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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].

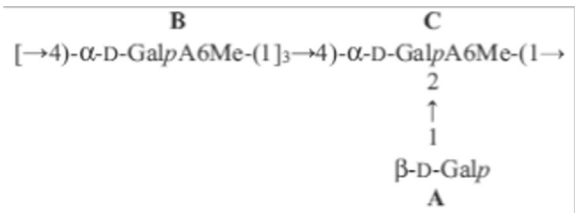
Protection of the liver from arsenic induced oxidative stress and worsening of antioxidant levels is achievable by curcumin treatment. This treatment is associated with amelioration of marker enzymes of hepatic function and restoration of normal histopathology of liver by limiting the hepatic deposition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