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Protection against arsenic-induced hematological and hepatic anomalies by supplementation of vitamin C and vitamin E in adult male rats

DOI 10.1515/jbcpp-2016-0020
Received February 20, 2016; accepted June 27, 2016

Abstract

Background: Chronic arsenic exposure via contaminated drinking water is a global environmental health problem associated with hematological, hepatic and many serious systemic disorders. This study on adult male rats evaluated the protective effects of vitamin E (VE) and vitamin C (VC) against arsenic-mediated hematological and hepatic toxicities.

Methods: Arsenic was administered orally as arsenic trioxide (3 mg/kg body weight/day), as a single dose for 30 consecutive days or along with VC/ascorbic acid (200 mg/kg body weight/day dissolved in water) and VE/α-tocopherol (400 mg/kg body weight/day dissolved in olive oil) as supplements. Multiple hematological and hepatic parameters were assessed.

Results: Arsenic exposure caused significant reduction of erythrocyte counts ($p<0.05$), leukocyte counts ($p<0.01$) and hemoglobin (Hb) levels ($p<0.01$). Arsenic exposure also led to marked echinocytic transformation of erythrocytes resulting in increased morphological index ($p<0.001$). Altered serum oxidative balance was observed with a higher oxidative stress index ($p<0.001$). The results also showed a significant increase of serum cholesterol ($p<0.05$), low-density lipoprotein ($p<0.001$) and triglycerides ($p<0.01$), and decreased high-density lipoprotein ($p<0.01$) along with total protein ($p<0.01$). A marked elevation of hepatic thiobarbituric acid reactive substance ($p<0.05$) along with decreased reduced glutathione ($p<0.001$) levels were also observed. Interestingly, co-administration of VC and VE significantly prevented all the arsenic-induced alterations ($p<0.05$) except Hb content and serum protein.

Conclusions: The present investigation offers strong evidence regarding the protective efficacy of co-administration of VC and VE against hematotoxicity and hepatotoxicity in adult male rats caused by chronic arsenic exposure.

Keywords: arsenic; echinocyte; erythrocyte; liver; vitamin C and E.

Introduction

Arsenic is a naturally occurring metalloid known to be a potent carcinogen and environmental toxin responsible for debilitating dermal toxicity and organ damages. Consumption of contaminated drinking water is the primary route of arsenic exposure. Arsenic contamination of drinking water is a serious health concern around the world, given that several countries report levels well above the permissible limit of 0.01 mg/L, as set by the World Health Organization [1]. In India, arsenic contamination of groundwater often reaches several times higher than the permissible limit [2, 3].

Arsenic toxicity is associated with severe metabolic disorders often leading to pathological conditions in multiple organs and systems [4]. The adverse effects of arsenic on hematopoietic and hematological systems are well documented along with its effect on the enzymes responsible for heme synthesis and degradation [5]. Arsenic in the post-ingestion phase undergoes biotransformation by

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methylation, which is responsible for reduction of erythrocyte and leukocyte counts [6]. It has been observed that the blood arsenic level of workers in some Indian glass plants is five-fold higher than that of the non-exposed group, and the exposure causes DNA damage in leukocytes [7]. Its exposure and consequent accumulation are responsible for oxidation of erythrocytes, resulting in membrane injury, methemoglobin formation, osmotic fragility and destruction [8]. The early events leading to distorted erythrocyte morphology, called echinocytic transformation, has been observed in humans as well as in an arsenic-induced murine model [6, 9].

The liver is the main metabolic port of entry and also a primary target site of arsenic toxicity. Patients with chronic exposure to arsenic exhibit symptoms of hepatomegaly, cirrhosis and abnormal function of liver enzymes [10]. The methylated forms of inorganic arsenic, monomethyl arsonous and dimethyl arsinous acid, react with intracellular oxygen and generate reactive oxygen species (ROS) [11], resulting in depletion of ROS-metabolizing enzymes in the hepatocytes [12]. The resulting oxidative imbalance plays a crucial role in the generation of hepatic disorders [13].

Mitigation of arsenic toxicity in spite of its inevitable exposure is a global challenge of present-day research. For this purpose, drugs like British anti-Lewisite and dimercaptosuccinic acid have been used; however, these medicines have massive side effects, which led to their minimum use [13, 14]. On the other hand, extensive research using different dietary supplements to mitigate metal toxicities showed minimal or no side effects. In 1931, Mayer and Sulzberger [15] suggested that adequate levels of vitamin C (VC)/ascorbic acid in the diet prevented or reduced the occurrence of arsenic-induced anaphylaxis. VC is a widely cited water-soluble antioxidant that prevents oxidative damage of cell membranes induced by oxidative radicals in an aqueous environment [12]. However, the hydrophilic nature of VC forces inactivation of its antioxidant potential in hydrophobic zones like membrane interiors. On the contrary, vitamin E (VE)/α-tocopherol, a hydrophobic molecule, is effective in this regard, being a major chain-terminating antioxidant, and it scavenges a wide array of antioxidant species [16]. Hence, a combination of VC and VE (VC+VE) is speculated to be more effective and has been supported by studies on lead- and cadmium-mediated oxidative damages in animal models [17, 18].

We have previously reported that administration of VC+VE can mitigate the toxic effects of arsenic on the male reproductive system of rats [19, 20]. The present study aims to investigate the efficacy of supplementation of VC+VE in protecting arsenic-mediated hematotoxicity and hepatotoxicity using adult rat as a model system.

Materials and methods

Chemicals

The following chemicals were used in this study: arsenic trioxide (Loba Chemie, Mumbai, India), VE/α-tocopherol (HiMedia, Mumbai, India), VC/ascorbic acid (Merck, Mumbai, India), olive oil (Merck, Mumbai, India) and salt mixture (SRL, Mumbai, India). All other chemicals were of analytical grade.

Preparation of aqueous solution of arsenic trioxide and selection of dose

Arsenic trioxide (75 mg/mL) was dissolved in 1 mL of 10% NaOH, and the volume was made up to 100 mL with distilled water after adjusting the pH to 6.8 with 1.0 N H_2SO_4. For chronic exposure to arsenic, an oral dose was selected (3 mg/kg body weight/day) for each rat, essentially as described previously [20].

Animal selection

Eighteen adult male Wister albino rats (weighing 130±10 g) were purchased from registered breeders, and kept in the departmental animal house under standard conditions (24±2 °C, 12 h light dark cycle, 60% humidity). The rats were allowed to acclimatize for a period of 15 days and were provided with a control diet composed of 71% carbohydrate, 18% protein, 7% fat and 4% salt mixture and vitamins [21]. All experiments were performed according to the permission given by the Institutional Animal Ethics Committee (permit no. 796/ac/03/CPCSEA/18-01, Dated 18.01.2013) under the ethical guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forest, Government of India.

Experimental design and collection of samples

The rats were randomly divided into three groups and were under treatment for 30 days. Group I animals (control) were given only vehicle. Group II animals (treated) were given arsenic trioxide orally at a dose of 3 mg/kg body weight/day/rat. Group III animals (supplemented) were orally given with the same dose of arsenic trioxide along with VE (400 mg dissolved in olive oil/kg body weight/day/rat) and VC (200 mg dissolved in water/kg body weight/day/rat).

Food, water intake and the body weight of rats were monitored throughout the experimental period. All animals were subjected to light ether anesthesia after the last arsenic treatment and an overnight fast. Blood was collected from the dorsal aorta and collected in three separate vials – (i) EDTA vial, (ii) heparinized vial and (iii) plain vial, under aseptic conditions. EDTA anticoagulated blood was

used for scanning electron microscopic (SEM) analysis of the erythrocytes and for counting of cellular components. Plasma is separated from the heparinized blood for the estimation of redox parameters. Serum was separated using a standard protocol, frozen and stored at –20 °C for the assessment of protein content and lipid profile. Rats were autopsied and livers were dissected out, precisely weighed and stored frozen (–20 °C) for biochemical estimation, leaving a small portion for histological work.

Counting of erythrocytes and leukocytes and estimation of hemoglobin

The total numbers of erythrocytes and leukocytes were counted, and estimation of hemoglobin (Hb) was done using an automated cell counter (Beckman Coulter, France).

Determination of plasma total antioxidant status and total oxidant status

The total antioxidant status (TAS) was measured according to the method of Re et al. [22] based on the inhibition of radical cation $ABTS^{+\cdot}$ and expressed as mM Trolox equivalent. The total oxidant status (TOS) was estimated according to the method of Erel [23] based on generation of a colored complex of ferric ion and expressed as μM H_2O_2 equivalent. The oxidative stress index (OSI) was calculated from the ratio of TOS and TAS according to Harma et al. [24], and expressed as arbitrary units. The result unit of TAS was changed to μM Trolox equivalent, and the OSI value was calculated as follows:

$$\text{OSI}=[(\text{TOS, } \mu\text{M } H_2O_2 \text{ equivalent}) \times 100/(\text{TAS, } \mu\text{M Trolox equivalent})].$$

Morphological studies of erythrocytes by using SEM

Blood was collected in the presence of ethylene diamine tetraacetic acid (EDTA). Approximately 1 mL of EDTA-treated blood was centrifuged at 1500 *g* for 3 min, and plasma was removed; to this, 1 mL of sterile mammalian Ringer fluid was added, and cells were mixed carefully and centrifuged again at 1500 *g* for 3 min as described previously [25]. Supernatant was discarded and cell pellet was rewashed as described above. Washed erythrocyte cell pellets were resuspended in Ringer fluid and used for SEM analysis, as described previously [25]. Briefly, erythrocytes were directly fixed overnight with 2.5% glutaraldehyde solution in 10 mM phosphate-buffered saline (pH 7.2), and stained overnight in 1% osmium tetroxide in the same buffer. Erythrocyte cell suspension was dehydrated in graded ethanol series. After drying with carbon dioxide by the critical point method and sputter coating with gold, samples were examined using a scanning electron microscope (Model VEGAII LSU; TESCAN, Czech Republic).

Analysis of erythrocyte cell morphology

Quantification of different morphologically distorted forms of erythrocytes was done using photomicrographs obtained after SEM analysis, and scores were given based on Bessis [26] and Ferrell and Huestis [27]. In short, discocytes were assigned a score of zero, echinocyte I (irregularly shaped erythrocyte without defined spicules) assigned a score of +1, echinocyte II (erythrocytes with central pallor and few spicules) assigned a score of +2, echinocyte III (spherical erythrocyte with multiple spicules) assigned a score of +3, spheroechinocytes (spherical erythrocytes with blunted spicules) assigned a score of +4 and spherocytes (spherical erythrocyte lacking central pallor and spicules) assigned a score of +5. About 200 erythrocytes were counted in each case. The morphological index was calculated as follows:

$$\text{Morphological index}=\frac{\sum(\text{morphological score})\times(\text{number of distorted cell type})}{\text{Total number of cells}}.$$

Determination of lipid profile

Lipid components such as total cholesterol (TC), high-density lipoprotein (HDL) and triglyceride (TG) were estimated in serum by using standard kits supplied by Span Diagnostic Limited (Surat, India). Very-low-density lipoprotein and low-density lipoprotein (LDL) were calculated from the value of TG, TC and HDL, as described by the company.

Assay of serum total protein

The total protein contents of different serum samples were measured following Biuret method using a standard kit from Span Diagnostic Limited.

Estimation of liver malondialdehyde level

Malondialdehyde (MDA) levels in the liver tissues from rats of all groups were measured biochemically following the method of Ohkawa et al. [28]. Tissue samples were homogenized in 0.1 M phosphate buffer (pH 7.4) at a tissue concentration of 50 mg/mL. The homogenizing mixture (0.5 mL) was mixed with 0.5 mL of 0.9% saline and 2 mL of mixture of thiobarbituric acid and trichloroacetic acid (TBA-TCA) (0.392 g TBA in 75 mL of 0.25 N HCl with 15 g of TCA, and volume was made up to 100 mL with 95% ethanol) and boiled for 10 min. The mixture was then cooled to room temperature and centrifuged at 4000 rpm for 10 min. Supernatants were subjected to spectrophotometric (JASCO v-530, Easton, MD, USA) analysis at 535 nm.

Quantification of reduced glutathione content of liver

A 10% (w/v) homogenate of liver tissue in 5% (w/v) mataphosphoric acid was centrifuged at 1000×*g* for 30 min at room temperature; the supernatant was carefully collected, deproteinized and used to determine glutathione (GSH) content by monitoring the absorbance at 412 nm in a spectrophotometer (JASCO v-530) by determining the rate of reduction of 5,5-dithiobis-2-nitrobenzoate to 2-nitro-5-thiobenzoate

[29]. The level of liver GSH was determined by using the standard curve of GSH and expressed as mg/g of tissue.

Histology of the liver

Collected tissues were fixed in buffered formol and embedded in paraffin with melting point at 56–58 °C. Routine hematoxylin/eosin (H/E) staining was done using sections of 5 μ thickness by using a high precision microtome (Model IEC Microtome, USA). Histomorphometric analysis was done microscopically (Zeiss, Thornwood, NY, USA).

Statistical analysis

The data were expressed as mean±standard error of the mean (SE). For statistical analysis, quantitative data of each parameter from different groups were analyzed by one-way analysis of variance followed by post hoc test (LSD). Mean±SE was calculated for each group, and the corresponding level of significance was calculated using SPSS statistical software (SPSS, Chicago, IL, USA). For all instances, $p<0.05$ was considered to be statistically significant.

Results

Status of erythrocytes, leukocytes and Hb

Chronic arsenic exposure resulted in significant alteration of erythrocyte and leukocyte counts with concomitant alteration in Hb concentration. Significant reductions of erythrocyte count ($p<0.001$) (Figure 1A) and leukocyte count ($p=0.002$) (Figure 1B) associated with parallel reduction of total Hb content ($p=0.003$) (Figure 1C) were noticed in arsenic-treated rats compared to those of control. Though remarkable increases in both the cases of erythrocyte counts ($p=0.023$) and leukocyte counts ($p=0.002$) due to VC+VE supplementation have been observed, significant differences ($p=0.025$ and $p=0.001$, respectively) with the normal values also exist (Figure 1A,B). However, VC+VE supplementation was unable to restore the Hb content up to a significant level (Figure 1C).

Plasma redox status

Generation of ROS due to arsenic toxicity causes alteration of the redox balance in blood as evidenced by a significant increase of TOS ($p<0.001$) in plasma of treated rats as compared to that of control. Co-supplementation of VC+VE significantly ($p<0.001$) restored the alterations but not at par with the control ($p<0.001$) (Figure 2A). Concomitant decrease of serum TAS was also noted ($p<0.001$) due to arsenic insult, as predicted, which reached the normal value ($p<0.001$) upon VC+VE supplementation (Figure 2B). The OSI was simultaneously increased ($p<0.001$) in arsenic-treated rats compared to that of controls. VC+VE co-administration resulted in significant improvement of OSI ($p<0.001$) but failed to achieve normal status ($p=0.005$) (Figure 2C).

Erythrocyte morphology

Hematological alterations were found to be accompanied by poikilocytosis, i.e. morphological alteration of erythrocytes. The major shape transformations in arsenic-treated

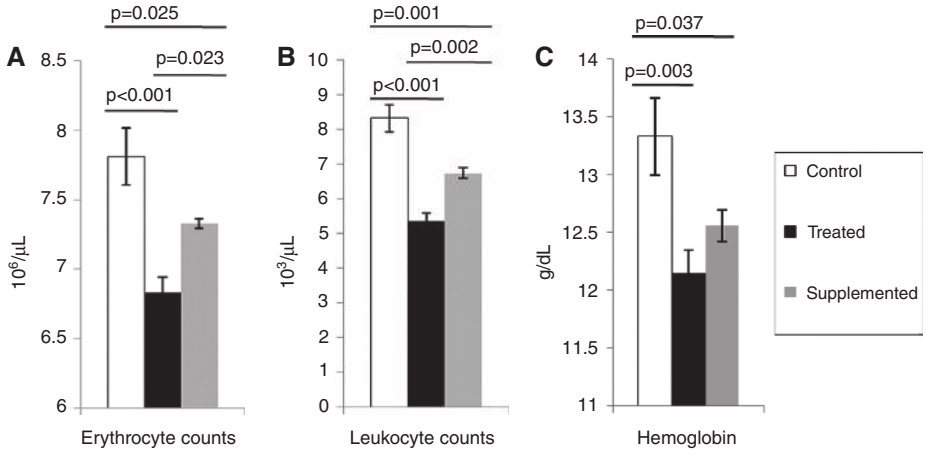

Figure 1: Effect of VC+VE supplementation on the RBC counts, WBC counts and total Hb content in arsenic-treated rats.
Bar diagrams depicting the variation in the erythrocyte (A), leukocyte (B) counts and total hemoglobin content (C) across the three groups as indicated.

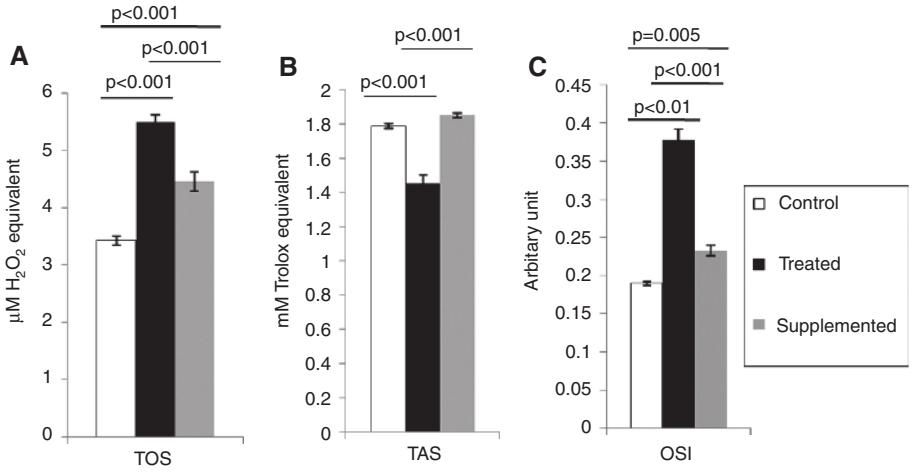

Figure 2: Effect of VC+VE supplementation on redox parameters in arsenic-treated rats.
Bar diagrams depicting the variation in the TOS (A), TAS (B) and OSI (C) across the three groups as indicated.

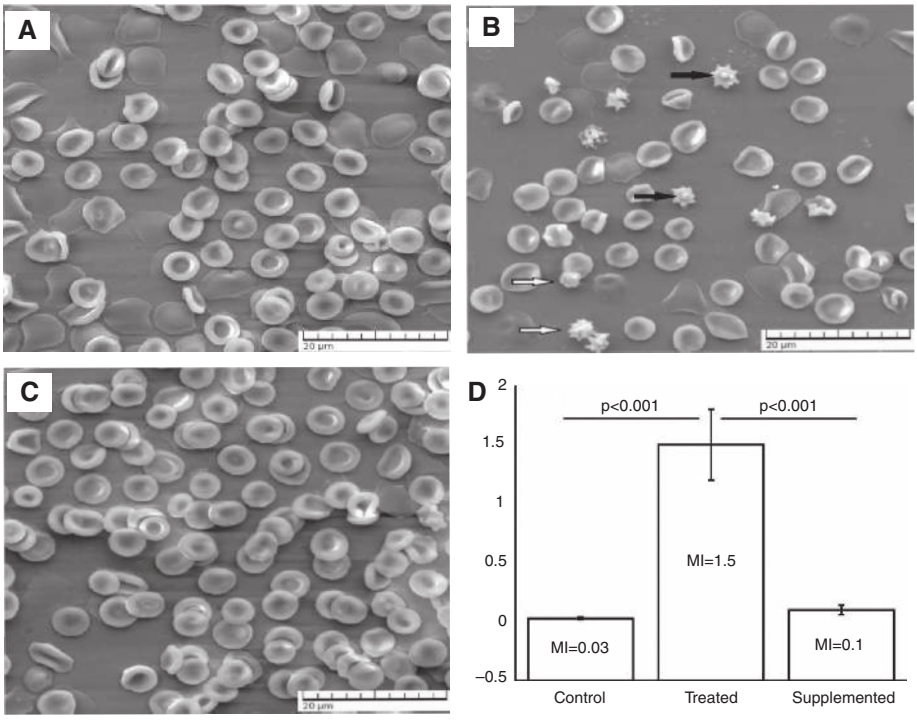

Figure 3: Effect of VC+VE supplementation on erythrocyte morphology in arsenic-treated rats.
SEM images of erythrocytes obtained from control (A), arsenic-treated (B) and VC+VE-supplemented (C) rats. Echinocytic and microcytic transformations of erythrocytes are indicated by black and white arrow, respectively. (D) The morphological index (MI) across the three groups.

rats were found to be echinocytes and spherocytes (Figure 3B) compared to healthy controls. Morphological analysis revealed that arsenic treatment resulted in higher echinocytes and spheroechinocytes (Figure 3B) and a significantly higher morphological index ($p<0.001$) in the rats compared to the control group where the predominant population was discocyte (Figure 3A). VC+VE supplementation significantly inhibited the poikilocytic response due to arsenic exposure in rats (Figure 3C). The animals of group III had lower numbers of echinocytes and spheroechinocytes and near-normal discocytes (about 93%), as observed in the peripheral blood smear. The morphological index was also restored to near normal compared to the arsenic-treated group ($p<0.001$) (Figure 3D).

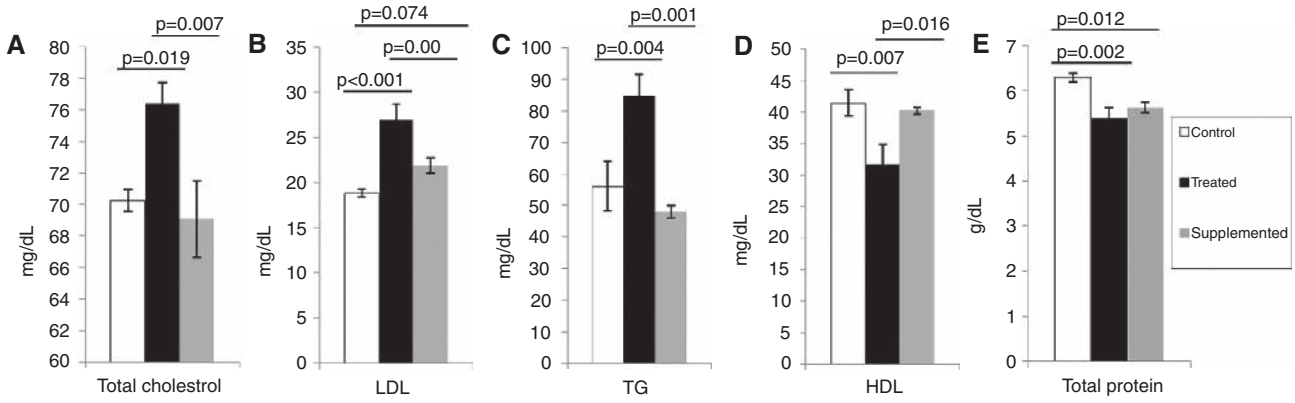

Figure 4: Effect of VC+VE supplementation on serum lipid profile and protein levels in arsenic-exposed rats.
Bar diagram depicting the variation in total cholesterol (A), LDL (B), TG (C), HDL (D) and total protein (E) levels across the three groups as indicated.

Serum lipid and protein status

Administration of arsenic in adult male rats caused a significant increase (p=0.019) of serum TC (Figure 4A). Elevated serum LDL (p<0.001), TG (p=0.004) along with low levels of HDL (p=0.007) were observed (Figure 4B–D) upon arsenic exposure. VC+VE administration in spite of arsenic treatment, however, restored all the values to the normal levels (p=0.007, p=0.007, p=0.001 and p=0.16, respectively) (Figure 4A–D). Arsenic toxicity also caused decrease of serum protein level (p=0.002), which may be due to arsenic-induced free radical-mediated damages on hepatic tissues. However, the vitamin supplementation did not show a significant effect on the restoration of the serum protein (Figure 4E).

Hepatic thiobarbituric acid reactive substance and GSH levels

Arsenic-mediated ROS-induced lipid peroxidation and GSH depletion as evidenced by marked increase of thiobarbituric acid reactive substance (TBARS) (p=0.018) and reduced GSH content (p=0.005) in liver tissue homogenate were observed. Administration of VC+VE in spite of arsenic effects restored the TBARS (p=0.045) and GSH (p<0.005) levels near normal in this metabolic organ when compared to the arsenic-intoxicated group (Table 1).

Histological changes

The liver of arsenic-treated animals showed a varied degree of histoarchitectural alterations, which include expansion of sinusoidal space, degeneration of hepatocytes associated with pyknotic cells, increased diameter of central vein, cytoplasmic blebbing and binucleation (Figure 5C,D). Co-administration of VC+VE managed to restore the alterations of the hepatic tissues (Figure 5E,F).

Discussion

Arsenic toxicity management is a priority area of research mainly because of the ubiquitous nature of the metalloid and the serious global issues that it causes. Survey reports for the last 20 years in India indicate that areas of the

Table 1: Effect of VC+VE supplementation on hepatic GSH and TBARS levels in arsenic-treated adult male rats.

Parameters	Experimental rats		
	Group I	Group II	Group III
Liver			
GSH, µg/g tissue	263.3±5.76	191.7±20.35[b]	248.2±1.86[d]
TBARS, nm/mg tissue	4.88±0.27	6.38±0.5[a]	4.92±0.42[c]

Values are expressed as mean±SEM. [a] and [b] indicate significance from the control group (Gr I) at the p<0.05, and p<0.001 probability level, respectively. [c] and [d] indicate significance from the arsenic group (Gr II) at the p<0.05 and p<0.01 and probability level, respectively.

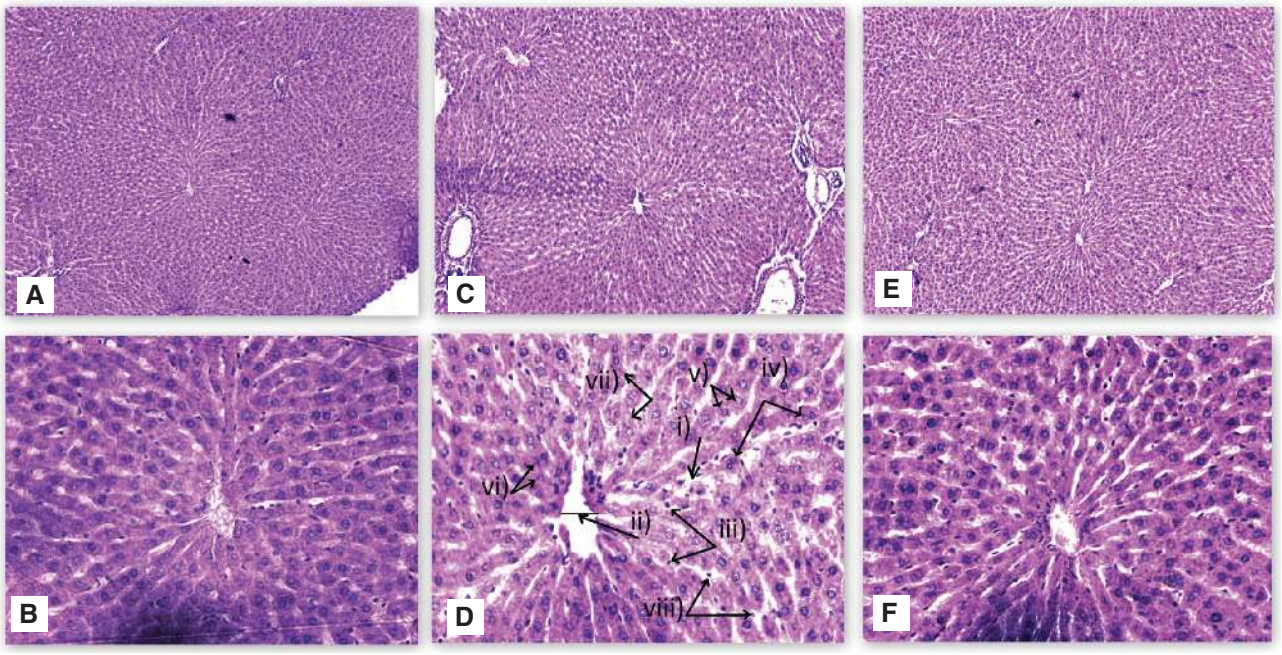

Figure 5: Representative hepatic histopathology by H/E staining as shown in panels A–F.
Normal liver sections from healthy control rat showing no necrotic and degenerative changes (A – 100×, B – 400×); expansion of sinusoidal space (i), increase in diameter of central vein (ii), pyknotic hepatocytes (iii), binucleation of hepatocytes (iv), moderate karyolisis (v), moderate karyorrhexis (vi), cytoplasmic blabbing (vii), free nuclei (viii) in arsenic-intoxicated male adult rats (C – 100×, D – 400×) and restoration of normal histological features in the hepatic tissues of VC+VE-supplemented rats (E – 100×, F – 400×).

Gangetic belt, particularly areas in Uttar Pradesh, Bihar, Jharkhand and West Bengal, contain arsenic-contaminated groundwater at levels much higher than the safe limit, posing deleterious effects to thousands of people and simultaneously putting millions at risk [30]. The first report of arsenic contamination of groundwater and its health effects from the Gangetic plain of West Bengal was published in 1984 [31] and since then a lot of research has been carried out for evaluating its mechanism and management.

This study examined the adverse effects of arsenic on hematological and hepatic systems, and the subsequent amelioration by the combined use of VC+VE in adult male rats. The literature states that arsenic-contaminated drinking water causes morphological alterations in erythrocytes [6]. Smelter workers exposed to arsenic have also reported liver cirrhosis as an occupational hazard [32]. Related studies also indicate distorted histoarchitecture of the liver due to ingestion of arsenic-contaminated drinking water [13, 33]. Recently, we have reported the protective ability of the combined supplementation of VC+VE on arsenic-induced reproductive toxicity [19, 20, 34]. With this background knowledge, we now report the corrective ability of these antioxidant vitamins on hematological and hepatic systems under arsenic insult.

Arsenic treatment for 30 days resulted in decreased erythrocyte and leukocyte counts with concomitant reduction of Hb concentration, in agreement to the epidemiological report [6] and animal studies [9]. As per available information, erythrocytes are the main targets of arsenic toxicity, which leads to hemolysis resulting in anemia [35]. In addition to anemia, another reason for the reduction of Hb is the interaction of arsenic with the Hb-synthesizing enzyme δ-aminolevulinic acid dehydratase, which further aggravates its toxic effects [36]. Moreover, it may also be due to apoptotic effects of arsenic on plasma cells [9]. Co-administration of VC+VE partially restored the erythrocyte and leukocyte counts but failed to enhance the blood Hb concentration up to a significant level, which is in agreement with recent reports that show that arsenic treatment of multiple myeloma is adversely associated with neutropenia, anemia and thrombocytopenia in spite of ascorbic acid co-administration [37]. This could be attributed to the multiple pathways by which arsenic affects blood Hb levels with strong/potential cumulative effects, a situation that makes the supplementation ineffective in restoring the normal or near-normal levels.

The reduction in erythrocyte count is accompanied by a marked echinocytic response in experimental animals treated with arsenic. The erythrocyte morphological

response to arsenic exposure in rats can, in part, be explained on the basis of metabolic impairment associated with arsenic toxicity. It is well established that arsenic exposure favors an oxidative milieu, due to increased ROS production and decreased GSH [6, 38] along with ATP depletion [6], both of which are known to produce poikilocytosis. Arsenic-induced oxidative stress results in oxidation of membrane lipid and protein components and activation of caspase-3 [38, 39]. Simultaneously, ATP depletion results in spectrin dephosphorylation, as well as ATP-dependent phospholipid transportation resulting in cytoskeletal and membrane phospholipid rearrangement [27, 38]. These membrane alterations were known to contact the inner leaflet associated with outer leaflet expansion of bilayer favoring a speculated echinocytic transformation [40]. This morphological response to arsenic toxicity is significantly prevented by VC+VE supplementation, which is in agreement with similar studies in other metal toxicity models, including cadmium, copper, zinc, mercury and lead [41, 42].

It is well documented that arsenic treatment alters the ROS levels, and the assessment of plasma TAS is in agreement with this. TOS also contributes significantly in this regard. Decrease in plasma TAS associated with increase in plasma TOS is indicative of a state of oxidative stress due to arsenic toxicity. These changes, in turn, elevate the OSI, further supporting the state of redox imbalance due to arsenic toxicity. These alterations were merely reversed in the vitamin-supplemented group, indicating protection against arsenic-induced redox imbalance.

With the exposure of inorganic arsenic in blood, the liver is the primary organ involved in biomethylation and hence consequent ROS generation leading to impairment of normal functions. It is evident from the literature that damages in liver tissues occur due to necrosis or apoptosis, leading to related histological manifestations [13, 43]. The serum lipid profile, which is considered to be an indicator of the functional status of the liver, showed significant variation in the arsenic-treated group. TC along with LDL and TG was increased with a decrease of serum HDL value, which is in agreement with earlier reports [44]. As per the literature, this hyperlipidemic condition imposed by arsenic is an outcome of oxidative stress [45], which, in turn, plays a crucial role in the development of hepatic disorders. The antioxidant vitamins effectively protect such oxidative imbalance in hepatic tissues as evidenced by the observed values of lipid profile, which were close to normal. In the present study, significant decrease of serum total protein was observed upon arsenic exposure, which is in agreement with an earlier report [36]. Such decrease in serum protein can be attributed to inhibition of protein synthesis as arsenic alters numerous sulfhydryl-containing proteins [13, 46]. It could also be attributed, in part, to the damaging effects of arsenic on hepatocytes [36], and histological observations support such a hypothesis as discussed later. The vitamins remain ineffective in restoring the total protein level, and further research is needed to explain this.

Lipid peroxidation, or molecular oxidation of lipid-based macromolecules [36], is enhanced by overproduction of ROS that increase the levels of lipid peroxidation end products like MDA and TBARS, which leads to degeneration of cellular macromolecules [47]. The results revealed higher TBARS level in the arsenic-treated group, suggesting increased lipid peroxidation [48]. VC+VE significantly prevented tissue lipid peroxidation in the supplemented group by lowering the TBARS level in hepatic tissue toward normal.

A non-enzymatic thiol-based antioxidative mechanism serves as the second line of internal defense against oxidative damages via glutathione, which oscillated between two forms; GSH (reduced) and GSSG (oxidized) in cells participate in many detoxification events. Arsenic has a high affinity for sulfhydryl groups, causing their depletion by actively binding to reduced glutathione [49], a phenomenon that was also observed in this study. Alternatively, GSH could also be used up in the process of arsenic methylation. Arsenic methylation in the liver involving GSH is essential for effective detoxification and ultimate urinary excretion [50]. In the present study, co-administration of VC+VE resulted in significant recovery of GSH in arsenic-intoxicated rats, indicating an effective protection by these vitamins.

Light microscopic study of treated liver tissues revealed degenerative changes in the hepatocytes and areas around the central vein. The sinusoidal spaces were expanded due to shrinkage of hepatocytes, leading to formation of pyknotic hepatocytes associated with increase in central vein diameter. The appearance of cytoplasmic blebbing along with binucleation of hepatocytes was observed, resulting in the accumulation of free nuclei. The changes were almost similar with the work carried out by Ferzand et al. [9]. However, moderate karyolysis (nuclear fading) and moderate karyorrhexis (nuclear fragmentation) of hepatocytes were also seen due to the toxicity. The supplementation yielded normal histological features, confirming protection from the effects of arsenic on hepatocytes by the combined application of VC+VE.

According to the literature and previous work from this laboratory, administration of both vitamins can protect cells from free radical-mediated damages by scavenging well before the radicals interact with the cellular compartments [19, 20]. Moreover, the improvement of

the activities of the glutathione system by the vitamins may have an additional role in eliminating the deleterious radicals [50]. These might be considered a plausible explanation for the protective approach of VC+VE on body systems, especially blood and the liver under arsenic insult. However, further in-depth studies are expected to substantiate these findings before the obtained effects are explored in managing human health and disease with reference to arsenic toxicity.

Acknowledgments: The authors gratefully acknowledge the financial support provided by the Faculty Research and Professional Development Fund from Presidency University.
Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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