Chemico-Biological Interactions 180 (2009) 20–30

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Modulatory role of *Emblica officinalis* fruit extract against arsenic induced oxidative stress in Swiss albino mice

Ambika Sharma, Mukesh Kumar Sharma, Madhu Kumar*

Cell and Molecular Biology Laboratory, Department of Zoology, University of Rajasthan, Jaipur 302004, India

ARTICLE INFO

Article history:
Received 19 November 2008
Received in revised form 22 January 2009
Accepted 23 January 2009
Available online 3 February 2009

Keywords:
Emblica officinalis
Arsenic
Serum transaminases
Glutathione-S-transferase
Histopathology
Oxidative stress

ABSTRACT

Arsenic, an important human toxin, is naturally occurring in groundwater and its accumulation in plants and animals have assumed a menacing proportion in a large part of the world, particularly Asia. Epidemiological studies have shown a strong association between chronic arsenic exposure and various adverse health effects, including cardiovascular diseases, neurological defects and cancer of lung, skin, bladder, liver and kidney. The protective role of the fruits of *Emblica officinalis* (500 mg/kg b.wt.) was studied in adult Swiss albino mice against arsenic induced hepatopathy. Arsenic treated group ($NaAsO_2$, 4 mg/kg b.wt.) had a significant increase in serum transaminases and lipid peroxidation (LPO) content in liver, whereas significant decrease was recorded in hepatic superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and serum alkaline phosphatase activity. Combined treatment of *Emblica* and arsenic (pre and post) declined the serum transaminases and LPO content in liver whereas significant increase was noticed in SOD, CAT, GST and serum alkaline phosphatase activities. Liver histopathology showed that *Emblica* fruit extract had reduced karyolysis, karyorrhexis, necrosis and cytoplasmic vacuolization induced by $NaAsO_2$ intoxication. Thus it can be concluded that pre- and post-supplementation of *E. officinalis* fruit extract significantly reduced arsenic induced oxidative stress in liver.

1. Introduction

Arsenicals are widespread in the environment as a result of natural or anthropogenic activities. Nearly 50 million people in Bangladesh and parts of West Bengal in India are drinking toxic level of arsenic daily knowingly or unknowingly [1,2]. Arsenic is the first metalloid to be identified as a human carcinogen. Exposure to arsenic contaminated drinking water causes several health problems [3], Blackfoot disease [4], hypertension [5], diabetes mellitus [6], disturbances in nervous system [7], cancers of liver, kidney, lung and bladder in humans [8]. Arsenic forms strong complexes with various sulfhydryl groups [9] and exert its toxicity by generating reactive oxygen species (ROS) such as superoxide, hydroxyl and peroxyl radicals during its metabolism in cells [10]. Arsenic exposure was shown to depress the antioxidant defense system [11] leading to oxidative damage to cellular macromolecules including DNA, proteins, lipids [12], wreak havoc in biological system by tissue damage, altering biochemical compounds and corroding cell membranes [13]. As the oxidative stress plays a central role in liver pathogenesis and progression, the use of antioxidants has been proposed as therapeutic agents as well as drug coadjuvants to counteract liver damages [14] and to protect the cellular machinery from peroxidative injury inflicted by ROS [15]. Our earlier reports showed the protective efficacies of *Mentha piperita* [11], *Spirulina fusiformis* [16,17] and *Oscimum sanctum* [18] against sodium arsenite and mercuric chloride induced oxidative stress.

Emblica officinalis Gaertn (Commonly known in India as Amla, Syn. *Phyllanthus emblica* L.; Family: Euphorbiaceae) is regarded as "one of the best rejuvenating herbs" in the Ayurveda: an Indian traditional medicinal science. Pozharitskaya et al. [19] demonstrated that *E. officinalis* extract contains several antioxidants such as emblicanin A and B, gallic acid, ellagic acid, ascorbic acid that possesses strong antioxidative activity. A recent study also shows that *Emblica* extract has potent antioxidant activity in term of free radical scavenging properties [20].

Amla is an important dietary source of minerals, amino acids, tannin and sugar. The fruit extract has many pharmacological activities for the treatment of a number of diseases [21] and is a constituent of many hepatoprotective formulations [22,23]. It protect against radiation [24]; possess antidiabetic activity [25]; inhibits clastogenicity of benzopyrene and cyclophosphamide [26]; gastroprotective [27]; cytoprotective and immunomodulatory [28]. New pharmacological activities viz. cytoprotective activity against chromium [29]; protects against oxidative stress in ischemic-reperfusion injury [30]; shows antivenom capacity [31]; ameliorates hyperthyroidism and hepatic lipid peroxidation [32];

* Corresponding author. Tel.: +91 141 2395524.
E-mail address: mamsjpr@yahoo.com (M. Kumar).

0009-2797/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.cbi.2009.01.012

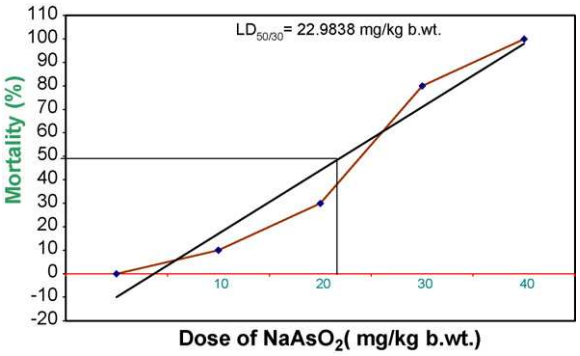


Fig. 1. Determination of $LD_{50/30}$ of sodium arsenite.

displays antiproliferative activity on MCF7 and MDA-MB-231 breast cancer cell lines [33]; shows antitussive activity [34] and induces apoptosis in Dalton's Lymphoma Ascites and CeHa cell lines [35] were also reported. It act as antibacterial [36]; anti-inflammatory agent [37] and modifies metal induced clastogenic effects [38].

In the present study, the objective is to elucidate the biochemical mechanism associated with hepatoprotective role of *E. officinalis* on arsenic-induced liver damages and oxidative stress in mice.

2. Materials and methods

2.1. Test system

Adult male Swiss albino mice (6–8 weeks old, weighing 25 ± 2 g) maintained in the animal house as inbred colony (Procured from IVRI, Izatnagar, India) under controlled conditions of temperature (25 ± 2 °C), relative humidity (50 ± 15%) and normal photoperiod (12 h light and 12 h dark). The animals were housed in standard polypropylene laboratory cages containing 5 cm deep layer of sawdust bedding. Mice were given standard mice feed (Hindustan Lever Ltd., India) and tap water ad libitum. Once in a fortnight tetracycline water was given as a preventive measure against infection. The ethical committee of Department of Zoology, University of Rajasthan, Jaipur (India) has approved to carry out the experimental protocol.

2.2. Test chemical

Arsenic in the form of $NaAsO_2$ (mol. wt. 129.9, CAS no. 7784-465) was obtained from standard commercial suppliers [Himedia, Mumbai, India Ltd., Batch no. 3-1621 RM 1847]. Chemical used for the present study was of analytical grade.

2.2.1. Optimum dose selection of arsenic

Arsenic ($NaAsO_2$) was administered at various dose levels, i.e. 2.5, 5, 10, 20, 40 mg/kg body weight (b.wt.) intraperitoneally (i.p.) in 0.9% NaCl. The animals were observed for 30 days for any sign of sickness and mortality and the acute toxicity was observed within 24 h. The $LD_{50/30}$ of $NaAsO_2$ was 22.98 mg/kg b.wt. (Fig. 1). On the basis of mortality data, the dose 4.0 mg/kg b.wt. (one tenth of lethal dose) was selected for the investigation. The dose selected was administered i.p. at once.

2.3. Plant material

Fresh fruits of *E. officinalis* were collected from the market at the harvest time. The plant was identified and a voucher specimen (No. RUBL 20131) was stored in the herbarium of Department of Botany, University of Rajasthan. The fruits were sliced, dried in shade, powdered and extraction was carried out with DDW (double distilled water) in soxhlet apparatus for 36 h. The extract was filtered and then vacuum evaporated to get powdered form.

2.3.1. E. officinalis drug tolerance study and optimum dose selection

The animals were administered *Emblica* fruit extract dissolved in DDW orally up to 30 days (100, 300, 500, 700 and 900 mg/kg. b.wt.) and LPO and GSH contents were measured in the liver. The optimum dose selection of *Emblica* fruit extract was done on the basis of maximum GSH and minimum LPO content. Among the doses, 500 mg/kg. b.wt. (or 0.5 ml/kg. b.wt.) was selected for the study (Fig. 2(A and B)).

2.3.2. Determination of radical scavenging activity of E. officinalis using DPPH* assay

The radical scavenger activity of the aqueous *Emblica* fruit extract was measured spectrophotometrically using DPPH (1,1-diphenyl-2-picryl hydrayzyl) radical [39]. Aqueous fruit extract (0.1 ml) of different concentrations (2, 4, 8, 16 and 32 μg/ml) were added to 3 ml of 0.001 M DPPH* solution in methanol. The solution was shaken and incubated at 37 °C for 30 min in the dark. The decrease in absorbance of DPPH* was measured against a blank at 517 nm. Percent (%) inhibition was calculated by comparing the absorbance values with and without extract as:

$$\%\text{Inhibition} = \frac{\text{Ao} - \text{Ae}}{\text{Ao}} \times 100$$

Ao = Absorbance without extract. Ae = Absorbance with extract.

In the present investigation, 50% Inhibitory concentration (IC50) of *Emblica* fruit extract was found to be 8.43 μg/ml and 97.07% as DPPH* percent inhibition activity was also observed (Fig. 2(C)). Thus, *E. officinalis* possess significant radical scavenging activity.

2.4. Experimental design

Mice selected from inbred colony were divided into 4 groups.

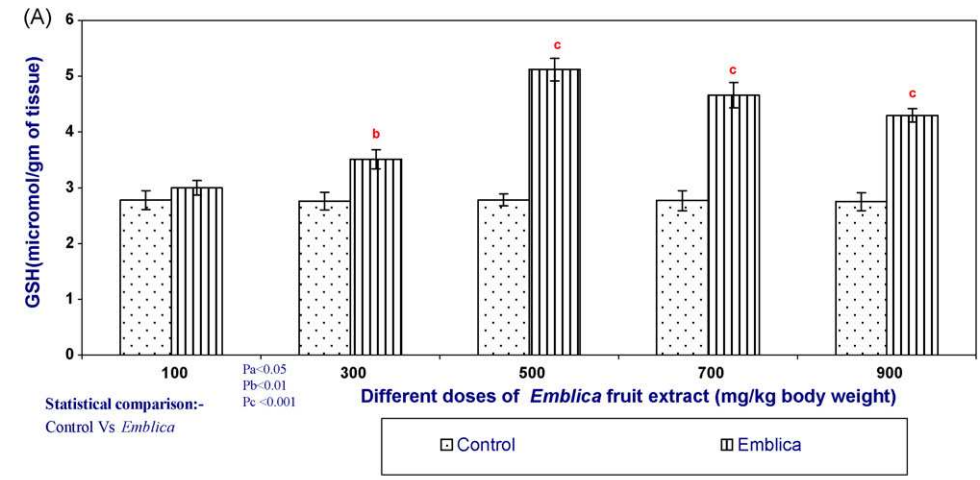
Groups	Number of animals	Treatment
I Control	6	Only vehicle DDW for 30 consecutive days
II $NaAsO_2$ treatment	6	Arsenic at 4 mg/kg b.wt. in 0.9%NaCl i.p. at once only
III *Emblica officinalis* treatment	6	*Emblica* fruit extract 500 mg/kg b.wt. orally in DDW for 30 consecutive days
IV *Emblica* + $NaAsO_2$ + *Emblica* (Combination)	6	*Emblica* fruit extract (orally 500 mg/kg b.wt.) was administered 10 days before $NaAsO_2$ (4 mg/kg b.wt.) and continued up to 30 days after arsenic treatment

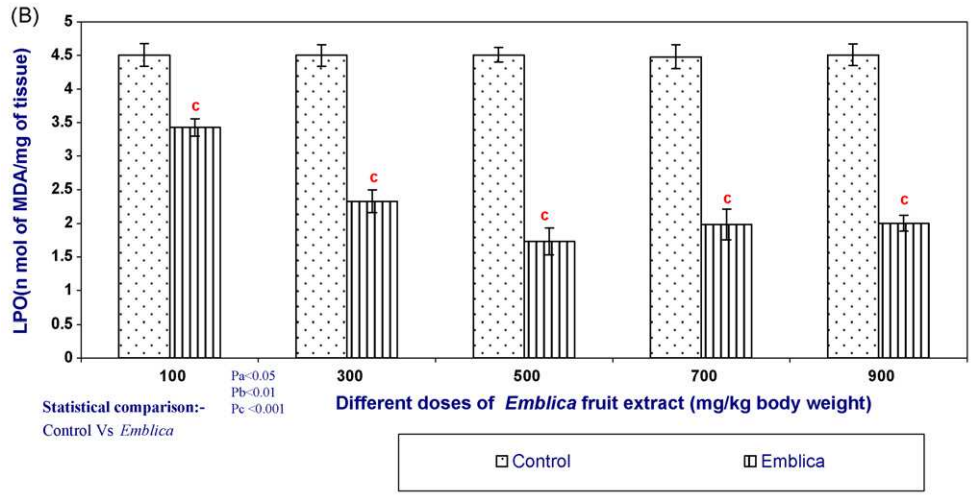
The animals from all the groups were sacrificed on 1, 3, 7, 15 and 30 days. Liver were removed, rinsed in cold saline, blotted, weighed, processed for histological and various biochemical assays. Fresh unhaemolysed serum was used for transaminases and alkaline phosphatase (ALP) activities.

2.5. Antioxidants assays

2.5.1. Serum glutamate oxaloacetate transaminase (SGOT) (2.6.1.1)

The SGOT activity in the serum was estimated by Reitman and Frankel [40] method. The oxaloacetate formed in the reaction is coupled with 2,4-dinitrophenyl hydrazine (DNPH) to give the corresponding hydrazone, which give brown colour in alkaline medium and this is measured colorimetrically at 505 nm. A standard curve obtained using different amounts of pyruvate and enzyme activity was expressed as unit/ml.





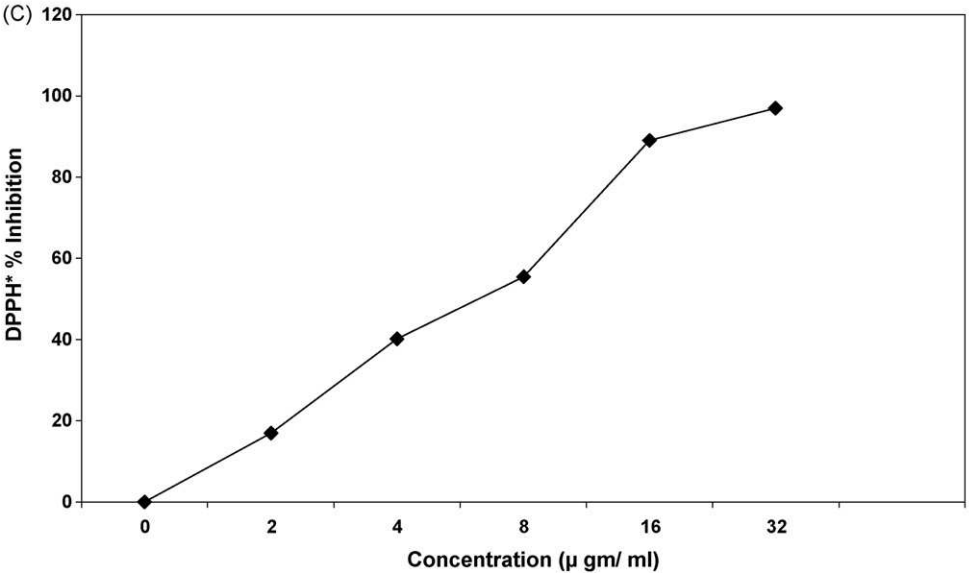


Fig. 2. (A) Variation in liver reduced in glutathione (GSH) content of different doses of *Emblica* fruit extract. (B) Variation in liver lipid peroxidation (LPO) level of doses of *Emblica* fruit extract. (C) *Emblica officinalis* fruit extract DPPH* radical scavenging assay.

2.5.2. Serum glutamate pyruvate transaminase (SGPT) (2.6.1.2)

SGPT activity was also estimated by the method of Reitman and Frankel [40] using DNPH as colour reagent. Pyruvate formed in the reaction is coupled with 2,4-DNPH to give the corresponding hydrazone, which give brown colour in alkaline medium and this is measured colorimetrically at 505 nm. A standard curve was obtained using different amounts of pyruvate and enzyme activity was expressed as unit/ml.

2.5.3. Serum alkaline phosphatase

Serum alkaline phosphatase activity was measured by the method of Kind and King [41] using commercially accessible kits from Span diagnostic limited, Surat, India. Alkaline phosphatase from serum converts phenyl phosphate to inorganic phosphate and phenol. Phenol reacts in alkaline medium with 4 amino antipyrine in the presence of an oxidizing agent, potassium ferricyanide and forms an orange-red coloured complex which can be measured

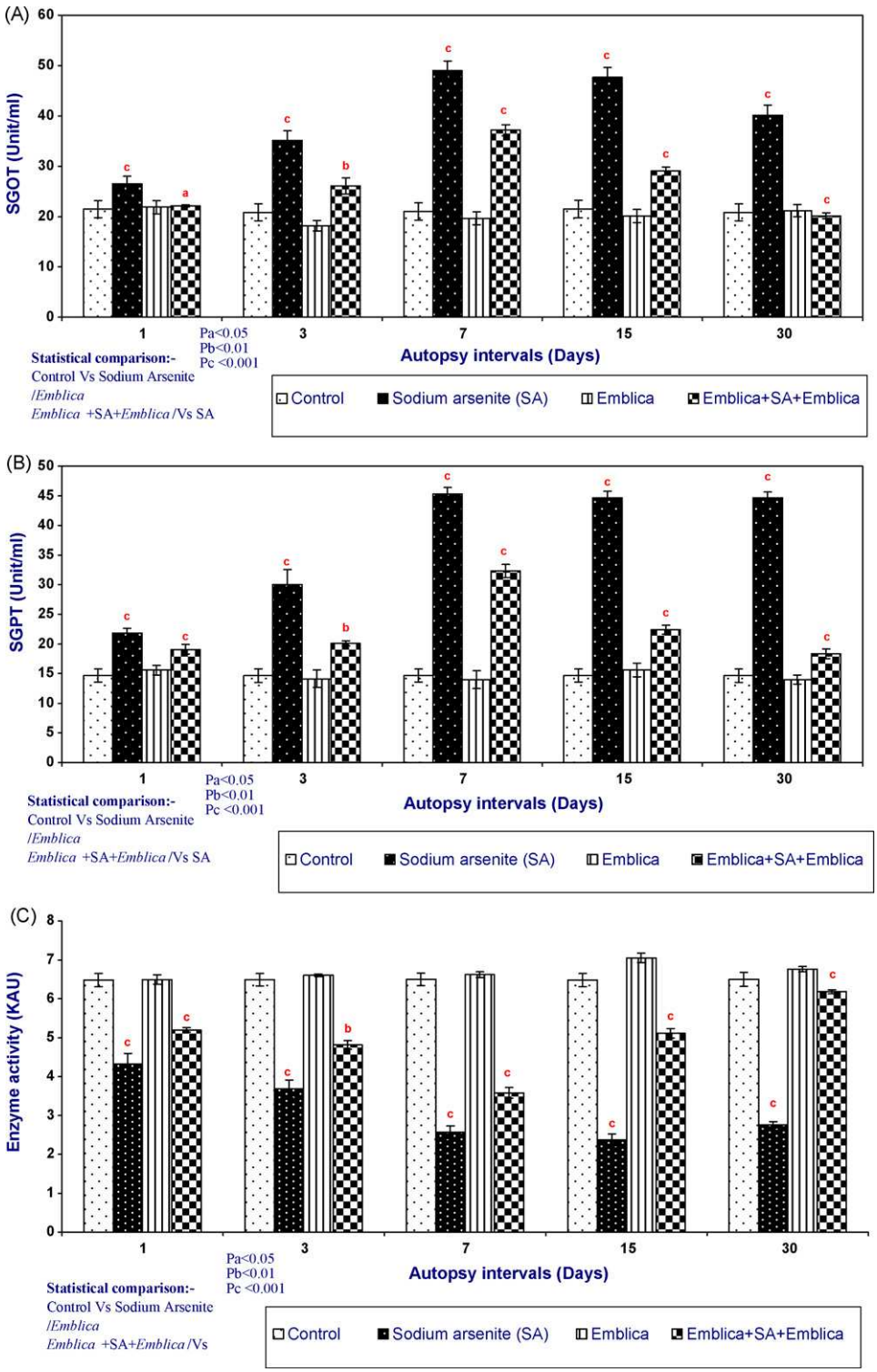


Fig. 3. (A) Variation in Serum glutamate oxaloacetate transaminase (SGOT) activity in different experimental groups. (B) Variation in Serum glutamate pyruvate transaminase (SGPT) activity in different experimental groups. (C) Variation in Serum alkaline phosphatase activity (KAU) in different experimental groups. (D) Variation in liver lipid peroxidation (LPO) content in different experimental groups. (E) Variation in liver superoxide dismutase (SOD) activity in different experimental groups. (F) Variation in liver catalase (CAT) activity in different experimental groups. (G) Variation in liver glutathione-S-transferase (GST) activity in different experimental groups.

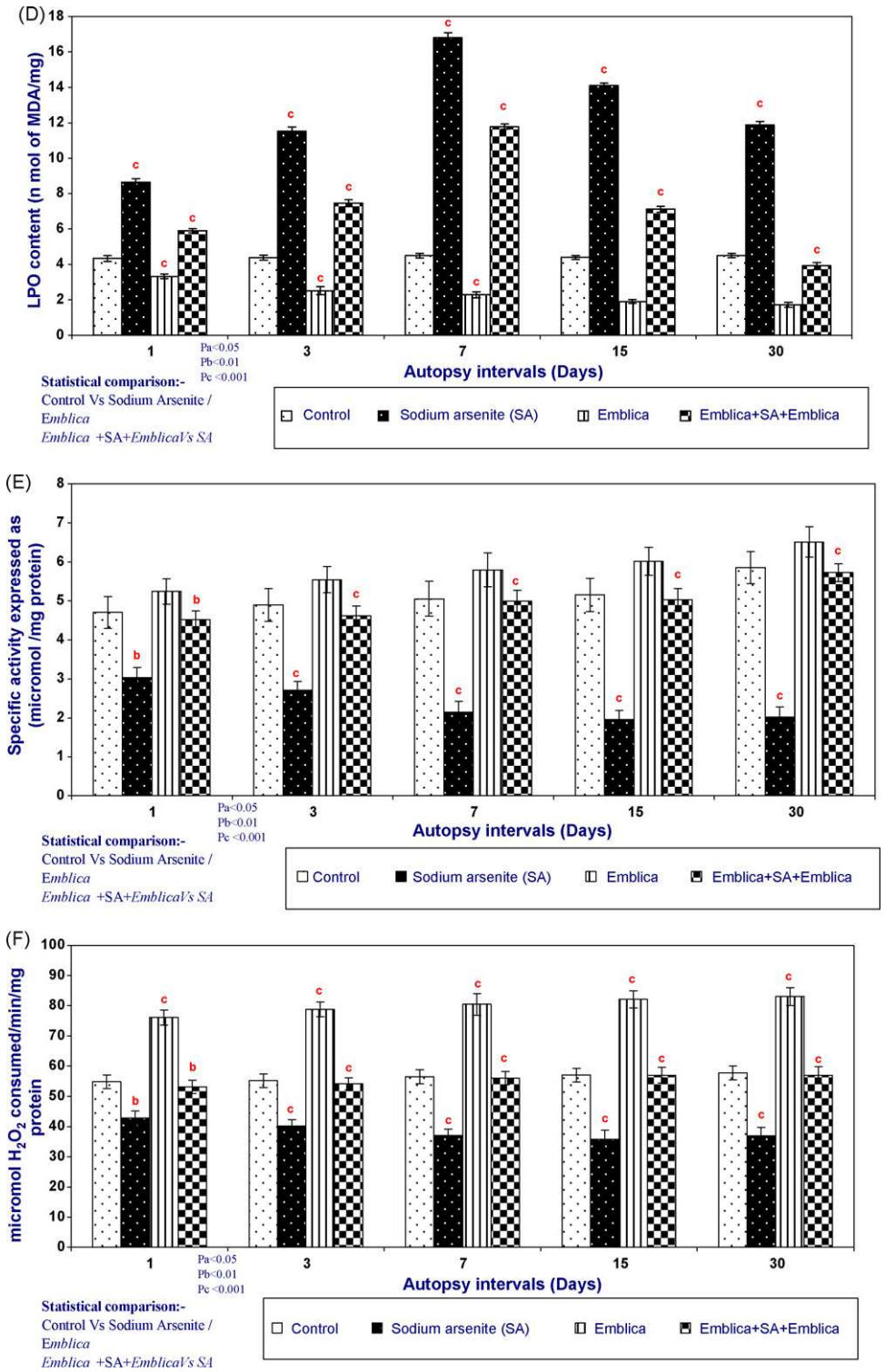


Fig. 3. (*Continued*)

colorimetrically at 510 nm. The colour intensity is proportional to the enzyme activity was expressed as King Armstrong Unit (KAU).

2.5.4. Lipid peroxidation (LPO)

Lipid peroxidation level in the liver was measured by the method of Ohkawa et al. [42] as thiobarbituric acid reactive substances (TBARS). The concentration of TBARS was expressed as *n* moles of malondialdehyde per mg of tissue using 1,1,3,3-tetramethoxypropane (TMP) as standard. A standard curve was prepared by using tetramethoxy propane (mol. wt. 164.20 purchased from Lancaster, England). 1–12 nmol of TMP was taken in corresponding no. of test tubes with all the reagents (minus sample) were added. Standard curve was plotted by taking absorbance against TMP concentration on abscissa. The absorbance was read at 532 nm using UV–vis systronic spectrophotometer.

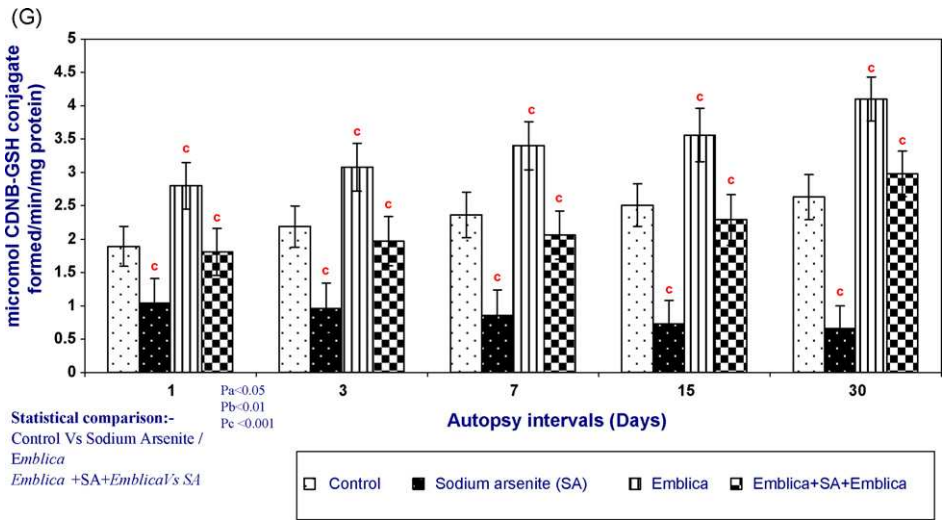


Fig. 3. (*Continued*).

2.5.5. Superoxide dismutase (SOD) (EC 1.15.1.1)

Hepatic superoxide dismutase was assayed by method of Marklund and Marklund [43], which involves inhibition of pyrogallol auto-oxidation in the presence of EDTA at pH 8. A single unit of enzyme activity is defined as the quantity of superoxide dismutase required to produce 50% inhibition of auto-oxidation of pyrogallol. The absorbance was read at 420 nm with a UV–vis Systronics spectrophotometer.

2.5.6. Catalase (CAT) (EC 1.11.1.6)

Catalase was estimated in the liver homogenate in a UV–vis spectrophotometer as describe by Aebi [44]. The reaction mixture (1 ml) contained 0.02 ml of suitably diluted cytosol in phosphate buffer (50 mM, pH 7.0) and 0.1 ml of 30 mM H_2O_2 in phosphate buffer. In the UV range H_2O_2 shows an increase in absorption with decreasing wavelength. The difference in absorbance at 240 nm per unit time is the specific activity of catalase, expressed in μ mol of H_2O_2 consumed/min/mg protein using 71 as molar extinction co-efficient.

2.5.7. Glutathione-S-transferase (GST) (EC 2.5.1.18)

The cytosolic glutathione-S-transferase activity was determined spectrophotometrically at 37 °C by method of Habig et al. [45]. The reaction mixture (1 ml) contained 0.334 ml of 100 mM phosphate buffer (pH 6.5), 0.033 ml of 30 mM CDNB and 0.033 ml of reduced glutathione. After pre-incubating the reaction mixture for 2 min the reaction was started by adding 0.01 ml of diluted cytosol and the absorbance was followed for 3 min at 340 nm. The specific activity of GST was expressed as μ mol of GSH-CDNB conjugate formed/min/mg protein using extinction co-efficient of 9.6 mM^{-1} cm^{-1}.

2.6. Histological examination

A portion of liver from autopsied animals was processed by routine histology procedure. Excised liver (marked according to the groups mentioned) was fixed in Bouin's fixatives for 24–48 h and then embedded in paraffin. 5 μm thick sections were prepared by using microtome and these sections were stained with hematoxylin and eosin [H.E.]. For histological alterations these slides were observed under light microscope.

2.7. Statistical analysis

The data for various biochemical parameters were expressed as mean + S.E. The values at each autopsy interval for each experiment was compared with control, i.e. Control (Group I) vs Arsenic (Group II)/*Emblica* (Group III); Arsenic (Group II) vs *Emblica* + $NaAsO_2$ + *Emblica* (Group IV).The statistical significance of variance was analyzed by Student's '*t*' test [46]. Significance level was set at $P < 0.05$, $P < 0.01$ and $P < 0.001$.

3. Results

3.1. Serum glutamate oxaloacetate transaminase (SGOT)

A highly significant ($P < 0.001$) elevation in serum glutamate oxaloacetate transaminase activity was observed in arsenic intoxicated mice, where *Emblica* treatment did not show any significant alteration. However, the combined treatment of *Emblica* with arsenic resulted in gradual recovery in SGOT activity. A highly significant decline in SGOT activity was observed in respect to arsenic treated animals (Fig. 3(A)).

3.2. Serum glutamate pyruvate transaminase (SGPT)

A highly significant ($P < 0.001$) elevation was observed in SGPT activity in arsenic intoxicated mice with respect to control. However *Emblica* treated group did not show any significant alterations. Whereas pre- and post-treatment of *Emblica* with arsenic resulted in significant decline in SGPT activity with respect to arsenic intoxicated mice (Fig. 3(B)).

3.3. Serum alkaline phosphatase

Sodium arsenite intoxication caused a significant depletion ($P < 0.001$) in serum alkaline phosphatase activity with respect to control. *Emblica* alone showed a significant elevation in serum alkaline phosphatase activity. In combined treatment of *Emblica* with arsenic, serum alkaline phosphatase activity was significantly increased with respect to sodium arsenite intoxicated mice (Fig. 3(C)).

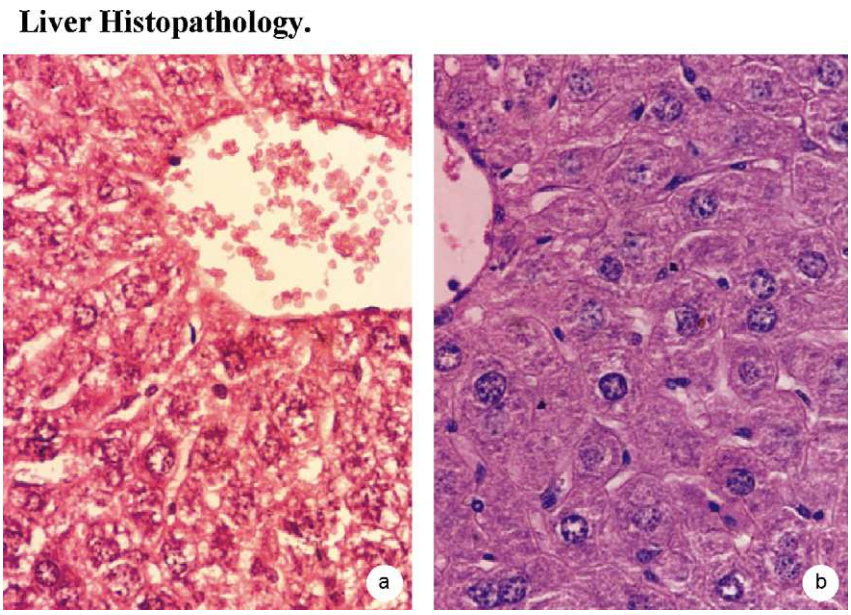


Fig. 4. [Day-1]: (a) $NaAsO_2$ group [Group-II]: hepatocytoplasmic vacuolization, nuclear changes karyorrhexis, occluded central vein. (b) Combination group [Group-IV]: maintained cytoplasmic granulation, enucleation at few places (H.E. 400×).

3.4. Lipid peroxidation (LPO)

A highly significant elevation in liver LPO level in arsenic intoxicated mice was found. Wherein *Emblica* alone treatment showed a significant decline. In combined treatment a highly significant depletion was recorded with respect to arsenic intoxicated mice (Fig. 3(D)).

3.5. Superoxide dismutase (SOD)

SOD catalyzes the conversion of superoxide anion into H_2O_2. $NaAsO_2$ intoxication caused a significant depletion ($P < 0.001$) in liver superoxide dismutase (SOD) activity with respect to control. *Emblica* alone showed a significant elevation in liver SOD activity. Whereas in combined treatment of *Emblica* with sodium arsenite SOD activity showed a highly significant elevation as compared to arsenic treated animals (Fig. 3(E)).

3.6. Catalase (CAT)

The primary role of catalase is to scavenge H_2O_2 that has been generated by free radical or by SOD in removal of superoxide anions. Sodium arsenite intoxication caused a significant depletion ($P < 0.001$) in liver CAT activity with respect to control. *Emblica* alone showed a significant elevation in liver CAT activity and in combined treatment of *Emblica* with arsenic a significant increase was observed with respect to sodium arsenite intoxicated mice (Fig. 3(F)).

3.7. Glutathione-S-transferase (GST)

GSTs are a multigene family of isozymes that catalyze the conjugation of cellular antioxidants to a variety of electrophilic compounds and thereby exert a critical role in cytoprotection against reactive oxygen species. Sodium arsenite administration

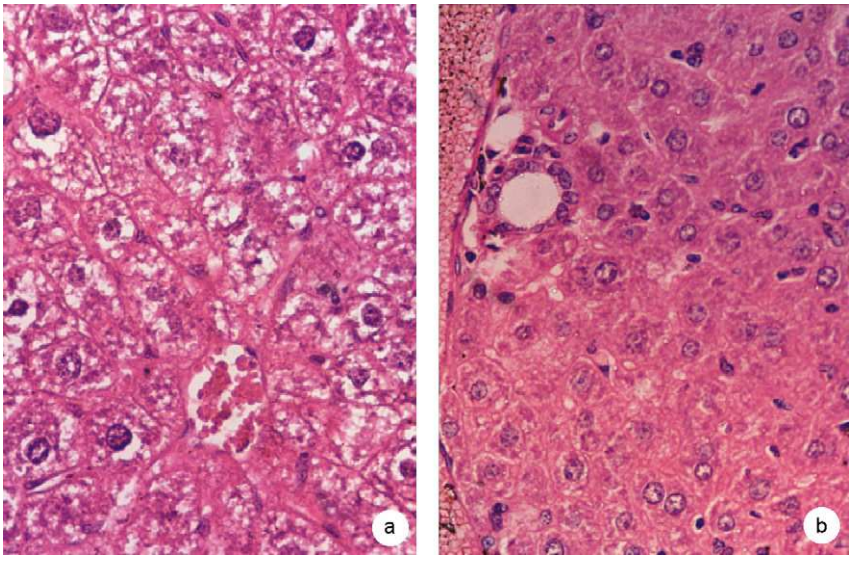


Fig. 5. [Day-3]: (a) $NaAsO_2$ group [Group-II]: more cytoplasmic vacuolization, occasional enucleation alongwith karyorrhexis. (b) Combination group [Group-IV]: maintained granular cytoplasm and normal hepatocytes (H.E. 400×).

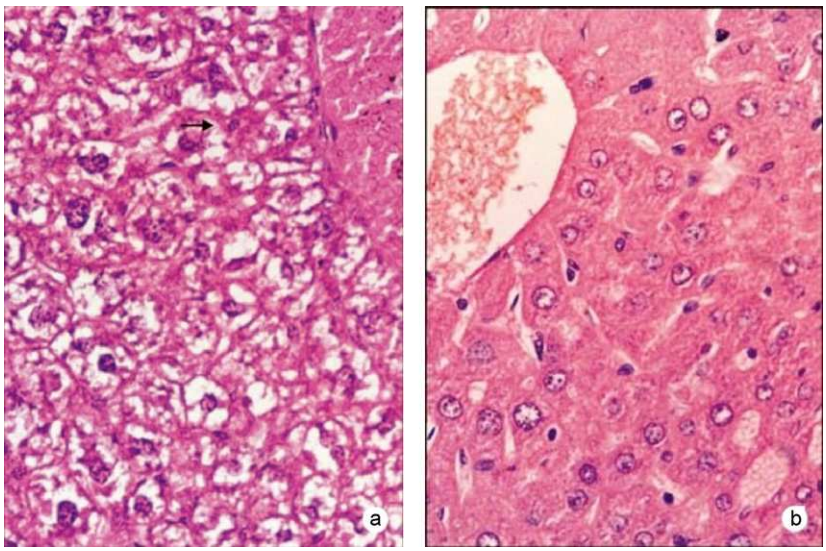


Fig. 6. [Day-7]: (a) $NaAsO_2$ group [Group-II]: karyolysis, karyorrhexis, centrilobular necrosis↑ and cytoplasmic vacuolization. (b) Combination group [Group-IV]: more or less normal hepatic architecture (H.E. 400×).

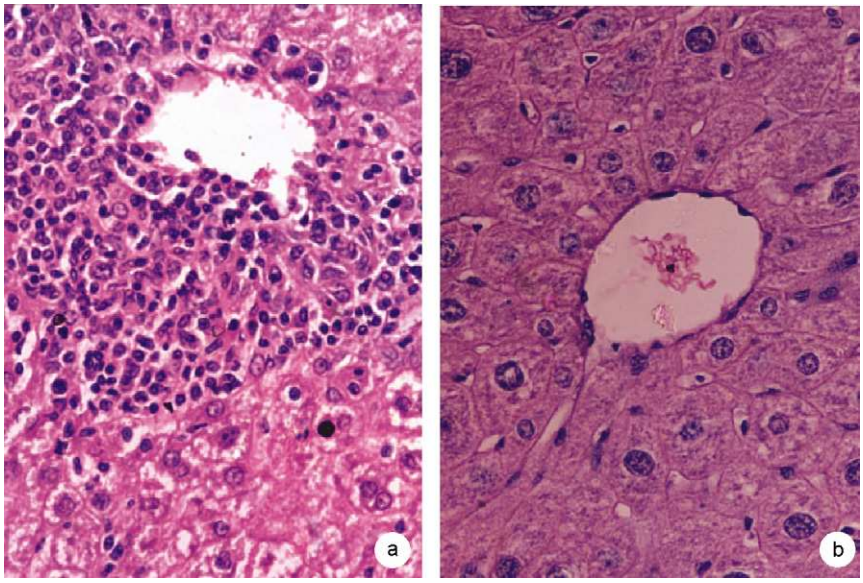


Fig. 7. [Day-15]: (a) $NaAsO_2$ group [Group-II]: extensive lymphocytic infilteration and pyknosis. (b) Combination group [Group-IV]: reparation with better developed hepatic parenchyma and pronounced sinusoidal spaces (H.E. 400×).

significantly ($P < 0.001$) depleted the level of GST in liver of Swiss albino mice with respect to control. *Emblica* alone treatment significantly increases liver GST level. Whereas in combination group GST activity was significantly increased with respect to sodium arsenite intoxicated mice (Fig. 3(G)).

3.8. Histopathology

In the present experimental design $NaAsO_2$ causes various pathological alterations such as karyolysis, karyorhexis, centrilobular necrosis and cytoplasmic vacuolization (Figs. 4–8(a)) in liver of Swiss albino mice, whereas the combination group (*Emblica* was administered before and after arsenic treatment) showed prominent recovery in the form of maintained hepatic histoarchitecture (Figs. 4–8(b)), such as reduced cytoplasmic vacuolization, karyolysis, karyorhexis, centrilobular necrosis and normal sinusoidal spaces (H.E. 400×).

4. Discussion

Our study revealed that *Emblica* fruit extract possessed strong antioxidant activity against arsenic intoxication.

Sodium arsenite is a well-known hepatotoxicant [11]. The present investigation revealed that arsenic intoxication caused significant increase in lipid peroxidation level, SGOT and SGPT activities alongwith the significant decrease in serum alkaline phosphatase, superoxide dismutase, catalase and glutathione-S-transferase activities in liver.

The main cause of the arsenic induced liver injury is the formation of free radicals and its metabolites. Arsenic binds with sulfhydryl groups of proteins and various enzymes and interferes with metabolism of essential antioxidant molecules responsible for metabolism and excretion of xenobiotics [47]. Arsenic compounds during their metabolism in cells generate reactive oxygen species. These ROS contribute to the pathogenesis of various acute and chronic liver diseases [48] and damages cellular macromolecules.

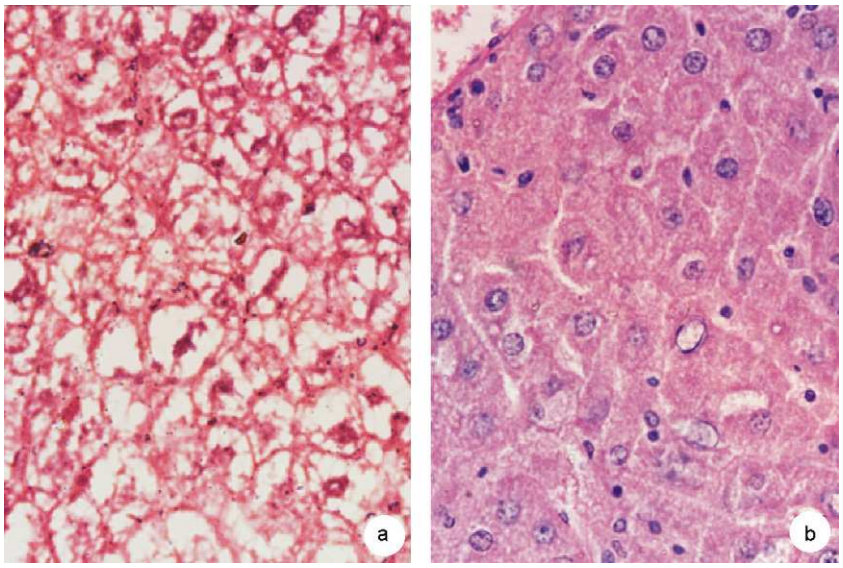


Fig. 8. [Day-30]: (a) $NaAsO_2$ group [Group-II]: necrosis in hepatocytes. (b) Combination group [Group-IV]: sign of reparation with normal hepatocytes (H.E. 400×).

Lipid peroxidation is widely accepted to be one of the principal causes of $NaAsO_2$ induced liver injury. Enhanced production of free radicals and inhibition of antioxidant enzymes have been suggested as possible mechanisms to explain arsenic induced oxidative damages [49,50]. ROS impairs cell membrane stability and causes cell death by lipid peroxidation [51]. An increase in free radicals causes overproduction of thiobarbituric acid-reactive substances (TBARS), the end product of the lipid peroxidation process which is commonly used as a marker of oxidative stress or a reliable method to assess the degree of peroxidative damage to cell membrane [52].

Free radical scavenging enzymes such as SOD, CAT, GPx and GST are the first line of defense against oxidative injury. Status of these antioxidant enzymes is appropriate indirect way to assess the prooxidant–antioxidant status in tissues. We have observed a decrease in the levels of antioxidants such as SOD, CAT, GST in liver of arsenic treated mice. The inhibition of antioxidant system may cause the accumulation of H_2O_2 or products of its decomposition. SOD and CAT are the two basic subcellular defense of antioxidant system that counteracts free radicals produced during xenobiotic exposure [53]. Arsenic has also been known to decrease the detoxification system produced by GST [54]. Thus, arsenic or its metabolic products might specifically target GST isoenzymes and the reduction in enzyme activity or expression may contribute to arsenic hepatotoxicity. Significantly decreased SOD and CAT with increased LPO were earlier reported in arsenic intoxicated rats at the end of exposure period [55–57,50].

The liver is a major site for the biotransformation, accumulation and excretion of exogenous chemicals. Hepatocytes may thus be expected to be the primary targets of toxic substances, providing an excellent biomarker of pollution [58]. The present investigation suggests that $NaAsO_2$ intoxication produces various pathological alterations in liver of Swiss albino mice such as pyknosis, karyolysis and karyorrhexis in the nuclei of hepatocytes. Routine histological studies on liver documented arsenic-induced changes characterized by dilated sinusoids, formation of intracellular edema, megalocytosis, vacuolation and appearance of hepatic cells with distorted nuclei [59]. Morphologic changes including inflammation, foci of apoptosis and necrosis, and hepatocellular degeneration were also observed in arsenic-treated mice [60].

Serum glutamate oxaloacetae tranaminase (SGOT or AST) and serum glutamate pyruvate transaminase (SGPT or ALT) are the reliable makers for liver function. Increased levels of these serum enzymes indicate the increased permeability and damage and/or necrosis of hepatocytes [61]. These membrane bound enzymes are released unequally in to bloodstream depending on the pathological phenomenon [62]. In our study, we have found that arsenic intoxication caused a significant increase in the activities of SGOT, SGPT which could be due to severe damage of membrane of hepatocytes. If the liver is injured, the liver cells spill the enzymes into blood, raising the enzyme levels in the blood and cause the liver damages.

The present study suggests that $NaAsO_2$ treatment significantly results in a decline of the serum alkaline phosphatase activity in mice which plays important role in cell membrane permeability. Damaged cell membrane leads to imbalance between synthesis and degradation of enzyme protein [63], thus lowering the enzyme activity. The decreased activity of alkaline phosphatase indicates disturbance in the structure of cell organelles and integrity of membrane transport system [64–66]. Arsenic exposed mice [67] and pigs [68] exhibited liver injury as reflected by reduced alkaline phosphatase activity. The results of functional tests suggest that arsenic leads to serious changes in liver. The increased formation of lipid peroxides and associated reactive oxygen species leads to damage in membrane integrity and other pathological changes in liver.

Free radical-induced oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficiency of natural antioxidant defenses. Antioxidant protects the cellular machinery from peroxidative injury inflicted by ROS [69]. The efficacy of any protective drug is essentially dependent on its capacity of either reducing the harmful effects or in maintaining the normal physiology of cells and tissues, which have been damaged by toxins. *Emblica* is a rich dietary source of vitamin C, minerals and amino acids and also contains a wide variety of phenolic compounds. The active constituents such as Phyllembic acid, Ellagic acid, Gallic acid, Tannoids [70] have been reported as powerful antioxidants. These are all responsible for the reversal of antioxidant levels in tissues of arsenic intoxicated mice. Polyphenolic compounds show powerful antioxidant properties, i.e. free radical scavenging activity [70]. It also contains hydrolysable tannins emblicanin A and B [71] punigluconin and pedunclagin. The prophylactic, curative and restorative effects of the fruits were thought to be mainly due to these factors.

Administration of *Emblica* significantly decreased the level of lipid peroxidation when compared with arsenic treated mice. Non-enzymatic antioxidant such as vitamin C, the richest amount in *Emblica*, plays an excellent role in protecting the cell from lipid peroxidation. Our results obviously indicate that *Emblica* fruit extract has radical scavenging activity, inhibit the lipid peroxidation damage and shows hepatoprotective potential against arsenic toxicity.

The antioxidants found in *Emblica* fruit extract maintains the endogenous antioxidants, thus reduces oxidative stress and alleviate the pathological changes caused by arsenic in liver.

E. officinalis is a constituent of various liver tonics used against acute viral hepatitis and other liver disorders [22,72]. Antioxidants such as ellagic acid [73] have been reported to protect liver injury and fibrosis induced by hepatotoxins. *E. officinalis* fruit extract neutralize the oxidizing potentials of reactive oxygen species generated thereby maintaining cell membrane integrity and viability.

E. officinalis extract shows efficacy as a good inhibitor against arsenic-induced oxidative stress as observed by decrease in lipid peroxidation and increase in SOD, CAT and GST activity and also maintained the structural integrity of the hepatocellular architecture. The protective action of fruit extract of the *E. officinalis* may be due to the presence of the known anti inflammatory flavanoid, luteolin (5,7,3%,4%-tetrahydroxyflavone) and its 3-O-glycoside derivatives, which provides maximum conjugation with free radical species, thus reducing the number of free radicals available and the extent of cellular damages.

In conclusion, the enhanced levels of antioxidant enzymes and reduced amount of lipid peroxides and serum transaminases are suggested to be the major mechanisms of *Emblica* fruit extract in preventing the liver damages induced by arsenic.

Conflict of interest statement

None.

Acknowledgement

Authors are gratefully acknowledge to the CSIR-New Delhi for providing financial assistance to Ambika Sharma as Senior research fellow, Letter No.: 09/149/(0489)/2008/EMR-I.

References

[1] F. McLellan, Arsenic contamination affects millions in Bangladesh, Lancet 359 (2002) 1127.
[2] R. MacDonald, Providing clean water: lessons from Bangladesh, Br. Med. J. 322 (2001) 626–627.
[3] D.N. Guha Mazumder, Chronic arsenic toxicity & human health, Indian J. Med. Res. 128 (2008) 436–447.
[4] C.H. Tseng, C.K. Chong, C.J. Chen, T.Y. Tai, Dose–response relationship between peripheral vascular disease and ingested inorganic arsenic among residents in Blackfoot disease endemic villages in Taiwan, Atherosclerosis 120 (1996) 125–133.
[5] M.S. Lai, Y.M. Hsueh, C.H. Chen, M.P. Shyu, S.Y. Chen, T.L. Kuo, M.M. Wu, T.Y. Tai, Ingested inorganic arsenic and prevalence of diabetes mellitus, Am. J. Epidemiol. 139 (1994) 484–492.
[6] C.J. Chen, Y.M. Hsueh, M.S. Lai, M.P. Shyu, S.Y. Chen, M.M. Wu, T.L. Kuo, T.Y. Tai, Increased prevalence of hypertension and long term arsenic exposure, Hypertension 25 (1995) 53–60.
[7] E. García-Chávez, I. Jiménez, B. Segura, L.M. Del Razo, Lipid oxidative damage and distribution of inorganic arsenic and its metabolites in the rat nervous system after arsenite exposure: influence of alpha tocopherol supplementation, Neurotoxicology 27 (2006) 1024–1031.
[8] T.K. Hei, M. Filipic, Role of oxidative damage in the genotoxicity of arsenic, Free Radic. Biol. Med. 37 (2004) 574–581.
[9] H.V. Aposhian, Biochemical toxicology of arsenic, Rev. Biochem. Toxicol. 10 (1989) 265–299.
[10] J. Liu, M. Kaduska, Y. Liu, W. Qu, R.P. Mason, M.P. Walker, Acute arsenic induced free radical production and oxidative stress related gene expression in mice, Toxicologists 54 (2000) 280–281.
[11] A. Sharma, M.K. Sharma, M. Kumar, Protective effect of *Mentha piperita* against arsenic-induced toxicity in liver of Swiss albino mice, Basic Clin. Pharmacol. Toxicol. 100 (2007) 249–257.
[12] H. Shi, X. Shi, K.J. Liu, Oxidative mechanism of arsenic toxicity and carcinogenesis, Mol. Cell Biochem. (2004) 25567–25578 (Review).
[13] H. Wiseman, B. Halliwell, Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer, Biochem. J. 313 (1996) 17–29.
[14] P. Vitaglione, F. Morisco, N. Caporaso, V. Fogliano, Dietary antioxidant compounds and liver health, Crit. Rev. Food Sci. Nutr. 44 (2004) 575–586.
[15] B. Halliwell, O.I. Arouma, DNA and Free Radicals, Ellis Horwood Ltd., Chichester, UK, 1993.
[16] M.K. Sharma, A. Sharma, A. Kumar, M. Kumar, *Spirulina fusiformis* provides protection against mercuric chloride induced oxidative stress in Swiss albino mice, Food Chem. Toxicol. 45 (2007) 2412–2419.
[17] M. Kumar, M.K. Sharma, A. Kumar, *Spirulina fusiformis*: a food supplement against mercury induced hepatic toxicity, J. Health Sci. 51 (2005) 424–430.
[18] M.K. Sharma, M. Kumar, A. Kumar, *Ocimum sanctum* aqueous leaf extract provides protection against mercury induced toxicity in Swiss albino mice, Indian J. Exp. Biol. 40 (2002) 1079–1082.
[19] O.N. Pozharitskaya, S.A. Ivanova, A.N. Shikov, V.G. Makarov, Separation and evaluation of free radical-scavenging activity of phenol components of *Emblica officinalis* extract by using an HPTLC-DPPH* method, J. Sep. Sci. 30 (2007) 1250–1254.
[20] P.K. Jain, V. Ravichandran, S. Sharma, R.K. Agarwal, The antioxidant activity of some medicinal plants, Turk. J. Biol. 32 (2008) 197–202.
[21] R.N. Chopra, I.C. Handa, I.D. Kapur, Indigenous Drugs of India, second ed., U.N. Dhar and Sons, Calcutta, 1958.
[22] D.S. Antarkar, B.V. Ashok, J.C. Doshi, A.V. Athavale, K.S. Vinchoo, M.R. Natekar, P.S. Thathed, V. Ramesh, N. Kale, Double-bind clinical trial of Arogyawardhani—an ayurvedic drug in acute viral hepatitis, Indian J. Med. Res. 72 (1980) 588–593.
[23] S. De, B. Ravishankar, G.C. Bhavsar, Plants with hepatoprotective activity—a review, Indian Drugs 30 (1993) 355–363.
[24] P. Scartezzini, E. Speroni, Review on some plants of Indian traditional medicine with antioxidant activity, J. Ethnopharmacol. 71 (2000) 23–43.
[25] M.C. Sabu, R. Kuttan, Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property, J. Ethnopharmacol. 81 (2002) 155–160.
[26] N. Sharma, P. Trikha, M. Athar, S. Raisuddin, Inhibitory effect of *Emblica officinals* on the in vivo clastogenicity of benzo pyrene and cyclophosphamide in mice, Hum. Exp. Toxicol. 19 (2000) 377–384.
[27] A.J. Al-Rehaily, T.A. Al-Howiriny, M.O. Al-Sohaibani, S. Rafatullah, Gastroprotective effects of 'Amla' *Emblica officinalis* on in vivo test models in rats, Phytomedicine 9 (2002) 515–522.
[28] M. Sai Ram, D. Neetu, B. Yogesh, B. Anju, P. Dipti, T. Pauline, S.K. Sharma, S.K. Sarada, G. Ilavazhagan, D. Kumar, W. Selvamurthy, Cyto-protective and immunomodulating properties of Amla (*Emblica officinalis*) on lymphocytes: an in-vitro study, J. Ethnopharmacol. 81 (2002) 5–10.
[29] M. Sai Ram, D. Neetu, P. Deepti, M. Vandana, G. Ilavazhagan, D. Kumar, W. Selvamurthy, Cytoprotective activity of Amla (*Emblica officinalis*) against chromium (VI) induced oxidative injury in murine macrophages, Phytother. Res. 17 (2003) 430–433.
[30] S. Rajak, S.K. Banerjee, S. Sood, A.K. Dinda, Y.K. Gupta, S.K. Gupta, S.K. Maulik, *Emblica officinalis* causes myocardial adaptation and protects against oxidative stress in ischemic-reperfusion injury in rats, Phytother. Res. 18 (2004) 54–60.
[31] M.I. Alam, A. Gomes, Snake venom neutralization by Indian medicinal plants (*Vitex negundo* and *Emblica officinalis*) root extracts, J. Ethnopharmacol. 86 (2003) 75–80.
[32] S. Panda, A. Kar, Fruit extract of *Emblica officinalis* ameliorates hyperthyroidism and hepatic lipid peroxidation in mice, Pharmazie 58 (2003) 753–755.
[33] E. Lambertini, R. Piva, M.T. Khan, I. Lampronti, N. Bianchi, M. Borgatti, R. Gambari, Effects of extracts from Bangladeshi medicinal plants on in vitro proliferation of human breast cancer cell lines and expression of estrogen receptor alpha gene, Int. J. Oncol. 24 (2003) 419–423.
[34] G. Nosal'ova, J. Mokry, K.M. Hassan, Antitussive activity of the fruit extract of *Emblica officinalis* Gaertn. (Euphorbiaceae), Phytomedicine 10 (2003) 583–589.
[35] N.V. Rajeshkumar, M.R. Pillai, R. Kuttan, Induction of apoptosis in mouse and human carcinoma cell lines by *Emblica officinalis* polyphenols and its effect on chemical carcinogenesis, J. Exp. Clin. Cancer Res. 22 (2003) 201–212.
[36] S.H. Godbole, G.S. Pendse, Antibacterial property of some plants, Indian J. Pharm. 22 (1960) 39–42.
[37] M.Z. Asmawi, H. Kankaanranta, E. Moilanen, H. Vapaatalo, Anti-inflammatory activities of *Emblica officinalis* Gaertn. leaf extracts, J. Pharm. Pharmacol. 45 (1993) 581–584.
[38] H. Dhir, R.K. Roy, A. Sharma, G. Talukdar, Modification of clastogenicity of lead and aluminium in mouse bone marrow cells by dietary ingestion of *Phyllanthus emblica* fruit extract, Mutat. Res. 241 (1990) 305–312.
[39] M. Gulluce, F. Sahin, M. Sokmen, H. Ozer, D. Daferera, A. Sokmen, M. Polissiou, A. Adiguzel, H. Ozkan, Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. longifolia, Food Chem. 103 (2007) 1449–1456.
[40] S. Reitman, S. Frankel, A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases, Am. J. Clin. Pathol. 28 (1957) 56–63.
[41] P.R.N. Kind, E.J. King, Estimation of plasma phosphatase by determination of hydrolysed phenol with anti-pyrine, J. Clin. Pathol. 7 (1954) 322–326.
[42] H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 95 (1979) 351–358.

[43] S. Marklund, G. Marklund, Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, Eur. J. Biochem. 47 (1974) 469–474.
[44] H. Aebi, Catalase in vitro, Methods Enzymol. 105 (1984) 121–126.
[45] W.H. Habig, M.J. Pabst, W.B. Jakoby, Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation, J. Biol. Chem. 249 (1974) 7130–7139.
[46] G.J. Bourke, L.E. Daly, J.C. Mc Gilvary, In Interpretation and Uses of Medical Statistics, third ed., Blackwell Scientific Publication, Oxford, 1985.
[47] B. Hultberg, A. Andersson, A. Isaksson, Interactions of metals and thiols in cell damage and glutathione distribution: potential of mercury toxicity by dithiothreitol, Toxicology 56 (2001) 93–100.
[48] D.B. Zorov, M. Juhaszova, S.J. Sollott, Mitochondrial ROS-induced ROS release: an update and review, Biochim. Biophys. Acta 1757 (2006) 509–517.
[49] S.X. Liu, M. Athar, I. Lippai, C. Waldren, T.K. Hei, Induction of oxyradicals by arsenic: implication for mechanism of genotoxicity, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 1643–1648.
[50] O. Ramos, L. Carrizales, L. Yanez, J. Mejia, L. Batres, D. Ortiz, F. Barriga, Arsenic increased lipid peroxidation in rat tissues by a mechanism independent of glutathione levels, Environ. Health Perspect. 103 (1995) 85–88.
[51] E.J. Morcillo, J. Estrela, J. Cortijo, Oxidative stress and pulmonary inflammation: pharmacological intervention with antioxidants, Pharmacol. Res. 40 (1999) 393–404.
[52] M. Cini, R.Y. Fariello, A. Bianchetti, A. Moretti, Studies on lipid peroxidation in the rat brain, Neurochem. Res. 19 (1994) 283–288.
[53] B. Halliwell, Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet 344 (1994) 721–724.
[54] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, Oxford University Press, 2000, pp. 148–149.
[55] S. Maiti, A.K. Chatterjee, Differential response of cellular antioxidant mechanism of liver and kidney to arsenic exposure and its relation to dietary protein deficiency, Environ. Toxicol. Pharmacol. 8 (2000) 227–235.
[56] S.J. Flora, Arsenic induced oxidative stress and its reversibility following combined administration of N-acetyl cysteine and meso-2,3 dimercaptosuccinic acid in rats, Clin. Exp. Pharmacol. Physiol. 26 (1999) 865–869.
[57] K. Ramanathan, B.S. Balakumar, C. Panneerselvam, Effects of ascorbic acid and alpha tocopherol on arsenic induced oxidative stress, Human Exp. Toxicol. 21 (2002) 675–680.
[58] T. Braunbeck, A. Völkl, Toxicant induced cytological alterations in fish liver as biomarkers of environmental pollution a case-study on hepatocellular effects of dinitro-o-cresol in golden ide (Leuciscus idus melanotus), in: T. Braunbeck, W. Hanke, H. Segner (Eds.), Fish in Ecotoxicology and Ecophysiology, Verlag Chemie, Weinheim, 1993, pp. 55–80.
[59] S. Datta, D.R. Saha, D. Ghosh, T. Majumdar, S. Bhattacharya, S. Mazumder, Sublethal concentration of arsenic interferes with the proliferation of hepatocytes and induces in vivo apoptosis in *Clarias batrachus* L., Comp. Biochem. Physiol. C Toxicol. Pharmacol. 145 (2007) 339–349.
[60] Y. Xie, K.J. Trouba, J. Liu, M.P. Waalkes, D.R. Germolec, Biokinetics and subchronic toxic effects of oral arsenite arsenate, monomethylarsonic acid and dimethylarsinic acid in v-Ha-ras transgenic (Tg. AC) mice, Environ. Health Perspect. 112 (2004) 1255–1263.
[61] D.M. Goldberg, C. Watts, Serum enzyme changes as evidence of liver reaction to oral alcohol, Gastroenterology 49 (1965) 256–261.
[62] P. Sillanaukee, Laboratory markers of alcohol abuse, Alcohol 31 (1996) 613–616.
[63] M.J. Hardonk, J. Koudstaal, Enzyme histochemistry as a link between biochemistry and morphology, Prog. Histochem. Cytochem. 8 (1976) 1–68.
[64] M. Humtsoe, R. Davoodi, B.G. Kulkarni, B. Chavan, Effect of arsenic on the enzymes of the Rohu carp, Labeo Rohita (Hamilton 1822), Raffle Bull. Zool. 14 (2007) 17–19.
[65] S. Karatas, M. Kala, Accumulation of the lead in the gill, liver, kidney and brain tissues of Tilapia Zilli, Turk. J. Vet. Anim. Sci. 26 (2002) 471–477.
[66] S.S. Roy, Some toxicological aspects of Chlorpyrifos to the intertidal fish Boleopthalamus Dussumieri, Ph.D. Thesis, University of Mumbai, India, 2002, pp. 52–71.
[67] R. Gupta, D.K. Dubey, G.M. Kannan, S.J. Flora, Concomitant administration of *Moringa oleifera* seed powder in the remediation of arsenic-induced oxidative stress in mouse, Cell Biol. Int. 31 (2007) 44–56.
[68] G.M. Kannan, S.J. Flora, Combined administration of N-acetylcysteine and monoisoamyl DMSA on tissue oxidative stress during arsenic chelation therapy, Biol. Trace Elem. Res. 110 (2006) 43–59.
[69] B. Halliwell, O.I. Arouma, DNA and Free Radicals, Ellis Harwood, New York, 1993, pp. 211–222.
[70] M. Monagas, B. Hernandez-Ledesma, C. Gomez-Cordoves, B. Bartalome, Commercial dietary ingredients from *Vitis vinifera* L. leaves and grape skins: antioxidant and chemical characterization, J. Agric. Food Chem. 54 (2006) 319–327.
[71] S. Ghosal, V.K. Tripathi, S. Chauhan, Active constituents of *Emblica officinalis*: Part 1—the chemistry and antioxidant effects of two new hydrolysable tannins, Emblicanin A and B, Indian J. Chem. 35B (1996) 941–948.
[72] S.S. Handa, A. Sharma, K.K. Chakraborti, Natural products and plants as liver protecting drugs, Fitoterapia 57 (1986) 307–321.
[73] K.C. Thresiamma, R. Kuttan, Inhibition of liver fibrosis by ellagic acid, Indian J. Physiol. Pharmacol. 404 (1996) 363–366.