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Curcumin supplementation protects from genotoxic effects of arsenic and fluoride

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ABSTRACT

The present study was aimed to evaluate curcumin as a potential natural antioxidant to mitigate the genotoxic effects of arsenic (As) and fluoride (F) in human peripheral blood lymphocytes. The study was divided into nine groups consisting of negative control, positive control treated with ethyl methane sulphonate (EMS; 1.93 mM) and curcumin control with only curcumin (1.7 μM) in blood culture. As (1.4 μM) and F (34 μM) were added alone as well as in combination, to the cultures, with and without curcumin. Cultures were analysed for chromosomal aberrations (both structural and numerical) and primary DNA damage via comet assay as the genotoxic parameters after an exposure duration of 24 h. Results revealed that curcumin efficiently ameliorates the toxic effect of As and F by reducing the frequency of structural aberrations (>60%), hypoploidy (>50%) and primary DNA damage. In conclusion, curcumin mitigates the genotoxic effects of the two well known water contaminants (As and F) effectively and efficiently at the given concentration *in vitro*.

1. Introduction

Curcumin (diferuloylmethane), the active principle of turmeric (*Curcuma longa*), is commonly used as a coloring agent in food, drugs and cosmetics, and has a wide range of effects. It has been mentioned as an important component in Chinese and Indian traditional medicinal system (Aggarwal et al., 2007). The EPR spin trapping technique demonstrated that curcumin quenches singlet oxygen effectively, leading to adduct formation, in comparison to scavenging hydroxyl radical (Das and Das, 2002). Its antioxidant effect is 8× more powerful than vitamin E and is significantly more effective in preventing lipid peroxide formation (Sreejayan and Rao, 1994; Reddy and Lokesh, 1992). Curcumin can significantly reduce the frequencies of micronucleated polychromatic erythrocytes in mice exposed to γ-radiation (Abraham et al., 1993), and it is also indicated to be an antimutagen against environmental mutagens *in vitro* and an antitumor drug *in vivo* (Nagabhushan and Bhide, 1992). The property of curcumin to increase the activity of glutathione peroxidase, catalase and G6PDH in ddY mice has been implicated to possibly play a protective role against chemical toxicity and carcinogenesis (Iqbal et al., 2003). Thus, in the present study, curcumin was selected as the most potential natural compound to prevail over arsenic and fluoride induced genotoxicity.

Arsenic (As) and fluoride (F) both are known water contaminants raising big health issues in India and world over (ATSDR, 2003, 2005). As is found to alter mitochondrial function by altering cytochrome *c* oxidase and citrate synthase activity (Partridge et al., 2007). Further, immunohistochemical study reported the appearance of 8-oxodG, not only in tumor tissues but also in keratosis and normal tissues, of arsenic related skin tumors (An et al., 2004). These results suggested the induction of oxidative stress playing an important role in arsenic toxicity. Arsenic genotoxicity has been analysed extensively in a wide range of *in vivo* and *in vitro* studies and the overall conclusion is that there is a clear induction of genotoxic effects, including an increase in micronucleus (MN) frequency and a decrease in the proliferation index that reflects its toxic potential (Hayakawa et al., 2005; Mandal et al., 2004). On the other hand F has been a topic of conflict as far as the ROS generation is concerned. He and Chen (2006) demonstrated that fluoride can induce oxidative stress as well as DNA damage and lead to apoptosis and cell cycle change in rat oral mucosal cells. Contradictory to this, Jung et al. (2006) demonstrated fluoride induced apoptotic morphological changes in Human Gingival Fibroblasts with no affect on production of ROS. But there are ample evidences in favour of genotoxic potential of fluoride in humans, *in vitro* as well as *in vivo*, exhibited by increased frequency of chromosomal aberrations, micronuclei induction, sister chromatid exchanges, etc. (Rao and Tiwari, 2006; Aardema et al., 1989).

Abbreviations: As, arsenic; F, fluoride; CA, chromosomal aberrations; NOS, nitric oxide synthase; ROS, reactive oxygen species; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; DDW, double distilled water; DMSO, dimethyl sulphoxide; PBLC, peripheral blood lymphocyte culture.

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Taking all these evidences into consideration this study was designed to confirm the genotoxic potential of As and F *in vitro*, and to further evaluate the protective role of curcumin upon As and F induced genotoxicity.

2. Materials and methods

2.1. Test substances

Arsenic trioxide was obtained from Himedia Lab., Mumbai, 2.5 mM stock solution of As_2O_3 was prepared and sterilized with 0.22 μm filter. The working solution was prepared by diluting the stock 100× with sterilized DDW. Sodium fluoride was obtained from Qualigens fine chemicals, Mumbai, India. A 5 mg/ml solution was prepared in double distilled water and then sterilized by passing through 0.22 μm filter. Curcumin was procured from HiMedia Lab, Mumbai, India and a 1 mg/ml solution was prepared in 1:1 solution of dimethyl sulfoxide (DMSO) and water. Ethyl methane sulphonate was obtained from HiMedia Lab, Mumbai, India, 240 mg/ml solution prepared in DMSO.

2.2. Sample collection

Blood was collected from 15 normal healthy individuals within the age group of 20–30 years in heparinised vacuutainers. (Greiner bio-one, Austria).

2.3. Experimental design

The study included nine groups, as described below and the exposure time for test chemicals in all the groups was 24 h (Table 1).

2.4. Peripheral blood lymphocyte culture (PBLC)

Peripheral blood lymphocytes were cultured (Hungerford, 1965) in RPMI-1640 (Hi Media Lab.Mumbai, India) supplemented with 10% heat inactivated fetal calf serum (FCS) from Hi Media Lab, Mumbai, India, 100 mg/l streptomycin (Sarabhai Piramal pharma Pvt. Ltd., Vadodara, India) and 100 units/ml penicillin (Alembic Ltd., Vadodara, India). Hundred microgram of phytohaemagglutinin (PHA), (L8902) obtained from Sigma–Aldrich Chemicals Pvt. Ltd., St. Louis, MO, was added to stimulate lymphocytes. These closed cultures were incubated at 37 °C for another 48 h and then the *in vitro* treatment was given for 24 h. At 69th hour 2 μg colchicine (HiMedia Lab, Mumbai, India) was added to 7 ml culture and centrifuged to after 30 min. The pellet formed was incubated, at 37 °C, with hypotonic solution (0.075 M KCl) for 20 min, then fixed with fixative (1:3 acetomethanol). Final cell suspension was made after two washes with fixative, and 3–4 drops of this suspension was added to wet chilled slides and flamed.

2.5. Analysis of chromosomal aberrations

Slides were labelled and stained with 2% giemsa for analysis (Stassen et al., 1953) Chromosomal aberrations can either be structural or numerical. Numerical aberrations were analysed by counting chromosome number in 100 metaphase plates of each group. Plates with chromosome number <44 were not included in analysis. Structural aberrations include breaks and gaps. Slides were scanned for 100 plates per group.

2.6. Comet assay

Base slides were prepared by dipping it in 1% normal melting point agarose (NMA) (500 mg/50 ml double distilled water), wiped from the back side and dried at room temperature. The cultures treated for 24 h (as described in PBLC) were then centrifuged and the pellet was suspended in 0.5 ml calcium magnesium free phosphate buffer saline (PBS). To 80 μl of 0.75% low melting point agarose (LMA), 20 μl of cell suspension was added and mixed properly. Eighty microlitre of this was added to the base slide, covered with the coverslip and refrigerated for 10–15 min. Eighty microlitre of LMA (without cells) was layered on the slide and kept in refrigerator after replacing the coverslip, to harden the gel. The slides were put into cold lysing solution [2.5 M NaCl, 100 mM EDTA, 10 mM Tris (pH 10.0), 1% sodium lauryl sarcosinate along with fresh addition of 1% Triton X-100and 10% DMSO], in dark at 4 °C for 16–18 h. The slides were then washed with double distilled water (DDW) and electrophoresed in alkaline electrophoresis buffer (300 mM NaOH and 1 mM EDTA, pH 13) 30 min, at 25 V, 300 mA after an incubation of 40 min. Slides were neutralized by washes with 0.4 M Tris, pH 7.5, placed into 100% ethanol and then air-dried (Tice et al., 1997).

2.7. Analysis of comet parameters

Slides were stained with 0.02 mg/ml ethidium bromide (Sigma). Comet tail lengths were scored (50 cells/slide) under an Axiophot Microscope (Zeiss) by using the Comet score software. All measurements were done on triplicate slides. The parameters analysed were comet tail length, percent DNA in tail and percent DNA in head.

2.8. Statistical analysis

Percentage amelioration was calculated by using the following formula (Rao and Tiwari, 2006)

$$\text{Percentage amelioration} = \frac{(\text{Pro oxidant group} - \text{Antioxidant group}) \times 100}{\text{Control} - \text{pro oxidant group}} \quad (1)$$

The significance levels were determined by students *t*-test wherein, values for all the groups were compared to control group. Also pro oxidant groups were compared with the respective antioxidant groups.

The data was also analysed by ANOVA.

3. Results

Chromosomal aberrations: the results verified the genotoxic effects of As and F indicated by increased frequency of structural ($p < 0.001$) and numerical aberrations ($p < 0.001$). Addition of curcumin along with arsenic and fluoride (individually and in combination), brought about significant decrease in the pro oxidant induced chromosomal breaks and gaps ($p < 0.001$, $p < 0.01$). Similarly a remarkable fall was observed in arsenic and fluoride led hypoploidy in human peripheral blood cultures ($p < 0.001$). The percent amelioration calculated for both the cases was more than 50% (Table 2).

Comet assay: increased comet length ($p < 0.01$, $p < 0.05$) and percent DNA in comet tail visibly indicated As and F induced DNA damage at the given dose ($p < 0.001$). This DNA damage by As and F was escorted with the decreased percent DNA in comet head (Fig. 2; $p < 0.001$). Noteworthy decrease was observed in the comet length (Fig. 1; $p < 0.01$, $p < 0.05$) as well as percent DNA in comet tail (Fig. 2; $p < 0.01$; $p < 0.05$), in cultures supplemented with curcumin along with As and F. The above data was further supported by the convincing rise in percent DNA in comet head (Fig. 2; $p < 0.001$, $p < 0.05$). Though the values for percent DNA in comet head and tail were not comparable to control after curcumin

Table 1
Description of the experimental design.

Experimental groups	Treatment	Doses
Group I	Control (Con)	
Group II	Arsenic (As)	As_2O_3 (1.4 μM)
Group III	Fluoride (F)	NaF (34 μM)
Group IV	Arsenic + fluoride (As + F)	As_2O_3 (1.4 μM) + NaF:(34 μM)
Group V	Arsenic + curcumin (As + Cur)	As_2O_3 (1.4 μM) + Cur (7.7 μM)
Group VI	Fluoride + curcumin (F + Cur)	NaF (34 μM) + Cur (7.7 μM)
Group VII	Arsenic + fluoride + curcumin (As + F + Cur)	As_2O_3 (1.4 μM) + NaF(34 μM) + Cur (7.7 μM)
Group VIII	Curcumin (Cur)	Cur (7.7 μM)
Group IX	Ethyl methane sulphonate (EMS)	EMS (1.93 mM)