RESEARCH COMMUNICATION

Indian Spice Curcumin may be an Effective Strategy to Combat the Genotoxicity of Arsenic in Swiss Albino Mice

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Abstract

Inorganic arsenic (As) is considered as a human carcinogen because it is associated with cancers of skin, lung, liver and bladder in exposed populations. Consumption of As contaminated ground water in the long term causes oxidative stress. Generation of reactive oxygen species (ROS) beyond the body's endogenous antioxidant balance results in severe imbalance of the cellular antioxidant defense mechanisms. The present study was conducted to investigate the antioxidative effects of curcumin against sodium arsenite (As III) induced oxidative damage in Swiss albino mice. Bio-monitoring with comet and micronucleus assays revealed that the increase in genotoxicity caused by As III was counteracted when mice were orally administered 5, 10 or 15 mg curcumin per kg body weight daily. ROS generation, lipid peroxidation and protein carbonyl content, which were elevated by As III, were all reduced by curcumin treatment. Curcumin also exhibited protective action against the As III induced depletion of antioxidants like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST) and glutathione (GSH) in mouse liver tissue. Thus the present work provides direct evidence for an involvement of curcumin in reducing As III induced oxidative stress in Swiss albino mice by virtue of its antioxidant potential and trapping of free radicals.

Key Words: Oxidative stress - sodium arsenite - Swiss albino mice - curcumin - antioxidants

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Introduction

Inorganic arsenic (As) is considered a high priority health hazard, particularly because of its potential to be a human carcinogen. Humans are exposed to As primarily from air, food and from drinking water which may be contaminated by arsenical pesticide, natural mineral deposits or improperly disposed arsenical chemicals. The major regions affected with As contaminated ground water is the river basin of Ganga, Brahmaputra and Meghna in India and Bangladesh (Guhamazumdar, 2008). In West Bengal, arsenic levels in thousands of village wells range between 50-3200 mg/l which is far above from the U.S. Environmental Protection Agency (EPA) adopted arsenic standard of 10 μg/l in drinking water (Mo et al., 2006; Bhattacharya et al., 2003).

Arsenic has been correlated convincingly with cancers of the skin, lung, liver, kidney, and urinary bladder (Huff et al., 2000). However the mechanism by which it causes cancer is not completely understood. One possible mode of action for arsenic toxicity and carcinogenicity is oxidative stress formed by reactive oxygen species (ROS) which are important component of cell signaling and deregulation of ROS alters expression of genes (Ruiz-Ramos et al.,2008). ROS also results in DNA damage, protein damage and lipid peroxidation which may initiate cancer by enhancing cell proliferation (Buchet et al., 1980). Arsenic was found to increase protein carbonyl content and lower protein sulphydryls in brain of male Wistar rats (Samuel et al., 2005). Arsenic-induced oxidative stress has been associated with free radical metabolism, and known to cause depletion of glutathione and diminished activities of some enzymes, such as SOD, CAT, GPx, GR and GST in growing pigs (Wang et al., 2006).

Scientists have been focusing on chemopreventive approach to ameliorate the effect of arsenic toxicity using natural compounds, particularly polyphenols, many of which are endowed with excellent chemopreventive properties. Curcumin, a yellow pigment obtained from turmeric (*Curcumina longa*), is a dietary polyphenol that has been reported to possess anti-inflammatory and antioxidant properties (Suzuki et al., 2009). In vivo antioxidative effects of curcumin have already been investigated. According to the study of Watanabe et al, curcumin can significantly suppresses trichloroethylene (TCE) induced oxidative stress in mouse liver by scavenging various free radicals and increasing activities of antioxidative enzyme, such as Cu/Zn-SOD, catalase, glutathione reductase, glutathione peroxidase (GPx) and D-glucose-6-phosphate dehydrogenase (G6PD) (Watanabe et al., 2000) . Efficacy of curcumin appears to be related to induction of glutathione S-transferase enzymes (Sharma et al., 2004). Curcumin pre & post treatment in rats having isoprenaline induced myocardial

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ischemia, increased the activities of antioxidant enzymes and decreased the level of lipid peroxide (Manikandan et al., 2004). Curcumin-pretreatment restored increasing level of Fe-NTA induced protein carbonyl contents to normal in mice (Okazaki et al., 2005).

The present study was designed to investigate the role of curcumin in reducing As III induced oxidative damage on DNA, protein and lipid and in inducing antioxidants like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST) and glutathione (GSH) in Swiss albino mice.

Materials and Methods

Chemicals:

2-thiobarbituric acid (TBA) [CAS No.504-17-6], glutathione reductase (GR) [CAS No. 9001-48-3], histopaque 1077, ethidium bromide [CAS No. 1239-45-8], Triton-X 100 [CAS No.9002-93-1], 2, 7 dichlorofluorescein diacetate (DCFH-DA) [CAS No. 2044-85-1], Hepes buffer solution [CAS No. 7365-45-9], cytochalasin B [CAS No. 14930-96-2] were obtained from Sigma-Aldrich St Louis, MO, USA. Agarose, RPMI-1640, Hank's balanced salt solution [Cat No. 24020117], L glutamine [Cat No. 25030149], heat inactivated foetal bovine serum [Cat No. 2013-04] were obtained from Invitrogen. Kits for estimation of glutathione, glutathione reductase, glutathione s-transferase and protein carbonyl were procured from Cayman Chemicals. Sodium arsenite and trichloroacetic acid (TCA) was procured from Spectrochem India Pvt Ltd, Mumbai, India. 2-mercaptoethanol was purchased from Loba Chemie, Mumbai. Giemsa's solution was procured from Merck. Curcumin capsules (500mg/capsules) were procured from Indsaff, India. Methanol and glacial acetic acid were purchased from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Antibiotics were purchased locally.

Maintenance of animals:

The animal experiments were started after obtaining approval from Institutional Animal Ethical Committee. Normal male Swiss albino 4-5 weeks old mice weighing 19-20g were obtained from institutional breeding house which showed a steady increase in weight of up to 28-30 g during the period of study. The Swiss albino mice were divided into groups of 5 animals each. The animals were maintained in animal house under standard conditions (23±2ºC, relative humidity 57±2 %, 12/12 h hiopentone sodium in overdose (100mg kg^{-1} body weight).

Experiments:

For dose dependent studies- Control group (group I) was fed only with normal tap water as drinking water (tested free of As III whose data has not been enclosed) for 22 days. Group II, III and IV were given different doses of As III (50, 250, 500 μg/l) along with drinking water for the entire period of 22 days. The dose 1000 μg/l was also taken but could not be continued due to severe induction of genotoxicity.

Simultaneous treatment for genotoxicity tests: Control group (group I) was administered normal tap water (free of As) for 22 days. Group II was given only As III (500 μg/l) via drinking water for 22 days. Groups III, IV and V were administered a daily dose of 100 μl olive oil containing 0.1, 0.2 and 0.3 mg curcumin respectively (5, 10 and 15 mg curcumin kg^{-1} body weight respectively) by gavage and As III (500 μg/l) as the only drinking agent continuously for 22 days.

Pre treatment for genotoxicity tests: Control group (group I) was given normal tap water as drinking water for 22 days. Group II was given As III free water for first 11 days and As III (500 μg/l) plus water for subsequent 11 days. Groups III, IV and V were given same dose of 100 μl olive oil containing 0.1, 0.2 and 0.3 mg curcumin (5, 10 and 15 mg curcumin kg-1 body weight) daily by gavage and water free of As III for first 11 days. For the next 11 days, the animals were treated in two different sets A and B. Set A was continued with curcumin along with As III (500 μg/l) and in set B curcumin was withdrawn and only As III was continued.

Simultaneous treatment for antioxidant tests: Control group (group I) was administered normal tap water (free of As) for 22 days. Group II was given only As III (500 μg/l) via drinking water for 22 days. Each animals of group III (Simultaneous or S group) was given a daily dose of 15 mg curcumin kg^{-1} body weight (dose was selected on the basis of IC^{50}) by gavage and As III (500 μg/l) as the only drinking agent continuously for 22 days.

Pre treatment for antioxidant tests: Control group (group I) was administered normal tap water (free of As) for 22 days. Group II was given As III free water for first 11 days and As III (500 μg/l) plus water for subsequent 11 days. Animals of group III (Pretreatment or P group) were administered 10 mg curcumin kg^{-1} body weight (dose was selected on the basis of IC^{50}) by gavage and water free of As III for first 11 days. For the next 11 days, the animals were continued with mode A pre-treatment where curcumin 10 mg kg^{-1} body weight was given along with As III (500 μg/l).

Analysis of As in blood samples

Arsenic in blood samples were analyzed by flow injection hydride generation atomic absorption spectroscopy (FI-HG-AAS) after microwave digestion.

Comet Assay

DNA damage (single strand breaks) was measured by single cell gel electrophoresis (SCGE) or comet assay (Singh et al., 1988). Briefly, peripheral blood cells collected from heparinised whole blood of Swiss albino mice (withdrawn from the tail vein), was suspended in 0.6% (w/v) low melting agarose. Subsequently cells were layered over a frosted microscopic slide previously coated with a layer of 0.75% normal melting agarose. The slides were then immersed in a lysis buffer of pH 10 and left overnight. Subsequently slides were transferred into a horizontal electrophoresis chamber containing alkaline solution (300 mM NaOH, 1mM Na_2EDTA; pH 13.0). A pre-soaking of 20 min was done in order to unwind DNA. Electrophoresis was then carried out for 20 min (300 mA, 20 V). Slides were washed thrice with neutralizing

buffer (Tris Buffer 0.4 M, pH 7.5) followed by staining with ethidium bromide (final concentration 40 μg/ml). Finally slides were examined under a Nikon fluorescence microscope and subjected to image analysis using comet assay software programme (CASP). DNA damage was quantitated by tail moment measurement. It was calculated by multiplying the total intensity of the comet tail by the tail length, measured from the centre of the comet head.

In vivo MN assay

Spleenic lymphocytes (SL) were cultured according to the following method (Kim et al., 1997). SL were separated from the spleen of 3 swiss albino / 5 mice (male) on Ficoll Hypaque gradients, washed twice in Hank's balanced salt solution and resuspended in RPMI 1640 medium containing Hepes buffer, 15% heat inactivated foetal bovine serum, L glutamine, 2-mercaptoethanol and antibiotics. The lymphocytes were seeded (5 x 10^5 cells / ml) in 55 mm culture plates with phytohaemagglutinin (PHA; 30 μg/ml) and incubated at 37ºC in a humidified atmosphere containing 5% CO_2. After 48 hr of incubation, all plates were treated with cytochalasin B (5μg / ml) for next 20-22 hr. Lymphocytes were then collected by centrifugation and fixed for 10 min in a mixture of methanol: glacial acetic acid (3:1). Finally, the cells were dropped on moist grease free slide and made air dry. Finally, cells were stained with 10% Giemsa and observed under 40x magnification of light microscope. Genotoxicity was calculated in term of numbers of micronuclei generated per 1000 binucleated cells.

Preparation of homogenate

Animals were euthanised and dissected. The entire liver was perfused immediately with ice cold 0.9% NaCl. It was trimmed free of extraneous tissue rinsed in chilled 0.15 M Tris-HCl buffer (pH 7.4). The liver was blotted dry, weighed quickly and homogenized in 0.15 M Tris-HCl buffer (pH 7.4) to yield a 10% (w/v) homogenate. It was then centrifuged at 3500 rpm for 10 min at 4°C. The pellet represented the nuclear fraction and the supernatant was subjected to centrifugation at 20,h000 rpm for 20 min at 4°C. The resultant pellet and the supernatant represented the mitochondrial fraction and the cytosolic (including microsomal fraction) fraction respectively. ROS generation was observed in all the fractions as well as whole homogenate.

Estimation of protein

The protein concentrations of the plasma samples were measured according to Lowry's method (Lowry et al., 1951).

Determination of intracellular ROS production

Measurement of in vivo ROS generation was done according to Balasubramanyam, 2003 with slight modifications (Balasubramanyam et al., 2003). Tissue homogenate either in whole or in fraction was suspended in HEPES buffered saline (HBS, pH 7.4 containing 140 mM NaCl, 5 mM KCl, 10 mM HEPES, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose) and loaded with 10 μM DCFH-DA to make a final volume of 3 ml. The samples were incubated in dark for 45 min. ROS levels were measured using spectrofluorimeter (Waters, USA 474 Scanning Fluorescence Detector, with an excitation set at 485 nm and emission at 530 nm) as a change in fluorescence because of the conversion of non-fluorescent DCFH-DA to the highly fluorescent compound 2', 7'-dichlorofluorescein (DCF). The non-fluorescent dye passively diffuses into the cells where the acetates are cleaved by intracellular esterases. The resulting diol is retained by the cell membrane and ROS oxidizes this diol to the fluorescent form DCF.

Lipid peroxidation

Lipid peroxidation was estimated according to Varshney et al 1990 as mentioned in our earlier publications (Sinha et al., 2007).

Measurement of protein carbonyl content

Protein carbonyl was estimated by the reaction with 2,4,-dinitrophenylhydrazine (DNPH) which resulted into a Schiff base. This ultimately produces the corresponding hydrazone, which was analyzed spectrophotometrically. The analysis was done according to the manufacturer's protocol (Cayman). For each point two 2.0 ml plastic tubes were taken, of which one was the sample tube (S#) and the other control tube (C#). 800 μl of DNPH was added to S# tube and 800 μl of 2.5M HCl to C# tube. Both S# and C# tubes were incubated in dark for 1 hr with brief vortexing after every 15 min during incubation. 1ml of 20% TCA was added to each tube and vortexed. The tubes were placed on ice and incubated for 5 minutes. The tubes were then centrifuged at 10,000 g for 10 minutes at 4°C. The supernatant was discarded and the pellet was resuspended in 1ml of 10% TCA. The tubes were placed on ice for 5 minutes. Tubes were again centrifuged at 10,000 g for 10min at 4°C. The supernatant was discarded and pellet was resuspended in 1ml of (1:1) ethanol/ethyl acetate mixture. The pellet was manually suspended with spatula, vortexed thoroughly and centrifuged at 10,000 g for 10 min at 4°C. This step was repeated twice more. After the final wash the protein pellets were resuspended in guanidine hydrochloride by vortexing. The tubes were then centrifuged at 10,000 g for 10 min at 4°C. Absorbance was measure at 360-385 nm in a plate reader (TECAN-infinite M200).

Measurement of catalase (CAT) activity

Catalase was assayed according to Aebi et al 1984 as mentioned in our earlier publications (Sinha et al., 2007).

Superoxide dismutase (SOD) analysis

SOD was assayed by the method of Marklund and Marklund, 1974 as mentioned in our earlier publications (Sinha et al., 2007).

Estimation of glutathione reductase (GR)

GR is essential for the GSH redox cycle, which maintains adequate levels of reduced cellular GSH. The assay was done according to kit protocol (Cayman). The background or non enzymatic wells (three) contained 120 μl of assay buffer (50 mM potassium phosphate, pH

7.5, containing 1mM EDTA) and 20 μl GSSG (9.5 mM). Plasma samples if necessary were diluted with sample buffer (50 mM potassium phosphate, pH 7.5, containing 1mM EDTA and 1mg/ml BSA) prior to assay. The sample wells of the 96 well microplate contained 100 μl of assay buffer, 20 μl GSSG and 20 μl of sample in triplicates. The reaction was initiated 50 μl NADPH in all wells. The absorbance was read at 340 nm using a plate reader (TECAN- infinite M200) to obtain at least 5 points.

Estimation of Glutathione S-Transferase (GST)

The assay was done according to kit manufacturer's protocol (Cayman). The background or non enzymatic wells (three) contained 170 μl of assay buffer (100 mM potassium phosphate, pH 6.5, containing 0.1%Triton X-100) and 20 μl GSH (9.5 mM). Plasma samples if necessary were diluted with sample buffer (100 mM potassium phosphate, pH 6.5, containing 0.1%Triton X-100, 1mM GSH and 1mg/ml BSA) prior to assay. 150 μl of assay buffer, 20 μl GSH and 20 μl of sample were added in triplicates in the sample wells of a 96 well microplate. The reaction was initiated 10 μl 1-choloro-2, 4-dinitrobenzene (CDNB) in all wells. The absorbance was read once very minute at 340 nm using a plate reader (TECAN- infinite M200) to obtain at least 5 points.

Estimation of Glutathione Peroxidase (GPx)

The activity of GPx was measured by the procedure described by Paglia and Valentine, 1967 as mentioned in our earlier publications (Sinha et al., 2007).

Estimation of glutathione (GSH)

The assay was done according to kit manufacturer's protocol (Cayman). The plasma samples were first deproteinated. An equal volume of meta phosphoric acid was added to each sample and mixed on a vortex mixture. They were allowed to stand at room temperature for 5 min and centrifuged at > 2000 g for at least 2 mins. The supernatant was collected and stored at -20ºC until further use. Before the assay the samples are treated with 50 μl of a 4 M triethanolamine solution per ml of the supernatant. Eight standards of GSSG (25 μM) and MES buffer (0.4M 2-(N-morpholino) ethanesulphonic acid, 0.1mM phosphate and 2mM EDTA, pH 6.0 diluted with equal volume of water before use) were made which would finally give GSH concentration ranging between 0-16 μM for the standard curve. 50 μl of standards and 50 μl of samples were added in triplicate in the 96 well microplate.150 μl of freshly prepared assay cocktail was added. The assay cocktail was prepared by mixing 11.25 ml MES buffer, 0.45 ml reconstituted co factor mixture (NADP+ and glucose-6-phosphate mixed with 500 μl of water), 2.1 ml of reconstituted enzyme mixture (0.2 ml of glutathione reductase and glucose-6-phosphate dehydrogenase were mixed with 2ml of MES buffer), 2.3 ml water and 0.45 ml reconstituted DTNB (5,5'-dithiobis-2-nitrobenzoic aid, Ellman's reagent). The plate was incubated in dark on an orbital shaker. Absorbance was measured at 414 nm using a plate reader (TECAN- infinite M200) at 5 minutes interval for 30 minutes.

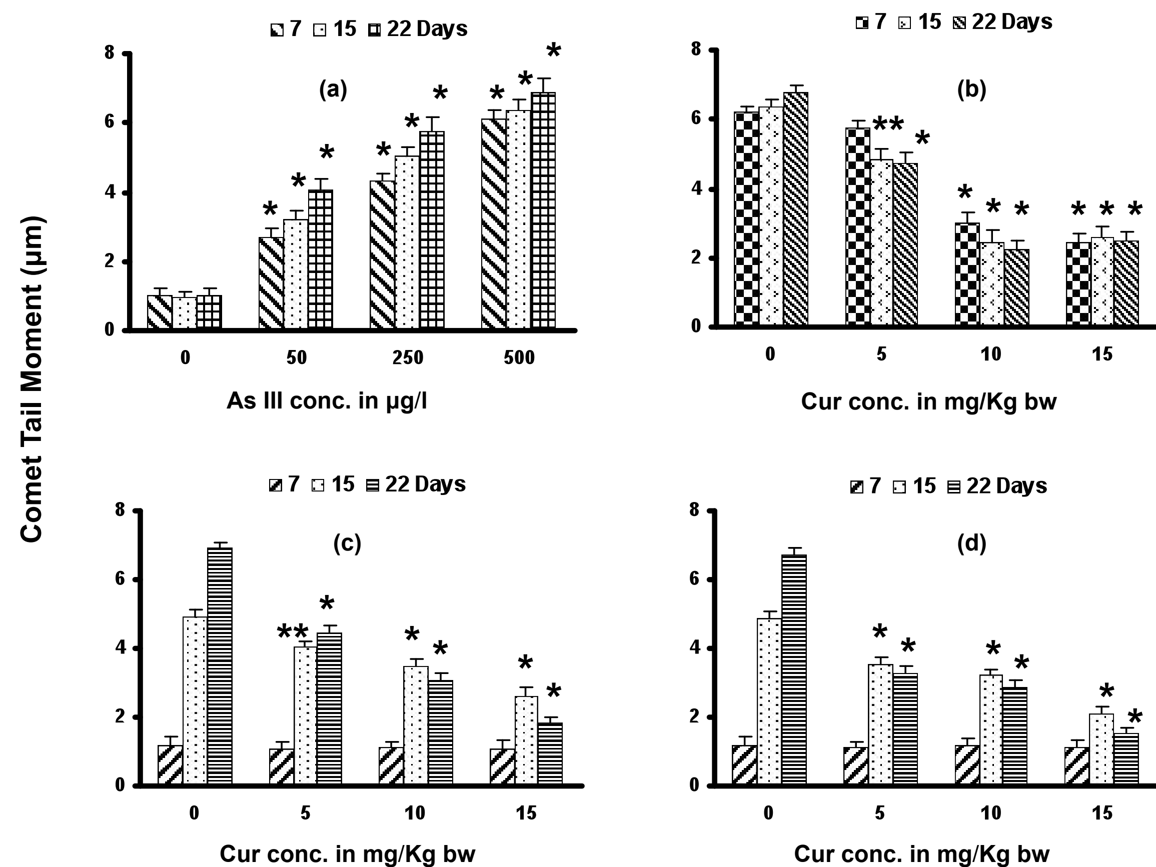

Figure 1. Comet Tail Moment. Dose and time dependent increase in (*p<0.001) in peripheral blood of Swiss albino mice by Arsenic (As) III (a); reduction of arsenic III (500 μM) induced comet tail moment (*p<0.001**p<0.005) during simultaneous treatment with three different concentration of curcumin (5, 10, 15 μg/Kg bw) in a time dependant manner in Swiss albino mice (b); decrease in comet tail moment (*p<0.001-**p<0.005) by pre-treatment treatment [mode A : curcumin was continued along with As III (500 μg/l) for next 11 days] with three increasing concentrations of curcumin in reducing arsenic III (500 μg/l) induced DNA damage on 15th and 22nd day (c) ; decrease in comet tail moment (*p<0.001) by pre-treatment [mode B : curcumin was withdrawn and only As III was continued for next 11 days] in reducing arsenic III (500 μg/l) induced DNA damage on 15th and 22nd day (d).

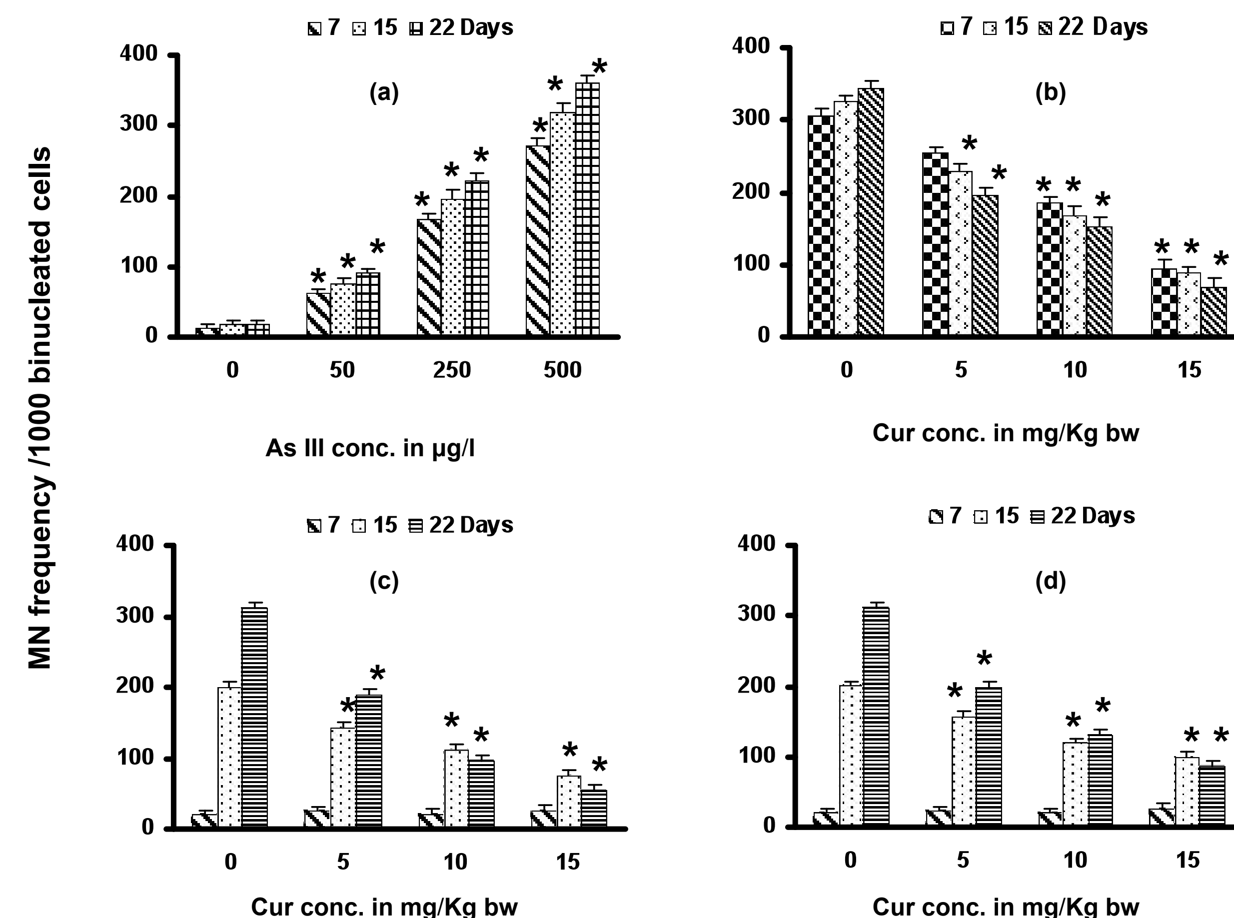

Figure 2. Induction of MN. Frequency /1000 binucleated cells (*p<0.001) in a dose (50, 250 and 500 μg/l of As III) and time (7, 15 and 22 days) dependant manner in comparison to control in Swiss albino mice (a), significant reduction of arsenic III (500 μg/l) induced MN frequency (*p<0.001) during simultaneous treatment with three different concentration of curcumin (5, 10, 15 μg/Kg BW) in a time dependant manner in mice (b), decrease in MN frequency (*p<0.001) by curcumin pre-treatment (mode A) on 15th and 22nd day (c), decrease in MN frequency (*p<0.001) by curcumin pre-treatment (mode B) on 15th and 22nd day (d).

Statistical analysis

Statistical analysis was performed with SPSS 10.0 (one way ANOVA followed by Dunett t-test, where significance level was set at 0.001). ANOVA was performed to account for intra-group variation and t-test for inter group comparisons.

Results

Comet assay (SCGE) and Micronucleus (MN) assay were used for assessment of arsenic (As) induced genotoxicity. It was expressed by comet tail moment and MN frequency/1000 binucleated cells, in a dose and time dependent manner after 22 days of As III exposure of Swiss albino mice. The treatment was not continued beyond the time period mentioned above as no further change in As III induced genotoxicity was observed. Four doses 50μg/l, 250μg/l, 500μg/l, 1000μg/l were selected on the basis of the prevailing range of arsenic found in ground water of West Bengal. The first three doses gave a prominent induction of comet tail moment and high frequency of MN in a time dependent manner (p<0.001) with respect to control (Fig. 1a & 2a) except 1000 μg/l which showed severe genotoxicity with deformed comet pattern. Henceforth subsequent experiments were done with 500 μg/l. Blood sample of mice was analyzed in atomic absorption spectroscopy within 2 hours of water (containing 500 μg As III/l) intake. Data showed that the level of As in 100μl of blood was 15.2 ng. Reversal of As III (500μg/l) induced genotoxicity was studied by simultaneous treatment and pre-treatment with 5, 10 and 15 mg curcumin kg^{-1} body weight (bw) in a time dependent manner. All three doses of curcumin reduced As III (500 μg/l) induced comet tail moment significantly (p<0.001-0.005) during simultaneous treatment (Fig. 1b). Similarly MN frequency was also decreased significantly (p<0.001) by administration of different doses of curcumin (Fig. 2b). Pre-treatment mode A (Fig. 1c, 2c) and mode B (Fig. 1d, 2d) with three doses of curcumin (5, 10 and 15 mg kg^{-1} bw) showed regression of comet tail moment and MN frequency. In pre-treatment mode A, the reduction of comet tail moment with curcumin was observed at the level of p<0.001-0.005 while pre-treatment mode B

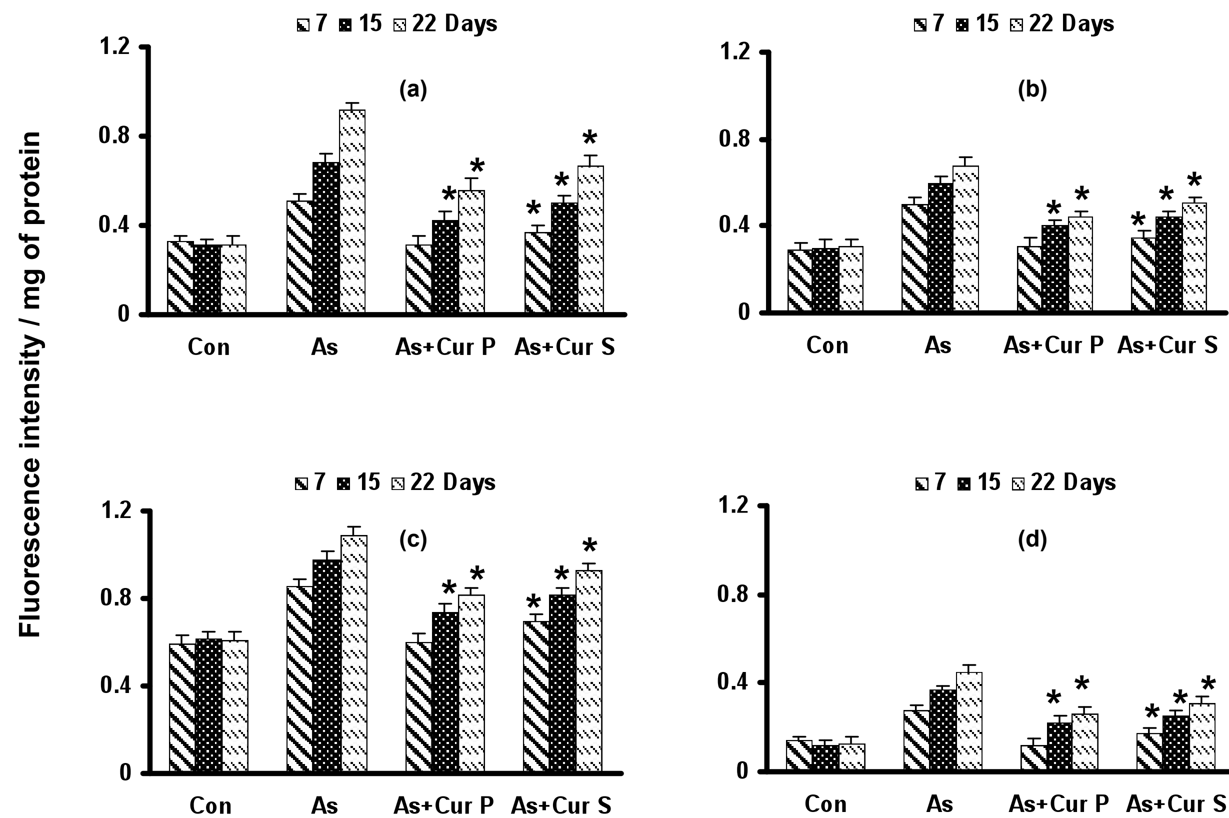

Figure 3. Quenching of As III (500 μg/l) generated ROS. (*p<0.001) in - total liver (a), nuclear fraction (b), mitochondrial fraction (c), cytosolic & microsomal fraction by pretreatment mode B and simultaneous treatment with curcumin (10 μg/Kg BW) (d).

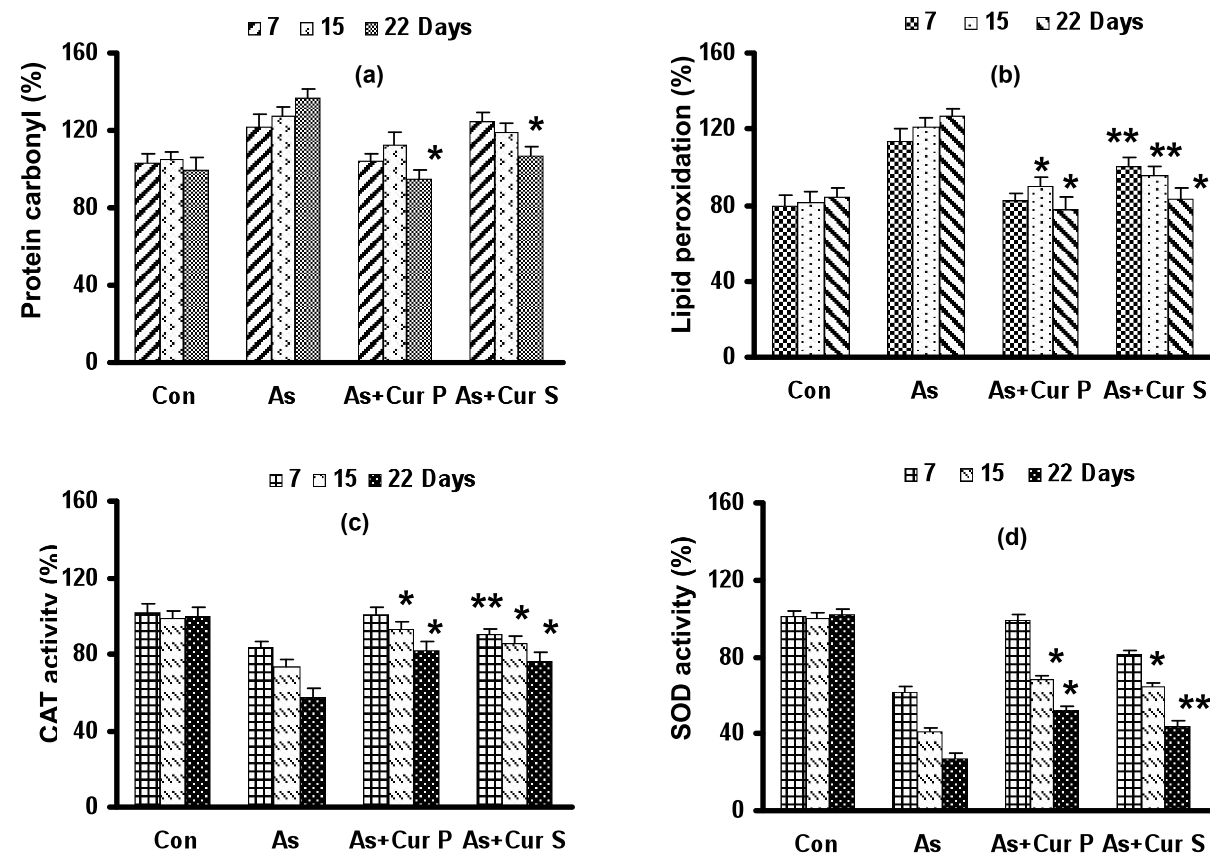

Figure 4. Protein carbonyl and Lipid Peroxidation Findings. Decrease in As III (500 μg / l) induced protein carbonyl (*p<0.001) and lipid peroxidation (*p<0.001) during simultaneous treatment and pretreatment (mode A) with curcumin (a) & (b) respectively. Reduction was significant in case of both simultaneous treatment and pretreatment (mode A) on 22nd day. Enhancement of antioxidant enzymes catalase (**p<0.005*p<0.001) (c) and SOD (**p<0.005*p<0.001) activity (d) which was depleted by As III (500 μg / l) by pretreatment (mode A) with curcumin of concentration 10 μg/Kg BW and simultaneous treatment with curcumin of concentration 15 μg/Kg BW with respect to control

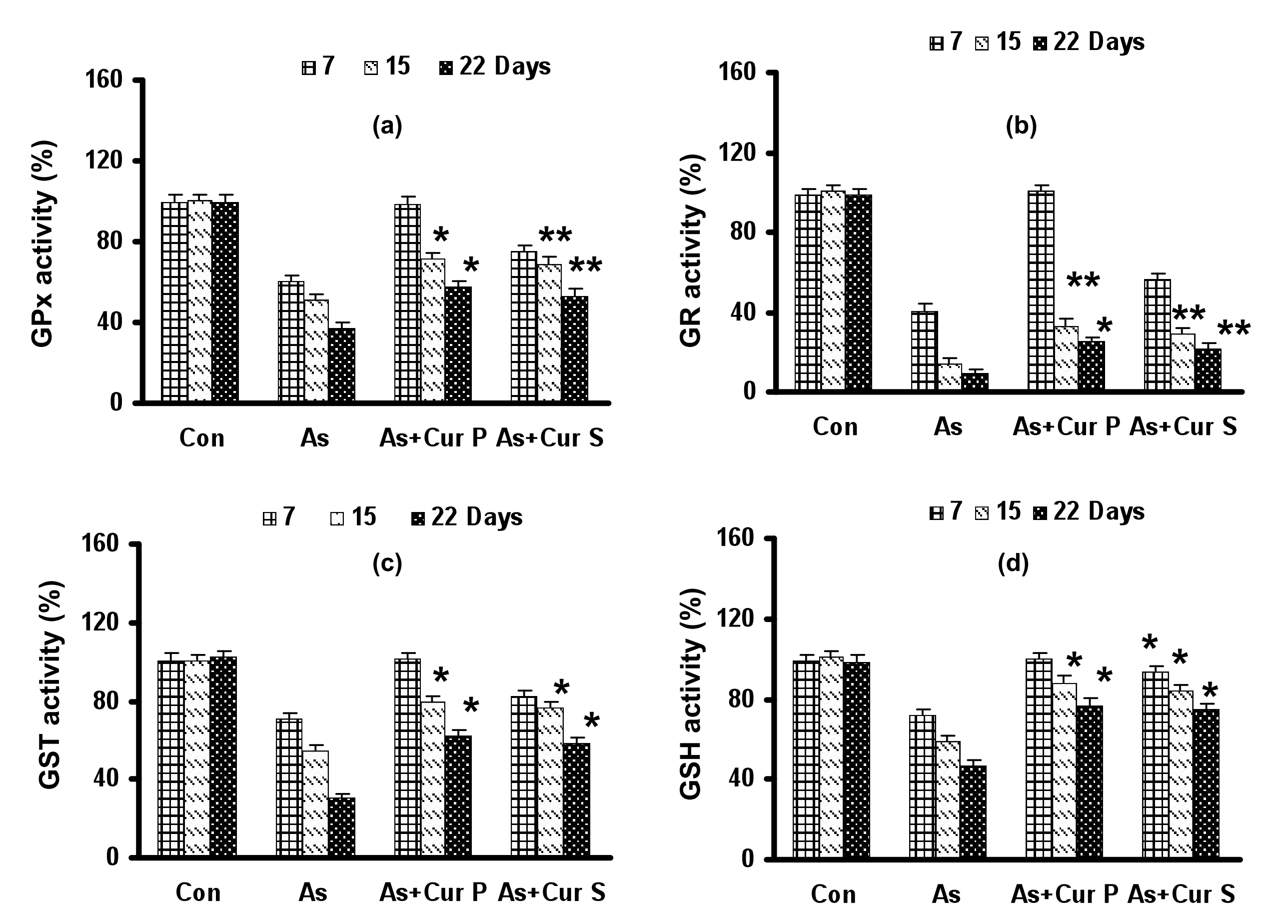

Figure 5. Antioxidant Enzyme Activity. Enhancement of antioxidant enzymes GPx (*p<0.001-**p<0.005) (a), GR (*p<0.001-**p<0.005) (b), GST (*p<0.001) (c) and antioxidant GSH (*p<0.001) (d) activity which was depleted by As III (500 μg / l) by pretreatment (mode A) with curcumin of concentration 10 μg/Kg BW and simultaneous treatment with curcumin of concentration 15 μg/Kg BW with respect to control

reduced comet tail moment at the level of p<0.001 on 15th and 22nd day. MN frequency was also decreased at the level of p<0.001 by pre-treatment mode A and mode B on 15th and 22nd day. As the pre treatment mode A proved more effective than mode B, the subsequent pre treatment experiments were done with the protocol followed for mode A.

The oxidative stress caused by As III was monitored by measuring ROS generation in the liver of Swiss albino mice. In our experiments curcumin was effective in quenching of As III (500 μg/l) generated ROS in all the fractions like total liver homogenate (Fig 3a), nuclear fraction (Fig 3b), mitochondrial fraction (Fig 3c), cytosolic and microsomal fraction (Fig 3d) of the liver tissue during simultaneous and pre-treatment mode of experiment. ROS generation was much more reduced during simultaneous treatment (p>0.001) in a time dependent manner and during pre treatment mode A (p>0.001) experiments in between 15th to 22nd day compared to arsenic exposed group. Our experiment also resulted that protein carbonyl contents (Fig. 4a) which was increased with the increase in days of As III exposure, was reduced only on 22nd day during simultaneous treatment (p<0.001) and pre-treatment mode A (p<0.001). Lipid peroxidation (Fig. 4b) which was high enough in As III treated (500 μg/l) group, was decreased during simultaneous treatment (p<0.005-0.001) in a time dependent manner and during pre-treatment mode A (p<0.001) on 15th and 22nd day. As III exposure caused depletion of antioxidative enzymes like CAT (Fig. 4c), SOD (Fig. 4d), GPx (Fig. 5a), GR (Fig. 5b), GST (Fig. 5c) and antioxidants like GSH (Fig. 5d). The animals of simultaneous treatment group receiving As III (500 μg/l) along with 15 mg curcumin kg^{-1} bw for 22 days showed elevation of antioxidant activities (p<0.005-0.001) than the animals receiving only As III (500 μg/l) for the same period. The pre-treatment group receiving As III free water for first 11 days and As III (500 μg/l) plus water for the next 11 days exhibited diminished antioxidant activities. Elevation of antioxidants such as CAT (p<0.001-0.005), SOD (p<0.001-0.005), GPx (p<0.001-0.005), GR (p<0.001-0.005), GST (p<0.001) and GSH (p<0.001) was monitored during pre-treatment with 10 mg curcumin kg^{-1} bw for first 11 days and As III (500 μg/l) plus 10 mg curcumin kg^{-1} bw for the next 11 days.

Thus curcumin proved to be an effective antioxidant to combat the pro-oxidant effects of As III in Swiss albino mice.

Discussion

Carcinogenesis is a multi-step process that ultimately culminates in cancer and the entire process might span over several years (Ghosh et al., 2007). Chronic arsenic toxicity due to drinking of arsenic contaminated ground water produces cancers in skin, lung, liver and urinary

bladder (Guhamazumdar, 2008). Oxidative stress, chromosomal abnormality and altered growth factors are possible modes of action in arsenic induced carcinogenesis (Yu et al., 2006). In our study we have observed that sodium arsenite (As III) increased generation of ROS, formation of protein carbonyl and lipid peroxidation in the liver of Swiss albino mice. These results were supported by the previous research work where production of ROS in high fluxes caused lipid peroxidation and oxidation of amino acid residues on proteins, forming protein carbonyls (Samuel et al., 2005). Another work revealed that arsenic may cause oxidative damage to erythrocyte membrane by decreasing glutathione content coupled with enhancement of malondialdehyde and protein carbonyl levels (Biswas et al., 2008). We also found that As III caused depletion of antioxidative enzymes and antioxidants which was in agreement with previous study reported by Flora et al in 2007 that exposure to arsenic significantly depleted delta-aminolevulinic acid dehydratase (ALAD) activity, GSH level, glutathione peroxidase (GPx), SOD and CAT activities and increased TBARS levels in liver tissues of mice (Flora and Gupta, 2007).

Researchers have observed that curcumin, the active ingredient of turmeric plant (*Curcuma longa* Linn) has protective effect against cisplatin, hydrocortisone, nicotine, lead, ethanol and irradiation induced damage in in vivo and in in vitro test systems (Ragunathan et al., 2007). It also exerts a genoprotective effect against DNA damage induced by high concentrations of copper cations (Corona-Rivera et al., 2007). In our investigation pre-treatment and simultaneous treatment with curcumin prevented As III (500 μg/l) induced DNA damage by reducing comet tail moment in whole blood and MN frequency in cultured spleenic lymphocytes (SL) of Swiss albino mice. Curcumin retarded generation of streptozotocin induced reactive oxygen species in islets (Meghana K et al., 2007). Our data showed that pre-treatment and simultaneous treatment with curcumin efficiently quenched generation of ROS by As III in all fraction of liver tissue. This protective effect of curcumin is due to its antioxidant action and trapping of free radicals (Ragunathan et al., 2007). According to Sreejayan and Rao the antioxidant properties of curcumin is for the presence of the phenolic hydroxyl and methoxyl groups on the phenyl ring and the structural feature of 1,3-diketone systems (Sreejayan and Rao, 1996, 1997). Curcumin treatment abolished the Cd-induced lipid peroxidation (125% of controls) and GSH depletion (67% of controls) in mice liver (Eybl et al., 2004). We observed that As III (500 μg/l) induced lipid peroxidation, protein carbonyl content and GSH depletion was inhibited by curcumin treatment. According to the literature survey curcumin induced activities of antioxidant enzymes (like glutathione S-transferase, superoxide dismutase and catalase) and the levels of SH-groups in rat's plasma and tissues which was decreased by sodium arsenite (El-Demerdash et al., 2009). There was evidence that curcumin pre-treatment increased the activities of SOD, CAT and GPx significantly along with GSH levels in human lymphocytes against gamma-radiation induced cellular damage (Srinivasan et al., 2006). We obtained that curcumin not only exhibited free radical scavenging properties but also enhanced the activities of other antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-s-transferase (GST).

Present study therefore highlights the efficacy of curcumin in preventing the DNA damage and quenching of ROS induced by As III in Swiss albino mice. As evident from our study, this has been possible because of induction of antioxidant enzymes by curcumin, which were depleted by As III.

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