Biology and Medicine

Amer et al., Biol Med (Aligarh) 2016, 8:1
http://dx.doi.org/10.4172/0974-8369.1000266

Research Article Open Access

Protective Role of Some Antioxidants on Arsenic Toxicity in Male Mice: Physiological and Histopathological Perspectives

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Received date: December 5, 2015; **Accepted date:** January 4, 2016; **Published date:** January 8, 2016

Abstract

The present study aimed to investigate the protective effects of some natural and artificial antioxidants on the hepato-renal injuries induced by arsenic toxicity. Sixty adult male albino mice weighing 30-40 g were subjected to a sub-lethal dose of sodium arsenate (40 mg/kg body weight) to investigate hematological, biochemical and histopathological alterations resulting from arsenic-induced hepato-renal toxicity. Arsenic-exposed mice were also co-treated with different antioxidants including green tea, garlic and vitamin C to reveal their potential protective role. The antioxidants induced normalization of all blood parameters that showed significant declines by arsenic toxicity. ALT and AST activities were significantly increased in sodium arsenate treated group compared to all other groups. The enzymatic activities did not acquire insignificant differences in antioxidants-treated groups compared to the control mice. Creatinine and urea levels were significantly increased in arsenate treated mice and become normal in mice co-treated with different antioxidants. Histolopatholgical findings in liver sections from arsenate treated mice were represented by venous congestion, sinusoidal dilatation, mononuclear cell infiltration and periportal fibrosis. Simultaneously, renal sections from mice in the same groups revealed interstitial hemorrhages and mononuclear cell infiltration, glomerulonephritis and proximal tubular necrosis. The hepatic and renal histopathological alterations were greatly reduced particularly in groups received combined antioxidants treatment. In conclusion, the antioxidants used in this study exhibited potential protective capacity for the hepato-renal induced arsenic toxicity in male mice.

Keywords: Arsenic toxicity; Antioxidants; Kidney; Liver; Histopathology; Mice

Introduction

Toxicity, aging and diseases are among the main causes of free radicals production inside the cell [1]. The main source of arsenic toxicity is the drinking of ground water contaminated with an inorganic form [2]. The metal commonly found in two oxidation states trivalent arsenite and pentavalent arsenate. These inorganic arsenicals have been considered more toxic than organoarsenicals [3]. Arsenic compounds are most readily absorbed from the gastrointestinal tract [4]. These arsenicals can bind to sulfhydryl groups of glycolysis and tricarboxylic acid cycle enzymes inhibiting their pathways and the pentavalent arsenicals can interfere with the mitochondrial oxidative phosphorylation enzymes [3].

Generally, oxidative stress of arsenic is due to the production of free radicals like super oxide and hydrogen peroxide which were supposed to initiate lipid peroxidation [5]. As has also been implicated, arsenic induced oxidants, such as superoxide anions and hydroxyl peroxide, are suggested to damage macromolecules in cells or can act as second messengers leading to alteration of gene expression and subsequent enhancement of cell proliferation [6].

Liver is considered as the first target organ in arsenic metabolism where the element is subjected to methylation [3]. Cytotoxic and physiological dysfunctions in the liver, caused by arsenic toxicity, are associated with oxidative DNA damage, enhanced cell proliferation, altered DNA methylation, genomic instability and general heptotoxicity [7-11]. Kidney also has been considered as the second target organ for arsenic toxicity. Pentavalent arsenic and organic arsenic are rapidly and completely eliminated via kidney [12].

Recent trials in controlling and treating diseases prefer natural antioxidants to be utilized in diets. Garlic (*Allium sativum)* possesses many important nutritive and antioxidant substances as selenium, sulfur-containing compounds and vitamins (A, B, C and E). Several studies explained garlic ability to eliminate arsenic from blood and soft tissues [13,14]. Consumption of tea has been associated with antimutagenic and possible anti-carcinogenic effects [15]. It had been shown that black tea when used along with freshly prepared solution of ferrous sulphate can reduce the cytotoxic effects of inorganic arsenic in mice [16]. Vitamin C inhibits lipid peroxidation by scavenging the aqueous reactive oxygen species (ROS) [17]. It acts as an antioxidant molecule chelating heavy metals [3]. Vitamin C as well as any other water soluble antioxidants scavenge ROS and play an important role in the regulation of intracellular redox state [5].

Several researchers investigated the histopathological effects of arsenicals on the liver of rats [10], mice [18] and goat [19]. Other investigations have showed the effect of arsenicals on renal tissues [10,19-21].

The protective role of natural antioxidants and their combinations have met a limited attention in the past. The present study is therefore, designed to investigate some physiological and histological parameters of liver and kidney for evaluating the protective role of some natural (green tea and garlic) and synthetic (Vitamin C) antioxidants in combating sodium arsenate toxicity in male mature mice.

Materials and Methods

Sixty adult albino male mice were purchased from King Fahd Center for Medical Research at King Abdulaziz University of Jeddah City in Saudi Arabia at an initial age of 7-8 weeks and a mean body weight of 38.40 ± 1.0 g. Animals were kept at room temperature (25 ± 2°C) in a light controlled room with an alternating 12 h light/dark cycle. The mice were maintained in polycarbonate cages with stainless steel wire-bar lids. The European Community Directive (86/609/EEC) and National rules on animal care have been followed. Animals were allowed to become acclimatized to the laboratory conditions for 10 days before starting the experiment.

Animals were randomly divided into 6 groups with 10 animals per a group and they were treated daily for 30 days. Group I received tap water normally (control). Group II received sodium arsenate (Na3AsO4). This chemical was dissolved in tap water (0.04 mg/ml) in such a way that it resulted in a dose of 40 mg/kg body weight per day. This dose was nearly similar the sublethal doze determined by Devaraju et al. [22] for male albino mice. In group III (Na3AsO4-green tea), mice were administrated the same dose of Na_3AsO_4 mixed with 625 mg/kg bw green tea [23]. Group IV (Na3AsO4- natural garlic) received the same dose of Na_3AsO_4 mixed with 33 g/900 mL water extract of garlic and the garlic dose was used by Chowdhury et al. [13]. Group V (Na_3AsO_4 + green tea + garlic) treated with the same dose of Na_3AsO_4 and a mixture of 625 mg green tea/kg bw and 33 g garlic/900 mL water. Mice in group VI (Na_3AsO_4 + vitamin C) received the same dose of Na_3AsO_4 mixed with 150 mg vitamin C [24]. Arsenate and antioxidants were dissolved in tap water and were available for animals along the day in drinking water. Animals were deprived of food for 12 h after 30 days of treatment and before collecting the blood and organ samples.

Blood samples of the anesthetized mice were collected from the medial retro-orbital venous plexus immediately with capillary tubes. The blood samples were centrifuged at 3000 rpm for 5 min and plasma were collected. Using cell counter (Sysmex, model KX21N), white blood cells (WBCs) in thousands per cubic milliliter and red blood cells (RBCs) in millions per cubic millimeter were measured. Hemoglobin (Hb) was measured in grams per deciliter (g/dL). The packed cell volume (PCV) was also determined. Mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were also calculated. Platelets were measured in thousands per cubic millimeter. Alanine aminotransferase (ALT or GPT), aspartate aminotransferase (AST or GOT) activities and creatinine and urea levels were measured calorimetrically according to the available kits.

Liver and kidney pieces from 5 individuals per a group were fixed in 10% neutral buffered formalin solution immediately after sacrificing the animal and were then preserved in 70% ethanol until embedding. The specimens were then dehydrated in a graded series of alcohol and embedded automatically by using a Shandon-Duplex-Processor. Finally the prepared specimens were embedded in paraplast blocks. Sections were cut using a Leitz rotatory microtome at 5µm thickness, deparaffinized in xylene and rehydrated in descending grades of ethanol down to distilled water. After staining they were dehydrated in ascending grades of ethanol, cleared in xylene and covered with Eukit®. Haematoxylin and Eosin (H&E) staining was conducted to investigate the general histological structures according to protocols in Bancroft et al. [25]. Digital photomicrographs were taken using an imaging system of light microscope (Leica DM LB, Leica Microsystems, Wetzlar, Germany) and digital camera (Leica EC3, Leica Microsystems).

The results were evaluated using ANOVA test in SPSS version 11.0 for estimating the significant differences among the different groups. LSD was also applied for the paired comparison. Values were presented as means ± S.E.

Results

Parameter	Groups						ANOVA	
	I	II	III	IV	V	VI	F-value	Sig.
WBCs ($10^3/mm^3$)	3.9 ± 0.3*** (5)	4.3 ± 0.34*** (8)	4.04 ± 0.5*** (6)	2.9 ± 0.3*** (5)	6.9 ± 0.8 (7)	3.8 ± 0.4*** (8)	7.8	***
RBCs ($10^6/mm^3$)	10.2 ± 0.37** (5)	8.9 ± 0.16 (10)	9.4 ± 0.28 (6)	11.2 ± 0.27** (5)	11.1 ± 0.2** (7)	3.8 ± 0.4*** (8)	9.7	***
Hb (g/dl)	15.7 ± 0.4*** (3)	11.7 ± 0.64 (6)	18.7 ± 0.7*** (6)	19.4 ± 0.4*** (6)	18.3 ± 0.3*** (7)	18.7 ± 0.2*** (8)	37.6	***
PCV (%)	47 ± 0.7** (4)	38.4 ± 1.10 (12)	18.7 ± 0.7*** (6)	48.2 ± 0.97*** (5)	48.5 ± 1.7*** (6)	43.3 ± 0.97** (8)	7.2	***
MCV (fl)	44.6 ± 1.1* (4)	37.1 ± 2 (6)	51 ± 2.1*** (6)	43 ± 1.7* (5)	43.6 ± 1.5* (6)	41.1 ± 2.6 (8)	4.6	***
MCH (pg)	15.1 ± 0.63 (3)	12.8 ± 0.45 (5)	19.8 ± 0.9*** (6)	17.1 ± 0.7*** (5)	16.4 ± 0.2*** (7)	17.5 ± 0.8*** (8)	10.3	***
MCHC (g/dl)	33.5 ± 0.4*** (3)	24 ± 2.3 (4)	37.6 ± 1.4*** (5)	17.1 ± 0.7*** (5)	38 ± 1.5*** (6)	43.1 ± 1.3*** (8)	18.2	***
ALT (U/L)	152.6 ± 19.1*** (5)	292.7 ± 29.1 (6)	181.3 ± 25.3*** (4)	156.3 ± 20*** (4)	163.7 ± 20.2*** (5)	159.7 ± 9*** (5)	6.9	***

AST (U/L)	267.9 ± 16.2*** (7)	412.7 ± 27.1 (12)	281.7 ± 26.9** (4)	313.8 ± 9.4* (3)	275.6 ± 25.3*** (4)	284.6 ± 9.8** (5)	6.4	***
Creatinine (mg/dl)	2.2 ± 0.23*** (3)	15.6 ± 0.66 (8)	2.2 ± 0.14*** (3)	2.1 ± 0.07*** (3)	2.3 ± 0.10*** (5)	2 ± 0.10*** (5)	19.9	***
Urea (mg/dl)	49.3 ± 2.5*** (6)	80 ± 4.3 (8)	53.4 ± 11.8* (3)	52.7 ± 12.7* (3)	51.8 ± 10.1** (5)	40.6 ± 4.4*** (7)	5.9	***

Table 1: Effect of green tea, garlic and vitamin C co-treatments on sodium arsenate induced alternations in male mice hematology, liver (ALT and AST) and kidney (creatinine and urea) functions. Data are presented as means ± standard errors and numbers within brackets refer to the sample size. The significant differences among groups were shown when it was available according to ANOVA-test (***=significance difference at P<0.001). For the hematological parameters, the shaded boxes refer to the group to which other groups were compared and the symbols refer to the significant difference (*, **, *** denote significant difference at P<0.05, P<0.01 and P<0.001, respectively). For liver and kidney functions, LSD significant differences between group II and each of other groups were shown as symbols above the mean values of the corresponding group.

Table 1 represents the effect of antioxidant co-treatments on sodium arsenate induced alterations of mice hematology, liver (ALT and AST) and kidney (creatinine and urea) functions. RBCs, Hb, PCV, MCH and MCHC had decreased significantly in the treated group as compared to the control one. These parameters were increased in antioxidant co-treatments (groups III to VI) as compared arsenate group II. The number of WBCs did not show a significant alteration after exposure to sodium arsenate and antioxidant co-treatments. The coupled administration of green tea and garlic in a mixture with arsenate induced WBCs number to be increased significantly (P<0.001) than in the other groups. The RBCs count decreased significantly in group II compared to groups I, IV, V (P<0.01) and VI (P<0.001). The RBCs count was increased significantly (P<0.01) in groups IV, V and VI. The Hb content showed a significant decrease (P<0.001) in group II compared to the control and the antioxidants co-treated groups. The antioxidants co-treated groups showed significant increases in the Hb content compared to the control group. PCV showed significant decreases in group II compared to the antioxidants co-treated groups and the control one. MCV showed significant decreases in group II compared to the control group (P<0.05), group III (P<0.001) and group V (P<0.05). Group III showed significant increases in MCV (P<0.01) compared to groups IV, V and VI. The antioxidants co-administration induced a significant increase (P<0.001) in the MCH compared to the group II. MCHC showed significant decreases (P<0.001) in group II compared to all other groups.

Liver functions were determined in this study by measuring plasma activities of ALT and AST. ALT activity (Table 1) was significantly increased (P<0.001) in sodium arsenate treated group II compared to all other groups. AST activity (Table 1) was significantly increased (P<0.001, P<0.01 and P<0.05) in group II compared to all other groups.

As shown in Table 1, creatinine and urea levels were significantly increased (P<0.001) in arsenate treated mice when they were compared to control animals. Co-administration of either natural or synthetic antioxidants significantly normalized creatinine and urea levels. Compared to the control group, there was no significant changes in the creatinine and urea levels in mice co-treated with green tea (group III), garlic (group IV), green tea and garlic (group V) and vitamin C (group VI).

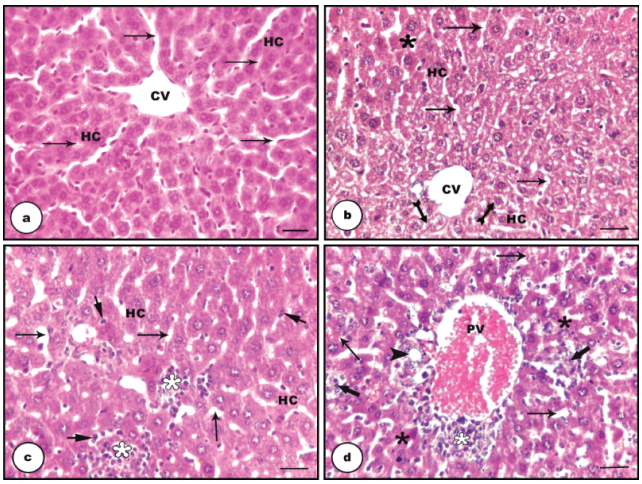

Figure 1: a, photomicrograph of H&E stained section of liver from control mice showing central vein (CV) from which radiate the hepatic cords (HC) separated by hepatic sinusoids (arrows); b, photomicrograph of H&E stained hepatic section from mouse exposed to sodium arsenate displaying a central vein (CV) from which radiate the hepatic cords (HC) separated by dilated hepatic sinusoids (arrow) displaying less affected hepatocytes (asterisks) away from the CV and hepatocytes suffering from centrilobular hepatoxicity (forked tail arrows) next to CV; c, photomicrograph of H&E stained hepatic section from mouse exposed to sodium arsenate showing hepatic cords (HC) separated by dilated sinusoids (arrow) with proliferation of kupffer cells (long head arrow) and mononuclear leukocytic infiltration (white asterisks); d, photomicrograph of H&E stained hepatic section from mouse exposed to sodium arsenate exhibiting periportal mononuclear leukocytic infiltration (white asterisks), necrotic foci (thick arrows) and hyalinized hepatocytes (black asterisks) separated by congested hepatic sinusoids (arrow) surrounding severly congested portal vein (PV) and bile ductule (arrowhead). Scale bar = 30 μm.

Hematoxyline and eosin-stained liver sections of control mice showed normal lobular pattern of a central vein (CV) from which the hepatic cords (HC) separated by radiated blood sinusoids. Portal tracts (PT) bordered the hepatic lobule. Hepatocytes were large and polyhedral in shape with slightly eosinophilic granular cytoplasm.

They varied in size and have large rounded vesicular single or double nuclei with prominent nucleoli (Figure 1a).

Hepatocytes of group II animals showed obvious histological changes in the form of distorted hepatic architecture, dilatation and congestion of the CV and hepatic sinusoids (HS) (Figure 1b-d). They showed proliferation of kupffer cells. Some hepatocytes showed features of degeneration in the form of cellular swelling with highly vacuolated cytoplasm (vacuolar degeneration) and deeply stained pyknotic nuclei. Other hepatocytes exhibited hyalinized cytoplasm with pale nuclei and prominent nucleoli (Figure 1b). Some sections showed focal areas of complete degeneration and mononuclear cell infiltration (Figure 1c, d).

Liver sections from mice of group III (Figure 2) and group IV (Figure 3) showed histological changes somewhat similar to that shown in animals of group II (Figure 1b, c, d).

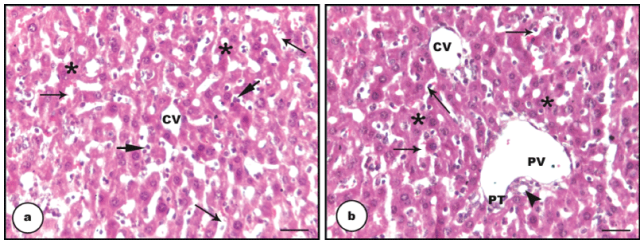

Figure 2: photomicrograph of H&E stained sections of liver from mice exposed to sodium arsenate and treated concurrently with green tea extract showing: a, central vein (CV) around which the disorganized and hyalinized hepatic cords (asterisks) separated by dilated and slightly congested hepatic sinusoids (arrow) with proliferation of kupffer cells (long head arrow); b, hyalinized hepatic cords (asterisks) surrounding a portal tract (PT) displaying a dilated portal vein (PV), bile ductule (arrowhead) and dilated congested hepatic sinusoids (arrow). Scale bar = 30 µm.

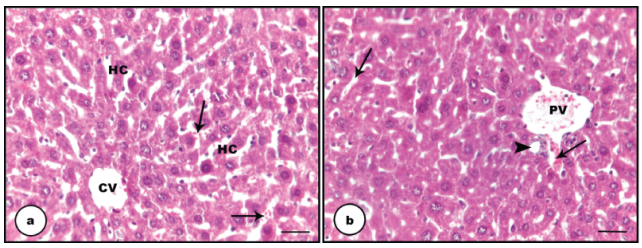

Figure 3: Photomicrographs of H&E stained sections of liver from mice exposed to sodium arsenate and treated concurrently with garlic extract showing: a, central vein (CV) from which radiate the hepatic cords (HC) separated by slightly congested hepatic sinusoids (arrows); b, portal tract displaying portal vein (PV), bile ductule (arrowhead) and hepatic sinusoids (arrow). Scale bar = 30 µm.

These changes included distortion in the hepatic architecture, dilatation and congestion of the CV and HS as well as proliferation of kupffer cells. Some hepatocytes showed highly vacuolated cytoplasm (vacuolar degeneration) and deeply stained pyknotic nuclei. Several hepatocytes exhibited hyalinized cytoplasm with pale nuclei and prominent nucleoli (Figures 2 and 3). The liver sections from mice treated with a combination of green tea and garlic (Figure 4) or with vitamin C (Figure 5) displayed hepatocytes somewhat similar to those from the control (Figure 1a). However, few hepatocytes displayed vacuolated cytoplasm and pyknotic nuclei (Figures 4 and 5).

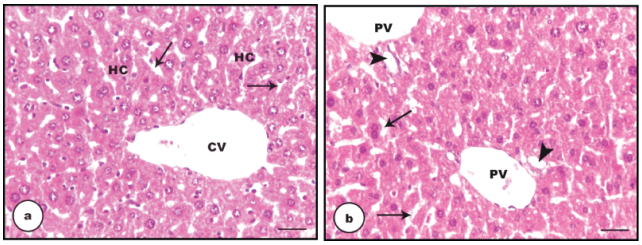

Figure 4: Photomicrograph of H&E stained sections of liver from mice exposed to sodium arsenate and treated concurrently with a combination of green tea and garlic extract displaying: a, central vein (CV) from which radiate the hepatic cords (HC) separated by hepatic sinusoids (arrows); b, portal tract displaying portal vein (PV), bile ductule (arrowhead) and hepatic sinusoids (arrows). Scale bar = 30 µm.

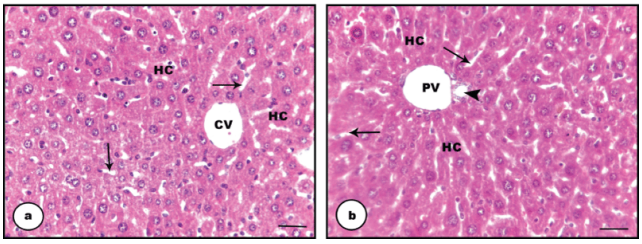

Figure 5: Photomicrograph of H&E stained sections of liver from mice exposed to sodium arsenate and treated concurrently with ascorbic acid showing more or less normal hepatic histological architecture: a, central vein (CV) from which radiate the hepatic cords (HC) separated by hepatic sinusoids (arrow); b, portal tract displaying portal vein (PV), bile ductule (arrowhead) surrounded by hepatic cords (HC) separated by hepatic sinusoids (arrow). Scale bar = 30 µm.

Hematoxyline and eosin-stained kidney sections displayed that the renal cortex from group I was mostly occupied by renal corpuscles (RC) which was surrounded by proximal and distal convoluted tubules (Figure 6a). The medulla was occupied by proximal straight tubules (PST), distal straight tubules (DST), collecting ducts (CD) and thin tubules (TT) of the nephron's loop (Figure 6b).

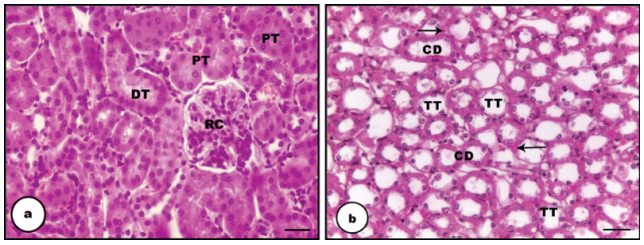

Figure 6: Photomicrograph of H&E stained sections of kidney from control mice showing: a, renal cortex of renal corpuscle (RC), proximal (PT) and distal (DT) tubules; b, renal medulla of thin tubules (TT), collecting ducts (CD) and inter-tubular blood capillaries (arrows). Scale bar = 30 µm.

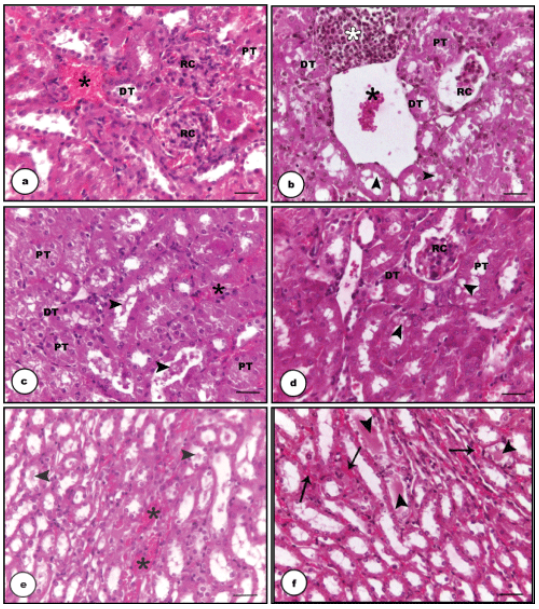

Figure 7: Photomicrographs of H&E stained sections of kidney from mice exposed to sodium arsenate: a, renal cortex displaying marked interstitial hemorrhage (black asterisk), obliterated renal corpuscles (RC) with mesangial hypercellularity and degenerating proximal (PT) and distal (DT) tubules; b, renal cortex showing shrunken renal corpuscle (RC), mononuclear leukocytic infiltration (white asterisk), highly congested vessel (black asterisk) and degenerating PT and DT presenting fatty change (arrowhead); c, a medullary Supplementary files ray at renal cortex showing interstitial hemorrhage (black asterisk) and degenerating PT and DT surrounding a collecting duct enclosing proteinceous casts (arrowheads); d, renal cortex displaying distorted renal corpuscle (RC) as well as degenerating PT and DT exhibiting almost hyalinized cytoplasm and scattered cells displaying fatty change (arrowheads); e, renal medulla of thin tubules and collecting ducts showing interstitial hemorrhages (black asterisks) and intra-tubular casts (arrowheads); f, renal medulla of thin tubules and collecting ducts displaying interstitial hemorrhages (arrows) and intra-tubular hyaline casts (arrowheads). Scale bar = 30 µm.

The glomerular capillaries of some RC in renal sections of animals of group II displayed congestion and dilatation. Other RC showed hypercellularity and obliteration of the capsular space (Figure 7a).

Some RC showed shrunken profiles surrounded by widened capsular space (Figure 7b). Interstitial hemorrhages were also observed among the renal tubules (Figure 7a). Some sections displayed remarkable mononuclear cell infiltration, especially at the perivascular areas (Figure 7b). There was swelling of some cells of the proximal convoluted tubules (PCT) leading to diminution or even obliteration of the tubular lumina. Cytoplasmic vacuolation (Figure 7b-d) and deeply stained nuclei were observed compared to that from the control group. Destruction of the brush borders of the PCT was also detected. The distal convoluted tubules (DCT) showed degenerative changes in the form of pyknotic nuclei and vacuolated cytoplasm. The medulla showed interstitial hemorrhages among the tubular structures and hyaline casts within the medullary tubules (Figure 7c-f).

The co-treatment with green tea (Figure 8), garlic (Figure 9), both green tea and garlic (Figure 10) or vitamin C (Figure 11), reduced the injurious effect of sodium arsenate on the renal structures. The congestion and dilatation of glomerular capillaries were milder than the case in mice receiving sodium arsenate only (Figure 7). The hypercellularity of RC and obliteration of the capsular space disappeared. The interstitial hemorrhages and mononuclear cell infiltration were rarely seen (Figures 8- 11). The degenerative changes in the renal tubular cells were greatly reduced, particularly, in groups V (Figure 10) and VI (Figure 11). Pyknosis and cytoplasmic vacuolation of the tubular cells were scarcely seen. Most cells displayed acidophilic cytoplasm and round vesicular nuclei. The interstitial hemorrhages among the tubular structures within the medulla and hyaline casts within the medullary tubules disappeared particularly in groups V (Figure 10) and VI (Figure 11).

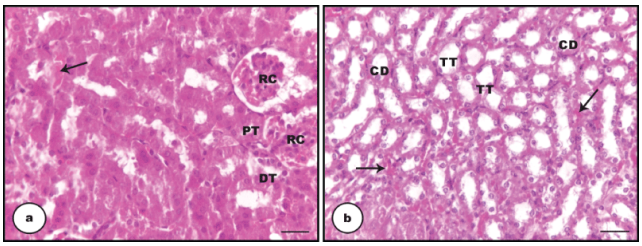

Figure 8: Photomicrographs of H&E stained sections of kidney from mice exposed to sodium arsenate and treated concurrently with green tea extract: a, renal cortex displaying renal corpuscles (RC), milder interstitial hemorrhage (arrow) and slightly regenerating PT and DT; b, renal medulla of thin tubules (TT) and collecting ducts (CD) showing milder interstitial hemorrhages (arrows) compared to renal sections from mice exposed to sod. arsenate only. Scale bar = 30 µm.

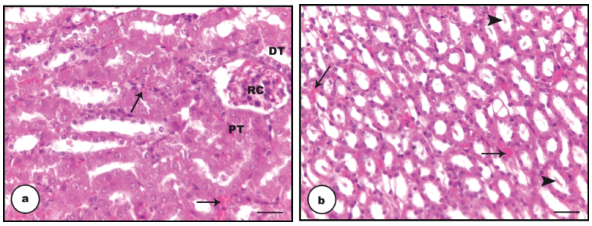

Figure 9: Photomicrographs of H&E stained sections of kidney from mice exposed to sodium arsenate and treated concurrently with garlic extract showing: a, renal cortex displaying renal corpuscles (RC), milder interstitial hemorrhage (arrow) and slightly regenerating PT and DT; b, renal medulla showing milder interstitial hemorrhages (arrows) and intra-tubular desquamations (arrowheads). Scale bar = 30 µm.

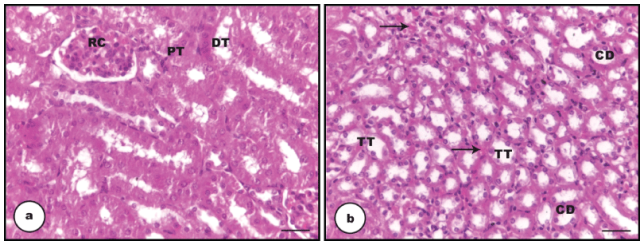

Figure 10: Photomicrographs of H&E stained sections of kidney from mice exposed to sodium arsenate and treated concurrently with a combination of green tea and garlic extracts: a, renal cortex showing renal corpuscle (RC) as well as more or less normal PT and DT; b, renal medulla of thin tubules (TT) and collecting ducts (CD) showing milder interstitial hemorrhages (arrows). Scale bar = 30 µm.

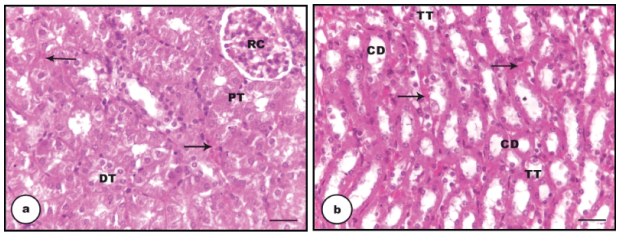

Figure 11: Photomicrographs of H&E stained sections of kidney from mice exposed to sodium arsenate and treated concurrently with ascorbic acid showing more or less normal renal histological architecture and mild interstitial hemorrhages (arrows): a, renal cortex of renal corpuscle (RC), proximal (PT) and distal (DT) tubules; b, renal medulla of thin tubules (TT) and collecting ducts (CD). Scale bar = 30 µm.

Discussion

The pathway of arsenic found in drinking water was explained by Karmakar et al. [4]. The authors stated that absorption of arsenic primarily occurs through the gastrointestinal tract after its ingestion causing gastrointestinal lesions. These lesions increase the permeability of the small blood vessels through which arsenic enters into the blood and binds to haemoglobin. After that arsenic reaches the liver and its biotransformation takes place and finally passes to kidney to be excreted out.

The current study demonstrated that drinking tap water contaminated with sub-lethal dose of sodium arsenate for 4 to 6 weeks is considered as an acute toxicity. Acute exposure of male mice to arsenic produced harmful effects on the animal physiology, hematology and organs histology. The liver as a major target organ of arsenic toxicity and carcinogenesis [2,26] showed abnormal increase in its enzymes (ALT and AST) and these findings are supported by several investigations. Yasmin et al. [11] found that the activities of both ALT and AST were significantly higher in arsenic treated mice indicating liver dysfunction. Fowler et al. [27] also stated that arsenic is known to produce disturbance in liver function. The increase in plasma levels of both enzymes are reliable determinants of liver parenchymal injury [7] and could be due to the leakage of these enzymes from the liver cytosol into the blood stream [8]. Other investigations have also revealed similar hepatotoxicity of arsenicals [9-11].

Usually, leucopenia could be induced by acute, intermediate and chronic exposure to arsenic [3]. However, the present study and other previous studies [11,28] have revealed that white blood cells had no significant difference between control and arsenic treated groups. The most reasonable explanation of this result is that the acute exposure of arsenic did not affect the WBCs count of the animal where leucopenia starts to appear when exposure to arsenic has become prolonged. Rousselot et al. [29] found that WBCs decreased when mice were given higher dose of arsenic for long period. However, the application of the high arsenic dose in this study was for short period.

The significant decreases in MCV in group II implied that RBCs had become microcytic [30] and were not being able to carry enough oxygen for tissue respiration [31]. This could result in ATP deficit (energy reduction), general body weakness and death; which was not observed in this study due most probably to the short period of exposure. Some studies have shown a moderate hemolytic anemia linked to arsenic exposure in mice [32] and this could be in agreement with the significant Hb decrease in the group II. The present study together with Gupta et al. [9] found that RBCs and hemoglobin levels are decreased when the concentration of arsenic increased. This could be due to binding ability of arsenic to hemoglobin that leads to inhibition of heme synthesis pathway.

The present study tested the protective effect of some natural antioxidants like green tea and garlic as well as the synthetic vitamin C (ascorbic acid). Similarly, several investigations have examined the protective effect of these antioxidants against arsenic toxicity [5,10,15,16,33] but one did not examine the combined effect of natural antioxidants which was tested herein for the first time. All these investigations revealed that green tea, garlic and vitamin C possess an antioxidant character enhancing the inside body antioxidants in scavenging the free radicals liberated by the arsenicals and normalize the enzymatic and non-enzymatic biology. In agreement with these studies, the present study examined the protective role of the water extracts of green tea, garlic and vitamin C against a dose of 40 mg/Kg body weight sodium arsenate. The protective effect of these antioxidants was shown in the form of normalization of the enzymatic

and non-enzymatic activities by significant increases in blood parameters, significant normalization of liver and kidney functions.

Arsenic toxicity is postulated to be primarily due to the binding of arsenic (III) to sulfhydryl group containing enzymes [3]. This heavy metal liberates the reactive oxygen species (ROS) in the mitochondria of liver and kidney cells and these free radicals are the main reason of the organ toxicity and injury. The internal body antioxidant (glutathione GSH) helps in both enzymatic and nonenzymatic reduction of pentavalent arsenicals to trivalent and in its complexation during methylation process [34]. Arsenic changes the redox status of glutathione and its related enzymes and this may lead to the inactivation of these enzymes and could affect the detoxification processes and other critical cellular processes. The present antioxidants could potentiate the activities of free radical scavengers, superoxide dimutase, and catalase glutathione peroxidase thereby preventing microsomal lipid peroxidation, liver fibrosis, liver necrosis and hepatic inflammation.

All blood parameters showed significant declines due to arsenic toxicity and the applied antioxidants induced general hematological protection. Erythrocytes and the blood parameters may be susceptible to oxidative damage due to the presence of haem iron, polyunsaturated fatty acid and oxygen, which may initiate the reactions that induce oxidative changes in RBCs.

The present study is unique in examining the combined effect of the two natural antioxidants (green tea and garlic). Their obvious effect could be shown in WBCs where these cells showed high significant increase in the mice treated with both antioxidants. This result supports the immunomodulatory effect of garlic [13] and green tea [15,16].

In agreement with previous studies regarding arsenic-induced hepatotoxicity [10,19,21], liver sections from arsenate treated mice showed moderate to marked venous congestion, sinusoidal dilatation, multiple foci of mononuclear cell infiltration and parenchymal disorganization. Additionally, necrosis, choliangiofibrosis and periportal fibrosis were also observed. Hepatic necrosis may be due to oxidative stress induced by arsenic that further involved in the cellular protein degradation. Shrinkage and necrosis of hepatocytes as a result of arsenic toxicity could increase the permeability and leakage of cellular material resulting in sinusoidal expansion [35-37]. Consistent with the present study, Reddy et al. [18] revealed that there was hepatocyte damage and hepatic fibrosis due to long-term arsenic toxicity in mice. In accordance with previous studies on arsenic induced hepatotoxicity in rats treated with ascorbic acid [10] and with N-acetyl cysteine [21] and goats supplemented with spirulina [19] significant hepatoprotective effects were observed. Moreover, the histological abnormalities related to arsenic-induced hepatotoxicity in mice were greatly prevented by co-treatment with curcumin and confined only to mild sinusoidal dilation [18].

In agreement with the finding of Qureshi et al. [20], the renal corpuscles in animals treated with sodium arsenate showed congestion and dilatation of glomerular capillaries. Renal corpuscles displayed hypercellularity and obliteration of the capsular space. Qureshi et al. [20] reported that maternal exposure to sodium arsenate during gestation period in albino mice resulted in significant renal lesions in the offspring. As observed by Cullen et al. [38] and Ferzand et al. [36], arsenic toxicity induced gloumerular and capillary damage and this could induce an increase in glomerular filtration and capillary permeability resulting in leakage of proteins. Shrinkage of glomeruli and increase in capsular spaces may result from leakage of liquid material into the capsular spaces [39]. Additionally, the current study showed severe tubular necrosis which might be due to degradation of leaking cytoplasmic material and denaturing of protein components causing cytoplasmic vacuolation [36]. In agreement with previous studies regarding arsenic-induced nephrotoxicity in rats [10,21], mice [20] and goats [19], sections of kidney from arsenate treated mice in the current study showed glomerulonephritis, proximal tubular necrosis, epithelial damage and loss of nuclei, interstitial hemorrhages and mononuclear cell infiltration, as well as hyaline casts within the medullary tubules. In accordance with previous studies on arsenic-induced nephrotoxicity in rats treated with ascorbic acid [10], in mice treated with Vitamin C and E [20] and in goats supplemented with spirulina [19], significant reno-protective effects were observed.

It had also been documented that co-administration of two antioxidants like Vitamins C and E reduces arsenic burden of liver and kidney as Vitamin C acts as a detoxifying agent by forming poorly ionized but soluble complexes [40]. This evidence supported the protective effect of both natural antioxidants co-administration (green tea and garlic). Both antioxidants showed the highest protective effect on arsenic induced injuries of both liver and kidney.

The co-administration of green tea and garlic may imitate the action of vitamin C and E [30] in preventing the endothelial damage and congestion of efferent arterioles, thus reducing the glomerular hypertrophy. Meanwhile, vitamin C, used in the present study, showed similar renoprotective activity. The treatment with vitamin C considerably reduced the tubular degenerative changes, epithelial cell vacuolation and tubular atrophy after chronic arsenic exposure.

In conclusion, the present study evaluated the protective role of water extracts of green tea, garlic and vitamin C against a toxic dose of 40 mg/Kg body weight sodium arsenate. The protective effect of these antioxidants was shown in the form of normalization of enzymatic and non-enzymatic activities represented by normalization of blood parameters, and liver and kidney functions. There was also a reasonable consistency between the biochemical and the histopathological findings. It was obvious that arsenic-induced toxicity was clearly seen in both kidney and liver tissues and the natural and synthetic antioxidants showed hepato-renal protective capacities.

Acknowledgement

The authors are thankful to Prof. Dr. Mohamed El-Sayed Alkafafy, at the Department of Biotechnology, College of Science, for accomplishing and interpretation of the histological studies in the current work.

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