen toxicity. The exact mechanisms of arsenic toxicity are not fully understood, but, arsenic is found to increase the generation of reactive oxygen and nitrogen species (ROS/RNS) resulting in the lipid

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https://doi.org/10.1016/j.lfs.2018.11.050
Received 24 August 2018; Received in revised form 17 November 2018; Accepted 21 November 2018
Available online 22 November 2018

peroxidation, protein oxidation and DNA damage [13]. Arsenic induces the production of various free radicals including peroxyl radicals (ROO·), singlet oxygen ($^1O_2$), superoxide ($O_2\cdot^-$), hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), nitric oxide (NO·) and dimethylarsinic peroxyl radicals [$(CH_3)_2AsOO\cdot$]. Under physiological conditions, arsenite is metabolized to arsenate which this reaction is associated with the formation of H_2O_2. Then, H_2O_2 is decomposed to hydroxyl radicals via Haber–Weiss reaction; hydroxyl radicals are highly reactive ROS reacting with biological macromolecules [2]. Furthermore, arsenite inhibits the activity of antioxidant enzymes including catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-*S*-transferase (GST) and superoxide dismutase (SOD) as well as the content of reduced glutathione (GSH) [14]. These indicate that arsenic could induce oxidative stress by increasing free radical generation and decreasing antioxidant capacity of cells, which contributes to the oxidative damage of tissues. Current therapeutic approaches for arsenic intoxications include the use of chelating agents such as dimercaptosuccinic acid (DMSA) or British Anti Lewisite (BAL), which these compounds promote the excretion of arsenic from the body and prevent arsenic-induced tissue damage. However, these compounds have a number of toxic effects which limit their usage at therapeutic doses for an extended period of time [15]. The limitations of conventional therapeutic strategies highlight the need for clinically safe and efficacious new agents to reduce arsenic-induced toxicity. Recent studies have shown effectiveness of alternative and herbal medicine in the reduction of deleterious effect of arsenic on various organs and tissues [16–19].

Gallic acid (3,4,5-trihydroxicbenzoic acid, GA) is an important phenolic compound which could be extracted from fruits including mango, grapes, walnut, areca nut and different types of berries as well as from red wine, green tea and oak bark. Gallic acid has been reported to possess various pharmacological properties including anti-inflammatory, anticancer, antimicrobial, antioxidant and free radical scavenging activities. Recent studies indicate that this phenolic substance could protect brain, testis, liver and kidney tissues from oxidative damage and ameliorate the incidence of heart infarction [20–24]. The anti-inflammatory effects of GA result from the inhibition of nuclear factor-κB (NF-κB) activity and subsequent reduction of pro-inflammatory cytokines including interleukin-1β (IL-1β), IL-6, IL-8 and tumor necrosis factor α (TNF-α) as well as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) [25–28]. Furthermore, GA exhibits the antioxidant effect through increasing the activity of cellular antioxidant enzymes including CAT, GPx and SOD [24]. Considering the important etiological role of oxidative stress in arsenic-induced cardiac-, spleen- and hemato-toxicity, and antioxidant properties of GA, it was thought that GA may be a promising agent to reduce arsenic-induced oxidative damage. Current work was therefore carried out to investigate the effects of GA on sodium arsenite (SA)-induced oxidative stress in heart and spleen tissues and alteration of hematological parameters.

2. Materials and methods

2.1. Chemicals

Reduced glutathione (GSH), gallic acid HCl (dissolved in normal saline), 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), bradford reagent, bovine serum albumin (BSA) were obtained from Sigma–Aldrich Chemical Company (St. Louis, MO), USA. Sodium arsenite (SA, NaAsO2, dissolved in normal saline) and all other materials used in this experiment were purchased from Merck Company (Darmstadt, Germany). Doses of SA and GA used in this study were based on the findings of previous studies [22,24,29,30].

2.2. Animals

Thirty-five adult male Wistar strain rats (200–250 g in weight) were obtained from the animal house of Ahvaz Jundishapur University of Medical Science, Iran. Animals were housed in polypropylene cages at a standard condition; under a 12-h dark/light cycle at 20 ± 2 °C with free access to standard rat chow and drinking water. This investigation was performed according to the decision of Animal Experiments Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Ethic code: IR.AJUMS.REC.1395.126).

2.3. Experimental design

Animals were randomly divided into five experimental groups. Group I served as control and received normal saline (2 ml/kg/day, p.o.) for 21 days. Group II received SA (10 mg/kg/day p.o.) for 14 days. Group III and IV were treated with GA (10 and 30 mg/kg/day, respectively) dissolved in normal saline for 7 days prior to receive SA, which treatment was continued up to 21 days in parallel with SA administration. Group V received GA (30 mg/kg/day, p.o.) for 21 days.

2.4. Sample collection

After 24 h from the last administration, blood samples were taken from the jugular vein of animals under ketamine/xylazine (60/6 mg/kg, i.p.)-induced anesthesia. Collected blood was divided to two samples which one sample was centrifuged at 3000 rpm for 10 min to separate serum and another sample was tested for complete blood count. Animals were sacrificed by rapid decapitation. Heart and spleen tissues were isolated and washed with normal saline. One part of tissues was fixed in 10% phosphate buffered formalin for histological examination and the remained part was homogenized (1/10 w/v) in 0.1 M ice-cold Tris-HCl buffer (pH 7.4) for biochemical analysis; the protein concentration was determined using Bradford assay [31].

2.5. Hematological assays

Collected blood was used for the determination of white blood cells (WBC), red blood cells (RBC), hematocrit (HCT), hemoglobin (HGB), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and platelets (PLT). To prevent coagulation, samples were inverted in an EDTA-coated tube for several times. Measurements were conducted using a Sysmex K4500 hematology analyzer.

2.6. CK-MB assay

Creatine kinase-MB (CK-MB) activity was estimated in serum using commercially available CK-MB assay kit (BioAssay Systems, USA) adopting the Bishop method [32].

2.7. Tissue biochemical parameters

2.7.1. Malondialdehyde (MDA) assay

The MDA level, as index of lipid peroxidation, was determined using MDA assay kit (Teb Pazhouhan Razi (TPR), Tehran, IRAN). Briefly, heart and spleen tissues homogenates were centrifuged at 13000*g* for 10 min. Butylated hydroxytoluene (BHT) was added to supernatants of samples. Samples (100 μM) were transferred to tubes containing the solution of sodium dodecyl sulfate. The solution of TBA (0.5%, w/v), sodium hydroxide and acetic acid was then added to each reaction mixture. Tubes were heated in boiling water for 60 min to form a pink color. The mixtures were cooled at room temperature and the absorbance was measured at 532 nm using Synergy HT Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA). The MDA content of tissues was determined using MDA standard curve and expressed as

Table 1
The effect of GA on hematological parameters in rats exposed to SA.

Variables	Control	SA	GA 10 mg/kg + SA	GA 30 mg/kg + SA	GA 30 mg/kg
WBC	24.72 ± 2.20	16.52 ± 1.38***	18.33 ± 1.70	20 ± 1.83$^{\#}$	25.24 ± 2.34
RBC	9.24 ± 0.70	7.97 ± 0.56*	8.50 ± 0.74	8.85 ± 1.05	9.33 ± 0.79
HGB	16.20 ± 1.26	14.03 ± 0.81**	14.07 ± 0.80	15.90 ± 0.92$^{\#}$	16.18 ± 1.10
HCT	45.90 ± 2.79	41.89 ± 2.18*	42.90 ± 1.47	44.16 ± 1.91	45.20 ± 2.52
MCV	53.71 ± 2.18	59.82 ± 2.59***	57.55 ± 2.45	54.82 ± 1.74$^{\#\#}$	53.21 ± 1.94
MCH	14.29 ± 1.40	18.64 ± 1.71***	18.68 ± 1.44	15.44 ± 1.28$^{\#\#}$	14.74 ± 0.89
MCHC	38.64 ± 3.12	36.02 ± 2.85	37.30 ± 2.95	38.26 ± 3.02	38.99 ± 2.71
PLT	833.6 ± 62.13	413.2 ± 31.67***	656.9 ± 54.87$^{\#\#\#}$	726.2 ± 56.98$^{\#\#\#}$	808.6 ± 59.13

WBC, white blood cells as $\times 10^3\,\mu l^{-1}$; RBC, red blood cells as $\times 10^6\,\mu l^{-1}$; HGB, hemoglobin as $g\,dl^{-1}$; MCV, mean cell volume as fl.; MCH, mean cell hemoglobin as pg; MCHC, mean cell hemoglobin concentration as $g\,dl^{-1}$; PLT, platelet count as $\times 10^5\,\mu l^{-1}$; HCT, hematocrit as %. Values are means ± SD (n = 7). Data were analyzed by one-way ANOVA test followed by Tukey's post hoc test for multiple comparisons. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$; significant changes with respect to control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$; significant changes with respect to SA-treated group.

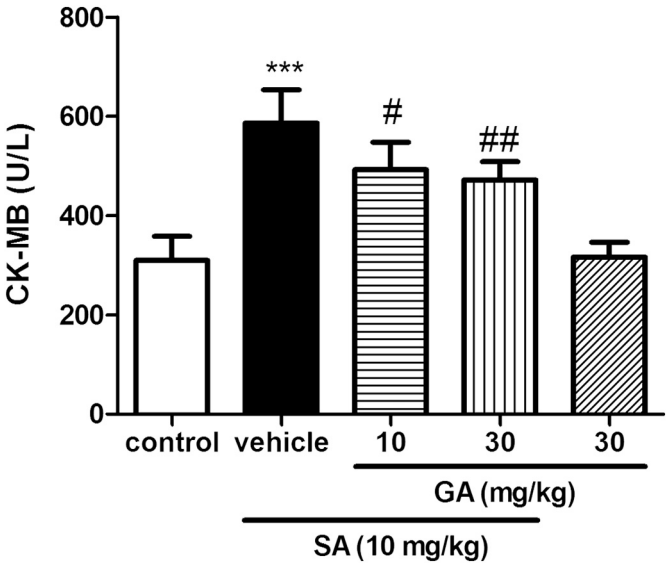

Fig. 1. The effect of GA on the CK-MB level in the serum of rats exposed to SA. Control animals received normal saline as the solvent of GA and SA for 21 days. The animals received GA (10 and 30 mg/kg/day) for 7 days prior to receive SA. Animals were exposed to SA (10 mg/kg/day p.o.) for 14 days. SA significantly increased serum CK-MB level (***$p < 0.001$) while GA significantly decreased SA-induced elevation of CK-MB level (10 mg/kg; $^{\#}p < 0.05$ and 30 mg/kg; $^{\#\#}p < 0.01$). GA did not significantly change CK-MB level in normal rats compared to control group. Values are means ± S.D. (n = 7). Data were analyzed by one-way ANOVA test followed by Tukey's post hoc test for multiple comparisons.

nmol/mg of protein.

2.7.2. NO· assay

The level of NO· in heart and spleen tissues was evaluated using the Griess reaction; nitrite and nitrate concentrations were measured as stable end products of NO· [33]. The homogenates of heart and spleen tissues were centrifuged at 1000*g* for 10 min. To deproteinization of samples, 40 µl of zinc sulfate ($ZnSO_4$, 30% (w/v)) was added to 800 ml samples and mixed using vortex. The solutions were then centrifuged at 4000*g* for 10 min. For nitrate estimation, supernatants were mixed with 2.5–3 g cadmium granules and kept at room temperature for 2 h. The equal volumes of sample and Griess reagent (5% phosphoric acid containing 0.1% NEDD, 1% sulfanilamide) were mixed. The solution was allowed to stand at room temperature for 10 min and the absorbance was measured at 540 nm using UV–visible Spectrophotometer (UV-160A, Shimadzu, Japan). The NO· level was determined using sodium nitrite (NaNO2) standard curve and expressed as µmol/mg of protein.

2.7.3. GSH assay

The content of GSH of heart and spleen tissues homogenates was measured as previously described [34]. Tissues homogenates were mixed with Tris–EDTA buffer (pH = 8.6) and DTNB reagent (10 mM in methanol) and were incubated at room temperature for 20 min to yield a yellow color. The absorbance of obtained yellow color was measured at 412 nm using a spectrophotometer (UV-1650 PC, Shimadzu, Japan). The GSH concentration in the tissues was determined using GSH standard calibration curve and expressed as nmol/mg of protein.

2.7.4. The activity of GPx, CAT and SOD assay

The activity of GPx enzyme was determined using GPx kit (Randox Labs, Crumlin, UK). Briefly, heart and spleen tissues homogenates were centrifuged for 15 min at 4 °C at 10,000*g*. The supernatants were collected, transferred to a clean tube and kept on ice. 50 µL of samples were added to wells and then 40 µL of colorimetric reaction mixture containing assay buffer (33 µl), NADPH solution (3 µl), GR solution (2 µl) and GSH solution (2 µl) was mixed with samples and incubated at room temperature for 15 min. 10 µL cumene hydroperoxide solution was added to start the GPx reaction. The output was measured at 340 nm using a microplate reader. The activity of GPx was expressed as units of enzyme per mg of protein (U/mg protein).

The activity of CAT was determined as previously described [35]. Briefly, the supernatants of heart and spleen tissues were mixed with phosphate buffer (200 µl) and H_2O_2 (0.066 M, 250 µl). The absorbance was monitored for 60 s at 240 nm and the activity of CAT activity was calculated using a molar extinction coefficient of $43.6\,M^{-1}\,cm^{-1}$ and expressed in terms of mole H_2O_2 decomposed per min per mg of protein (U/mg protein).

The activity of SOD was assessed by commercial kit (ZellBio GmbH, Germany) according to the protocol of manufacturer. The supernatants of heart and spleen tissues were mixed with water-soluble tetrazolium working solution, dilution buffer, and enzyme working solution. After incubation in 37 °C for 20 min, the absorbance was measured at 450 nm. The activity of SOD was expressed as units of enzyme per mg of protein.

2.8. Histopathological examination

The small pieces of heart and spleen tissues were fixed in 10% phosphate buffered formalin for 72 h, dehydrated in graded ethanol and embedded in paraffin and serial sections (5 µm) were prepared and then stained with hematoxylin and eosin. 10–15 microscopic fields in each section were qualitatively examined in a blinded method by light microscope (Olympus, Tokyo, Japan) connected to camera (Digital Microscope BMZ-04-DZ) in a magnification 100 X.

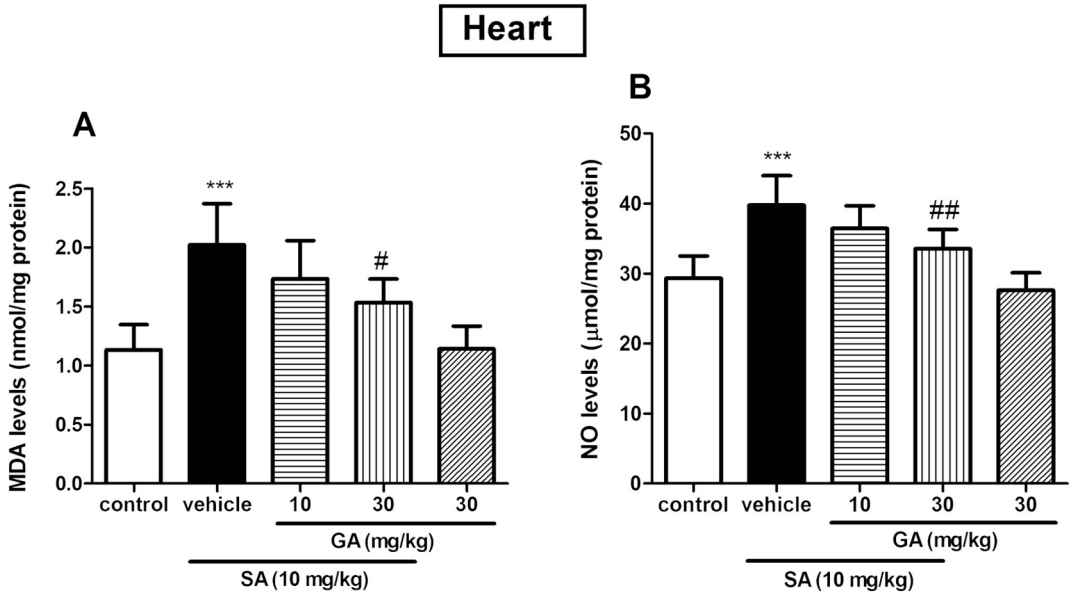

Fig. 2. The effect of GA on MDA and NO· levels in the heart tissue of rats exposed to SA. Control animals received normal saline as the solvent of GA and SA for 21 days. The animals received GA (10 and 30 mg/kg/day) for 7 days prior to receive SA. Animals were exposed to SA (10 mg/kg/day p.o.) for 14 days. SA significantly increased MDA and NO· levels (***$p < 0.001$) in heart tissue. Treatment with GA (30 mg/kg) significantly decreased SA-induced elevation of MDA ($^{\#}p < 0.05$) and NO· levels ($^{\#\#}p < 0.01$). Values are means ± S.D. (n = 7). Data were analyzed by one-way ANOVA test followed by Tukey's post hoc test for multiple comparisons.

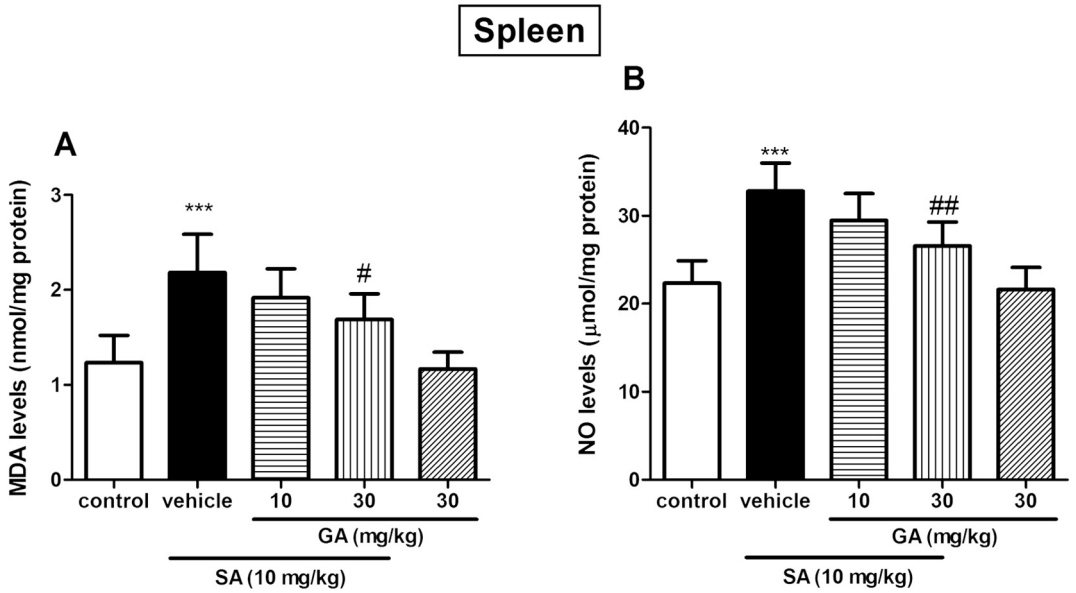

Fig. 3. The effect of GA on the MDA and NO· levels in the spleen tissue of rats exposed to SA. Control animals received normal saline as the solvent of GA and SA for 21 days. The animals received GA (10 and 30 mg/kg/day) for 7 days prior to receive SA. Animals were exposed to SA (10 mg/kg/day p.o.) for 14 days. SA significantly increased the level of MDA and NO· (***$p < 0.001$) in spleen tissue and GA (30 mg/kg) could significantly reduce SA-induced increased level of MDA ($^{\#}p < 0.05$) and NO· ($^{\#\#}p < 0.01$). Values are means ± S.D. (n = 7). Data were analyzed by one-way ANOVA test followed by Tukey's post hoc test for multiple comparisons.

2.9. Statistical analysis

Results were expressed as mean ± standard deviations (SD). Statistical significance was analyzed by one-way ANOVA test followed by Tukey's post hoc test for multiple comparisons and $p < 0.05$ was considered significant.

3. Results

3.1. Effect of GA on hematological parameters in SA induced toxicity in rats

The obtained results showed that SA significantly decreased WBC, RBC, HGB, HCT and PLT as well as increased MCV and MCH compared to the control group ($p < 0.05$). Treatment with GA (30 mg/kg) significantly inhibited SA-induced changes of WBC, HGB, PLT, MCV and MCH ($p < 0.05$). No significant change was shown in hematological parameters in the group that only received GA (30 mg/kg) compared to

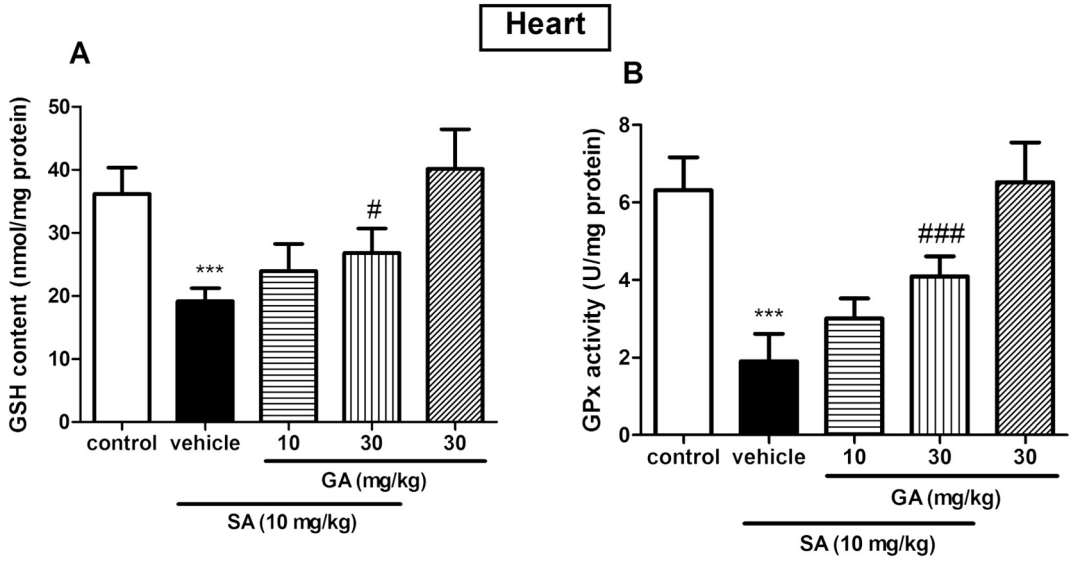

Fig. 4. The effect of GA on the GSH content and the GPx activity in the heart tissue of rats exposed to SA. Control animals received normal saline as the solvent of GA and SA for 21 days. The animals received GA (10 and 30 mg/kg/day) for 7 days prior to receive SA. Animals were exposed to SA (10 mg/kg/day p.o.) for 14 days. SA significantly decreased GSH level and GPx activity compared to the control group (***$p < 0.001$). Treatment with GA (30 mg/kg) could significantly decrease SA-induced reduction of GSH level and GPx activity ($^{\#}p < 0.05$ and $^{\#\#\#}p < 0.001$, respectively). Values are means ± S.D. (n = 7). Data were analyzed by one-way ANOVA test followed by Tukey's post hoc test for multiple comparisons.

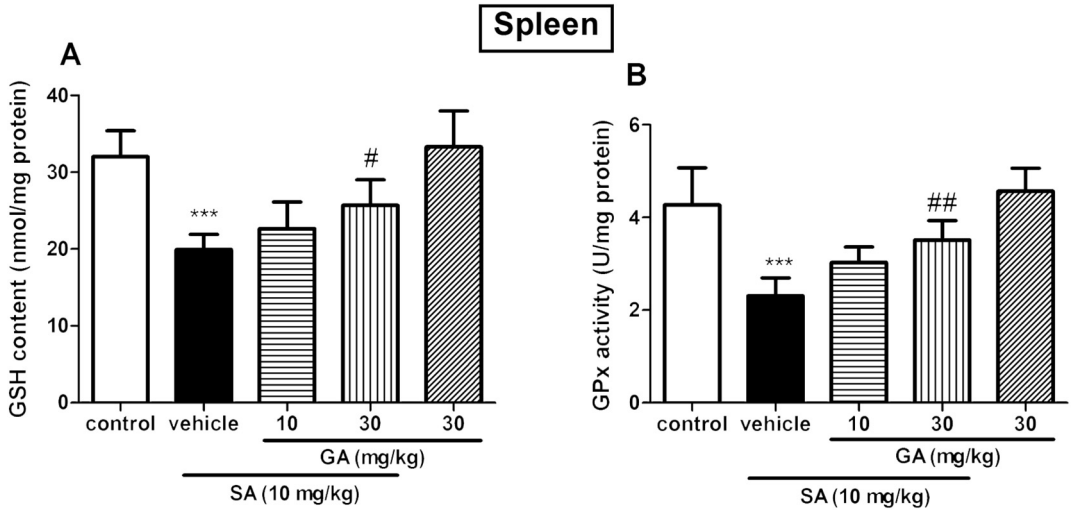

Fig. 5. The effect of GA on the GSH content and the GPx activity in the spleen tissue of rats exposed to SA. Control animals received normal saline as the solvent of GA and SA for 21 days. The animals received GA (10 and 30 mg/kg/day) for 7 days prior to receive SA. Animals were exposed to SA (10 mg/kg/day p.o.) for 14 days. The level of GSH and the activity of GPx significantly decreased in the spleen of rats treated with SA, while treatment with GA (30 mg/kg) significantly increased GSH level and GPx activity compared with SA group ($^{\#}p < 0.05$ and $^{\#\#}p < 0.01$, respectively). Values are means ± S.D. (n = 7). Data were analyzed by one-way ANOVA test followed by Tukey's post hoc test for multiple comparisons.

the control group (Table 1).

3.2. The effect of GA on CK-MB level in the serum of rats exposed to SA

Results showed that SA significantly increased serum CK-MB level ($p < 0.001$) and treatment with GA significantly decreased SA-induced elevation level of CK-MB (10 mg/kg; $p < 0.05$ and 30 mg/kg; $p < 0.01$). Administration of GA in normal rats did not significantly change CK-MB level compared to the control group (Fig. 1).

3.3. The effect of GA on MDA and NO· levels in heart and spleen tissues of rats exposed to SA

In heart and spleen tissues, SA significantly increased MDA and NO· levels compared to the control group ($p < 0.001$). Treatment with GA (30 mg/kg) significantly decreased the level of MDA (heart and spleen; $p < 0.05$) and NO· (heart and spleen; $p < 0.01$) levels compared to the SA-treated group. Results showed that GA did not significantly change the level of MDA and NO· in tissues of animals receiving normal saline and GA (Figs. 2 and 3).

3.4. The effect of GA on GSH level and SOD, GPx and CAT activity in heart and spleen tissues of rats exposed to SA

The level of GSH and the activity of GPx, SOD and CAT significantly decreased in heart and spleen tissues of animals exposed to SA compared to the control group ($p < 0.001$). Treatment with GA (30 mg/kg) significantly increased the GSH level (heart and spleen; $p < 0.05$) and

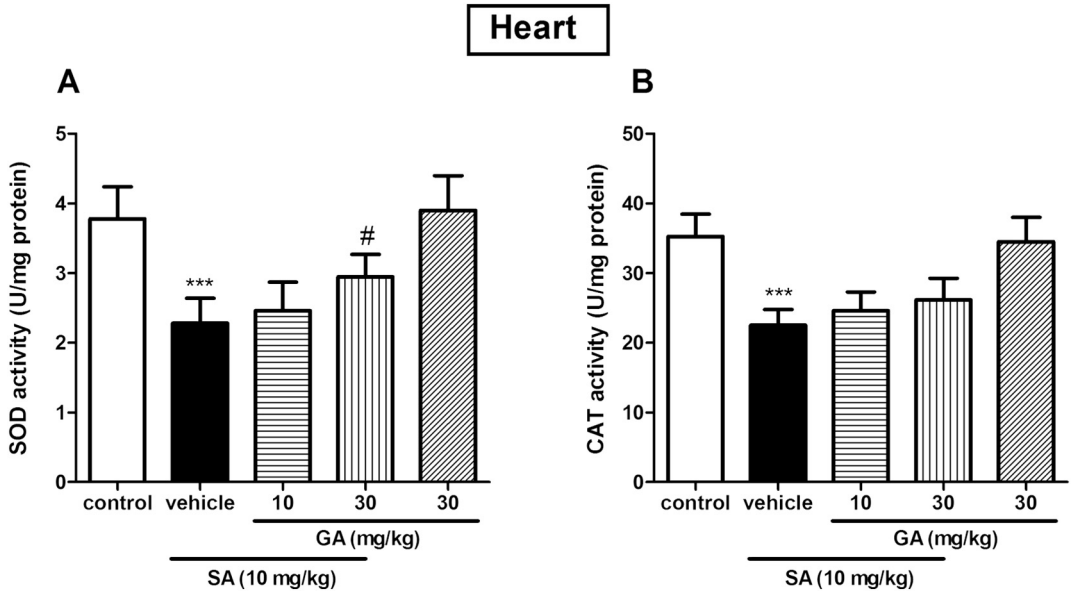

Fig. 6. The effect of GA on the SOD and CAT activity in the heart tissue of rats exposed to SA. Control animals received normal saline as the solvent of GA and SA for 21 days. The animals received GA (10 and 30 mg/kg/day) for 7 days prior to receive SA. Animals were exposed to SA (10 mg/kg/day p.o.) for 14 days. The activity of SOD and CAT significantly decreased in the heart of rats exposed to SA, while treatment with GA (30 mg/kg) significantly increased SOD activity compared with SA group ($^{\#}p < 0.05$). Treatment with GA (30 mg/kg) could not significantly change CAT activity in the heart tissue of animal receiving SA. Values are means ± S.D. (n = 7). Data were analyzed by one-way ANOVA test followed by Tukey's post hoc test for multiple comparisons.

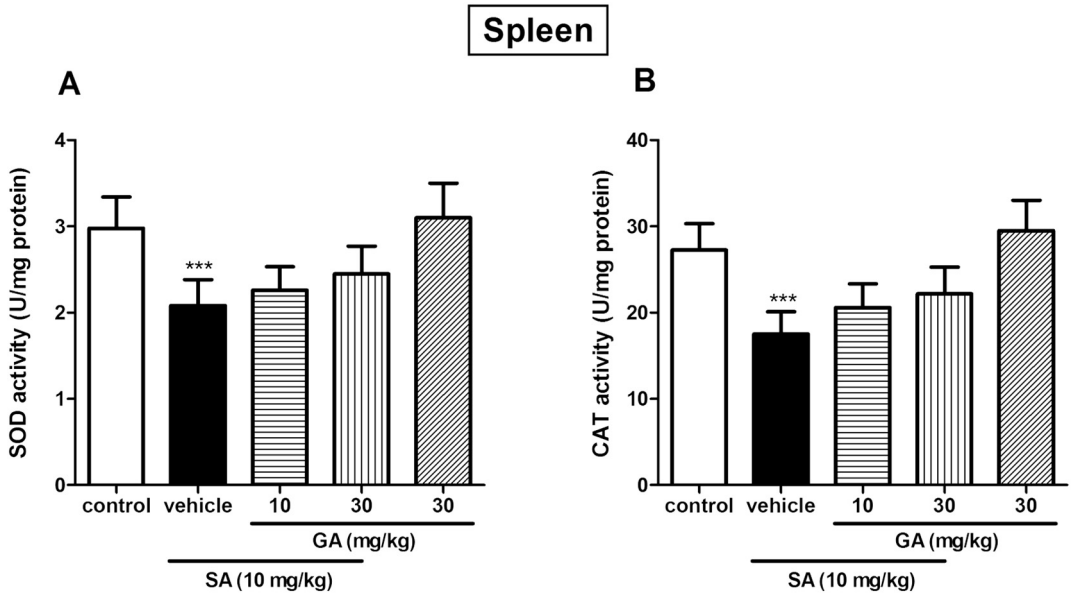

Fig. 7. The effect of GA on the SOD and CAT activity in the spleen tissue of rats exposed to SA. Control animals received normal saline as the solvent of GA and SA for 21 days. The animals received GA (10 and 30 mg/kg/day) for 7 days prior to receive SA. Animals were exposed to SA (10 mg/kg/day p.o.) for 14 days. The activity of SOD and CAT significantly decreased in the spleen of rats exposed to SA. Treatment with GA (30 mg/kg) could not significantly change SOD and CAT activity in the spleen tissue of animal receiving SA. Values are means ± S.D. (n = 7). Data were analyzed by one-way ANOVA test followed by Tukey's post hoc test for multiple comparisons.

GPx activity (heart; $p < 0.001$ and spleen; $p < 0.01$) compared to the SA-treated group. GA (30 mg/kg) significantly increased the activity of SOD in the heart tissue of animals receiving SA ($p < 0.05$) but not in the spleen tissue. Furthermore, GA could not improve the activity of CAT in heart and spleen tissues of animals receiving SA. Results showed that GA did not significantly change the activity of GPx, SOD and CAT in tissues of animals receiving normal saline and GA (Figs. 4–7).

3.5. The effect of GA on histological changes in heart and spleen tissues of rats exposed to SA

The heart tissue in the control and GA (30 mg/kg) groups did not show any histological changes. Cardiomyocytes showed normal structure with oval nuclei located in the center of the cells (Fig. 8A and E). In contrast, SA-treated group showed degenerative changes in cardiomyocytes including condensed nuclei and hypereosinophilic cytoplasm which are the indication of necrosis. These histological changes were associated with the infiltration of inflammatory cells (Fig. 8B).

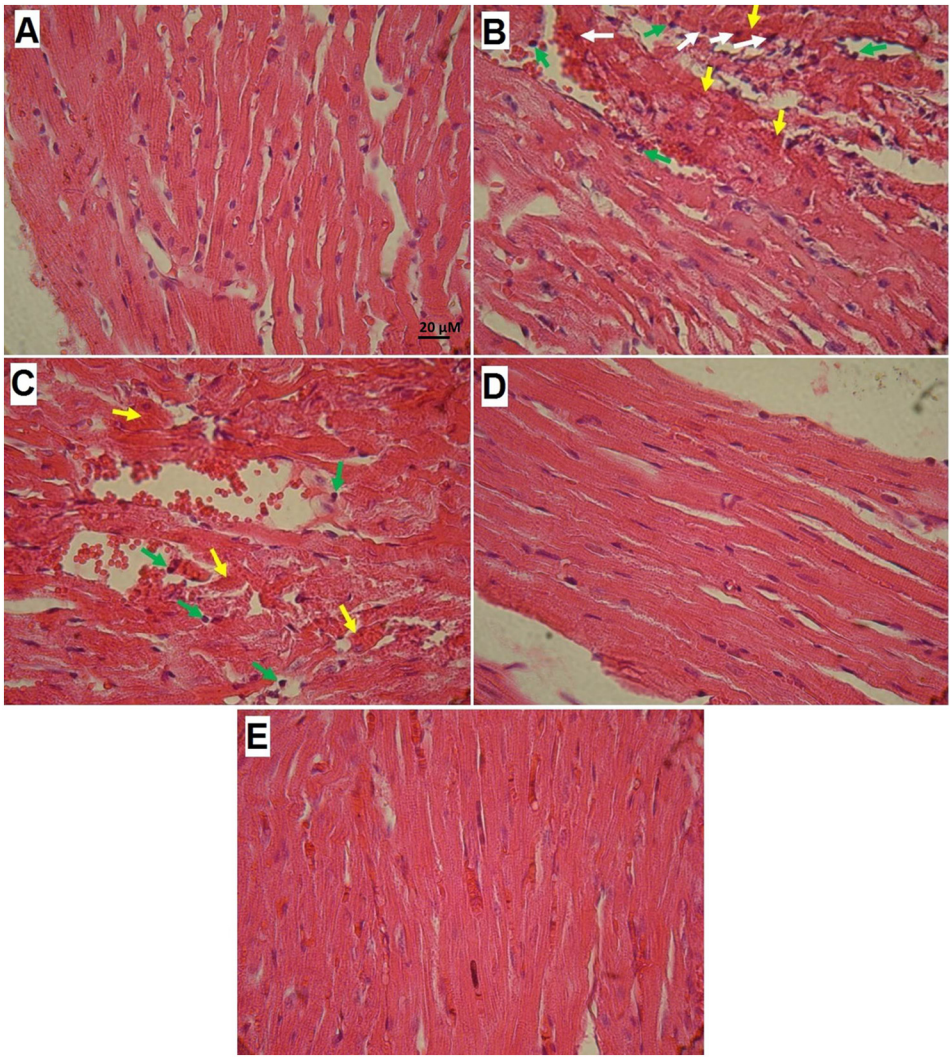

Fig. 8. The effect of GA on histological changes in the heart tissue of rats exposed to SA. Control animals received normal saline as the solvent of GA and SA for 21 days. The animals received GA (10 and 30 mg/kg/day) for 7 days prior to receive SA. Animals were exposed to SA (10 mg/kg/day p.o.) for 14 days. Control and GA (30 mg/kg)-treated groups (A and E, respectively): in these groups, heart tissue did not show any histological changes. Cardiomyocytes showed normal structure with oval nuclei located in the center of the cells. SA-treated group (B): in these groups, the heart tissue showed some pathological changes including infiltration of inflammatory cells (green arrow) and cardiomyocytes with condensed nuclei (white arrow) and hypereosinophilic cytoplasm (yellow arrow) indicating some degree of necrosis. GA 10 mg/kg + SA treated group (C): this group showed moderate cardiomyocyte necrosis (yellow arrow) with mild infiltration of inflammatory cells (green arrow). GA 30 mg/kg + SA treated group (D): this group showed improvement in the structure of cardiomyocytes. Cardiomyocyt necrosis and infiltration of inflammatory cells were not observed in this group. Sections were stained with hematoxylin and eosin (100×). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Moderate cardiomyocyte necrosis and mild infiltration of inflammatory cells were observed in rats treated with 10 mg/kg GA (Fig. 8C), while treatment with 30 mg/kg GA dramatically improved the structure of cardiomyocytes and reduced infiltration of inflammatory cells (Fig. 8D).

In the spleen tissue, the control group did not show any histological changes (Fig. 9A). The SA-treated group showed marked splenic myelosis (myeloid hyperplasia), accompanying by megakaryocytic and diffuse hemosiderin deposition (Fig. 9B). SA-induced structural changes of tissue moderately improved by treatment with 10 mg/kg GA (Fig. 9C) and dramatically improved by 30 mg/kg GA (Fig. 9D). The group treated with normal saline and GA showed no pathological changes compared to the control group (Fig. 9E).

4. Discussion

The present study was investigated the protective effects of GA (10 and 30 mg/kg/day, p.o. for 21 days) on SA (10 mg/kg/day, p.o. for 14 days)-induced spleno-, cardio- and hemato-toxicity in rats. Arsenic has been reported to promote spleen- and cardiac-toxicity via induction of oxidative stress [9,19,36]. Arsenic compounds induce oxidative stress in cells through methylation to reactive metabolites, induction of Haber–Weiss reaction, impairment of mitochondrial function, enhancement of NADPH oxidase activity and interaction with cellular antioxidant [37–40]. Arsenic compounds also increase the activity of inducible isoform of NO· synthase (iNOS) contributing to the excessive generation of $O_2\cdot^-$ and NO· radicals [41–43]. Reactive radicals directly subject proteins, lipids, DNA, membranes and organelles of cell resulting in the protein oxidation and lipid peroxidation. Among the different aldehydes formed as secondary products during lipid peroxidation, MDA is widely used as a convenient biomarker for lipid peroxidation [44]. Therefore, excessive level of MDA indicates ROS-dependent tissue damage [45]. Our results showed that SA increased the level of MDA and NO in the heart and spleen tissues, which this indicated that SA induces the production of ROS/RNS contributing to the lipid peroxidation and its further tissue damage. In addition to the lipid peroxidation, our data showed that SA decreased the content of GSH and the activity of SOD, GPx and CAT in heart and spleen tissues, which these effects accompanied by histological changes in these tissues. Administration of SA resulted in the degenerative changes in cardiomyocytes, associated with the increased serum level of CK-MB. These obtained results are in agreement with previous studies indicating that arsenic compounds decrease antioxidant defenses; arsenite reduces the activity of antioxidant enzymes including SOD (converts $O_2\cdot^-$ to H_2O_2), CAT (converts H_2O_2 to water), GPx (converts H_2O_2 to water by oxidation of GSH), GR (converts oxidized glutathione disulfide (GSSG) to GSH) [46]. Long term arsenic exposure increases the risk of ischemic heart disease, which this effect is associated with the reduction of serum α- and β-carotene levels [47]. Arsenic causes depletion of serum and tissue level of GSH, the most important antioxidant directly reacting with ROS and RNS. Short term exposure of rats with arsenic reduces GSH concentration in the cardiac tissue, which this is accompanied by

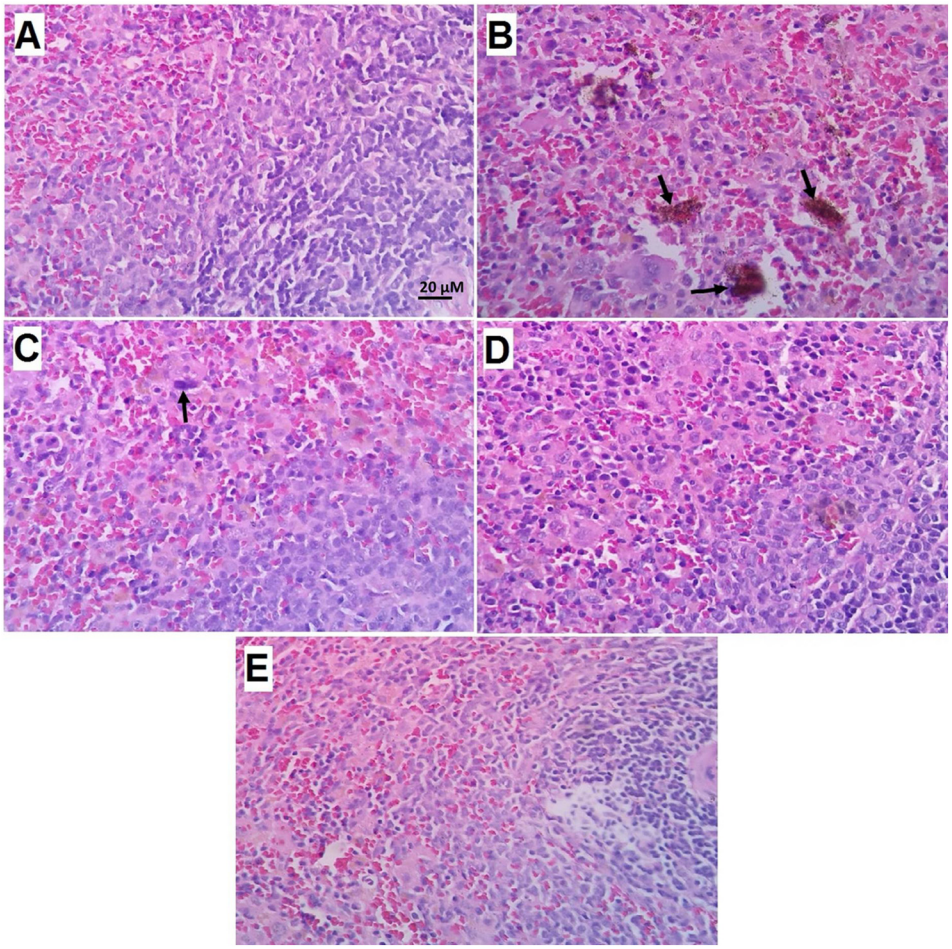

Fig. 9. The effect of GA on histological changes in the spleen tissue of rats exposed to SA. Control animals received normal saline as the solvent of GA and SA for 21 days. The animals received GA (10 and 30 mg/kg/day) for 7 days prior to receive SA. Animals were exposed to SA (10 mg/kg/day p.o.) for 14 days. Control and GA (30 mg/kg)-treated groups (A and E, respectively): the spleen tissue showed normal morphology in these groups. SA-treated group (B): this group showed marked splenic myelosis (myeloid hyperplasia) accompanying by megakaryocytic and diffuse hemosiderin deposition (black arrow). GA 10 mg/kg + SA-treated group (C): this group showed mild histological changes including moderate hemosiderin deposition (black arrow) in the spleen tissue. GA 30 mg/kg + SA (D): 30 mg/kg GA dramatically improved SA-induced histological changes in the spleen tissue. Sections were stained with hematoxylin and eosin (100×).

the elevation level of lipid peroxidation [16]. The imbalance between the pro-oxidants and antioxidants levels in tissues leads to the induction of oxidative stress [48]; in vivo and in vitro studies have demonstrated that oxidative stress results in the alteration in genes expressions, calcium handling and cell death in myocardium leading to the myocardial remodeling and heart failure [49]. Therefore, SA-mediated histological alteration in heart and spleen tissues may result from excessive level of oxidative stress induced by SA.

Current results showed that GA ameliorated SA-induced oxidative stress in heart and spleen tissues through reducing the level of MDA and NO· and increasing the level of GSH and the activity of anti-oxidant enzymes including GPx and SOD; these effects were associated with the improvement of histological changes induced by SA. These results are in line with previous studies indicating the protective effect of GA in oxidative stress conditions [50,51], which this results from direct antioxidant activity of GA or its stimulatory effect on the activity of antioxidant enzymes [52]. Gallic acid reduces the lipid peroxidation and improves the activity of antioxidant enzymes including SOD, catalase, GPx, GR and GST as well as increases the level of GSH in the heart tissue of isoproterenol-treated rats [53]. Gallic acid reduces the activity of lysosomal enzymes and maintains the integrity of lysosomal membrane in the serum and heart of isoproterenol-treated rats; this may result from antioxidant and free radical scavenging activity of GA [54]. Furthermore, GA improves antioxidant capacity and reduces lipid peroxidation in ischemia/reperfusion and diabetes-induced myocardial damage [55,56]. Evaluation of the effect of GA on the spleen of septic mice has shown that pretreatment with GA reduces the MDA level and improves the activity of antioxidant enzymes including SOD and CAT [52].

The cardio-protective effect of GA has been shown in previous studies indicating that GA reduces functional and structural changes in cardiac tissue of streptozotocin-induced diabetic rats; this is accompanied by the increased activity of antioxidant enzymes and the reduced level of lipid peroxidation in the heart tissue [56]. Treatment with GA reduces the serum level of cardiac injury markers including CK, CK-MB, lactate dehydrogenase, alanine transaminase, troponin-T and aspartate transaminase in rats with isoproterenol-induced myocardial infarction. The protective effect of GA on myocardium results from the elevation of antioxidant capacity and the reduction of lipid peroxidation products, contributing to the inhibition of isoproterenol-induced myocardial damage and subsequent leakage of cardiac injury markers. This is confirmed by histopathological findings indicating the reduction of infarction area and infiltration of inflammatory cells in the heart tissue of animals treated with GA [57]. Treatment with GA also reduces the spleen enlargement in mice after injection of WEHI-3 leukemia cells; this may be due to the promotion of macrophage phagocytosis activity by GA [58].

Our CBC results also showed that SA reduced RBC, WBC, HBG, HCT and PLT as well as increased MCH and MCV. However, treatment with GA improved SA-induced changes in hematological parameters. These results are in agreement with previous studies indicating that arsenic exposure changes hematological parameters [59,60]. SA-induced alteration of hematological parameters may result from excessive level of oxidative stress in blood cells or the interference of SA with the activity of bone marrow [10,61]. Considering that spleen is the largest filter of RBCs and hosts macrophages, dendritic cells, plasma cells and lymphocytes [62], the injury of this organ by SA-induced oxidative stress may also affect hematological parameters. The beneficial effect of GA to prevent oxidative stress-induced hematological changes in rats has also been shown in previous studies, which this effect has been associated

with the reduction of oxidative stress markers [63]. Therefore, the protective effect of GA against SA-induced hematological changes in current study may result from its ability to the reduction of oxidative stress and elevation of antioxidant enzyme capacity.

5. Conclusions

Present study demonstrated that sodium arsenite (SA) increased lipid peroxidation and NO· production as well as decreased the antioxidant capacity leading to the induction of oxidative stress in heart and spleen tissues; these effects were associated with the alteration of hematological and histopathological parameters. Inhibition of oxidative stress may be a therapeutic approach for preventing SA-induced spleno-, hemato- and cardio-toxicity. Results from our study revealed that the protective effect of gallic acid (GA) on SA-induced toxicity may result from the reduction of lipid peroxidation and NO· production as well as elevation of antioxidant capacity. These results suggest that GA can be used as a promising agent to improve arsenic and its metabolites-induced toxicity.

Acknowledgments

This work was supported by Deputy of Research of Ahvaz Jundishapur University of Medical Sciences, Iran (Grant number 94S123).

Conflict of interest

The authors declare that they have no conflict of interest.

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