

THE PROTECTIVE EFFECT OF CURCUMIN AGAINST SODIUM FLUORIDE-INDUCED OXIDATIVE STRESS IN RAT HEART

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Abstract - In the present study the cardioprotective effects of curcumin, a herbal polyphenolic compound, against sodium fluoride (NaF)-induced toxicity in rat heart was evaluated. Fifty rats were divided into five experimental groups containing 10 rats each. Group I received standard water and diet and was used as a normal group; groups II and III were pretreated with curcumin intraperitoneally for 7 days prior to NaF intoxication. Group IV was pretreated with vitamin C, a standard antioxidant, intraperitoneally for 7 days prior to NaF intoxication and used as a positive control group. The animals in group V were intoxicated with NaF for the same time and used as a control group. There was a significant increase in lipid peroxidation along with a decrease in superoxide dismutase activity in the homogenates of tissues of the NaF-treated animals. Curcumin pretreatment in animals prior to fluoride intoxication normalized the levels of biochemical parameters measured.

Key words: Curcumin, oxidative stress, sodium fluoride, enzymatic antioxidant

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INTRODUCTION

Curcumin (Fig. 1) is the bioactive natural product in *Curcuma longa* Linn. Curcumin extracted from *Curcuma longa* Linn is widely used as a food additive (Kçhler, 1887) and has a long history as a food additive in Chinese, Indian and Iranian traditional medicines (Ammon and Wahl, 1991; Zargari, 1992). In the traditional medicine of India, a cream of *Curcuma longa* Linn. called "Ayurveda" is used for the treatment of eye diseases, wounds, bites, burns and various dermal diseases (Thakur et al., 1989). In India, *Curcuma longa* Linn. powder is taken with milk and used as an antitussive and cure of respiratory damage (Thakur et al., 1989). Roasted *Curcuma longa* Linn. is a component used for protection against intestinal diseases in children (Thakur et al., 1989). Also, this

natural product is used as a drug for the treatment of dental and digestive diseases and to relieve the hallucinatory effects of addictive compounds (Tilak et al., 2004). In food technology, curcumin is used as a yellow food additive to flavor different types of curries and mustards (Shishodia et al., 2005). Recent reports on the use of natural products in Western medicine have drawn the attention of food scientists to this polyphenolic compound. Research has shown that curcumin has different beneficial effects such as anti-inflammatory, antioxidant, chemopreventive and chemotherapeutic. These beneficial effects have been demonstrated both in cell cultures and in experimental animal models and have been useful for human clinical trial studies (Hatcher et al., 2008). The fluoride common in water and food sources often causes harmful effects to the human body (Cic-

ek et al., 2005). The most important toxic effects of fluoride in humans are dental and skeletal fluorosis, which are common in regions with elevated exposure to fluoride (Shivarajashankara et al., 2001). Fluoride is known to cross the cell membranes and to enter the soft tissues (Jacyszyn and Marut, 1986). Histological and biochemical abnormalities of some tissues have been reported in experimental animals that have been intoxicated with different doses of fluoride (Karaoz et al., 2004; Aydin et al., 2003; Chinoy and Patel, 1999; Chinoy and Patel, 1998; Ghosh et al., 2002; Guan et al., 1998). However, the mechanism of fluoride toxicity in the human body is still unclear. Free radical generation, lipid peroxidation and changes in the antioxidant protection systems are considered to play an important role in fluoride intoxication (Sharma and Chinoy, 1998). Several reports show that fluoride causes oxidative injuries, lipid peroxidation and abnormalities in antioxidant enzyme activities *in vivo* or *in vitro* (Shanthakumari et al., 2004; Shan et al., 2004). Fluoride intoxication of human organs and tissues has long been known and has been examined histologically and biochemically many times in *in vivo* models. Reactive oxygen species produced by oxidative injuries have been involved in tissue injury. Numerous enzymatic pro-oxidants are known to participate in the production of free radicals. These produced free radicals are inhibited by enzymatic antioxidants, i.e. superoxide dismutase, glutathione peroxidase and catalase. Under some circumstances, these antioxidant protection systems are disturbed by the excessive production of free radicals, inactivation of detoxification mechanisms and antioxidant consumption in live tissues. The breakdown product of the major chain reactions, i.e. malondialdehyde, leads to the oxidation of polyunsaturated fatty acids, and so serves as a credible marker of free radical-induced lipid peroxidation in tissues (Slater, 1989). The cell has some procedures to allay oxidative stress, either by repairing the damage or by directly reducing oxidative damage via enzymatic and non-enzymatic antioxidant protection systems. Non-enzymatic antioxidants, such as edible antioxidants, can also act to overcome oxidative damage, being part of the antioxidant protection system. Numerous studies have reported

that polyphenolic compounds such as curcumin can reduce lipid peroxidation induced by toxicant and oxidative substances (Altuntas et al., 2002; Gultekin et al., 2001). Thus, the purpose of this study was to estimate the oxidative stress of fluoride intoxication and the cardioprotective effect of curcumin against fluoride-induced oxidative stress in rat heart.

MATERIALS AND METHODS

Animals

The study was performed on male Wistar rats (*Rattus norvegicus albinus*) of approximate body weight 200-250 gr, housed in ventilated cages at $24 \pm 2^\circ\text{C}$ with a 12 h light/dark cycle and $60 \pm 5\%$ humidity. The rats were fed with standard laboratory animal feed, manufactured by the pasture institute, Tehran, Iran. Water was provided *ad libitum*. Experiments were performed between 10:00 and 14:00. All experiments were performed according to the norms of the Ethical Committee of the University of Mazandaran, Babolsar which is in accordance with the national guidelines for animal care and use.

Chemicals

Bovine serum albumin (BSA) and a kit for protein estimation were purchased from the ZiestChem Company (Tehran) o, Iran. Curcumin, 5,5-dithiobis(2-nitrobenzoic acid) [DTNB, (Ellman's reagent)], glacial acetic acid, heparin, nitro blue tetrazolium chloride (NBT), potassium dihydrogen phosphate (KH_2PO_4), reduced glutathione (GSH), sodium dihydrogen phosphate (NaH_2PO_4), sodium fluoride (NaF), trichloroacetic acid (TCA), thiobarbituric acid (TBA), and hydrogen peroxide were obtained from Sigma-Aldrich Chemical Company, (St. Louis, MO), USA. Other chemical reagent were of analytical grade or purer.

Animal treatments

Animals were randomly divided into five groups of 10 animals each. Group I was kept as a normal control receiving isotonic saline (0.5 mL, i.p.) for

7 consecutive days, and animals of groups II and III were treated with the curcumin (10 and 20 mg/kg body weight) intraperitoneally for 7 days followed by NaF (600 ppm through drinking water) in drinking water for next 7 days. Animals of group IV were treated with vitamin C (10 mg/kg body weight) intraperitoneally for 7 days, followed by NaF treatment for the next 7 days (600 ppm through drinking water) and used as a positive control group. Animals in group V were treated with NaF (600 ppm through drinking water) for the same time and used as a control group. After the last treatment, the animals were anesthetized with ketamine (60 mg/kg) and xylazine (5 mg/kg) given intraperitoneally. The rats' hearts were removed and kept at -60°C before biochemical estimation (Sinha et al., 2007).

Preparation of tissue homogenate

Whole heart tissue was homogenized in KH_2PO_4 buffer (100 mM) containing EDTA (1 mM) (pH 7.4) (1:10 w/v) and centrifuged (12000 g, 30 min, 4°C). The supernatant was used for biochemical estimations.

Determination of protein content

The protein content was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as standard.

Biochemical estimation

Estimation of lipid peroxidation

Lipid peroxidation in terms of thiobarbituric acid reactive substance formation was determined by the method of Esterbauer and Cheeseman (1990). Tissue homogenates containing 1 mg protein were mixed with trichloroacetic acid (1 ml, 20%), thiobarbituric acid (2 ml, 0.67%) and incubated for 1 h at 100°C . After cooling, the precipitate was removed by centrifugation. The absorbance of the reaction mixtures was measured at 535 nm using a blank containing all the reagents except the tissue homogenates.

Determination of superoxide dismutase activity

Superoxide dismutase was examined according to 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 a clear supernatant of tissue homogenates (0.1 ml, 1:10 w/v). The change in absorbance of samples was recorded at 560 nm.

Determination of catalase activity

The enzyme catalase converts hydrogen peroxide into oxygen and water. Catalase activity was measured by the method of Bonaventura et al., (1972). Proteins contained in tissue homogenate (5 μg) were mixed with hydrogen peroxide (2.1 ml, 7.5 mM) and a time scan was performed for 10 min at 240 nm at 25°C . The disappearance of peroxide depending on the catalase activity was observed; one unit of catalase activity was defined as the amount of enzyme that reduces 1 μmol of hydrogen peroxide/minute.

Determination of reduced glutathione activity

Reduced glutathione level was determined according to the method of Ellman (1959). Here, the tissue homogenate (720 μl) was double diluted and trichloroacetic acid (5%) was added to precipitate the protein content of the tissue homogenates. After centrifugation (10000 g, 5 min) the supernatant was taken, 5, 5-dithiobis (2-nitrobenzoic acid solution (Ellman's reagent) was added to it and the absorbance was measured at 417 nm. A standard curve was constructed using the different known levels of reduced glutathione solution. With the help of this standard curve, the reduced glutathione level of the homogenate was calculated.

Statistical analysis

The values are presented as means \pm S.D. Differences between group means were estimated using

one-way analysis of variance followed by Duncan's multiple range tests. Results were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

The malondialdehyde levels for all experimental groups are shown in Fig. 2. Lipid peroxidation in heart tissue homogenates of the sodium fluoride-intoxicated group (59.36 ± 2.19 nmol/g tissue) increased compared to the normal group (43.51 ± 1.47 nmol/g tissue). The groups that were treated with curcumin and vitamin C for 7 days before sodium fluoride intoxication showed a reduction in the malondialdehyde levels (54.44 ± 2.07 nmol/g tissue for 10 mg/kg of curcumin, 47.11 ± 1.94 nmol/g tissue for 20 mg/kg of curcumin and 44.52 ± 1.73 nmol/g tissue for vitamin C). The activity of superoxide dismutase in the heart tissue homogenates of the rat of normal, sodium fluoride, and different doses of curcumin and vitamin C treated groups are shown in Fig. 3. Curcumin treatment before sodium fluoride intoxication for 7 days increased the superoxide dismutase activity (71.42 ± 1.94 U/mg protein for 10 mg/kg and 101.63 ± 3.02 U/mg protein for 20 mg/kg). A similar effect was obtained from the vitamin C treatment (Fig. 3). Catalase activity determined in the heart tissue homogenates of rats are shown in Fig. 4. The activity of catalase in the heart tissue homogenates of the rats that were intoxicated with sodium fluoride through drinking water (29.17 ± 1.01 $\mu\text{mol}/\text{min}/\text{mg}$ protein) was much lower than the normal group (45.36 ± 2.27 $\mu\text{mol}/\text{min}/\text{mg}$ protein). In the curcumin-treated rats that received curcumin at doses 10 and 20 mg/kg for 7 days before sodium fluoride intoxication, the catalase activity were higher than in the sodium fluoride intoxicated group (34.23 ± 1.73 $\mu\text{mol}/\text{min}/\text{mg}$ protein for 10 mg/kg and 43.60 ± 2.19 $\mu\text{mol}/\text{min}/\text{mg}$ protein for 20 mg/kg). Treatment with vitamin C prevented catalase activity abnormalities (Fig. 4). Fig. 5 shows reduced glutathione levels in the heart tissue homogenates of rats of different groups. Intoxication with sodium fluoride caused a significant reduction in the glutathione level in the homogenates of rat heart (3.81 ± 0.15 $\mu\text{g}/\text{mg}$ protein). Treatment with curcumin before sodium fluoride intoxication

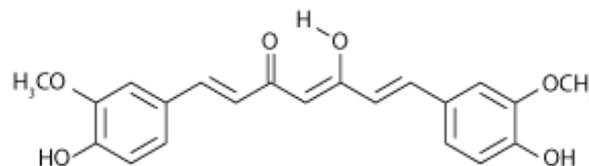


Fig. 1. Chemical structure of curcumin

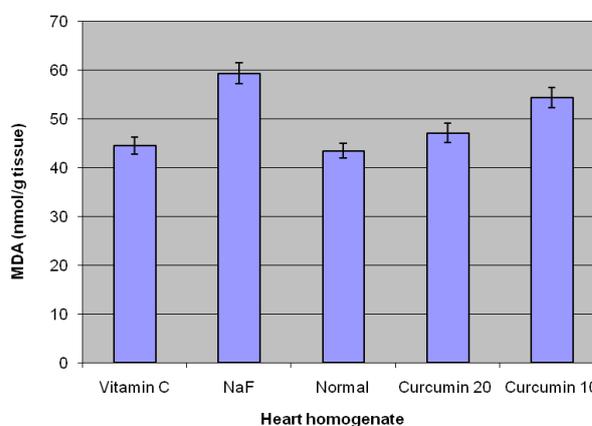


Fig. 2. The effect on malondialdehyde level in homogenate of rat heart tissues. Data are mean \pm S.D. values ($n = 10$). Significant different between normal vs. sodium fluoride treated group ($p < 0.001$). There is no significant difference between the normal and curcumin 10-treated group ($p > 0.05$).

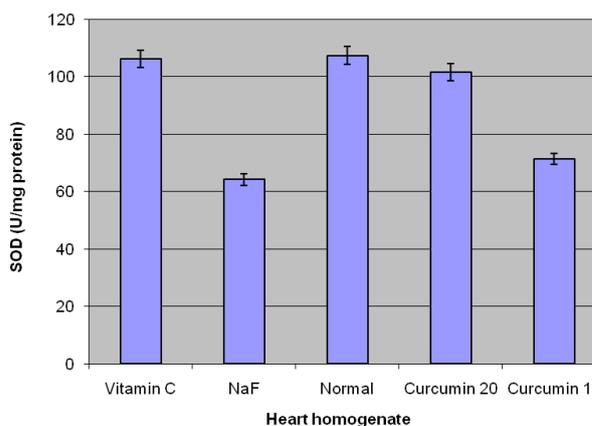


Fig. 3. The effect of sodium fluoride intoxication on superoxide dismutase activity in rat heart. Data are mean \pm S.D. values ($n = 10$). Significant difference between the normal vs. sodium fluoride and curcumin 20-treated rats ($p < 0.001$). There is no significant difference between the normal vs. curcumin 20 and vitamin C treated rats ($p > 0.05$).

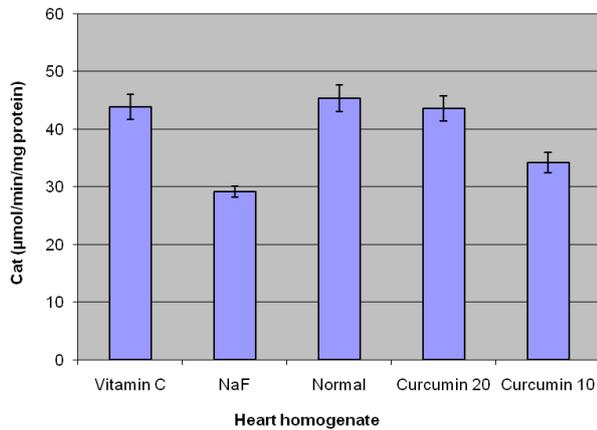


Fig. 4. Effect treatment on catalase levels in sodium fluoride intoxicated rats. Data are mean \pm S.D. values ($n = 10$). Significant difference between normal vs. sodium fluoride and curcumin 10-treated rats ($p < 0.001$). There is no significant difference between the normal vs. curcumin 20 and vitamin C treated rats ($p > 0.05$).

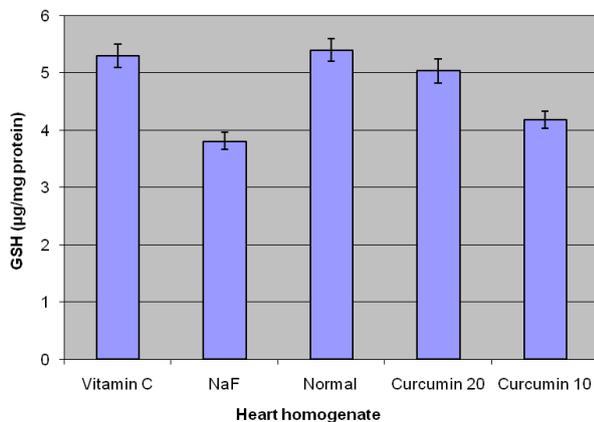


Fig. 5. The toxic effect of sodium fluoride on the levels of reduced glutathione in the heart homogenate of rat. Data are mean \pm S.D. values ($n = 10$). Significant difference between the normal vs. sodium fluoride, curcumin 10-treated rats ($p < 0.001$). There is no significant difference between the normal vs. curcumin 20 and vitamin C treated rats ($p > 0.05$).

caused increasing reduction of glutathione ($4.19 \pm 0.15 \mu\text{g}/\text{mg}$ protein for 10 mg/kg and $5.04 \pm 0.21 \mu\text{g}/\text{mg}$ protein for 20 mg/kg). Treatment with vitamin C before sodium fluoride intoxication prevented abnormalities in the level of reduced glutathione ($5.30 \pm 0.21 \mu\text{g}/\text{mg}$ protein).

The purpose of this study was to examine the cardioprotective effect of curcumin on sodium fluoride intoxication in rat heart. Therefore, different antioxidant-related parameters were examined, namely, TBARS, the activity of the enzymatic antioxidant SOD, catalase, as well as the level of the cellular GSH. Sodium fluoride caused significant oxidative stress in the homogenate of rat heart and this could be protected by the intraperitoneal administration of curcumin prior to sodium fluoride intoxication. During aerobic metabolism as well as exposure to different environmental substances such as radiation and redox cycling materials (Babior, 1992; Mates et al., 1999; Yamagishi et al., 2001), reactive oxygen species such as superoxide anion, hydrogen peroxide and the hydroxyl radical are produced *in vivo* due to successive reduction of oxygen. In some diseases such as cardiovascular, diabetes, neurodegenerative diseases, etc. free radicals have been shown to play an important role as mediators of disease progression (Mazzetti et al., 1996; Bartsch and Nair, 2000). Oxidative damage occurs when the production of free radicals exceeds the antioxidant defense systems causing injury to macromolecules such as DNA, proteins and lipids (Esterbauer and Cheeseman et al., 1990; Halliwell and Gutteridge, 2007; Gul et al., 2000; Sheweita et al., 2001). Antioxidant defense systems in humans manage to scavenge free radicals for the protection of the body against oxidative damages. The antioxidant defense systems contain various enzymatic antioxidants such as SOD, catalase, etc. together with the agents which are able to scavenge free radicals, like GSH (Sheweita et al., 2001). In addition, different antioxidants agents like ascorbic acid, vitamin E, etc. are also known as inhibitors of these free radicals (Esterbauer and Cheeseman, 1990; Liu et al., 1995). When the parameters of oxidative damage were examined in the homogenates of heart tissues of experimental male rats, we observed that rats intoxicated with sodium fluoride at 600 ppm through drinking water for 7 days exhibited significantly abnormal activities of SOD, catalase, as well as GSH levels and lipid peroxidation. Curcumin treatment at doses of 10 and 20 mg/kg body weight before sodium fluoride intoxication restored the activity of enzymatic antioxidants, i.e. SOD, catalase, and normalized the level

of GSH and lipid peroxidation in the homogenates of rat heart tissues. Treatment with vitamin C before sodium fluoride intoxication also showed similar effects like the curcumin treated animals whereas a sample solvent had no beneficial effects.

CONCLUSION

The presented results suggest that curcumin possesses a potential cardioprotective effect against sodium fluoride intoxication in male rats. This study is the first to show that the cardioprotective effect of curcumin, a natural bioactive compound, against sodium fluoride intoxication in the homogenates of rat heart tissues might be considered for clinical trials. The results can be viewed as a starting point for further applications of this natural compound in the pharmaceutical industry after performing clinical researches.

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