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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 [1]. Various gastrointestinal ailments, encephalopathy and peripheral neuropathy are the consequences of acute arsenic poisoning [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 [4–6] including metabolic disorders, reproductive hazards, infertility etc. due to the consumption of arsenic contaminated water [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 (H_2O_2) and hydroxyl free radicals (OH•), which are capable of direct or indirect cellular DNA and protein breakdown [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 [12], which may trim down the expression of tumor suppressor genes. Progression of DNA repair is also sluggish in response to arsenic intoxication [13]. Over-expression of certain cellular apoptotic gene may be the consequence of arsenic induced activation of transcription factor NF-κβ and C-reactive protein (CRP) through ROS generation [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 [15]. Colorectal tumorogenesis via ROS-mediated Wnt/β-catenin signaling pathway is promoted in a rat model by the ingestion of drinking water arsenic [16]. It is evident that the level of a specific marker of oxidative DNA damage known as 8-hydroxy-2/-73-deoxy-guanosine (8-OHdG) is increased by carcinogenic metal and that suggesting the ROS involvement in the DNA damage process [17].

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In relation to arsenic therapeutics several earlier observations had indicated for some herbal products, and phytochemicals remediation of arsenic-induced tissue toxicity [18], but there is a paucity of information regarding the satisfactory level of efficacy of such herbal cures.

Curcumin or diferuloyl methane, the main active component of Curcuma longa is expansively used as a therapeutic agent in traditional Indian medicine [19] and is a leading compound for formulating new chemotherapeutic agents for treatment of several health hazards [20,21]. Anti-carcinogenic pro-oxidant property of curcumin makes it competent therapeutic agent in the treatment of numerous diseases, including pancreatic malignancy, Myelodysplasia, multiple myeloma, colon carcinoma, dementia, Alzheimer's disease and psoriatic skin [22,23]. A wide variety of useful pharmacological effects of curcumin has been found to be fairly safe in both animals and humans as confirmed by FDA [24]. A recent report claims that curcumin reduces the hepatotoxic effect of arsenic and restores normal histopathology of liver by limiting lipid peroxidation, glutathione (GSH) depletion [25]. Diazinon-induced toxicity in blood, liver, and erythrocyte of male Wistar rats could be also improved by curcumin [26]. Evidence suggests that curcumin protects the liver from fibrogenesis by attenuating hepatic oxidative stress [27].

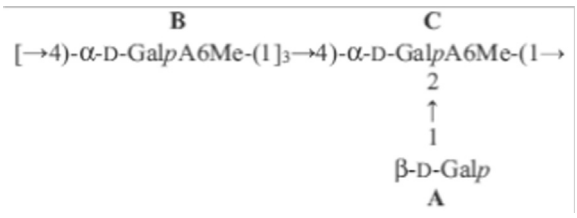
Protection of the liver from arsenic induced oxidative stress and worsening of antioxidant levels is achievable by curcumin treatment. This treatment is associated with amelioration of marker enzymes of hepatic function and restoration of normal histopathology of liver by limiting the hepatic deposition of arsenic.

The plant *Momordica charantia* from the family of Cucurbitaceae commonly known as bitter gourd or Karela. It is an economically important medicinal plant, extensively grown in India and other parts of the Indian subcontinent. Fruit and seed extract of *M. charantia* have anti-HIV, antimicrobial, antitumor, anti-inflammatory, immune stimulator [28–30] and anti-diabetic properties [31,32]. It may also protect β cells by enhancing insulin sensitivity and reduce oxidative stress [33]. In lieu of above context the present study is first time intended to explore the combined therapeutic role of pure curcumin and a CCPS isolated from *Momordica charantia* and its attainable mechanism against arsenic-induced oxidative stress, mutagenic DNA-breakage and hepatic damage in vitro experimental model. Our experiment is unreservedly important to generate new insights into the development of the easily acceptable treatment strategy against arsenic intoxication when conventional intramuscular chelating therapy against arsenic have several moderate to severe side effects [34].

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 D-galactose and D-methyl galacturonate with a molar ratio of 1: 4 with following configuration as described by Panda et al. [36]:



2.2. Treatment for liver tissue maintaining in vitro assay system

Wistar rats were initially anesthesized by ketamine and liver tissue was collected from anesthesized 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 CPSCEA 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,

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

Groups 3: H_20_2 group (100 mM/2 g liver slices),

Groups 4: As3+ treated group (0.6 ppm/2 g liver slices) + H_20_2 group (100 mM/2 g liver slices),

Groups 5: As3+ treated group (0.6 ppm/2 g liver slices) + H_20_2 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) + H_20_2 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) + H_20_2 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 ($\varepsilon = 1.56 \times 105\ mol^{-1}\ cm^{-1}$).

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 H_2O_2. Formed chromic acetate was measured at 570 nm with modification according to Hadwan [40]. The catalase preparation was permissible for splitting H_2O_2 for different duration. The reaction was terminated at different time intervals by adding of a dichromate-acetic acid mixture. Remaining H_2O_2 was determined as chromic acetate. One unit of activity was noted as a mole of H_2O_2 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 $O_2^{\bullet -}$ 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 $O_2^{\bullet -}$ 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_2O_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 agarose 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 bromophenol 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_2O, 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 rinsing of the gels with water and observed 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_2O_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).

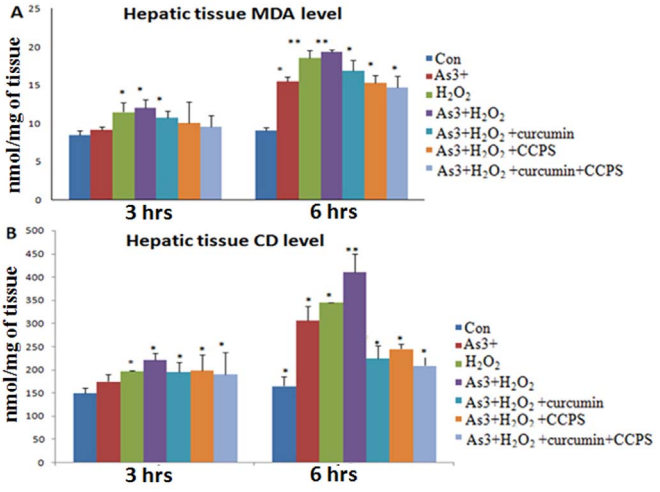


Fig. 1. (A & B). Protective effect on malondialdehyde (MDA) and conjugated dine (CD) in liver tissue by curcumin and CCPS in arsenic induced toxicated rats. Each bar represents mean ± SE, N = 6. ANOVA followed by two tailed student's 't' test were used to find out statistical significance at $p < 0.001$. Bar with different upper scripts differ from each other.

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

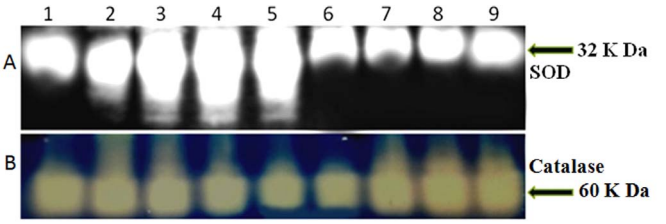


Fig. 3. (A & B) SOD and catalase activity in liver tissue on polyacrylamide gel. lane distribution Lane 1: control; Lane 2: (0.2) ppm arsenic; Lane 3: (0.4) ppm arsenic; Lane 4: (0.6) ppm arsenic; Lane 5: (0.8) ppm arsenic; Lane 6: CCPS; Lane 7: Curcumin; Lane 8: arsenic + CCPS; Lane 9: arsenic + curcumin.

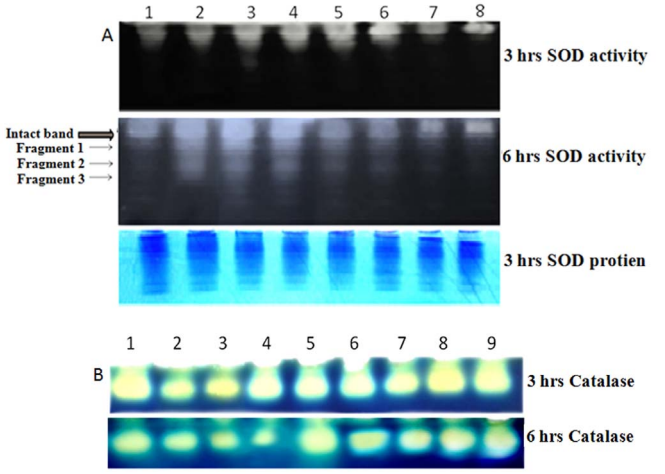


Fig. 4. (A) SOD activity in liver tissue on polyacrylamide gel. Lane distribution Lane 1: Control; Lane 2: Arsenic; Lane 3: H_2O_2; Lane 4: Arsenic + H_2O_2; Lane 5: Arsenic + H_2O_2 + curcumin; Lane 6: Arsenic + H_2O_2 + CCPS; Lane 7,8: Arsenic + H2O2 + curcumin + CCPS.Fig. 4. (B) Catalase activity in liver tissue on polyacrylamide gel. Lane distribution; Lane 1: Control; Lane 2: Arsenic; Lane 3: H_2O_2; Lane 4: Arsenic + H_2O_2; Lane 5: Arsenic + Curcumin; Lane 6: arsenic + CCPS; Lane 7: Arsenic + H_2O_2 + curcumin; Lane 8 & 9: Arsenic + H_2O_2 + CCPS.

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

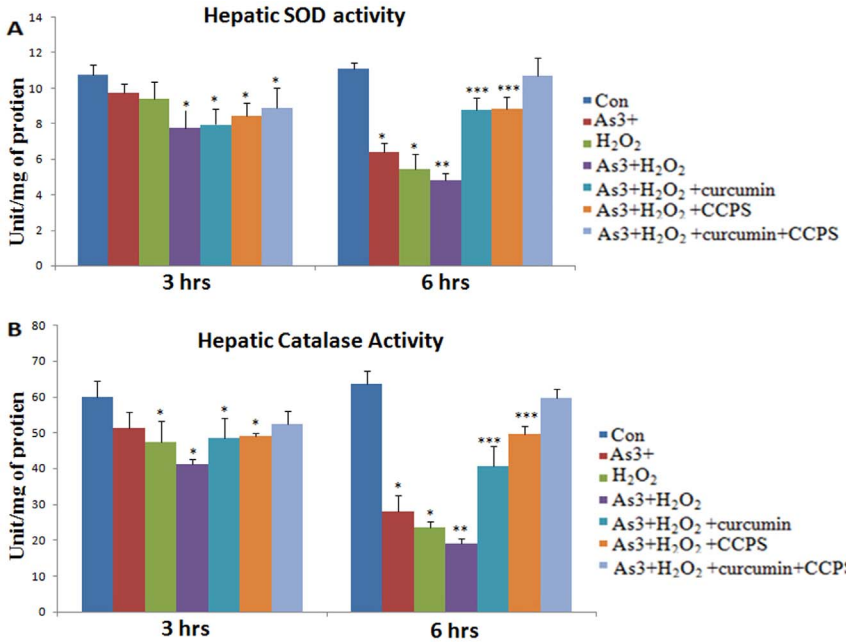


Fig. 2. (A & B) SOD and Catalase activity show a differential pattern in liver tissue. Each bar represents mean ± SE, N = 6. ANOVA followed by two tailed student's *t*-test were used to find out statistical significance at $p < 0.001$. Bar with different upper scripts differs from each other.

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 H_2O_2 [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 H_2O_2 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 (H_2O_2) has been previously reported using SDS-PAGE [53]. Moreover, As^{3+} 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 As^{3+} mediated inhibition of SOD may be due to the indirect effect of As^{3+} where As^{3+} induced elevation of H_2O_2 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 H_2O_2 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/H_2O_2 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/H_2O_2 treated liver tissue (Fig. 3A and 4A).

Compared with control, hepatic catalase expression reduced 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/H_2O_2 treated liver tissue (Fig. 3B). The expression of liver catalase reduced remarkably to 0.6 ppm of As^{3+} 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 As^{3+} induced diminution of catalase activities in liver tissue. (Fig. 4B). This indicates that an impairment of H_2O_2 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/H_2O_2 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 As^{3+}. Administration of CCPS of *MC* and curcumin caused a significant increase in liver peroxidase activity compared to the As^{3+}-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 As^{3+} exposure for 3 h and 6 h in the corresponding lane. Co-treatment with these two plant products to arsenic exposed cells exhibited a

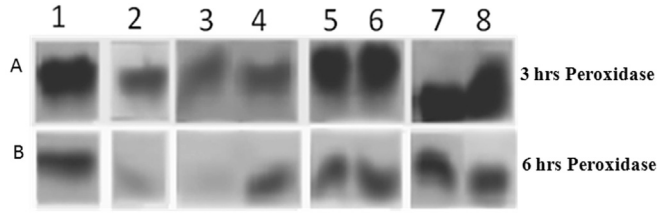


Fig. 5. (A & B). Electrozymogram showing total peroxidase activities in cell free extract [A] protected better with CCPS and curcumin in different duration. Lane distribution Lane 1: Control; Lane 2: Arsenic; Lane 3: H_2O_2; Lane 4: Arsenic + H_2O_2; Lane 5: Arsenic +Curcumin; Lane 6: arsenic + CCPS; Lane 7: Arsenic + H2O2 + curcumin; Lane 8: Arsenic + H_2O_2 + CCPS;.

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 H_2O_2 accumulation during the programmed cell death [58].

In vitro DNA agarose gel electrophoresis demonstrated that As^{3+} (Fig. 6) could damage hepatic DNA at a concentration of 0.6 ppm (Fig. 6) and even at lower concentration (data not shown).

When As^{3+} showed a conspicuous degradation of DNA (Lane 2) in hepatic cells compared with the effect seen in unexposed hepatic cells (Lane 1); H_2O_2 and or H_2O_2 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 As^{3+} 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 As^{3+} 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 As^{3+} and H_2O_2 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

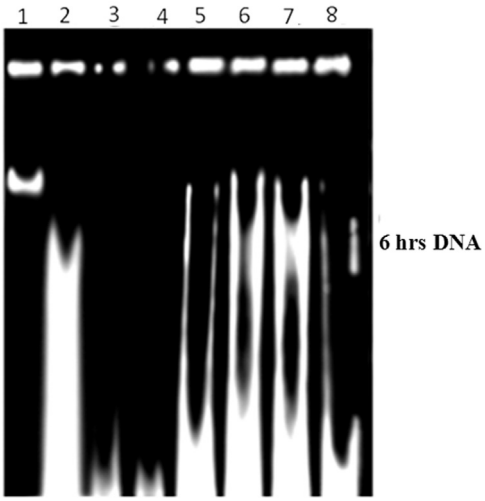


Fig. 6. Arsenic-induced changes in DNA degradation in hepatic cells: protective effects of curcumin or CCPS or combination of both. Representative electrophoretogram Lane distribution; Lane 1: Control; Lane 2: Arsenic; Lane 3: H_2O_2; Lane 4: Arsenic + H_2O_2; Lane 5: Arsenic + Curcumin; Lane 6: Arsenic + CCPS; Lane 7: Arsenic +H2O2+curcumin; Lane 8: Arsenic + H_2O_2 + 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.

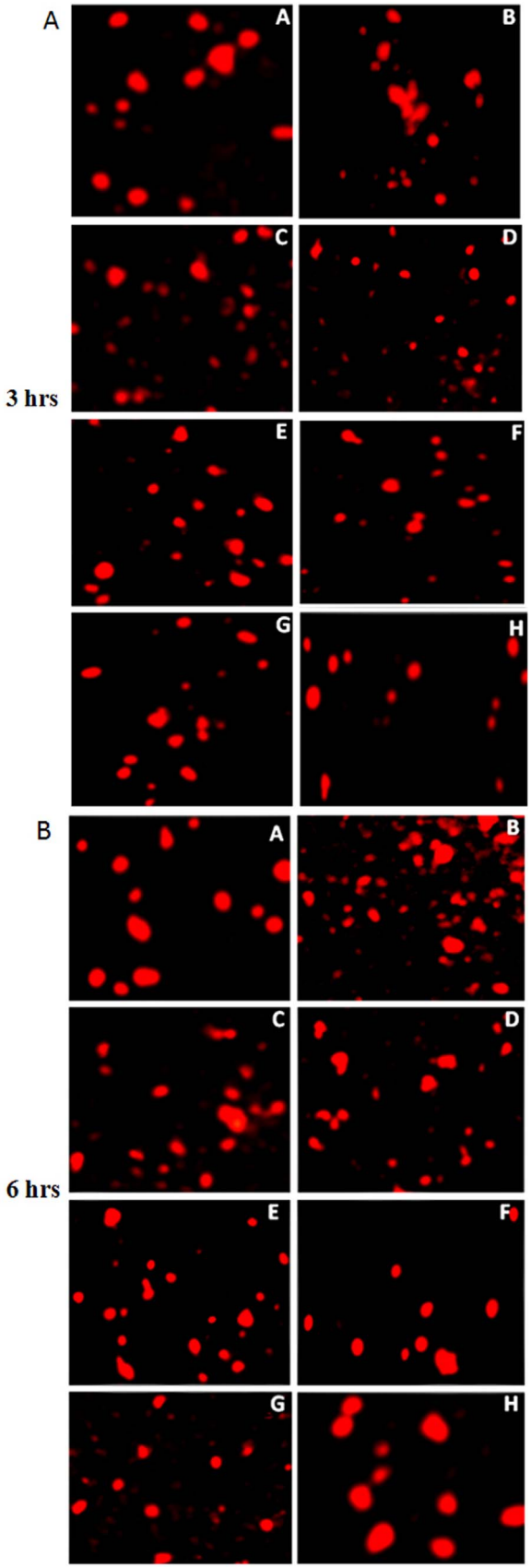


Fig. 7. (A & B) Curcumin and CCPS (*MC*) effects on the comet assay in liver cell 3 & 6 h exposure. Lane distribution; Lane A: Control; Lane B: Arsenic; Lane C: H_2O_2; Lane D: Arsenic + H_2O_2; Lane E: Arsenic + Curcumin; Lane F: Arsenic + CCPS; Lane G: Arsenic + H_2O_2 + curcumin; Lane H: Arsenic + curcumin + CCPS + H_2O_2.

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]. Gguanine 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-20-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 As^{3+} 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.

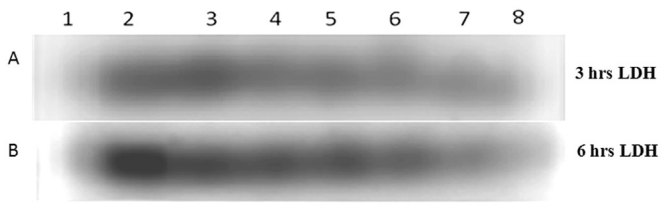


Fig. 8. (A & B). Liver LDH activity illustrated electrozymographically for the extent of cellular damage. Lane distribution: Lane 1: Control; Lane 2: Arsenic; Lane 3: Arsenic + H_2O_2; Lane 4: arsenic + CCPS; Lane 5: Arsenic + Curcumin; Lane 6: Arsenic + H2O2 + CCPS; Lane 7: Arsenic + H_2O_2 + curcumin; Lane 8: Arsenic + H_2O_2 + curcumin + CCPS.

Additionally, a recent study confirmed that phenolic compounds of curcumin contribute a crucial role by the donation of H-atom from a phenolic group of curcumins [77] and that makes curcumin as an antioxidant with superb features. Currently Palipoch and his group reported that pre-treatment with curcumin attenuates ROS production by down-regulating the expression of NADPH oxidase gene in cisplatin-exposed liver cells [78] *Momordica charantia* is well known for its several pharmacological properties exhibited by its bioactive compounds and phytochemicals e.g. momorcharins, saponins, alkaloids, glycosides, goyaglycosides, cryptoxanthin etc. [79] Here, we extracted a CCPS from *Momordica charantia* fruit that is composed of D-galactose and D-methyl galacturonate [36] with a molar ratio of 1: 4. And it was examined against arsenic mediated hepatotoxicity in vitro in combination with curcumin. We found that CCPS of *Momordica charantia* fruit was able to counteract ROS generation by restoring the enzymatic antioxidants partially but significantly in arsenic treated hepatic cells (Figs. 1–5). Hepatic DNA was protected moderately in response to this polysaccharide in arsenic exposed liver tissue. Different investigators explored that *Momordica* fruit extract could effectively reduce carcinogen-induced lipid peroxidation in liver and DNA damage in lymphocytes and significantly strengthen the liver antioxidant system via the restoration of glutathione-S-transferase, glutathione peroxidase and catalase [80].

Till date, there is a lack of information about the efficacy of this fruit extract against arsenic induced tissue toxicity in vivo and in vitro model. This is the first time we demonstrated that CCPS of *Momordica* may be fruitful in the attenuation of arsenic induced toxicity. Thus, it was tenable to postulate that the supplementation of curcumin and CCPS during arsenic treatment was likely to reduce As-induced liver disorders [81]. The present study has, for the first time, to the best of our knowledge, demonstrated the defensive consequence of curcumin and CCPS conjointly against the hepatotoxic condition in rats following short duration arsenic exposure at higher concentration. Although it is yet to be explored from the present investigation, the exact mechanism of action of these two bioactive components regulating the antioxidant defense system against arsenic. Hence, further study is important to explore the actual mechanistic to judge the involvement of S-adenosine-methionine in tissue detoxification of arsenic.

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Appendix A. Transparency document

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

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