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Cytoprotective Effects of Curcumin on Sodium Fluoride-Induced Intoxication in Rat Erythrocytes

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Abstract Curcumin is well known for its potent antioxidant activity. The result of numerous studies showed that antioxidants can protect against fluoride-induced toxicity. In the present study, protective effects of curcumin against sodium fluoride-induced toxicity in rat erythrocytes were evaluated. Curcumin (10 and 20 mg/kg) and vitamin C (10 mg/kg) were administrated intraperitoneally for 1 week followed by sodium fluoride (600 ppm) treatment for next week. Erythrocytes were isolated and superoxide dismutase and catalase activities as well as the levels of reduced glutathione, and lipid peroxidation were measured. The level of malondialdehyde in sodium fluoride treated rats (595.13 ± 20.23 nmol/g Hb) increased compared to the normal rats (315.44 ± 9.76 nmol/g Hb). Animals which were pretreated with curcumin at 20 mg/kg for 1 week prior to sodium fluoride intoxication showed significant reduction in the malondialdehyde level (320.22 ± 9.28 nmol/g Hb). Also, pretreated with curcumin (20 mg/kg) and vitamin C restored the superoxide dismutase and catalase activities and modified the level of reduced glutathione compared with control group ($p > 0.01$).

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Curcumin is the active polyphenolic compound in the *Curcuma longa* Linn. It is used for the treatment of dental diseases, digestive disorders such as dyspepsia and acidity, indigestion, flatulence, ulcers, and to alleviate the hallucinatory effects of marijuana and other psychotropic drugs (Tilak et al. 2004). In food industries, curcumin is used in perfumes and as a natural yellow food additive to flavor various types of curries and mustards (Tilak et al. 2004; Shishodia et al. 2005). Research revealed that curcumin has wide range of beneficial effects including: anti-inflammatory, antioxidant, chemopreventive and chemotherapeutic activity. These activities have been exhibited both in cultured cells and in animal models and have paved the way for ongoing human clinical trials (Hatcher et al. 2008). The popular distribution of fluoride in drinking water and food may results in adverse health effects (Cicek et al. 2005). The most obvious early toxic effects of fluoride in human's body are dental and skeletal fluorosis, which are endemic in areas with elevated exposure to fluoride (Shivarajashankara et al. 2001). Fluoride is known to cross the cell membranes and to enter soft tissues (Jacyszyn and Marut 1986). Morphological, histological and biochemical changes of several tissues have been reported in animals that received different doses of fluoride (Chinoy and Patel 1998; Guan et al. 1998). However, the toxicity kinetics and pathogenesis of fluoride-induced toxicity on the whole body is still unclear. The generation of free radicals, lipid peroxidation and altered antioxidant protection systems are considered to play an important role in the toxic effects of fluoride (Sharma and Chinoy 1998; Rzeuski et al. 1998). Many reports showed that fluoride caused oxidative stress,

lipid peroxidation and reduced antioxidant enzyme activities in vivo or in vitro (Shanthakumari et al. 2004; Shan et al. 2004). Reactive oxygen species induced by oxidative stress have been implicated in tissue injury. The main reactive oxygen species that have to be considered are superoxide anion, which is mostly generated by the mitochondria, hydrogen peroxide produced from oxygen by the action of superoxide dismutase and peroxynitrite, which is generated by the reaction of oxygen with nitric oxide. Many pro-oxidant enzymes are known to participate in the production of reactive oxygen species. These continuously produced reactive oxygen species are scavenged by superoxide dismutase, glutathione peroxidase and catalase. Under some circumstances, these endogenous antioxidant protection systems are probably perturbed because of overproduction of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants in tissues. Malondialdehyde is the breakdown product of the major chain reactions, leading to oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of oxidative stress-mediated lipid peroxidation in tissues (Slater 1989). A cell has several methods of alleviating the effects of oxidative stress either by repairing the injury (i.e. injured nucleotides) or by directly decreasing the occurrence of oxidative injury by means of enzymatic and non-enzymatic antioxidants. Non-enzymatic antioxidants, i.e., curcumin, can also act to overcome the oxidative stress, being part of the antioxidant system. Many studies have reported that polyphenolic compounds such as flavonoids can reduce lipid peroxidation caused by toxicant and oxidative substances (Altuntas et al. 2002). Thus, the aim of the present study was to assess the injury and oxidative stress of fluoride intoxication and a possible protective effect of curcumin against fluoride intoxication in rat erythrocytes.

Materials and Methods

In the present study male Wistar rats (*Rattus norvegicus albinus*) with approximately the body weight (200–250 g), housed in ventilated animal rooms at a temperature of 24 ± 2°C with a 12 h light/dark cycle and 60% ± 5% humidity were used. All experiments were performed according to the norms of the ethical committee of University of Mazandaran, Babolsar which is in accordance with the national guidelines for animal care and use.

Bovine serum albumin and a kit for protein estimation were purchased from ZiestChem Company, (Tehran, Iran). Curcumin, 5,5-dithiobis(2-nitrobenzoic acid), glacial acetic acid, heparin, nitro blue tetrazolium chloride, potassium dihydrogen phosphate, reduced glutathione, sodium dihydrogen phosphate, sodium fluoride, trichloro acetic acid, thiobarbituric acid, hydrogen peroxide were purchased from Sigma-Aldrich chemical company, (St. Louis, MO USA). Other chemical reagent were of analytical grade or purer.

Animals were randomly divided into five groups of 10 animals each. Group I was kept as normal control receiving sample solvent (0.5 mL, i.p.) for 7 consecutive days. Animals of groups II and III were treated with curcumin (10 and 20 mg/kg) intraperitoneally for 7 days followed by sodium fluoride in drinking water (600 ppm) for next 7 days. Animals of group IV were treated with vitamin C (10 mg/kg) intraperitoneally for 7 days followed by sodium fluoride in drinking water (600 ppm) for next 7 days and used as positive control group. Animals of group V were treated with sodium fluoride (600 ppm) for the same time and used as control group. After the last treatment, animals were anesthetized with ketamine (60 mg/kg) and xylazine (5 mg/kg) given intraperitoneally. Blood samples were collected via cardiac puncture in plain plastic tubes (Sinha et al. 2007).

After collecting blood sample in heparinized tube, it was centrifuged at 1,000*g* for 15 min. The buffy coat was removed and the packed cells of the bottom were washed thrice with phosphate buffer saline (0.9% sodium chloride in 0.01 M phosphate buffer, pH 7.4). A known amount of erythrocytes were lysed with hypotonic phosphate buffer. After removing the cell debris by centrifugation at 3,000*g* for 15 min, the hemolysate was obtained and it was used for following experiments. The protein concentration of the hemolysate was measured using Bradford method by commercial kit of ZiestChem Co. using bovine serum albumin as standard (Bradford 1976). Also, haemoglobin content of the solution measured by the method of Drabkin (Rice-Evans et al. 1991).

Lipid peroxidation in terms of thiobarbituric acid reactive substances formation was measured according to the method of Esterbauer and Cheeseman (1990). A sample containing 1 mg protein was mixed with 1 mL trichloro acetic acid (20%), 2 mL thiobarbituric acid (0.67%) and heated for 1 h at 100°C. After cooling, the precipitate was removed by centrifugation. The absorbance of the sample was measured at 535 nm using a blank containing all the reagents except the hemolysate.

Superoxide dismutase was examined according the method of Misra and Fridovich (1972). Reaction mixtures contained sodium carbonate (1 mL, 50 mM), nitroblue tetrazolium (0.4 mL, 25 μm) and freshly prepared hydroxylamine hydrochloride (0.2 mL, 0.1 mM). The reaction mixtures were mixed by inversion followed by the addition of clear hemolysate (0.1 mL, 1:10 w/v). The change in absorbance of reaction mixture was recorded at 560 nm.

The activity of catalase was assayed by the method described by Pari and Latha (2004). The inhibition percentage was evaluated following decrease in absorbance at

620 nm. The reaction mixture consisted of 0.4 mL of hydrogen peroxide (0.2 M), 1 mL of 0.01 M phosphate buffer (pH 7.0) and 0.1 mL of hemolysate. The reaction of the mixture was stopped by adding 2 mL of dichromate-acetic acid reagent (5% potassium dichromate prepared in glacial acetic acid). The changes in the absorbance were measured and recorded at 620 nm.

Reduced glutathione level was measured by the method of Ellman (1959). Here, the hemolysate (720 µL) was double diluted and 5% trichloro acetic acid was added to precipitate the protein content of the hemolysate. After centrifugation (10,000*g*, 5 min), the supernatant was taken, 5,5-dithiobis(2-nitrobenzoic acid) solution was added to it and the absorbance was recorded at 417 nm.

The values are presented as means ± S.D. Differences between group means were estimated using one-way ANOVA followed by Duncan's multiple range tests. Results were considered statistically significant when $p < 0.05$.

Results and Discussion

The malondialdehyde levels for all groups are shown in Fig. 1. In the erythrocytes, the lipid peroxidation in sodium fluoride intoxicated rats (595.13 ± 20.23 nmol/g Hb) increased compared to the normal group (315.44 ± 9.76 nmol/g Hb). Rats pretreated with curcumin and vit C for 7 days before intoxicate by sodium fluoride showed significant reduction in the malondialdehyde level. The superoxide dismutase activity in the rat erythrocytes normal, sodium fluoride treated, and different curcumin treated and vitamin C treated groups prior to sodium fluoride intoxication are shown in Fig. 2. In the erythrocytes, sodium fluoride intoxication caused a reduction of the superoxide dismutase activity (590.24 ± 17.11 U/g Hb) compared to the normal group (1625.34 ± 40.62 U/g Hb). Seven days treatment with curcumin prior to the sodium fluoride intoxication for 7 days significantly enhanced the superoxide dismutase activity. Similar result was obtained with the antioxidant vitamin C (Fig. 2). Catalase activity as measured from the erythrocytes of all of the rats is shown in Fig. 3. The catalase activity in the erythrocytes of sodium fluoride intoxicated rats (563.93 ± 18.47 U/g Hb) was much lower than that of normal group (1,237.27 ± 0.002 U/g Hb). In the curcumin pretreated groups, which received curcumin at doses of 10 and 20 mg/kg for 7 days before sodium fluoride, the catalase activity was significantly higher compared to the sodium fluoride treated group. Vitamin C treatment prior to sodium fluoride intoxication prevented the change in catalase activity (Fig. 3). Figure 4 shows the levels of reduced

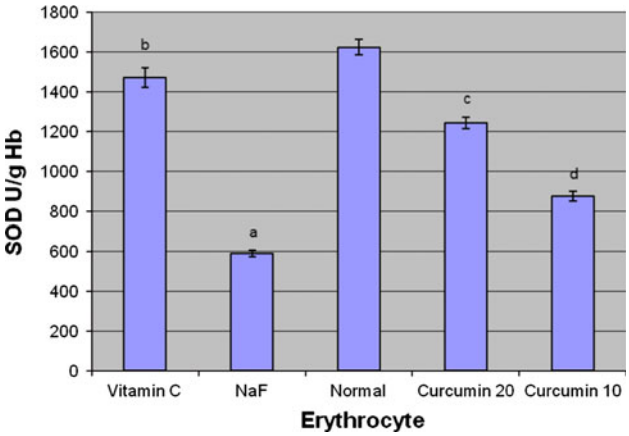

Fig. 2 Toxic effect of sodium fluoride on superoxide dismutase activity in the rat erythrocytes. Data are mean ± SD values (n = 10). [a]$P < 0.001$ versus normal group. [b]$P > 0.05$ versus normal group. [c]$P > 0.01$ versus normal group. [d]$P < 0.01$ versus normal group

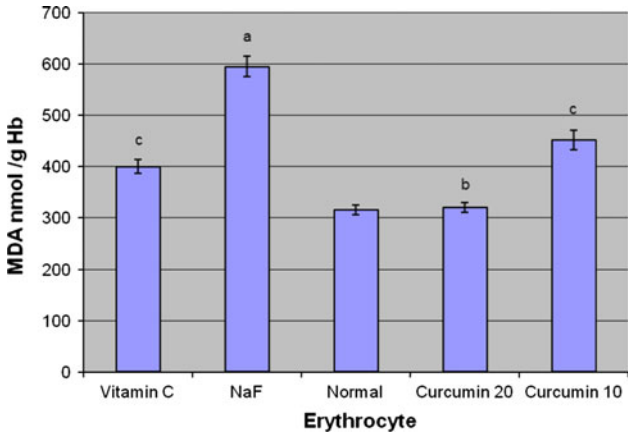

Fig. 1 Effect of curcumin and vitamin C on malondialdehyde level (MDA) in sodium fluoride-intoxicated rats. Data are mean ± SD values (n = 10). [a]$P < 0.001$ versus normal group. [b]$P > 0.05$ versus normal group. [c]$P < 0.05$ versus normal group

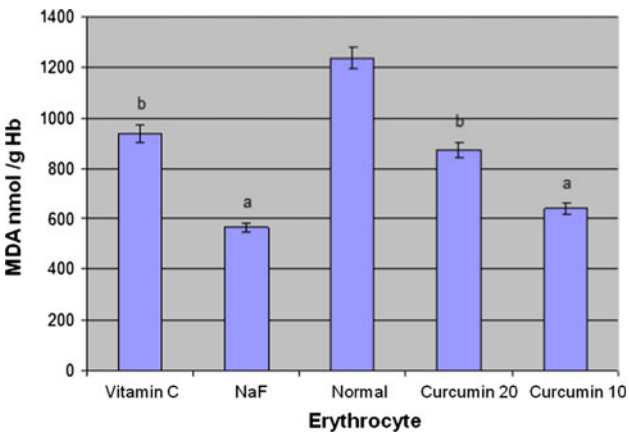

Fig. 3 Effect of vitamin C and curcumin (10 and 20 mg/kg) on catalase levels in sodium fluoride-intoxicated rats. Data are mean ± SD values (n = 10). [a]$P < 0.001$ versus normal group. [b]$P > 0.01$ versus normal group

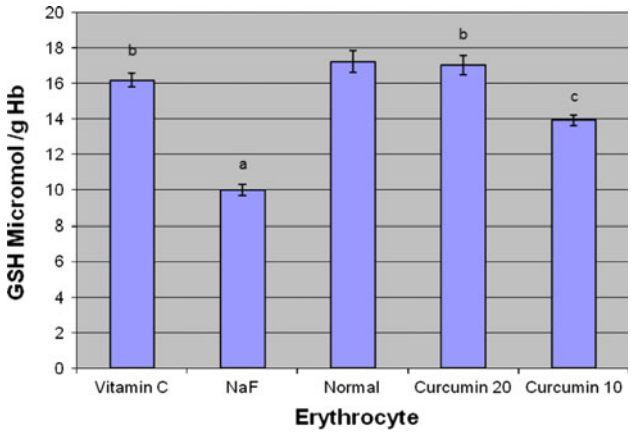

Fig. 4 Toxic effect of sodium fluoride on reduced glutathione levels in the rat erythrocytes. Data are mean ± SD values (n = 10). [a]$P < 0.001$ versus normal group. [b]$P > 0.05$ versus normal group. [c]$P < 0.05$ versus normal group

glutathione in rat erythrocytes of different groups. Sodium fluoride treatment caused a significant reduction in reduced glutathione level in erythrocytes compared to that of normal group. Pretreatment of the curcumin prior to the sodium fluoride intoxication increased the level of reduced glutathione. Vitamin C treatment prior to sodium fluoride intoxication prevented the change in reduced glutathione level. The aim of the present study was to investigate the possible effect of curcumin on sodium fluoride-induced oxidative stress in erythrocytes isolated from the blood of the rats. So, various prooxidant- antioxidant related parameters such as thiobarbituric acid reactive substances, the activity of the antioxidant enzyme superoxide dismutase, catalase, as well as the level of the cellular reduced glutathione were measured. The results of the present study showed that sodium fluoride caused significant oxidative stress in erythrocytes and it could be protected by the administration of curcumin prior to sodium fluoride treatment. During aerobic metabolism as well as exposure to various environmental agents such as radiation and redox cycling substances (Mates et al. 1999), reactive oxygen species namely, superoxide anion, hydrogen peroxide and hydroxyl radical are generated in vivo due to sequential reduction of oxygen. In a variety of disease such as cardiovascular diseases, diabetes, neurodegenerative diseases reactive oxygen species have been shown to play important role as mediators or in progress of diseases (Yamagishi et al. 2001). Oxidative stress occurs when production of reactive oxygen species exceeds the body's natural antioxidant defense mechanism causing damage to macromolecules such as DNA, proteins and lipids (Rice-Evans et al. 1991; Halliwell and Guttteridge 2007; Gul et al. 2000). Antioxidant defense mechanism operates in our body to scavenge reactive oxygen species for the protection from body against oxidative injuries. The antioxidant system includes different antioxidant enzymes namely superoxide dismutase, catalase, etc., together with the substances which are capable of reducing reactive oxygen species, like reduced glutathione (Gul et al. 2000). Besides, different antioxidants like ascorbic acid and vitamin E are also known as scavengers of these reactive oxygen species (Rice-Evans et al. 1991; Liu et al. 1995). When the parameters of the oxidative stress were evaluated in the erythrocytes, we observed that rats treated with sodium fluoride at a dose of 600 ppm for 7 days the activity of the antioxidant enzyme superoxide dismutase, catalase, reduced glutathione and the lipid peroxidation significantly reduced. Treatment with curcumin at the doses of 10 and 20 mg/kg body weight prior to the sodium fluoride administration restored the activity of superoxide dismutase, catalase and normalized the level of reduced glutathione and lipid peroxidation in erythrocytes. Treatment of vitamin C prior to sodium fluoride intoxication also showed similar effects like curcumin whereas a sample solvent has no preventive activity. Results from the pretreatment studies suggest that curcumin also possesses curative activity in erythrocytes against sodium fluoride intoxication in rats. Present study for the first time showed cytoprotective effect of curcumin, a natural flavonoid, against sodium fluoride-induced oxidative stress in rat erythrocytes. Therefore, its therapeutic effect in clinical situations may be considered. These results can be useful as a starting point of view for further applications of this flavonoid in pharmaceutical preparations after performing clinical studies.

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