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 sulphhydrlys 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
ischemia, increased the activities of antioxidant enzymes and decreased the level of lipid peroxide (Manikanand et al., 2004). Curcumin-pretreatment restored increased levels of Fe-NTA induced oxidative damage on DNA. Curcumin protects lipid and induced 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], histioplasca 1077, ethidium bromide [CAS No. 1234-45-4], [dithiothreitol (DTT)] 100 [CAS No.9002-93-1], 2,7 dichloroflourescein diacetate (DCFH-DA) [CAS No.2044-85-1]. Heps buffer solution [CAS No.7365-45-9], cytosinclusin [CAS No.1430-96-2] were obtained from Sigma-Aldrich, St Louis, MO, USA, Agrose, RPMI-1640, Hank’s balanced salt solution [Cas No.24200117]. L glutamine [Cat. No.25031-019], heat inactivated foetal bovine serum [Cat No. 2013-04] were obtained from Invitrogen, Mumbai, India, 2-mercaptoethanol was purchased from Loba Chemie, Mumbai. Giemsa’s solution was procured from Merck, Mumbai. Curcumin capsules (2 mg/capsule) were procured from DFNT Ltd, Mumbai. Sodium and trichloroacetic acid (TCA) was procured from Cayman Chemicals. Sodium arsenite and trichloroacetic acid (TCA) was procured from Sigma-Aldrich, Mumbai, India. Mitochondria, liver and plasma samples were procured 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. Batches of Swiss albino mice weighing 19-20g were obtained from institutional breeding house which showed a steady increase in weight of up to 28-30g 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±2°C, relative humidity 57±2%, 12/12 h ipiontene sodium in overdose (100g kg body weight).

Experiments:

For dose dependent studies- Control group (group I) was fed only with normal tap water as drinking water (tested to be non contaminated) and 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±2°C, relative humidity 57±2%, 12/12 h ipiontene sodium in overdose (100g kg body weight).

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The assay was done according to kit manufacturer’s protocol (Cayman). Plasma samples if necessary were diluted with sample buffer (100 mM potassium phosphate, pH 6.5, containing 1% Triton X-100 and 4% GSH (9.5 mM). Plasma samples if necessary were diluted with sample buffer (100 mM potassium phosphate, pH 6.5, containing 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 sample 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 (c) ; decrease in comet tail moment (*p<0.001) by pre-treatment [mode B : curcumin was withdrawn beyond the time period mentioned above as no further change in As III induced genotoxicity was observed. Four doses 50µg/kg, 250µg/kg, 500µg/kg, 1000µg/kg 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/kg which showed severe genotoxicity with deformed comet pattern. Henceforth subsequent experiments were done with 500 µg/kg. 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 mg. Reversal of As III (500 µg/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 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<0.001-0.005) during simultaneous treatment with three different concentration of curcumin (5, 10, 15 µg/kg bw) in a time dependent manner in Swiss albino mice (b); dose and time dependent increase in (**p<0.001) in peripheral blood of Swiss albino mice (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).
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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) on 15th and 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<0.001) 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-**p<0.005), SOD (p<0.001-**p<0.005), GPx (p<0.001-**p<0.005), GR (p<0.001-**p<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

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 antioxidative 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.
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 vivo and 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 splenetic 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 aromatic rings and the structural feature of 1,3-diketone systems (Sreejayan and Rao, 1996, 1997). Curcumin treatment abolished the Cu-induced lipid peroxidation (125% of controls) and GSH depletion (67% of controls) in mice liver (Eby 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-Demerash 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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References
