Electrozymographic evaluation of the attenuation of arsenic induced degradation of hepatic SOD, catalase in an in vitro assay system by pectic polysaccharides of *Momordica charantia* in combination with curcumin

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**ABSTRACT**

*Momordica charantia* (MC) fruit known as bitter gourd, is of potential nutritional and medicinal value. The objectives of the present in vitro study were to evaluate the efficacy of bioactive pectic polysaccharides (CCPS) of MC along with another well-known bioactive compound curcumin in the abrogation of hepatocellular oxidative stress persuaded by sodium arsenite. Electrozymographic method was developed for the assessment of superoxide dismutase (SOD) and catalase activities of liver tissues maintained under an in vitro system. A significant association of CCPS of MC in combination with curcumin was found in the alleviation of oxidative stress induced by sodium arsenite in liver slice. Generated data pointed out that CCPS of MC and curcumin separately or in combination can offer significant protection against alterations in malondialdehyde (MDA), conjugated diene (CD) and antioxidative defense (SOD, CAT) markers. Furthermore, results of hepatic cell DNA degradation strongly supported that both these co-administrations have efficacy in preventing cellular damage. This is the first information of extracted polysaccharides from MC preventing arsenic induced damage in a liver slice of rat.

1. Introduction

Arsenic pollution is responsible for the ill health of vast populations worldwide. Human contact with inorganic and organic arsenic occurs most often from food and to a smaller extent of drinking water \cite{1}. Various gastrointestinal ailments, encephalopathy and peripheral neuropathy are the consequences of acute arsenic poisoning \cite{2,3}. Persistent arsenic toxicity results in multisystem disease and is associated with cancer of the skin and internal organs and with several non-malignant adverse health effects \cite{4–6} including metabolic disorders, reproductive hazards, infertility etc. due to the consumption of arsenic contaminated water \cite{7–9}.

Arsenic is one of the most comprehensively studied metals that instigate reactive oxygen species (ROS) generation and upshot in oxidative stress. An over burden of free radicals in response to arsenic ingestion lead to cell damage and death through the commencement of oxidative sensitive signaling pathways and that are ultimately escalating the generation of ROS, such as intracellular peroxide, superoxide anion radical (O2⁻), hydrogen peroxide (H₂O₂) and hydroxyl free radicals (OH⁻), which are capable of direct or indirect cellular DNA and protein breakdown \cite{10,11}.

The liver is the metabolic dock of entry of arsenic and is the major destination of arsenic toxicity. Arsenic confines the chemotherapeutic effectiveness of liver tissue resulting in secondary toxicity. DNA damage is the outcome of arsenic interceded chromosomal aberrations, sister-chromatid exchange and interference in the DNA methylation process \cite{12}, which may trim down the expression of tumor suppressor genes. Progression of DNA repair is also sluggish in response to arsenic intoxication \cite{13}. Over-expression of certain cellular apoptotic gene may be the consequence of arsenic induced activation of transcription factor NF-κB and C-reactive protein (CRP) through ROS generation \cite{14}. Mitochondrial ROS-driven as well as caspase-dependent apoptosis by the release of cytochrome-c and activation of liver BAD/Bcl-2 in association with a deprivation of cellular thiol level are also directed by chronic arsenic poisoning \cite{15}. Colorectal tumorigenesis via ROS-mediated Wnt/β-catenin signaling pathway is promoted in a rat model by the ingestion of drinking water arsenic \cite{16}. It is evident that the level of a specific marker of oxidative DNA damage known as 8-hydroxy-2'-deoxy-guanosine (8-OHdG) is increased by carcinogenic metal and that suggesting the ROS involvement in the DNA damage process \cite{17}.
2. Materials and methods

2.1. Preparation of the pectic polysaccharide isolated from Momordica charantia

The small pieces of fresh fruits of *Momordica charantia* (1.5 kg) were washed with water, and boiled with distilled water for 10 h. The entire extract was settled overnight at 4 °C and then filtered through linen cloth. The filtrate was then centrifuged at 8000 rpm for 45 min at 4 °C. The supernatant was collected and precipitated in ethanol (1:5, v/v). The precipitated pectic polysaccharide of *Momordica charantia* (CCPS) was collected through centrifugation, washed with ethanol, and freeze dried [35]. The crude polysaccharide was isolated and purified through Sepharose-6B to obtain α-D-galactose and D-methyl galacturonate with a molar ratio of 1: 4 with following configuration as described by Panda et al. [36]:

| B | C |
|---|---|
| [-]D-GalpA6Me(1→4)-α-D-GalpA6Me(1→2) | α-D-GalpA6Me(1→2) |
| β-D-Galp | 1 |

2.2. Treatment for liver tissue maintaining in vitro assay system

Wistar rats were initially anesthetized by ketamine and liver tissue was collected from anesthetized Wistar female rat following the standard protocol of institutional ethical guideline (Ethical clearance no.-IEC/11/7-Met/16), and finally euthanized the animal using barbiturate overdose as per CPSCA guideline. The samples were kept into a separate sterile bag and transferred to the laboratory in an insulated ice unit having −20 °C temperature. Liver slices were pooled and weighed to produce 20 g samples. Thin liver slices were washed in ice cold Krebs's solution. Liver slices were randomly distributed in several groups having 7 in each.

**Groups 1:** Control group,

**Groups 2:** As3+ treated group (0.6 ppm/2 g liver slices),

**Groups 3:** H2O2 group (100 mM/2 g liver slices),

**Groups 4:** As3+ treated group (0.6 ppm/2 g liver slices) + H2O2 group (100 mM/2 g liver slices),

**Groups 5:** As3+ treated group (0.6 ppm/2 g liver slices) + H2O2 group (100 mM/2 g liver slices) + Curcumin group (20 mg/2 g liver slices),

**Groups 6:** As3+ treated group (0.6 ppm/2 g liver slices) + H2O2 group (100 mM/2 g liver slices) + CCPS group (2 mg/2 g liver slices),

**Groups 7:** As3+ treated group (0.6 ppm/2 g liver slices) + H2O2 group (100 mM/2 g liver slices) + Curcumin group (20 mg/2 g liver slices) + CCPS group (2 mg/2 g liver slices),

2.3. Estimation of malondialdehyde and conjugated dienes levels

Liver slices were homogenized (20% w/v) in ice-cold phosphate buffer (0.1 mol/L, pH 7.4) and centrifuged at 15,000×g in 4 °C for 3 min and supernatant was collected for the estimation of malondialdehyde (MDA) and conjugated dienes (CD).

MDA was determined from the reaction of thiobarbituric acid with MDA. The amount of MDA formed was measured [37] by taking the absorbance at 530 nm (ε = 1.56 × 105 mol−1 cm−1).

Conjugated dienes were determined by a standard method. The lipids were extracted with chloroform–methanol (2:1), and centrifuged at 1000×g for 5 min. Residue of the lipid was dissolved in 1.5 ml of cyclohexane, and the amount of hydroperoxide formed was measured at 233 nm [38].

2.4. Spectrophotometric assay of superoxide dismutase (SOD) and catalase activities

Liver slices were homogenized in 100 mmol/L chilled Tris-HCl buffer containing 0.16 mol/L KCl (pH 7.4) to make a tissue concentration of (10% w/v) and followed by centrifugation at 10,000 g for 20 min at 4 °C. The reaction mixture was prepared by mixing 800 μL of TDB (Merck), 40 μL of 7.5 mmol/L NADPH (Sigma), 25 μL of EDTA-MnCl2 and 100 μL of the tissue supernatant. The activity of SOD in this mixture was monitored at 340 nm from the rate of oxidation of NADPH [39].

The activity of catalase was measured spectrophotometrically. Here dichromate in acetic acid was transformed into per chromic acid and finally to chromic acetate when heated in the presence of H2O2. Formed chromic acetate was measured at 570 nm with modification according to Hadwan [40]. The catalase preparation was permissible for splitting H2O2 for different duration. The reaction was terminated at different time intervals by adding of a dichromate-acetic acid mixture. Remaining H2O2 was determined as chromic acetate. One unit of activity was noted as a mole of H2O2 consumed/min/mg protein.

2.5. Assessment of SOD and catalase by native gel electrophoresis

Hepatic slices were homogenized (20% w/v) in ice cold PBS (1.0 M, pH 7.4) following the centrifugation at 10,000×g for 20 min at 4 °C.
The SOD activity gel (12%) assay system was used on the principle of inhibition of the reduction of NBT by SOD and the capacity of O$_2$•$^-$ to interact with NBT reducing the yellow tetrazolium within the gel to a blue precipitate will develop a clear area of achromatic bands competing with NBT for the O$_2$•$^-$ developed at the active site of SOD [41]. SOD was separated by electrophoresis of the supernatant with little modification containing 60 μg protein on 12% native PAGE. Finally, gels were incubated with 2.3 mM NBT, 28 μM riboflavin and 28 mM TEMED for 20 min in dark. Achromatic bands of SOD were visible against a dark blue background following the exposure of the gels under fluorescent light. An identical gel was stained with coomassive brilliant blue to verify the intensities of the corresponding SOD protein bands.

Catalase eliminated the peroxides from the area of the gel it occupied. Exclusion of peroxide did not allow for the potassium ferricyanide (a yellow substance) to be reduced to potassium ferrocyanide that reacted with ferric chloride to form a Prussian blue precipitate. Tissue extracts containing 60 μg proteins were electrophoresed on 8% PAGE. Gels were soaked for 10 min in 0.003% and finally incubated with a stunning mixture of 2% potassium ferricyanide and 2% ferric chloride. Bluish yellow bands were prominent against a blue, green background.

2.6. Assessment of peroxidase by native gel electrophoresis

Liver slices were homogenized (20% w/v) in ice cold PBS (0.1 M, pH 7.4) centrifuged at 10,000 × g for 20 min at 4 °C. 50–100 μg protein per sample is loaded in 8% native gel. Then gel run with a power supply of 40 mA. The gel is stained by staining solution [42]. The composition of staining solution is Benzidine powder- 100 mg, Glacial acetic acid is 4 and half ml, 30% H$_2$O$_2$. After running 8% gel the gel is incubated to the reagent mixture till the brown color develop.

2.7. Assessment of total lactate dehydrogenase (LDH) in liver slices

For an electrozymography study of the enzyme agaroase gel of 1.2% in 50 mM Tris–HCl buffer pH 8.2 was used and 20 μl sup of liver slices were loaded into the different slots gel. The gel was electrophoresed at 170 V until the bromphenol blue has migrated to within 1 mm of the positive electrode end of the gel. Agarose gel was developed with slight modification in the presence of H$_2$O$_2$, 1.0 M Tris, tetrazolium-blue, phenazine-methosulphate, Na-lactate and NAD and then incubated at 37 °C to develop color reaction for 30 min following the exposure of the gels under light exposure [43].

2.8. DNA degradation study

Rat liver tissue was used for DNA preparation and added 500 μl lysis buffer (50 mM Tris pH 8.0, 20 mM EDTA, 10 mM NaCl, 1% SDS, 0.5 mg/ml proteinase K) to cell pellet for 15 min at 4 °C and centrifuged in the cold at 12,000 × rpm for 20 min. The supernatant was collected and treated with 1:1 mixture of phenol: chloroform with gentle agitation for 5 min and precipitated in two equivalence of cold ethanol and one tenth equivalence of sodium acetate [44]. After spinning down and decantation, the precipitate was resuspended in 30 μl of deionized water–RNase solution (0.4 ml water + 5 μl of RNase) and 5 μl of loading buffer for 30 min at 37 °C. The 0.8% agarose gel with ethidium bromide was run at 65 V and documented in gel documentation system.

2.9. Comet assay

According to the Singh and colleagues’ method with some minor modifications to the alkaline comet assay was performed [45]. A total of 75 ml of low melting point agarose (0.6%) in PBS at 37 °C was added to a 25 ml of cell suspension (105 cells). The mixture was then placed onto a glass slide pre-coated with 1% agarose, and a coverslip was placed on top. Following the solidification of agarose the coverslips were removed and the slides were soaked in ice cold lysis buffer (2.5 mM NaCl, 85 mM EDTA, 10 mM Trizma base, 1% Triton X-100, 10% DMSO and 1% sodium lauryl sarcosinate, adjusted to pH 10 for 1 h at 4 °C. The slides were washed thrice in PBS at room temperature after lysis. Next, 50 ml of buffer (control) or T4 endo V (Epicentre) (4 U/slide) in buffer was transferred to the slides. Coverslips were put on and the slides were incubated at 37 °C for 45 min. The coverslips were then removed and the slides were washed in water twice more to remove excess salt if any. Slides were then placed in a submarine gel electrophoresis chamber (Bio-Rad, USA) filled with alkaline electrophoresis buffer (0.3 M NaOH and 1 mM EDTA) for 25 min. Electrophoresis was performed for 30 min at 25 V and the current was adjusted to 300 mA by raising the buffer level. Slides were then neutralized with PBS and stained with a solution of 10 mg/ml ethidium bromide for 5 min washing in water excess stain was removed. Slides were read using a fluorescence microscope (Nikon, Eclipse LV100 POL), with the Vis Comet (Impuls Bild analyse) software.

3. Results and discussion

Arsenic is a powerful hepatotoxic agent. Numerous investigations have been shown the therapeutic effects of some medicinal plants on arsenic induced hepatotoxicity. *Momordica charantia* and *Curcuma longa* are widely consumed in the daily diet in Asian countries. *Momordica charantia* is frequently illustrated for its antioxidative and therapeutic efficacy against various health disorders [46]. Curcumin from *Curcuma longa* have been showing potential of preventing arsenic induced hepatotoxicity in vitro and in vivo [47]. However, there is scanty information regarding the combined hepatoprotective effect of CCPS from *Momordica* and curcumin against arsenic. In this present study the protective mechanism of CCPS and curcumin on arsenic induced hepatotoxicity was investigated with an approach to understand the intracellular events in liver tissue of rat maintained in a short duration in vitro model.

Considering end products of lipid peroxidation levels, we observed a significant elevation in the liver MDA and CD level in arsenite, As$_3^+$ + H$_2$O$_2$ induced group compared to the control group (Fig. 1A and B). But comparatively present results advocated that treatment of As$_3^+$ exposed liver tissues with curcumin and CCPS alone and or combination reverse the As$_3^+$ induced elevation of MDA and CD levels significantly (Fig. 1A and B). Furthermore, the combined mode of treatment with curcumin and CCPS had shown more intense recovery of these end products of lipid peroxidation in arsenic treated rats (Fig. 1A and B).

![Fig. 1. (A & B). Protective effect on malondialdehyde (MDA) and conjugated dine (CD) in liver tissue by curcumin and CCPS in arsenic induced intoxicated rats. Each bar represents mean ± SE, N = 6. ANOVA followed by two tailed student’s ‘t’ test were used to find out statistical significance at p < 0.001. Bar with different upper scripts differ from each other.](image-url)
The present study exhibited an extensive increase in the level of ROS production in the As$^{3+}$ treated group and this $As^{3+}$ mediated oxidative damage was associated with hepatotoxicity. However, exposure to $As^{3+}$ has been already established for the increased production of ROS [48]. Furthermore, short duration $As^{3+}$ exposure in vitro system was demonstrated to lead to hepatic dysfunction, as confirmed by the increased free radicals as evident from the significantly elevated level of MDA and CD (Fig. 1A and B). This incidence indicated that tri-valent form of arsenic harvests reactive oxygen species in association with $H_2O_2$ which ultimately led to the formation of several lipid peroxides and conjugated diene as the end products [49]. However, there is a common consensus that ROS result in oxidative damage, are important in the progression of hepatocellular degeneration [50]. However, co-treatment with curcumin and CCPS led to a pronounced recovery in the $As^{3+}$ induced oxidative injury. These results indicated the possible antioxidant efficacy of curcumin and CCPS.

In the present study, a significant decrease in hepatic SOD and catalase activities was noticeable in arsenic-treated versus the control group in a dose dependent manner. A distinct restoration of both enzymes (Fig. 2A) by the CCPS of MC and curcumin was observed following the exposure to 0.6 ppm of arsenic.

We further analyzed the samples by electrozymogram study under native gel to judge the impression of oxidative stress by these stressors on modification of the enzyme. Considering this native gel electrozymographic impression of SOD, diffused and fragmented occurrence of the band was amplified in a dose dependent manner following an increased dose of arsenic for 6 h duration and this nature of the band (SOD) appearance was attenuated following the incubation with CCPS in arsenic treated hepatic cells (Fig. 3A).

Present liver-slice experimentation suggests that arsenite ($As^{3+}$) alone or in combination with $H_2O_2$ significantly inactivates the SOD activity time dependently and showed a 12–27 folds and 42–56 folds reduction in its activity after 3 h and 6 h exposure, respectively in the presence of arsenic (0.6 ppm) and $H_2O_2$ alone and or in combination (Fig. 4A) After 6 h incubation, strong inactivation of SOD was noticed and that highest inactivation by (1.0 M) $H_2O_2$ alone or in combination with $As^{3+}$ was found to be significantly restored by curcumin and CCPS in a duration-dependent manner (Fig. 4A). In fact, at 0.6 p.m. $As^{3+}$ alone the original nature of SOD bands were starting to lose, whereas $H_2O_2$ alone or in combination with $As^{3+}$ small fragments were newly formed gradually (Fig. 4A). The analogous protein to SOD showed no significant alteration in its expression (Fig. 4A). This proposes that the distortion and inactivation of SOD protein structure is much possible and significant than the probable transcriptional and/or translational suppression by $As^{3+}$. In arsenic treated group the band strength of the...
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protein was found to be a little weaker than that of control group (Fig. 4A). The fragmented nature of the band was progressively recovered finally following the treatment with curcumin and CCPS. The lessening of SOD activity might result in a potential failure of the clearance of superoxide anion which generates numerous downstream radical products in response to the reaction to H₂O₂ [51,52]. Observation of the study revealed that upon increasing amounts of arsenic there was an increased diffusion of SOD bands along with a fragmented appearance (Fig. 3A). Furthermore, the same nature of SOD expression was observed following the treatment of liver cells with arsenic and H₂O₂ alone or together in a duration dependent manner (Fig. 4A).

However, three additional fragmented bands were detected in (Fig. 4A), suggesting that a minor modification of the protein may be plausible following the exposure to these exogenous oxidative stressors. This is the first time we are reporting such fragmentation by PAGE without using SDS, though fragmentation of extracellular SOD induced by oxidative stress (H₂O₂) has been previously reported using SDS-PAGE [53].

Moreover, As³⁺ generally interacts with the thiol residue of enzyme directly but so far there is no such evidence that implies As³⁺ has direct interaction with SOD. Here the As³⁺ mediated inhibition of SOD may be due to the indirect effect of As³⁺ where As³⁺ induced elevation of H₂O₂ levels leads to SOD inactivation. The fragment of oxidized SOD could acquire an α-amino acid histidine in active form and aromatic groups along with charged and hydrophobic surface of its structure [54].

Previously reported evidences also suggest that depletion of SOD activity in response to oxidative stress by arsenic and H₂O₂ treatment may be due to the alteration of cysteine residues in its structure [55]. However, the depletion of this enzymatic antioxidant was partially but significantly restrained by the administration of CCPS and curcumin alone or in combination, is suggestive of a possible recovery in the extensive generation of hepatic free radicals during arsenic metabolism. It may be postulated that CCPS and curcumin may avert the modification of cysteine residue of SOD in the metal/H₂O₂ exposed hepatocytes because our experiment has shown that fragmentation of SOD was completely inhibited in response to the supplementation of CCPS and curcumin in arsenic/H₂O₂ treated liver tissue (Fig. 3A and 4A).

Compared with control, hepatic catalase expression remarkably with variable dose of arsenic (Fig. 3B). Co-administration with CCPS in this in vitro media containing hepatic slices significantly reinstates the decreased level of arsenic-induced catalase expression. Applying this electrozymographic and enzyme activity assay techniques, we documented that catalase activity was decreased following arsenic ingestion (Fig. 3B), which was evident from the faint band of catalase in arsenic/H₂O₂ treated liver tissue (Fig. 3B). The expression of liver catalase reduced remarkably to 0.6 ppm of As³⁺ for 6 h in comparison to 3 h duration (Fig. 4B).

Interestingly, curcumin, and CCPS alone or in combination for 6 h. duration more effectively antagonized the As³⁺ induced diminution of catalase activities in liver tissue. (Fig. 4B). This indicates that an impairment of H₂O₂ detoxification of liver may possible due to reduced activity of catalase. Earlier studies revealed that the inhibition of catalase in response to arsenic trioxide treatment resulted in the intracellular ROS accumulation. This diminution in catalase activity by arsenic is mediated via the modulation of its expression at the level of mRNA transcription [56]. Metal/H₂O₂ may penetrate the active site of catalase and interact with the amino acids asparagine and histidine in its active and thereby modulate its activity [57].

Hepatic peroxidase activity is designated to be an essential marker to assess the breakdown of peroxides; therefore, monitoring its level is essential. The effect of MC and curcumin on the peroxidase activity illustrated in the Fig. 5A and B indicated that hepatic peroxidase activity was significantly decreased when treated with As³⁺. Administration of CCPS of MC and curcumin caused a significant increase in liver peroxidase activity compared to the As³⁺ treated rats (Fig. 5A and B).

Moreover, electrozymographic analysis revealed that the expression of peroxidase was also reduced when liver slices exposed to 0.6 ppm of As³⁺ exposure for 3 h and 6 h in the corresponding lane. Co-treatment with these two plant products to arsenic exposed cells exhibited a significant increase in the band density by replacing the abolished band (Fig. 5A and B) alone or in combination, although exposure of 6 h duration exhibited more well-defined effect than that of 3 h exposure. This finding may strongly suggestive of H₂O₂ accumulation during the programmed cell death [58].

In vitro DNA agarose gel electrophoresis demonstrated that As³⁺ (Fig. 6) could damage hepatic DNA at a concentration of 0.6 ppm (Fig. 6) and even at lower concentration (data not shown).

When As³⁺ showed a conspicuous degradation of DNA (Lane 2) in hepatic cells compared with the effect seen in unexposed hepatic cells (Lane 1); H₂O₂ and or H₂O₂ plus arsenic (Lane 3, Lane 4) were highly damaging and were able to degrade DNA entirely (Fig. 6). Present results advocated that co-treatment of As³⁺ exposed liver tissues with curcumin (Lane 5) and CCPS (Lane 6) alone and or combination (Lane 7 & 8) partially but significantly reduced the degradation of DNA along with its degradation. In vitro experimentation in liver tissue also supports the curcumin and CCPS protection of DNA from the damage induced by As³⁺ which was clearly noted in the DNA degradation comet assay (Fig. 7). The extrusion of the broken DNA from a majority number of cells was clearly visualized. Protection of DNA by curcumin and CCPS against arsenite-induced intoxication was also reflected in a single cell DNA damage test in agarose gel electrophoresis. It was noticed that the cellular DNA was damaged to different degrees due to As³⁺ and H₂O₂ or its combined intoxication and that was completely vetoed with the application of curcumin and pectic polysaccharide for both duration of 3 h and 6 h.

The damage of antioxidant enzymes like SOD and catalase activity

Fig. 5. (A & B). Electrozymogram showing total peroxidase activities in cell free extract [A] protected better with CCPS and curcumin in different duration. Lane distribution Lane 1: Control; Lane 2: Arsenic; Lane 3: H₂O₂; Lane 4: Arsenic + H₂O₂; Lane 5: Arsenic + Curcumin; Lane 6: arsenic + CCPS; Lane 7: Arsenic + H₂O₂ + curcumin; Lane 8: Arsenic + H₂O₂ + CCPS.

Fig. 6. Arsenic-induced changes in DNA degradation in hepatic cells: protective effects of curcumin or CCPS or combination of both. Representative electrophoretogram Lane distribution; Lane 1: Control; Lane 2: Arsenic; Lane 3: H₂O₂; Lane 4: Arsenic + H₂O₂; Lane 5: Arsenic + Curcumin; Lane 6: Arsenic + CCPS; Lane 7: Arsenic + H₂O₂ + curcumin; Lane 8: Arsenic + H₂O₂ + CCPS; of ethidium bromide-stained agarose gel demonstrated counteraction of arsenic-induced genomic DNA degradation by supplementation with curcumin, CCPS, and combination of curcumin,CCPS.
has been delineated to correlate with oxidative stress and DNA damage prompted by ROS [59] which was further confirmed in the present experimentation where arsenic treatment induced degradation of liver DNA (Fig. 6) and injury to single cell DNA (Fig. 7A and B). Several studies have been reported that, arsenic increased the formation of reactive oxygen species (ROS) causing oxidative DNA damage such as single-strand breaks (SSBs) and that can be processed to double-strand breaks (DSBs) during replication, inhibition of DNA repair and enhancing mutagenicity and carcinogenicity [60]. DNA is continually attacked by reactive species. DNA lesions and guanine lesion are the most abundant. DNA lesion is represented by the formation of 8-OH-G, one of the major products of DNA oxidation [61]. Guanine has the least oxidation potential and it can be easily modified by reactive species [62]. Hydroxy-2′-deoxyguanosine (8-OHdG) or 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) is one of the predominant forms of free radical-induced oxidative lesions, and it is used as a biomarker for oxidative stress [63]. Curcumin reduced 8-hydroxy-2′-deoxyguanosine formation and enhanced the DNA repair capacity [64]. This may indicate a possible necrotic and apoptotic changes in liver tissue [65]. Essentially, accumulated ROS may finally ensure DNA damage by trivalent arsenicals and thereby suppresses the DNA repair systems as well as repair of oxidative DNA damage [66]. DNA breakage by oxidative stress in response to inorganic arsenic (III) treatment may affect hypomethylation of liver DNA at the cost of S-adenosyl methionine (SAM), a well-known methyl donor of arsenic metabolism [67]. To delineate the probable necrotic status of the tissue, we performed zymogram study of LDH, where we found that As³⁺ stimulated hepatic LDH significantly in contrast to control cells. The combination of CCPS and curcumin more efficiently restored the hepatic LDH activity and it was confirmed by the presence of comparatively more indistinct band in this combined group, whereas CCPS and curcumin group alone have been shown comparatively more distinct band (Fig. 8A and B). LDH, a cancer-specific biomarker of cytotoxicity is not generally amplified in patients without cancer [68]. A high level of liver lactate dehydrogenase was reflected in our present investigation in response to arsenic treatment for 6 h (Fig. 8B). Possible initiation of apoptotic tissue lesions may be recognized by the upregulated LDH level in liver cells. And this is in agreement with the findings of other investigators [69]. However, experimentation with different in vitro model explored curcumin as an effective antioxidant [70]. The results of our investigation revealed a reduced production of reactive oxygen species (ROS) where arsenic treated liver slices were exposed to curcumin. Reports of other investigators also suggested the antioxidant activity of curcumin [71] where it quenches ROS probably by the induction of mitochondrial enzymes of antioxidant defense system [72]. Investigators also explored the possible involvement of non-enzymatic antioxidants like vitamin C and E in the amelioration of oxidative damage in tissues. It has been suggested that curcumin administration improves the circulating level of vitamin C and E [73]. Previously we have been reported that vitamin C and E improve arsenic induced ovarian and uterine disorders [74,75]. Recently we explored that the Embelica officinalis, a rich source vitamin C abrogates arsenic induced hepatotoxicity and protects DNA damage [76] by reducing oxidative injury to the liver tissue in vivo.
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Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2017.06.002.

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