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Curcumin protects DNA damage in a chronically arsenic-exposed population of West Bengal

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Abstract

Groundwater arsenic contamination has been a health hazard for West Bengal, India. Oxidative stress to DNA is recognized as an underlying mechanism of arsenic carcinogenicity. A phytochemical, curcumin, from turmeric appears to be potent antioxidant and antimutagenic agent. DNA damage prevention with curcumin could be an effective strategy to combat arsenic toxicity. This field trial in Chakdah block of West Bengal evaluated the role of curcumin against the genotoxic effects of arsenic. DNA damage in human lymphocytes was assessed by comet assay and fluorescence-activated DNA unwinding assay. Curcumin was analyzed in blood by high performance liquid chromatography (HPLC). Arsenic induced oxidative stress and elucidation of the antagonistic role of curcumin was done by observation on reactive oxygen species (ROS) generation, lipid peroxidation and protein carbonyl. Antioxidant enzymes like catalase, superoxide dismutase, glutathione reductase, glutathione-S-transferase, glutathione peroxidase and non-enzymatic glutathione were also analyzed. The blood samples of the endemic regions showed severe DNA damage with increased levels of ROS and lipid peroxidation. The antioxidants were found with depleted activity. Three months curcumin intervention reduced the DNA damage, retarded ROS generation and lipid peroxidation and raised the level of antioxidant activity. Thus curcumin may have some protective role against the DNA damage caused by arsenic.

Keywords

arsenic, DNA damage, curcumin, antioxidant, oxidative stress

Introduction

Groundwater arsenic (As) contamination makes it an insidious environmental pollutant, which poses a global threat to human health. The world's two biggest cases of groundwater As contamination that have affected the greatest number of people are from Bangladesh and West Bengal, in India. A total of 42.7 million people in nine districts of West Bengal have been drinking As-contaminated water.[1] Thousands of wells have recorded As concentration in water, ranging from 50–3200 μg/L,[2] which is far above the WHO recommended limit of 10 μg/L.[3] Skin lesions such as hyperkeratosis and hyper pigmentation are hallmarks of chronic As exposure, which develop early after exposure compared with the cancerous outcomes.[4] As is a notorious environmental carcinogen that primarily causes skin, lung, bladder, liver and kidneys cancers,[5] but the effects may even appear after 20 years of exposure.[6] Oxidative stress to DNA is recognized as a mechanism underlying carcinogenic effects of As.[7] Excessive generation of ROS beyond body's antioxidant balance may lead to damage of macromolecules like proteins, lipid and DNA. Increased frequencies of cytogenetic alterations such as chromosomal aberrations, sister chromatid exchanges and micronuclei have been found in

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different in vitro and in vivo test systems.[8] Such genetic imbalance seeds the stepping stone of carcinogenesis.

While the clinical intersession accepts it as a public health problem only after the onset of the disease, interventions based on scientific knowledge for prevention of such environmental calamities may find their application to reduce the misery of the people in the endemic regions. Many plant-derived constituents including turmeric appear to be potent antimutagenic and antioxidants. Curcumin, an active ingredient of turmeric, showed modulatory effects on the levels of benzo[a]pyrene-induced DNA adducts in the livers of rats, by the newly developed [32]P-post labeling assay method.[9] Human clinical trials also indicated that curcumin showed no toxicity when administered at doses of 10 g/day.[10]

The present work was a field study that envisaged to establish a biomonitoring method of determining the level of chronic As exposure in asymptomatic individuals by studying the DNA damage in blood lymphocytes and its consequences, and secondly to use curcumin, a known chemopreventive ingredient of Indian spice turmeric, in providing protection against the toxic effects of arsenic.

The survey was conducted in the Chakdah block of Nadia district in West Bengal. A total of 286 volunteers from five villages of Chakdah block (Chowgaccha, Manpur, Mathpara, Silinda and Darrapur) were recruited under the study. These villages had high levels of arsenic in all the aquifers ranging between 95 and 210 μg/L. The proposed investigation included the measurement of the status of DNA damage by alkaline single cell gel electrophoresis (SCGE) or comet assay and by fluorescence-activated DNA unwinding (FADU) assay in blood lymphocytes. After determining the DNA damage, 50% of the volunteers were randomly selected and prescribed curcumin capsules blended with piperine (20:1) 500 mg twice daily (in capsules) for 3 months and the remaining 50% volunteers were similarly given a placebo. Piperine enhanced the serum concentration, extent of absorption and bioavailability of curcumin in both rats and humans with no adverse effects.[11] In this perspective, piperine was used in combination with curcumin for the present field trial. At the end of each month, it was investigated whether curcumin had any antigenotoxic effect against As, and blood samples from all the 286 volunteers taking part in the project were tested for hematology and liver function tests. Curcumin analysis in blood was done every month by high performance liquid chromatography (HPLC). To have a better insight into the underlying protective effect of curcumin, the antioxidant role of curcumin was also examined. The oxidative stress created by arsenic and elucidation of the antagonistic role of curcumin was done by observation on reactive oxygen species (ROS) generation, lipid peroxidation and protein carbonyl content. Several antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and glutathione (GSH) were also analyzed in this respect.

Prevention of DNA damage with antioxidant natural polyphenols like curcumin may be an effective strategy to combat against As-induced genotoxicity and thereby cancer. The present study was not a clinical trial but a field study to ascertain the possibility of using curcumin as a chemopreventive intervention against the adverse effects of chronic arsenic exposure through groundwater contamination.

Materials and methods

Chemicals

Curcumin [CAS No. 458-37-7], 2-thiobarbituric acid (TBA) [CAS No.504-17-6], 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], 1,1,3,3-tetramethoxy propane (TMP) [CAS No. 102-52-3] were obtained from Sigma-Aldrich St Louis, MO, USA. RPMI-1640 and agarose was obtained from Invitrogen. Kits for estimation of GSH, GR, GST and protein carbonyl were procured from Cayman Chemicals. Trichloroacetic acid (TCA) was procured from Spectrochem India Pvt Ltd, Mumbai, India. Tris HCl, ammonium chloride, mesoinositol, sodium phosphate, potassium phosphate, magnesium chloride, urea, sodium hydroxide, cyclohexane diamine tetra acetate (CDTA), sodium dodecyl sulphate (SDS), glucose, Folin Ciocaltaeu, H_2O_2, N-2-hydroxyethyl-piperazine-N-2-ethane sulphonic acid (HEPES buffer), NADP, reduced GSH, ethylenediaminetetraacetic acid-disodium salt (Na_2EDTA) were purchased from Sisco Research Laboratories Pvt Ltd, Mumbai, India. β-mercaptoethanol was purchased from Loba Chemie, Mumbai, India. Curcumin capsules (Trade name, Cur Plus) were procured from Indsaff, India.

Analysis of As in water samples and blood samples

Water samples and blood samples collected from the endemic villages was analyzed for their As level by flow injection hydride generation atomic absorption spectroscopy (FI-HG-AAS) after microwave digestion.

Selection of volunteers

Before commencement of project, human ethical committee clearance was obtained for carrying out the project work. A written informed consent form was obtained from each of the volunteers prior to their recruitment in the field study. The volunteers selected for the field trial were nonsmoker males or females aged 25–55 years, with no chronic disease, no external manifestation of arsenic toxicity, or abnormal blood report.

Sample collection

Blood samples collected were divided into two parts; one part was collected in EDTA vials and the other part in non-EDTA vials. The EDTA-containing samples were analyzed for DNA damage, ROS and cellular antioxidant defence mechanism. The EDTA-free blood was used for biochemical analysis.

Lymphocyte isolation and maintenance

Lymphocytes were isolated and maintained using the usual protocol.[12]

Analysis of curcumin in blood samples

The sample preparation and analysis of curcumin in blood plasma was done with slight modifications of the method developed by Heath et al. in 2003.[13]

Standard curcumin stock was prepared by dissolving 5 mg of curcumin powder in 25 mL of methanol to obtain a final concentration of 200 μg/mL. During sample preparation, a total of six independent plasma samples (each 200 μL) were taken and each was spiked with varying amounts of curcumin from previously prepared stock (200 μg/mL) to produce final curcumin concentration of 2 μg/mL to 10 μg/mL. To each sample, 80 μL deionized, HPLC-grade water was added and the volume was made up with HPLC-grade methanol to a total volume of 520 μL. Unknown samples (each 200 μL) were taken in two sets, one with spiking (external addition) with known concentration of curcumin prepared from stock and other without spiking

Extraction reagent, that is 95% ethyl acetate/5% methanol (500 μL), was added to each tube, followed by centrifugation at 13,500 rpm for 5 min. After centrifugation, the upper organic layer (500 μL) was carefully removed into clean microcentrifuge tubes. This organic layer was dried in a speed vacuum dryer (Speed Vac, SC 110, Savant). The extracted dried product was resuspended in 200 μL of prepared mobile phase reagent. The tubes were vortexed at medium speed for 30 sec and left at room temperature in dark for at least 10 min. After a repeat vortexing, the contents were transferred to an injection sample vial (180 μL) for HPLC assay.

HPLC analytical run

The HPLC system consisted of a 515 pump (Waters, USA), a waters 996 photo diode array (PDA). The Waters Millennium[32] chromatographic software (version 3.2) was utilized for integration and processing of the results (Milford, MA, USA). Curcumin in plasma was quantified by isocratic HPLC method using PDA detector at 425 nm. Chromatographic separation was accomplished using Novapak 3.9 × 150 mm, 5 μm C_{18} (Milford) column attached with a C_{18} (Milford) guard column. An aliquot (20 μL) was injected onto a reverse-phase column and eluted with a mobile phase containing a mixture of acetonitrile-methanol-water-acetic acid (41:23:36:1, v/v/v/v). Flow rate of mobile phase was 1.0 mL/min. The quantitation of curcumin is based on a standard curve obtained by spiking six independent plasma samples (200 μL each) with varying amounts of previously prepared curcumin stock solution (200 μg/mL) to produce final curcumin concentration ranging from 2 μg/mL to 10 μg/mL. Sample preparation for the standards were similar to that mentioned for the unknown sample.

SCGE or comet assay

As-induced DNA single strand breaks were assessed by comet assay or SCGE following the method of Singh,[14] with minor modifications. Cells (1×10^4) suspended in 0.6% (w/v) low-melting agarose were layered over a frosted microscopic slide previously coated with a layer of 0.75% normal-melting agarose. Subsequently, slides were immersed in a lysis buffer [NaCl (2.5 M), Na_2EDTA (0.1 M), Tris (10 mM), NaOH (0.3 M), Triton X-100 (1%) and DMSO

(10%) in a solution of pH 10] and left overnight for lysis of cell membrane and nuclear membrane. Next day, slides were presoaked in electrophoresis buffer (300 mM NaOH, I mM Na_2EDTA; pH 13.0) for 20 min in order to unwind DNA and subsequently followed by electrophoresis for 20 min (300 mA, 20 V). Slides were then washed thrice with neutralizing buffer (Tris 0.4 M, pH 7.5), stained with ethidium bromide (final concentration 40 μg/mL) and examined under a Nikon fluorescence microscope. The cells were subjected to image analysis using Comet Assay Software Program (CASP). DNA damage was quantified by tail moment measurement.

Fluorescence activated DNA unwinding

The method detects single and double-strand breaks by their effect on the rate of DNA unwinding in alkali, monitored by the fluorescence intensity of an intercalating dye.[15] A measured amount of isolated lymphocytes were lysed in 0.25 M meso-inositol, 1 mM $MgCl_2$ and 10 mM Na_2PO_4/NaH_2PO_4 (pH 7.2) followed by treatment with denaturation buffer, 9 M Urea/10 mM NaOH/25 mM CDTA (trans-1, 2-diaminocyclohexane-N,N,N′, N′–tetra-acetic acid)/0.1% SDS. All the reactions were done at 4°C. The partial alkaline DNA unwinding was activated by addition of alkali solution (200 mM NaOH, 40% denaturation buffer) for 40 min at 15°C and was stopped by the addition of neutralization solution (1 M glucose/15 mM 2-βmercaptoethanol). The remaining double-stranded DNA was then stained by addition of intercalating dye ethidium bromide (diluted 1:25000 in 13 mM NaOH) and incubation at room temperature. Fluorescence was measured at I_{ex} – 520 nm and I_{em} – 590 nm using a fluorescence spectrophotometer (Varian CARY Eclipse). Fluorescence intensity was inversely proportional to the number of DNA strand breaks present at the time of lysis.

Biochemical analysis

Hematological assessment. During the study period with curcumin, blood was subjected to hematological analysis. Total RBC, WBC count, hemoglobin content and platelet count was performed from the blood smear after Leishman's staining.

Liver and kidney function test. Alkaline phosphatase, serum glutamate-oxaloacetate transaminase (SGOT) and serum glutamate-pyruvate transaminase (SGPT) and bilirubin for liver function, and urea and creatinine for kidney function were estimated using commercially available kits by autoanalyzer.

Determination of intracellular ROS production

Measurement of intracellular ROS production was carried out according to Balasubramanyam et al.[16] Lymphocytes were loaded with 10 μM DCFH-DA 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) in the cells. Cells were 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 the dye prior to each experiment. The non-fluorescent dye passively diffused into the cells where the acetates were cleaved by intracellular esterases. The resulting diol was retained by the cell membrane. ROS oxidized this diol to the fluorescent form DCF.

Collection of plasma from blood

Blood samples with anticoagulant EDTA were centrifuged at 1000*g* for 10 min at 4°C. The top yellow plasma was aspirated without disturbing the white buffy layer. This plasma sample was used for lipid peroxidation, protein carbonyl, enzyme assays and total GSH estimation.

Estimation of protein

The protein concentrations of the plasma samples were done according to Lowry's method.[17]

Lipid peroxidation

Lipid peroxidation was analyzed by the method of Ohkawa et al.[18] The reaction mixture in a final volume of 3.0 mL contained the cell lysate, 100 μL of 10% SDS, 600 μL of 20% glacial acetic acid, 600 μL of 0.8% TBA and water. The mixture was placed in a boiling water bath for 1 hour and immediately shifted to crushed ice bath for 10 min. The mixture was centrifuged at 2500*g* for 10 min. The amount of thiobarbituric acid reactive substances (TBARS) formed was assayed by measuring the optical density of the supernatant at 535 nm against a blank devoid of the cell lysate. The activity was expressed as nmoles

of TBARS/mg of protein using 1,1,3,3,-tetramethoxypropane (TMP) as standard.

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 2.5 M HCl to C# tube. Both S# and C# tubes were incubated in dark for 1 hour, with brief vortexing after every 15 min during incubation. One milliliter of 20% TCA was added to each tube and vortexed. The tubes were placed on ice and incubated for 5 min. The tubes were then centrifuged at 10,000*g* for 10 min at 4°C. The supernatant was discarded and the pellet was resuspended in 1 mL of 10% TCA. The tubes were placed on ice for 5 min. Tubes were again centrifuged at 10,000*g* for 10 min at 4°C. The supernatant was discarded and pellet was resuspended in 1 mL 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 CAT activity

CAT was assayed according to Aebi et al.[19] as mentioned in our earlier publications.[12]

SOD analysis

SOD was assayed by the method of Marklund and Marklund,[20] with slight modifications as mentioned in our earlier publications.[12]

Estimation of 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 1 mM 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 1 mM EDTA and 1 mg/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 five points.

Estimation of 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, 1 mM GSH and 1 mg/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 by 10 µL 1-choloro-2, 4-dinitrobenzene (CDNB) in all wells. The absorbance was read once every minute at 340 nm using a plate reader (TECAN-infinite M200) to obtain at least 5 points.

Estimation of GPx

The activity of GPx was measured by the procedure described by Paglia and Valentine,[21] as mentioned in our earlier publications.[12]

Estimation of 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 –20°C 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, 0.1 m phosphate and 2 mM EDTA, pH 6.0 diluted with equal volume of water before use) were made which would finally give GSH concentration ranging between 0 and 16 µM for the standard curve. In the 96-well microplate, 50 µL

Table 1. Demographic details of selected villages in Chakdah Block of Nadia district in West Bengal, India

		Population		No. of volunteers recruited		As level (μg/L)	
Village code	Village	♂	♀	♂	♀	In water	In blood
1	Chowgaccha	112	116	18	34	95 ± 10	7.23 ±2.25
2	Manpur	153	128	14	38	210 ± 12	9.50 ± 2.37
3	Mathpara	153	126	16	33	175 ± 15	14.00 ± 2.53
4	Silinda	245	215	39	28	120 ± 11	12.60 ± 2.32
5	Darrapur	335	324	34	32	100 ± 13	14.00 ± 1.73

of standards and 50 μL of samples were added in triplicate; 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 cofactor mixture ($NADP^+$ and glucose-6-phosphate mixed with 500 μL of water), 2.1 mL of reconstituted enzyme mixture (0.2 mL of GR and glucose-6-phosphate dehydrogenase were mixed with 2 mL 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 min interval for 30 min.

Statistical analysis

Statistical analysis was performed with SPSS 10.0 (one-way ANOVA followed by Dunett *t* test, where significance level was set at .001). Dunett *t* test treats one group as a control and treats all other groups against it.

Results

Chakdah block of Nadia District in West Bengal is situated about 160 Km from Kolkata. This block has 30 villages. Five adjacent villages namely Chowgaccha, Manpur, Mathpara, Silinda and Darappur were selected after demographic survey and water analysis revealed that all the villages had high levels of As in the groundwater, ranging between 95 and 210 μg/L .The As level in water of each village is the average value of 10 wells/hand pumps found in that area. The As level in blood represented here is an average of the volunteers recruited in each village. The values have been represented along with the standard deviations. The demographic details of the villages have been shown in Table 1. The subsequent data presented here, in different sections are the average values recorded in all the villages surveyed.

Determination of the basal level of DNA damage was done by recruitment of 50 volunteers residing in Kolkata and 50 volunteers from sub-urban areas around Kolkata (control population) who were drinking As-free water. The water of these areas was tested free of arsenic (data not shown). The average values obtained from FADU and comet analysis of the control population were used for comparison with values of the affected individuals. The data clearly showed that the control values of FADU and comet did not show any significant difference either in presence or absence of curcumin (Figure 1a and b). A total population of 286 people was surveyed in all the 5 villages. The As level detected in the blood samples collected ranged between 7.23 and 14.0 μg/L (normal As level in blood defined as <0.7 μg/L as per Agency for Toxic Substances and Disease Registry[22]), whose details have been given in Table 1. These subjects exhibited extensive DNA damage ($p < .001$) of blood lymphocytes with respect to control population during the first 3 months in absence of curcumin. This was evident from the low percentage of double-stranded DNA (D%) as measured by FADU (Figure 1a) and very high comet tail moment values as observed in SCGE (Figure 1b). The mean difference of DNA damage during the first 3 months without curcumin was not significant ($p > .05$). Fifty percent of the population that is 143 people was randomly selected and curcumin intervention was started after the third month. Curcumin with piperine (20:1) at a dose of 2×500 mg/day was given for 3 months. With regular monitoring at the end of each month, it was observed that there was a remarkable decrease in DNA damage, which was evident from the increase in D% values and reduction of comet tail moment as represented in Figure 1a and b, respectively. In all the villages, the rest 50% of the population was given placebo, who did not show any change ($p > .05$) in DNA damage during the study period. The comparative profile of DNA damage with and without curcumin as measured

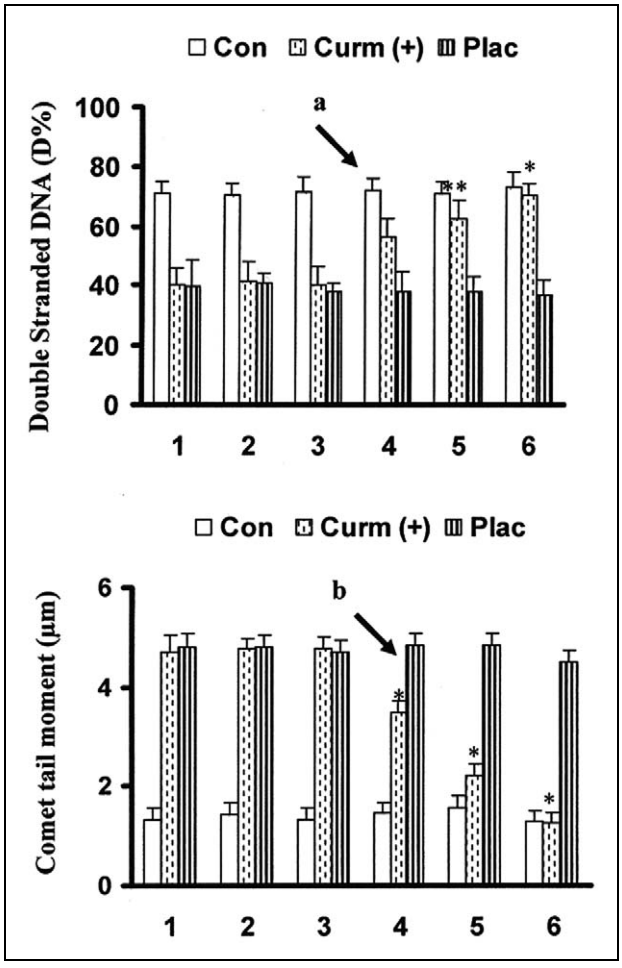

Figure 1. Comparative status of DNA damage - in a control population ($n = 100$) exposed to arsenic-free water, in chronic arsenic-exposed population ($n = 143$) with curcumin intervention for 3 months and in a chronic arsenic exposed population ($n = 143$) with administration of placebo for 3 months. Fluorescence-activated DNA unwinding (FADU) analysis revealed that the population receiving curcumin after 3 months showed significant increase ($^{*}p < .001$ to $^{**}p < .005$) in double-stranded DNA (D%) with respect to population receiving placebo (a). Comet assay exhibited that the population receiving curcumin after 3 months showed significant decrease ($^{*}p < .001$) in comet tail moment with respect to population receiving placebo (b). The arrows represented the beginning of curcumin or placebo administration.

by FADU and comet assay has been shown in (Figure 1a and b, respectively). During the fifth and sixth months, FADU analysis exhibited a significant increase in D% ($p < .001$–$.005$) with respect to placebo, whereas the comet pattern during the fourth, fifth and sixth months revealed a significant decrease of As-induced comet tail moment in presence of curcumin than placebo ($p < .001$).

During analysis of curcumin by HPLC, a basal level was obtained which was the average of the amount of curcumin obtained in the blood plasma (0.049μg/mL) before administration of curcumin without spiking. But the peak obtained for the basal level almost merged with the baseline noise. Therefore, for proper identification of the peak, the samples were spiked with a known concentration of curcumin. The analysis of the blood samples exhibited that the amount of curcumin obtained in the blood plasma increased after the first month and maintained a consistent level during the subsequent months of curcumin administration (Table 2).

The chronic exposure of As had created an oxidative stress in the individuals residing in the endemic areas, which was evident from the increase in ROS generation in comparison to the control population. ROS generation exhibited no significant changes in the control population receiving curcumin as well as on the individuals receiving placebo ($p > .05$). In contrast, the chronic As-exposed population receiving the curcumin administration for 3 months revealed a sharp quenching of ROS generation ($p < .001$) with respect to placebo receiving population (Figure 2a). High levels of lipid peroxidation and protein carbonyl formation was observed in the blood plasma of As-exposed population than control population ($p < .001$). Placebo showed effect neither on lipid peroxidation nor on protein carbonyl reduction ($p > .05$) (Figure 2b and c, respectively). Curcumin administration was successful in bringing down the levels of lipid peroxidation ($p < .001$) with respect to placebo, during the total period of intervention (Figure 2b). On the contrary, the protective effect of curcumin was not very significant against protein carbonyl (Figure 2c). The data depicted that only during 6 months, curcumin brought reduction of protein carbonyl, which was significant at $p < .005$ level with respect to placebo.

The chronic exposure of As was found to significantly deplete the activity of the antioxidant enzymes like CAT ($p < .001$), SOD ($p < .001$), GST ($p < .001$), GR ($p < .001$) and GPx ($p < .005$) as well as non-enzymatic antioxidants like GSH ($p < .005$) in the blood plasma of the exposed population in comparison to the normal level maintained in the blood. A 3-month curcumin administration at the dose stated above was found to induce the activity of both the enzymatic and non-enzymatic antioxidants. Significant induction of CAT ($p < .001$) and GSH ($p < .005$) was observed with curcumin during the total period of intervention (Figure 3a and 3c respectively).

Table 2. Curcumin level in blood plasma as detected by HPLC analysis

Village code	Village	Number of individuals (n)	Curcumin level in blood plasma (μg/mL) at months after curcumin administration		
			1	2	3
1	Chowgaccha	26	2.45 ± 0.16	2.67 ± 0.32	2.67 ± 0.34
2	Manpur	26	2.65 ± 0.04	2.56 ± 0.22	2.61 ± 0.43
3	Mathpara	25	2.49 ± 0.23	2.59 ± 0.12	2.76 ± 0.52
4	Silinda	34	2.72 ± 0.26	2.92 ± 0.12	2.78 ± 0.14
5	Darrapur	32	3.10 ± 0.08	2.74 ± 0.26	2.89 ± 0.38

SOD was significantly induced during 5th and 6th months of curcumin trial (Figure 3b). Figures 4a and 4b showed induction of GR ($p < .001$) and GST ($p < .001$–$.005$) during the total period of intervention whereas GPx was induced ($p < .005$) during 6th month of curcumin administration (Figure 4c).

The kidney and liver function tests as well as the hematological reports revealed that there was no abnormality recorded on the various parameters examined (Tables 3 and 4, respectively). Three months curcumin trial on individuals also did not display any significant change ($p > .05$; Tables 3 and 4).

Discussion

As contamination of drinking water is a public health issue worldwide and some of the worst effects of this environmental calamity has been reported from West Bengal, India. Environmental exposure to As is mainly in the form of arsenite (As III) and arsenate (As V) of which the former is more toxic.[23]

Body's endogenous antioxidant defense mechanism maintains equilibrium with production of free radicals or ROS. Overproduction of ROS can disturb this balance and result into oxidative stress, which in turn causes damage to important cellular components like proteins, DNA and membrane lipids. In the recent years, increasing experimental and clinical data has provided compelling evidences for the involvement of oxidative stress in large number of pathological states including carcinogenesis.[24] In mammals, As stimulated generation of ROS which in turn damaged proteins, lipids and DNA and probably is the direct cause of As-related carcinogenicity.[25] Inorganic As is one of the earliest known carcinogens. Although skin cancers are most frequent, strong epidemiological association exists between inorganic As ingestion and bladder, lung, kidney and liver cancers.[26]

Chromosomal instability is the basis of initiation of carcinogenesis. Various researchers have reported that frequencies of chromosomal aberrations, micronuclei and sister chromatid exchanges increase in human population chronically exposed to arsenic.[27-29] As III induced a dose-dependent increase in superoxide-driven hydroxyl radical production, which mediated its genotoxic activity.[25] Pi et al.[30] in 2002 observed that Chinese residents with high exposure of groundwater As contamination (400 μg/L) had significantly higher serum lipid peroxide levels than a control group drinking lesser As (20 μg/L). Arsenic-induced oxidative stress in growing pigs involved lipid peroxidation, depletion of GSH and decreased activities of some enzymes, such as SOD, CAT, GPx, GR and GST, which are associated with free radical metabolism.[31] As in blood was not only associated with an increased level of reactive oxygen radicals but was also inversely related to the antioxidant capacity in plasma of humans.[32]

The present field work was observed on a comparative basis with three different groups – control population drinking As free water ($n = 100$), chronically arsenic exposed population receiving curcumin ($n = 143$) and chronically arsenic exposed population receiving placebo ($n = 143$). It elicited that people residing in high endemic regions of Chakdah block of West Bengal, who were apparently without any clinical symptoms of chronic As exposure, had severe DNA damage in their blood lymphocytes compared to the control population. Blood plasma of the As-exposed population also manifested over production of ROS, increased lipid peroxidation and protein carbonyl formation. This excessive generation of ROS due to As might be related with the DNA damage, lipid peroxidation levels and protein carbonyl formation. Consistent to the findings of other researchers, it was found that chronic As exposure caused depletion of GSH and other antioxidant enzymes like CAT, SOD, GPx, GR, GST in the blood plasma of the exposed individuals.

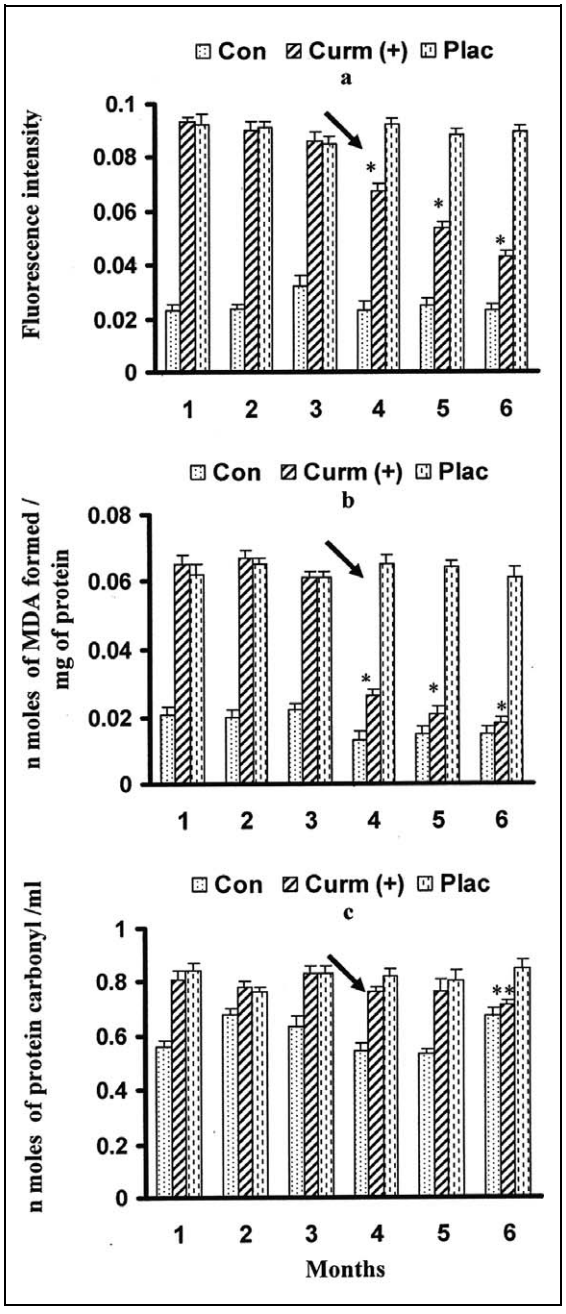

Figure 2. Comparative profile of reactive oxygen species (ROS) generation, lipid peroxidation and protein carbonyl formation in control group ($n = 100$), chronic arsenic-exposed population ($n = 143$) with curcumin intervention for 3 months and in a chronic arsenic-exposed population ($n = 143$) with placebo administration for 3 months. Significant quenching of ROS generation ($^{*}p < .001$) and inhibition of lipid peroxidation ($^{*}p < .001$) was observed among the individuals receiving curcumin than the placebo recipients (a and b, respectively) during the same period of trial. Curcumin showed significant effect ($^{**}p < .005$) on protein carbonyl formation with respect to placebo only during sixth month (c). The arrows represented the beginning of curcumin or placebo administration.

Table 3. Liver and kidney function reports of chronic As exposed population

Liver and kidney function tests	Normal range	Average values of the five villages
Urea	13–45 mg/dL	23.00 ± 4 mg/dL
Creatinine	0.7–1.5 mg/dL	0.86 ± 0.3 mg/dL
Alk. phosphatase	65–306 IU/L	165.28 ± 21 IU/L
Bilirubin	<1mg	0.72 ± 0.23 mg
SGOT	1–40 IU/L	26.64 ± 7 IU/L
SGPT	1–40 IU/L	22.36 ± 5 IU/L
Total protein	6.5–8.5 g/dL	7.10 ± 0.27 g/dL
Albumin	3.5–5.3 g/dL	4.34 ± 0.69 g/dL

Table 4. Hematological reports of chronic As-exposed population

Hematological analyses	Normal range	Average values of the five villages
WBC	4000–11000 cmm	7650 ± 356
RBC	4–6 million/μL	4.22 ± 1.32
Hb	12–15 gm %	12.45 ± 3.67
Platelet	1.5–4 lacs/cmm	1.51 ± 1.57
Lymphocytes	20%–40%	31.60 ± 6.00
Monocytes	2%–10%	2.00 ± 1.23
Neutrophil	40%–80%	58.63 ± 12.00
Eosinophil	1%–6%	3.80 ± 2.10
Basophil	1–2%	1.00 ± 0.23

Curcumin (diferuloylmethane) is derived from the rhizome part of the plant *Curcuma longa*, commonly called turmeric. Extensive research has shown that it can be used both as a chemopreventive and chemotherapeutic agent. In several systems, curcumin exhibited antioxidant and anti-inflammatory properties. Evidence suggests that curcumin can suppress tumor initiation, promotion and metastasis.[10] Due to polyphenolic structure and β-diketone functional group, curcumin is able to scavenge or neutralize free radicals by interacting with oxidative cascade, quench oxygen and chelate some metal ions inhibit peroxidation of membrane lipids thereby maintaining membrane integrity and their function.[33] Curcumin treatment decreased the frequencies of micronuclei and dicentric aberrations, reduced levels of TBARS and increased activities of SOD, CAT, GPx along with GSH levels in human lymphocytes damaged with gamma radiation.[34] Dietary supplementation of curcumin enhanced the activities of antioxidant and phase II metabolizing enzymes in mice.[35]

Curcumin remains in the body for 12 hours. Piperine has been reported as a bioavailability

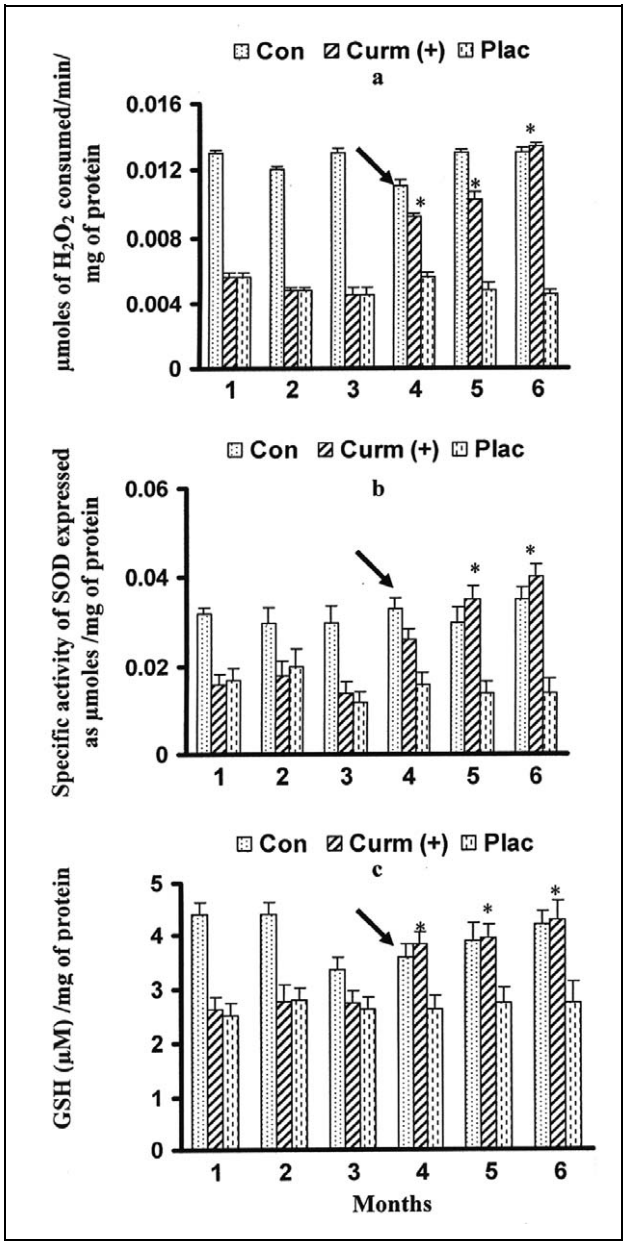

Figure 3. Comparison of antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD) and non-enzymatic antioxidant like glutathione (GSH) in control group ($n = 100$), chronic arsenic-exposed population ($n = 143$) with curcumin intervention for 3 months and in a chronic arsenic-exposed population ($n = 143$) with administration of placebo for 3 months. CAT, which was depleted due to chronic arsenic exposure, was significantly induced by curcumin ($^*p < .001$) during 3 months of the intervention with respect to placebo (a). SOD was also significantly induced during fifth and sixth months of curcumin intervention ($^*p < .001$) in comparison to placebo (b). GSH was significantly induced by curcumin during all the 3 months at $^*p < .001$ level with respect to placebo (c). The arrows represented the beginning of curcumin or placebo administration.

enhancer of curcumin during stress-induced behavioral, biochemical and neurological changes in rats.[36] Considering the above statistics, a dose of curcumin combined with piperine (20:1), 500 mg twice daily, was fixed for the volunteers for 3 consecutive months. Biomonitoring of the populations receiving curcumin as well as placebo revealed the antigenotoxic and antioxidant property of curcumin against the DNA damage and oxidative stress created by chronic As intoxication. The 3-month intervention with curcumin showed that there was a significant reduction in DNA damage when monitored at the end of each month. The ROS generation was effectively quenched in presence of curcumin, which in turn had a clear effect on the reduction of lipid peroxidation. However, curcumin did not have any significant effect on protein carbonyl. Curcumin induced significant enhancement in the activities of different antioxidant enzymes CAT, SOD, GPx, GR, GST along with GSH. GSH has a pivotal role in free radical scavenging and serves as a co-factor for several enzymes involved in overall antioxidant defense.[37] GSTs provide general protection against electrophilic xenobiotics, toxic metals and peroxides not only via conjugation or reduction with GSH but also by alleviating the oxidative stress and subsequent lipid peroxidation often associated with exposure to xenobiotics.[38] The induction of CAT and SOD enzymes by curcumin may have depleted the superoxide and hydroxyl ions, the main ROS involved in As-induced DNA damage. The effective quenching of ROS generation and overall impact in having a well-balanced antioxidant defense mechanism might have also been due to enhanced activities of GSH and GST induced by curcumin.

The HPLC analysis of the blood samples exhibited that the amount of curcumin obtained in the blood plasma increased after the first month and maintained a consistent level during the subsequent months of curcumin administration. Thus the attenuation of oxidative stress related to DNA damage could be partially explained with rise in curcumin level in blood samples.

The five villages surveyed in the Chakdah block of Nadia district in West Bengal showed that the population residing in this As-contaminated area had high risk of DNA damage and depleted antioxidant capacity of blood when compared to control population drinking As-free water. The comparison of the populations receiving curcumin and placebo established that curcumin had an effective role in regression of DNA damage and as an excellent antioxidant agent

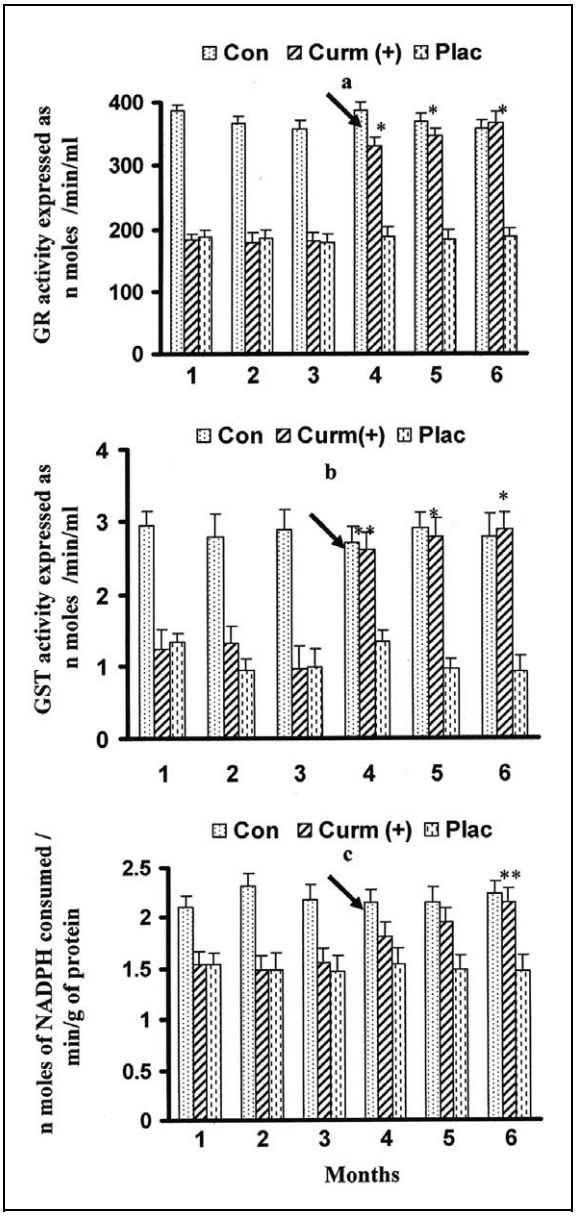

Figure 4. Comparison of antioxidant enzymes like, glutathione reductase (GR), glutathione-S-transferase (GST) and glutathione peroxidase (GPx) in control group ($n = 100$), chronic arsenic-exposed population ($n = 143$) with curcumin intervention for 3 months and in a chronic arsenic-exposed population ($n = 143$) with administration of placebo for 3 months. GR, which was depleted due to chronic arsenic exposure, was significantly induced by curcumin (*$p < .001$) during the total period of the intervention with respect to placebo (a). GST was also significantly induced after curcumin intervention (*$p < .001$ to **$p < .005$) during the fourth, fifth and sixth months in comparison to placebo (b). Significant induction of GSH (**$p < .005$) was observed during the sixth month of curcumin administration in comparison to placebo (c). The arrows represented the beginning of curcumin or placebo administration.

against the As-induced oxidative stress. Thus curcumin by its virtue of antioxidant property may protect the As-affected human population from undergoing genetic imbalance.

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