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Original Article

The Ameliorative Effect of Green Tea, Garlic and Vitamin C on Arsenic Toxicity in Male Mice: Biochemical and Histological Forensic Perspectives

التأثير الايجابي للشاي الأخضر والثوم وفيتامين ج على سمية الزرنيخ لدى ذكور الفئران: التغيرات البيوكيميائية والنسيجية من منظور جنائي.

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Abstract

Arsenic is a heavy metal with toxic effects on human health and is widely found in the environment. It is used in suicides and, hence, acquires forensic impact.

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 in liver and kidney. The mice were also co-treated with green tea, garlic and vitamin C to reveal the protective role of these herbal and synthetic antioxidants.

Arsenic induced significant declines in all blood parameters, while green tea, garlic and vitamin C ameliorated these affected hematological parameters. Alanine transaminase (ALT) and aspartate transaminase (AST) were significantly increased in the sodium arsenate treated group, while green tea, garlic and vitamin C ameliorated these increases in enzyme levels. Creatinine and urea were significantly increased in arsenic treated mice. These renal parameters become normal in mice co-treated with green tea, garlic and vitamin C. Arsenate-treated mice showed venous congestion, sinusoidal dilatation, mononuclear cell infiltration and periportal fibrosis in liver sections. Kidney samples from the same group revealed interstitial hemorrhages, mononuclear cell infiltration, glomerulonephritis and proximal tubular necrosis. Hepato-renal injuries were greatly reduced, particularly in animals that received both green tea and garlic.

The herbs used have a potential for ameliorating and protecting against the hepato-renal toxicity caused by arsenic and need further studies. This study revealed the possibility of using liver and kidney as indicators to ascertain arsenic poisoning in forensic caseworks.

Keywords: *Forensic Science, Arsenicals, Toxicity, Kidney, Liver, Mice, Green Tea, Garlic, Vitamin C.*

المستخلص

الزرنيخ هو معدن ثقيل ذو تأثير سام على صحة الإنسان ويوجد على نطاق واسع في البيئة. يتم استخدامه في الانتحار، ومن هنا يكتسب أثره الجنائي. تم تعريض عدد ستين ٦٠ من فئران ألبينو من الذكور البالغين والتي بلغ وزنها ٣٠-٤٠ غرام إلى جرعة شبه قاتلة من مادة زرنيخ الصوديوم (٤٠ ملغم / كغم من وزن الجسم) للتحقق من التغيرات الدموية والبيوكيميائية والنسيجية في الكبد والكلى. كما تمت معالجة الفئران أيضًا بالشاي الأخضر والثوم وفيتامين ج للكشف عن الدور الوقائي لمضادات الأكسدة العشبية والصناعية. تسبب الزرنيخ في حدوث انخفاضات كبيرة في جميع قياسات الدم، بينما ظهر أن الشاي الأخضر والثوم وفيتامين C يحسنان هذه القياسات الدموية. وقد ازداد بشكل كبير معدل أنزيم الألانين ترانساميناز (Alanine transaminase ALT) وأنزيم الأسبارتيت ترانسبارتيز (Aspartate transpartase AST) في المجموعة التي تم تعريضها لمادة زرنيخ الصوديوم، في حين أن الشاي الأخضر والثوم وفيتامين ج أدى إلى التقليل من هذه الزيادات في مستويات الإنزيم. وحدثت زيادة في معدلات الكرياتينين واليوريا بشكل كبير في الفئران المعالجة بالزرنيخ. هذه المقاييس الكلوية أصبحت بمعدلات طبيعية في الفئران التي تم معالجتها بالشاي الأخضر والثوم وفيتامين ج. وأظهرت الفئران التي تم معالجتها بالزرنيخ احتقان وريدي وتوسع جيبي وارتشاح خلوي على مستوى الخلايا أحادية النواة وتليف حويصلي في أقسام الكبد. وقد كشف فحص الكلية لنفس المجموعة وجود نزيف بين خلوي وارتشاح خلوي للخلايا وحيدة النواة، كما وجد التهاب في كبيبات الكلى والنخر أنبوبي الطرفية القريبة. حدث انخفاض بشكل كبير في إصابات الكبد والكلى، وخاصة في الحيوانات التي تلقت الشاي الأخضر والثوم. وكنتيجة فإن الأعشاب المستخدمة هنا، لديها القدرة على تحسين والحماية ضد سمية الكبد والكلى الناجمة عن الزرنيخ وتحتاج إلى مزيد من الدراسات. وكشفت هذه الدراسة عن إمكانية استخدام الكبد والكلى كمؤشرات للتأكد من التسمم بالزرنيخ في الحالات الجنائية.

الكلمات المفتاحية: علوم الأدلة الجنائية، مركبات الزرنيخ، التسمم، الكلى، الكبد، الفئران، الشاي الأخضر، الثوم، فيتامين ج.

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1. Introduction

Toxicity, aging and diseases are among the main health problems caused by free radicals inside cells [1]. Drinking water contaminated with inorganic arsenic is the main source of arsenic toxicity [2]. In fish and seaweed, arsenic is found in the form of arsenolipids and could be added to hot tea or food to kill people [3]. It can also be used in suicides [4]. The metal is commonly found as trivalent arsenite and pentavalent arsenate. These inorganic arsenicals are considered more toxic than organoarsenicals [5]. Arsenic compounds are most readily absorbed from the gastrointestinal tract [6]. They can bind to sulfhydryl groups of glycolysis and tricarboxylic acid cycle enzymes inhibiting their pathways. The pentavalent arsenicals can interfere with mitochondrial oxidative phosphorylation enzymes [5].

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 [7]. Arsenic also produces oxidants such as superoxide anions and hydroxyl peroxide, which damage the cellular macromolecules or can act as second messengers to alter gene expression and enhance cell proliferation [8].

The liver is the first target organ in arsenic metabolism in which the metal is subjected to methylation [5]. Arsenic causes hepatic cytotoxicity and physiological dysfunction in the form of DNA damage, enhanced cell proliferation, DNA methylation and genomic instability [9-13]. The kidney is considered the second target organ for arsenic toxicity. Pentavalent arsenic and organic arsenic are rapidly and completely eliminated via the kidney [14]. Recent studies revealed an oxidative stress of inorganic arsenicals on other organs such as the lung [15], heart [16] and brain [17].

Garlic (Allium sativum) possesses many important nutritive and antioxidant substances as selenium, sulfur-containing compounds, and vitamins (A, B, C and E). Several studies clarified the ability of garlic to eliminate arsenic from blood and soft tissues [18, 19]. Consumption of tea has been associated with antimutagenic and possible anticarcinogenic effects [20]. 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 [21]. Vitamin C inhibits lipid peroxidation by scavenging the aqueous reactive oxygen species (ROS) [22]. It acts as an antioxidant molecule chelating heavy metals [5]. Vitamin C, as well as any other water soluble antioxidants, scavenge ROS and play an important role in the regulation of intracellular redox state [7]. Several researchers investigated the histopathological effects of arsenicals on the liver and kidney of rats, mice and goats [12, 23-26].

The protective role of green tea and garlic or their combinations have received limited attention in the past. The present study therefore aimed to investigate some hepatic and renal biochemical and histological parameters to evaluate the protective role of green tea, garlic and vitamin C against arsenic toxicity in male mice.

2. Materials and Methods

Sixty adult male albino mice were purchased from the King Fahd Center for Medical Research at King Abdulaziz University in Jeddah,Saudi Arabia, at an initial age of 7-8 weeks and a mean body weight of 38.40 ± 1.0 g. The 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 were 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 group, and they were treated daily for 30 days. Group I received tap water and served as control. Group II received sodium arsenate (Na_3AsO_4) 40 mg/kg body weight (bw) per day [27]. Group III (Na_3AsO_4-green tea) administrated 40 mg/kg bw Na_3AsO_4 and 625 mg/kg bw green tea [28]. Group IV (Na_3AsO_4- garlic) received 40 mg/kg bw Na_3AsO_4 and 33 g/900 mL water garlic extract [19]. Group V (Na_3AsO_4+green tea+ garlic) was treated with 40 mg/kg bw Na_3AsO_4 and a mixture of 625 mg/kg bw green tea and 33 g garlic/ 900 mL water. Mice in group VI (Na_3AsO_4+vitamin C) received 40 mg/kg bw Na_3AsO_4 mixed with 150 mg vitamin C [29]. Doses were available for animals in the drinking water. After 30 days of the ex-

periment, mice were deprived of food and water for 12 h before collecting blood and tissue samples. All the experiments using mice were conducted following the ethics set by the Faculty of Sciences, King Saud University, KSA vide refrence no. 4/4/34.

Animals were anesthetized with diethyl ether, and blood was collected immediately from the medial retro-orbital venous plexus with capillary tubes. The blood samples were centrifuged at 3000 rpm for 5 min and plasma were collected. Using a cell counter (Sysmex, model KX21N), white blood cells (WBCs) in thousands/mL^3 and red blood cells (RBCs) in millions/mL^3 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/mL^3. ALT and AST activities and creatinine and urea levels were measured using a commercially available assay kit according to their manufacturer's protocol (Human Diagnostic, Germany; DiaSys Diagnostic Systems, Turkey; and Biosystems S.A., Spain).

Mice were sacrificed by cervical dislocation and liver and kidney specimens of 5 animals per group were collected. Tissues 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 (HE) staining was conducted to investigate the general histological structures according to protocols in Bancroft et al. [30]. 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 ± standard errors (S.E.).

3. Results

Table-1 represents the effect of green tea, garlic and vitamin C co-treatments on sodium arsenate induced alternations of mice hematology, liver (ALT and AST) and kidney (creatinine and urea) enzymes. RBCs, Hb, PCV, MCH and MCHC decreased significantly in the treated group as compared to the control group. These parameters were increased in green tea, garlic and vitamin C co-treatments (groups III to VI) as compared to arsenate-treated group II. WBCs did not show significant changes after exposure to arsenate together with green tea, garlic and vitamin C. When green tea and garlic were used in a mixture with arsenate, WBCs number increased significantly ($p < 0.001$). RBCs decreased significantly in group II compared to groups I, IV, V ($p < 0.01$) and VI ($p < 0.001$). RBCs increased significantly ($p < 0.01$) in groups IV, V and VI. Hb showed a significant decrease ($p < 0.001$) in group II compared to other groups, and it showed significant increases in groups III, IV, V and VI compared to the control group. PCV showed a significant decrease in group II compared to other groups. MCV showed a significant decrease 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. A significant increase ($p < 0.001$) in the MCH of groups III, IV, V and VI were shown compared to group II. MCHC showed a significant decrease ($p < 0.001$) in group II compared to all other groups.

ALT and AST (Table-1) was significantly increased in group II compared to all other groups. Creatinine and urea were significantly increased ($p < 0.001$) in group II compared to the control group. Co-administration of green tea, garlic and vitamin C significantly normalized creatinine and urea levels. There were no significant changes in 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) compared to the control group.

HE-stained liver sections of control mice showed a normal lobular pattern of a central vein (CV) from which the hepatic cords (HC) radiated and separated by blood sinu-

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.*

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)	10.8 ± 0.5*** (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)	48.1 ± 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)	39.9 ± 0.95*** (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 (gm/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 (gm/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	***

soids. 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 showed obvious histological changes in the form of distorted hepatic architecture, dilatation and congestion of the CV and hepatic sinusoids (HS) (Figure-1b, 1c, 1d). They showed proliferation of kupffer cells. Some hepatocytes showed signs 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, 1d).

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

cytes exhibited hyalinized cytoplasm with pale nuclei and prominent nucleoli (Figure-2a, 2b, 2c, 2d). The liver sections from mice treated with a combination of green tea and garlic (Figure-2e, 2f) or with vitamin C (Figure-2g, 2h) displayed hepatocytes somewhat similar to those from the control (Figure 1a). However, few hepatocytes displayed vacuolated cytoplasm and pyknotic nuclei (Figure-2e, 2f, 2g, 2h).

HE-stained kidney sections showed that the renal cortex from group I was mostly occupied by renal corpuscles (RC) and surrounding proximal and distal convoluted tubules (Figure-3a). 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-3b). The glomerular capillaries of some RC of group II displayed congestion and dilatation. Other RC showed hypercellularity and obliteration of the capsular space (Figure-3c). Some RC showed shrunken profiles surrounded by widened capsular space (Figure 3d). Interstitial hemorrhages were also observed among the renal tubules (Figure-3c). Some sections displayed remarkable mononuclear cell infiltration, especially at the perivascular areas (Figure-3d). There was swelling of some cells of the proximal convoluted tubules (PCT) leading to obliteration of the tubular lumina. Cytoplasmic vacuolation (Figure-3d, 3e, 3f) 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 (Fig-

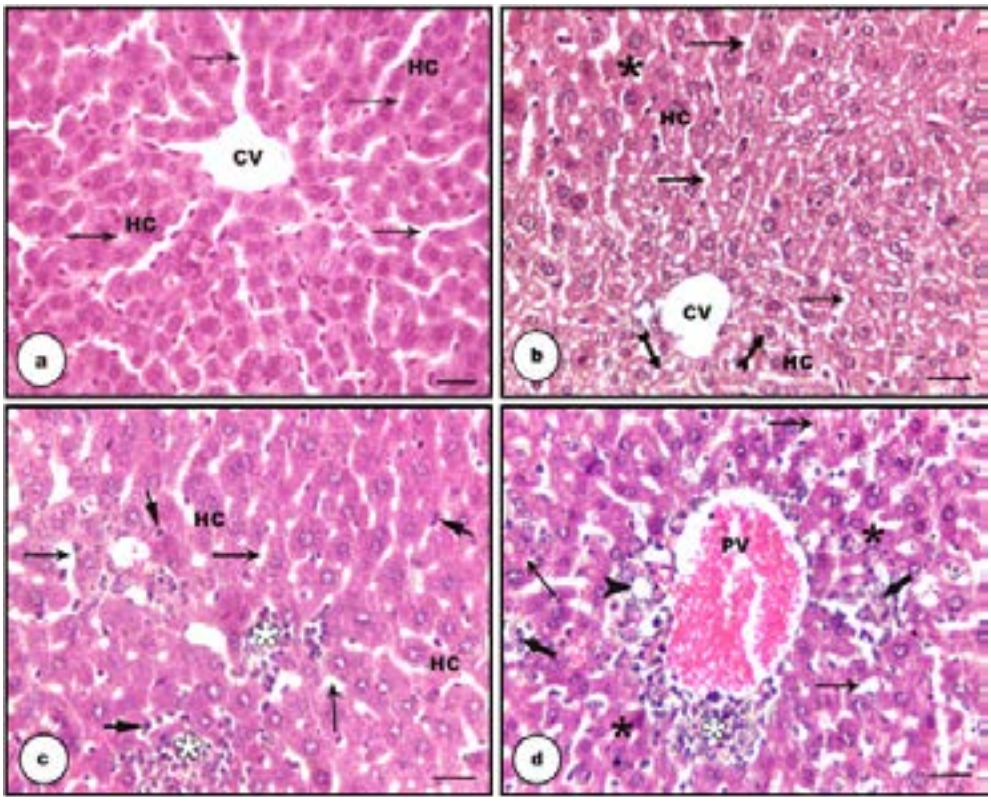

Figure 1- *HE-stained sections of liver from group I (a) and group II (b, c and d). (a) shows central vein (CV), radiated hepatic cords (HC) and hepatic sinusoids (arrows); (b) shows CV, radiated HC, dilated hepatic sinusoids (arrow) displaying less affected hepatocytes (asterisks) and hepatocytes suffering from centrilobular hepatoxicity (forked tail arrows); (c) shows hepatic cords (HC), dilated sinusoids (arrow) with proliferation of kupffer cells (long head arrow) and mononuclear leukocytic infiltration (white asterisks); (d) exhibits periportal mononuclear leukocytic infiltration (white asterisks), necrotic foci (thick arrows) and hyalinized hepatocytes (black asterisks) separated by congested hepatic sinusoids (arrow) surrounding severely congested portal vein (PV) and bile ductule (arrowhead). Scale bar = 30 μm.*

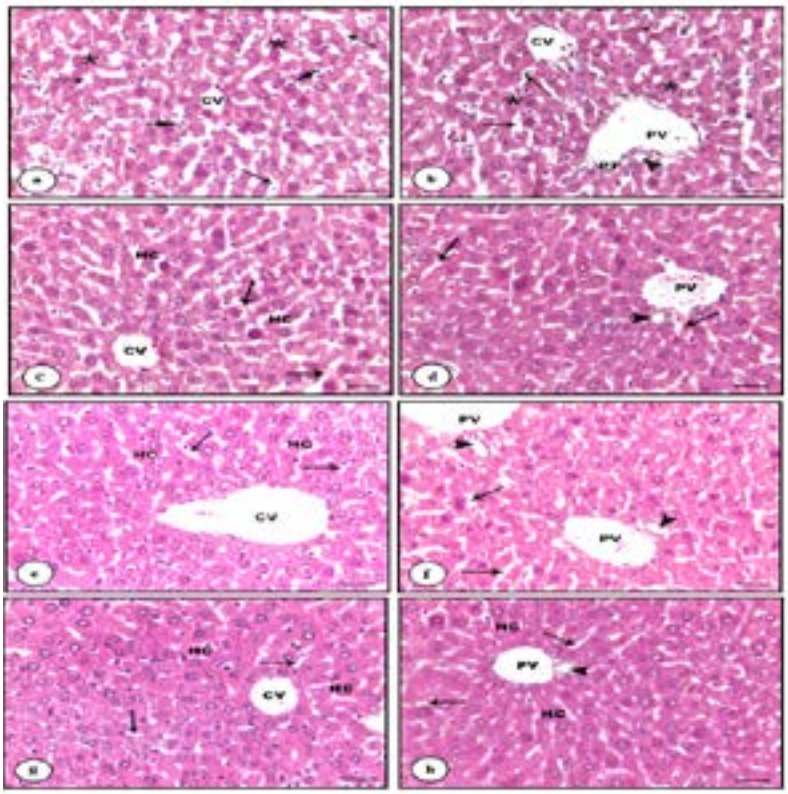

Figure 2- *HE-stained sections of liver from group II (a) and group III (b, c and d). (a) shows CV and hyalinized hepatic cords (asterisks), dilated and slightly congested hepatic sinusoids (arrow), proliferated kupffer cells (long head arrow); (b) shows hyalinized hepatic cords (asterisks), a portal tract (PT), a dilated PV, bile ductule (arrowhead) and dilated congested hepatic sinusoids (arrow). (c) A section of group IV showing CV, radiated HC and hepatic sinusoids (arrows); (d) shows portal tract displaying PV, bile ductule (arrowhead) and hepatic sinusoids (arrow). (e) A section of group V showing: CV, radiated HC and hepatic sinusoids (arrows); (f) portal tract displaying PV, bile ductule (arrowhead) and hepatic sinusoids (arrows). (g) A section of group VI showing normal hepatic histological architecture (CV and HC and hepatic sinusoids (arrow)); (h) portal tract displaying PV, bile ductule (arrowhead), HC and hepatic sinusoids (arrow). Scale bar = 30 µm.*

ure-3e, 3g, 3h).

The co-treatment with green tea (Figure-4a, 4b), garlic (Figure-4c, 4d), both green tea and garlic (Figure-4e, 4f) or vitamin C (Figure-4g, 4h) reduced the injurious effect of sodium arsenate on the renal structures. The congestion and dilatation of glomerular capillaries were milder than in group II (Figure-3). The hypercellularity of RC and obliteration of the capsular space disappeared. The interstitial hemorrhages and mononuclear cell infiltration were rarely seen (Figure 4). The degenerative changes in the renal tubular cells were greatly reduced, particularly in groups V (Figure-4e, 4f) and VI (Figure-4g, 4h). 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-4e, 4f) and VI (Figure-4g, 4h).

4. Discussion

The pathway of arsenic found in drinking water was explained by Karmakar et al. [6]. Absorption of arsenic occurs primarily through the gastrointestinal tract, causing gastrointestinal lesions. The lesions induce an increase in the permeability of small blood vessels through which arsenic enters into the blood and binds to haemoglobin. Arsenic bio-transformation occurs in the liver and its excretion finally happens through the kidney.

The target organs showing abnormalities in all parameters studied in this work could be used as biomarkers to ascertain poisoning due to arsenic toxicity and could be used as forensic indicators. An acute exposure of male mice to arsenic produced harmful effects on the animals' physiology, hematology and organs histology. The liver as a major

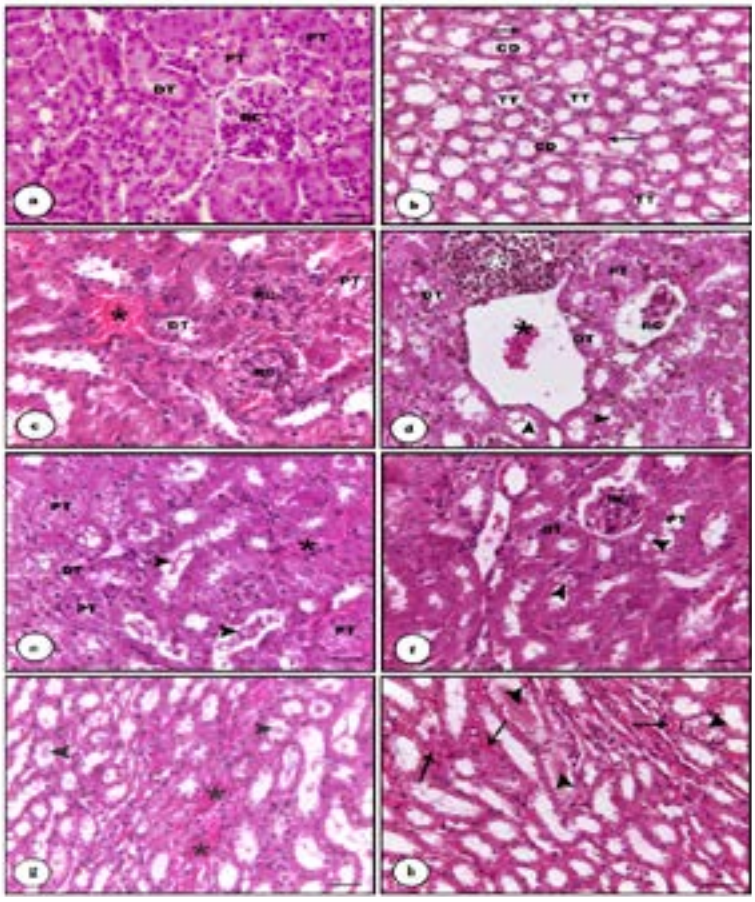

Figure 3- *HE-stained sections of kidney from control mice showing: (a) renal cortex of renal corpuscle (RC), proximal (PT) and distal (DT) tubules; (b) renal medulla of thin tubules (TT), collecting ducts (CD) and inter-tubular blood capillaries (arrows). Sections of kidney from mice of group II: (c) renal cortex displaying marked interstitial hemorrhage (black asterisk), obliterated RC with mesangial hypercellularity and degenerating PT and DT; (d) renal cortex showing shrunken RC, mononuclear leukocytic infiltration (white asterisk), highly congested vessel (black asterisk) and degenerating PT and DT presenting fatty change (arrowhead); (e) a medullary ray at renal cortex showing interstitial hemorrhage (black asterisk) and degenerating PT and DT surrounding a collecting duct enclosing proteinceous casts (arrowheads); (f) renal cortex displaying distorted renal RC as well as degenerating PT and DT exhibiting hyalinized cytoplasm and scattered cells displaying fatty change (arrowheads); (g) renal medulla of thin tubules and collecting ducts showing interstitial hemorrhages (black asterisks) and intra-tubular casts (arrowheads); (h) renal medulla of thin tubules and collecting ducts displaying interstitial hemorrhages (arrows) and intra-tubular hyaline casts (arrowheads). Scale bar = 30 µm.*

target organ for arsenic toxicity and carcinogenesis [2, 31] showed abnormal increase in ALT and AST enzymes and these findings are supported by several investigations. Yasmin et al. [13] found that the activities of both enzymes were significantly higher in arsenic treated mice and rats, indicating liver dysfunction and disturbance [32]. The increase in blood level of both enzymes is a reliable determinant of liver parenchymal injury [9] and could be due to their leakage from the liver cytosol into the blood stream [10]. Other investigations also referred to similar hepatotoxicity of arsenic [11-13].

Usually, leucopenia could be induced by acute, intermediate and chronic exposure to arsenic [5]. However, the current and other previous studies [13, 33] revealed no change in WBCs by acute exposure to arsenic, and leucopenia starts to appear when the exposure has become prolonged [34]. All blood parameters showed significant declines due to arsenic toxicity, and green tea and garlic as well as vitamin C induced general hematological protection. 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 obvious effect of green tea and garlic could be shown by a high significant increase of WBCs in mice treated with the aqueous extract of both herbs. This result supports the immunomodulatory

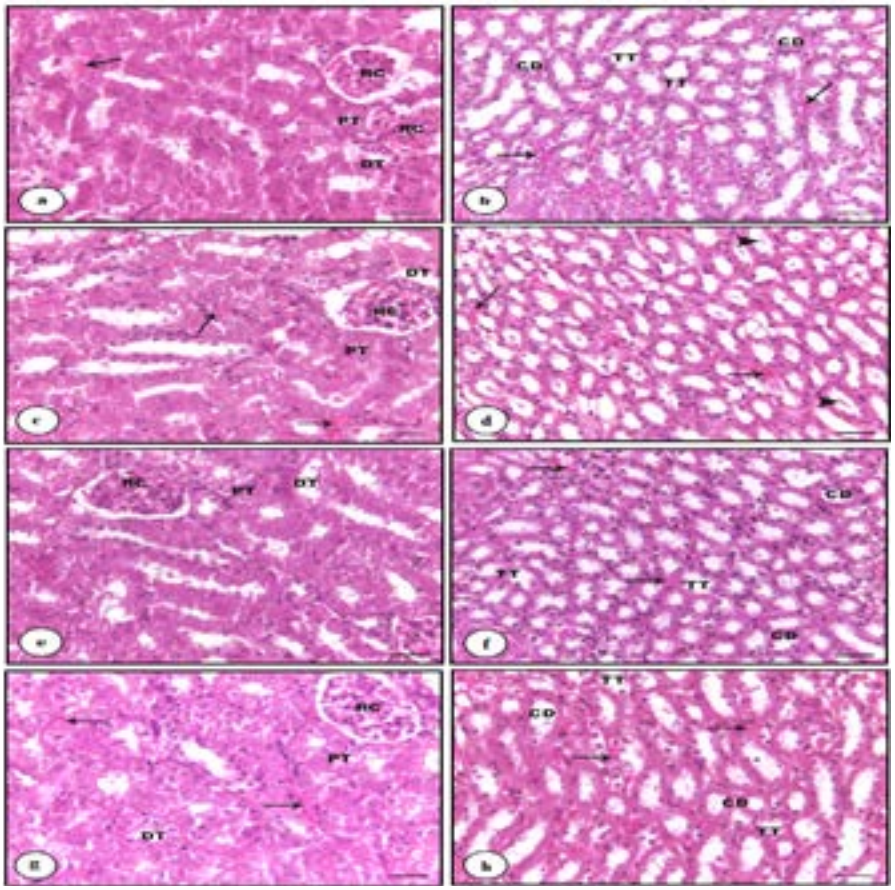

Figure 4- *HE-stained sections of kidney exposed to sodium arsenate and treated concurrently with green tea extract: (a) renal cortex displaying RC, milder interstitial hemorrhage (arrow) and slightly regenerating PT and DT; (b) renal medulla of thin tubules (TT) and collecting ducts (CD) showing milder interstitial hemorrhages (arrows) compared to renal sections from mice exposed to arsenic only. Sections of kidney exposed to sodium arsenate and treated concurrently with garlic extract showing: (c) renal cortex displaying RC, milder interstitial hemorrhage (arrow) and slightly regenerating PT and DT; (d) renal medulla showing milder interstitial hemorrhages (arrows) and intra-tubular desquamations (arrowheads). Sections of kidney exposed to sodium arsenate and treated concurrently with a combination of green tea and garlic extracts: (e) renal cortex showing RC as well as more or less normal PT and DT; (f) renal medulla of TT and CD showing milder interstitial hemorrhages (arrows). Sections of kidney exposed to sodium arsenate and treated concurrently with ascorbic acid showing more or less normal renal histological architecture and mild interstitial hemorrhages (arrows): (g) renal cortex of RC, PT and DT; (h) renal medulla of TT and CD. Scale bar = 30 µm.*

effect of garlic [19] and green tea [20, 21].

The significant decrease in MCV in group II indicated that RBCs had become microcytic [35] and were not able to carry enough oxygen for tissue respiration [36]. It may cause an ATP deficit, general body weakness and death but this was not observed, probably due to the short period of exposure to arsenic. Some studies showed that arsenic causes moderate hemolytic anemia in mice [37], which could be supported by significant decrease of Hb in group II. Similar to Gupta et al. [11], the present study revealed a decrease in RBCs and hemoglobin by increase of arsenic exposure. This could be due to the high affinity of arsenic to bind with hemoglobin causing an inhibition of the heme synthesis pathway.

Similar to several investigations [7, 12, 20, 21, 38], the present study examined the protective effect of green tea and garlic as well as vitamin C (ascorbic acid) against arsenic toxicity. However, this study examined the combined effect of green tea and garlic for the first time. Green tea, garlic and vitamin C possess an antioxidant ability to potentiate the inside body antioxidants [39] in scavenging the free radicals liberated by arsenicals. These herbs could normalize the enzymatic and non enzymatic biology and thereby prevent microsomal lipid peroxidation, liver fibrosis, liver necrosis and hepatic inflammation induced by arsenicals. In agreement with these studies, the present study

revealed that green tea, garlic and vitamin C normalized the blood parameters and liver and kidney enzymes.

In agreement with previous studies [12], hepatic sections from arsenate treated rats 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 was 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 [40-42]. Consistent with the present study, Noman et al. [23] 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 [12] and with N-acetyl cysteine [26] and goats supplemented with spirulina [24] showed significant hepatoprotective effects.

In agreement with the finding of Qureshi et al. [25], 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. [25] reported that maternal exposure to sodium arsenate during the gestation period in albino mice resulted in significant renal lesions in the offspring. As observed by Cullen et al. [43] and Ferzand et al. [41], 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 [44]. 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 [41]. In agreement with previous studies regarding arsenic-induced nephrotoxicity in rats [12, 26], mice [25] and goats [24], 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 [12], in mice treated with Vitamin C and E [25], and in goats supplemented with spirulina [24], significant reno-protective effects were observed.

It has also been documented that co-administration of two antioxidants like vitamins C and E reduces the arsenic burden on the liver and kidney, as vitamin C acts as a detoxifying agent by forming poorly ionized but soluble complexes [45]. This evidence supports the protective effect of both green tea and garlic co-administration. They 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 [35] in preventing the endothelial damage and congestion of efferent arterioles, thus reducing the glomerular hypertrophy. Meanwhile, in the present study vitamin C showed similar reno protective activity. The treatment with vitamin C considerably reduced the tubular degenerative changes, epithelial cell vacuolation and tubular atrophy after chronic arsenic exposure.

5. Conclusion

In conclusion, the present study examined the protective role of water extracts of green tea, garlic and vitamin C against a toxic dose of 40 mg/Kg body weight of sodium arsenate. The protective effect of these antioxidants was shown in the form of ameliorating effects on enzymatic and non-enzymatic parameters represented by blood profiles and liver and kidney functions. There was also a reasonable correlation between the biochemical and the histopathological findings. Arsenic-induced toxicity was clearly seen in both kidney and liver tissues and the natural and synthetic antioxidants showed hepato-renal protective capabilities. Further studies are necessary for measuring the concentrations of arsenic in liver and kidney. This could add a clue for identifying the fatal intentional poisoning by arsenic in forensic cases.

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Conflicts of Interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study, the collection, analyses, or interpretation of data, the writing of the manuscript, and in the decision to publish the results.

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