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Original article

Ameliorative effects of curcumin against sodium fluoride-induced hepatotoxicity

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Summary. *Objectives:* The present study was carried out to examine the possible protective effect of curcumin against fluoride-induced hepatotoxicity and oxidative stress. *Methods:* Hepatotoxicity was achieved after sodium fluoride exposure 600 ppm through drinking water during seven days. Different doses of curcumin were administered for seven days before sodium fluoride-induced toxicity. Vitamin C was also acquired as positive control. After the last treatment, thiobarbituric acid-reactive substances levels, activities of antioxidant enzymes including catalase and superoxide dismutase and also non enzymatic antioxidant reduced glutathione levels were determined in the rat liver. Moreover, some biochemical factors such as alkaline phosphatase, aspartate aminotransferase, alanine transaminase, triglyceride, high-density lipoprotein, low-density lipoprotein, total bilirubin, total protein and albumin were evaluated in serum samples. *Results:* Results showed that both vitamin C and curcumin reduced fluoride-induced abnormalities in serum biochemical factors. These compounds protected against imbalance of antioxidant enzymes and reduced lipid peroxidation levels in the rat liver. *Conclusion:* Combining results showed that curcumin possess protective effects against sodium fluoride-induced oxidative stress and hepatotoxicity.

Key words: curcumin, liver, oxidative stress, sodium fluoride

Introduction

Fluoride anion is widely distributed in the environment in several forms. Fluorine does not exist freely in environment (1). Fluorine anions in water sources are in ionic form and therefore quickly, completely and passively enter the intestinal mucosa and interfere with major metabolic pathways of living systems (2). Fluoride in low doses possesses significant prophylactic effect by inhibiting dental caries while in higher doses it causes dental and skeletal fluorosis (1). However, intoxication with high doses of fluoride is also harmful for other tissues (3). Fluoride enters the human body through water and food sources, toothpaste, mouth rinses, and other dental products (4). The fluorosis of human tissues is basically induced by burning coal, drinking water and tea (4). Fluorosis can induce many changes in enzyme activities, particularly metallo-enzymes, which can lead to severe disorders in different organ functions (5-7). Fluorosis, through excessive production of reactive oxygen species, causes reduction in some antioxidant enzyme activities (3, 8). Intoxication by fluoride causes abnormalities in various biochemical parameters (8-10). In soft tissues, fluoride toxicity increases free radical's generation and lipid peroxida-

tion (11). It has been reported that fluoride increases lipid peroxidation and disturbs antioxidant defense systems in the brain, erythrocytes and liver of rats (3, 11-13).

Researchers are looking for natural bioactive compounds that are able to show potent biological activities and curative effects against toxicant-induced damage (14-19). Curcumin, is a polyphenolic antioxidant, firstly isolated from rhizome of *Curcuma longa* L. This compound has been used as medicinal food from ancient time all over the world (20). In fact turmeric is widely used in cooking and gave Indian curry its flavor and yellow color. It is also used in mustard and to color butter and cheese. Structurally, due to presence of two electrophilic α,β-unsaturated carbonyl groups, curcumin is capable to react with glutathione (21). Also, there are two conjugated orthomethoxylated phenols and a β-diketone moiety (20, 21). Use of curcumin as a natural drug continues up to now. Numerous studies have shown that curcumin has wide range of beneficial effects, including anti-inflammatory, antioxidant, chemopreventive and chemotherapeutic activities (20). Hepatoprotective effect of curcumin against carbon tetrachloride-induced hepatotoxicity has been reported (20, 22). To our knowledge, there have been no scientific reports on hepatoprotective effects of curcumin against liver damage caused by fluoride-intoxication. In the present work, the possible ameliorative effect of curcumin against sodium fluoride-induced oxidative impairments in rat liver was examined.

Methods

Animals

Male Wistar rats weighing 200–250 g, purchased from Pasteur Institute of Iran-Amol Research Center, Amol (Mazandaran, Iran) were used in the present study. The animal experiments were processed following the internationally accepted ethical guidelines for the care of laboratory animals (DM42/2004-A). The rats were retained in animal rooms equipped with ventilation systems. The environmental temperature was kept at 24 ± 2°C and other conditions including 12 h light/dark cycle and 60 ± 5% humidity.

Reagent and kits

Commercial protein assessment kit and standard bovine serum albumin were bought from ZiestChem Company (Tehran, Iran). Other Kits for evaluation of other biochemical parameters were purchased from Sigma-Aldrich (USA). Curcumin, heparin, 5, 5-dithiobis (2-nitrobenzoic acid), nitro blue tetrazolium chloride, glacial acetic acid, potassium dihydrogen phosphate, trichloroacetic acid, sodium dihydrogen phosphate, reduced glutathione,sodium fluoride, thiobarbituric acid, hydrogen peroxide were bought from Sigma-Aldrich Chemical Company, (USA). Other solvents and reagents were purchased from Merck Chemical Company (Germany).

Animal treatments

Animals were randomly grouped into five equal groups (10 animals per each group). First group was kept as normal control which received 5% of dimethylsulfoxide solvent (1 ml/kg, intraperitoneally) for 7 consecutive days and then allowed for one week. Animals of second and third groups were administrated with two different doses of curcumin (10 mg/kg for second group and 20 mg/kg for third group, both dissolved in 5% of dimethylsulfoxide) intraperitoneally for one week and then exposed for seven days with sodium fluoride (600 ppm) via drinking water. Animals of group IV or positive control group were treated with vitamin C (10 mg/kg, dissolved in 0.9% saline) intraperitoneally for 7 days followed by 7 days sodium fluoride 600 ppm exposing. Animals of group V were treated with 5% of dimethylsulfoxide solvent (1 ml/kg, intraperitoneally) for one week and then treated with sodium fluoride (600 ppm through drinking water) for the same time and used as control group.

Anesthesia and tissue collection

At the end of the experimental period, the rats were anesthetized using mixture of xylazine (5 mg/kg) and ketamine (60 mg/kg) after withholding food for 12 h. The liver was removed. Blood samples were picked through cardiac puncture for the determination of biochemical parameters. Serum was separated using

10 minute centrifuging at 12000 g at 4 ˚C. Samples were frozen at -60ºC until biochemical assessments.

Tissue homogenizing

Briefly, liver tissue was homogenized in mixture phosphate buffer saline (100 mM, pH 7.4) and 1 mM ethylenediaminetetraacetic acid (1:10 w/v). Then, above mention mixture was centrifuged for one half at 12000 g (4˚C). The upper layer was collected and utilized for biochemical analysis.

Measurement of protein content

Bradford method was acquired for determination of protein content in homogenates of rat liver. Bovine serum albumin was used for drawing of standard curve (23).

Biochemical analysis

Estimation of lipid peroxidation

Briefly, in order to determination of Lipid peroxidation, one milliliter trichloroacetic acid (20%) and two milliliter thiobarbituric acid (0.67%) was added to tissue homogenates which contain one milligram of protein. The above mention reaction mixture was incubated at boiling water (100˚C). After one hour, reaction tube was cooled and centrifuged for eliminating of precipitate. The absorbance of reaction mixtures was recorded at λ = 532 nm in contrast of a blank which contains one milliliter trichloroacetic acid (20%) and two milliliter thiobarbituric acid (0.67%) (3).

Determination of superoxide dismutase activity

Reaction tube was contained two milliliter of 50 mM sodium carbonate, 0.4 milliliter of 0.1 mM freshly prepared hydroxylamine hydrochloride and 0.8 milliliter of 25 µM nitroblue tetrazolium. Then 0.2 milliliter of homogenates was added to above mention mixture. The absorbance of the reaction mixture was recorded at λ = 560 nm (3, 24).

Assessment of catalase enzyme activity

Briefly, the amount of homogenate which contain 5 µg proteins was mixed with 2.1 milliliter of 7.5 mM hydrogen peroxide. Then, a time scan at λ = 240 nm was carried out for ten min in room temperature. The vanishing of peroxide expressed the catalase activity. Each unit of catalase activity is known as the volume of enzyme which needed to reduce one micromole of hydrogen peroxide during one minute (7).

Estimation of reduced glutathione level

Briefly, homogenates (720 µl) were double diluted and then in order to precipitate the protein, 5 % trichloroacetic acid was added. The reaction mixture was centrifuged for 5 min at 12000 g. The upper layer was separated and mixed with 5, 5-dithiobis(2-nitrobenzoic acid solution. Absorbance of mixture was recorded at λ = 417 nm. Standard reduced glutathione solution was acquired for drawing of standard curve (7).

Serum chemistry

Alkaline phosphatase, aspartate aminotransferase, alanine transaminase, triglyceride, high-density lipoprotein, low-density lipoprotein, total bilirubin, total protein and albumin were measured in serum samples using commercially available kits.

Statistical analysis

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

Results

Data are summarized in Table 1. According to results, sodium fluoride exposure increase lipid peroxidation level of liver homogenates (53.11 ± 2.14 nmol MDA eq/g tissue) compared to the group I animals (25.03 ± 1.27 nmol MDA eq/g tissue). Seven days administration of curcumin or standard antioxidant (vitamin C) prior to sodium fluoride intoxication cause an increase in malondialdehyde levels (47.96 ± 1.98 nmol MDA eq/g tissue for 10 mg/kg of curcumin, 32.32 ± 1.54 nmol MDA eq/g tissue for 20 mg/kg of curcumin and 38.61 ± 1.66 nmol MDA eq/g tissue for vitamin C). Table 1 shows superoxide dismutase activities of treated groups. Administration of curcumin

increased the superoxide dismutase activities (45.03 ± 1.85 U/mg protein for 10 mg/kg and 52.46 ± 2.04 U/mg protein for 20 mg/kg). Vitamin C treatment leads to similar results (Table 1). Moreover, Table 1 shows activity of catalase in liver homogenates. Catalase activity in the liver homogenates of sodium fluoride-intoxicated rats (75.21 ± 3.69 μmol/min/mg protein) was fewer than group I rats (227.06 ± 6.49 μmol/min/mg protein). Activities of catalase also increased with curcumin administration (101.55 ± 4.56 μmol/min/mg protein for 10 mg/kg and 173.02 ± 5.12 μmol/min/mg protein for 20 mg/kg). Results of reduced glutathione levels are summarized in table 1. Exposure of sodium fluoride led to significant diminishing in reduced glutathione level (8.5 ± 0.57 μg/mg protein). Curcumin treatment increased the level of reduced glutathione (11.30 ± 0.65 μg/mg protein for 10 mg/kg and 15.38 ± 0.91 μg/mg protein for 20 mg/kg). Vitamin C also shows similar effect against sodium fluoride-induced decreasing of reduced glutathione (14.95 ± 0.83 μg/mg protein). Obtained data from serum biochemical parameters assessment are summarized in Table 2. Sodium fluoride for 7 consecutive days caused significant disturbance in serum parameters. Both curcumin and vitamin C showed mitigating effects against sodium fluoride-induced irregularities in biochemical parameters of the serum (Table 2).

Discussion

There are two different types of fluorosis: endemic fluorosis and industrial fluorosis. Endemic fluorosis is related to the high doses of fluoride in drinking water, whereas industrial fluorosis is chiefly due to fluorine pollution (3, 7). There are various types of fluoride-containing products and these are widely used in industry and medicine. Fluoride is distributed from plasma to all tissues and organs of the body (25). In the body, liver and kidneys have an important role in fluoride detoxification (25).

Oxidative stress has been implicated in the pathogenesis and progression of many diseases and intoxications (20, 26). Fluoride causes oxidative stress by increasing generation of reactive oxygen species, leading to cell damage and death (27, 28). A close correlation between chronic fluoride toxicity and oxidative stress has been previously reported (29, 30). It has also been reported that fluoride toxicity causes increases in lipid peroxidation of erythrocytes in humans and in blood and tissue of experimental animals (31). In the body, liver and kidneys have most important role in fluoride elimination and detoxification, and numerous studies that have demonstrated the correlation between fluoride intake and liver injury (3, 32).

Curcumin is the most active constituent of the dietary spice turmeric which constitutes up to 8% of most turmeric preparation (33). It is a polyphenol compound that has demonstrated antioxidant, anti-inflammatory and anti-cancer properties (34). However, its efficacy is influenced by low oral bioavailability, about 1% in rodents, and its rapid metabolism (35). It has been reported that intraperitoneal administration of curcumin metabolically converted to tetrahydrocurcumin, hexahydrocurcuminol, and hexahydrocurcumin (20).

Table 1. Effect of pretreatment on superoxide dismutase and catalase activities and reduced glutathione, TBARS levels in sodium fluoride induced oxidative stress in rat Liver.

Group	SOD (U/mg protein)	Cat (μmol/min/mg protein)	GSH (μg/mg protein)	TBARS (nmol MDA eq/g tissue)
Group I	56.30 ± 2.48	227.06 ± 6.49	17.01 ± 1.01	25.03 ± 1.27
Group II	45.03 ± 1.85 **	101.55 ± 4.56 **	11.30 ± 0.65 *	47.96 ± 1.98 **
Group III	52.46 ± 2.04 ****	173.02 ± 5.12 **	15.38 ± 0.91 ***	32.32 ± 1.54 ****
Group IV	53.77 ± 2.21 ****	183.34 ± 5.87 **	14.95 ± 0.83 ***	38.61 ± 1.66 **
Group V	37.23 ± 2.15 **	75.21 ± 3.69 **	8.5 ± 0.57 **	53.11 ± 2.14 **

*Values are mean ± SE (n = 10). Data for normal animals are considered as base-line data; there was no significant base-line difference between the groups. * P<0.01 versus Group I, ** P<0.001 versus Group I, *** P>0.05 versus Group I, **** P<0.05 versus Group I.*

Table 2. Effect of curcumin on serum biochemical parameters in sodium fluoride induced hepatotoxicity in rat.

Biochemical parameters	Group I	Group II	Group III	Group IV	Group V
Alkaline phosphatase (U/l)	329.54 ± 10.80	391.23 ± 12.96 ***	199.88 ± 5.15 *	387.11 ± 9.36 ***	608.51 ± 19.02 **
Aspartate aminotransferase (U/l)	134.89 ± 5.67	160.12 ± 6.54 ***	131.65 ± 5.18 ****	151.01 ± 6.33 ***	253.64 ± 7.41 **
Alanine transaminase (U/l)	17.47 ± 0.84	28.07 ± 1.39 *	19.75 ± 0.90 ****	29.77 ± 1.16 *	32.34 ± 1.67 **
Triglyceride (mg/dl)	104.36 ± 3.88	129.57 ± 3.91 ***	109.01 ± 2.44 ****	137.28 ± 4.09 ***	160.05 ± 5.44 *
High-density lipoprotein (mg/dl)	45.80 ± 1.93	44.22 ± 1.54 ***	46.06 ± 2.15 ***	44.64 ± 1.63 ***	41.31 ± 1.77 *
Low-density lipoprotein (mg/dl)	12.53 ± 0.50	12.41 ± 0.43 ****	10.50 ± 0.47 ****	13.88 ± 0.62 ****	21.06 ± 1.13 **
Total Bilirubin (mg/dl)	0.15 ± 0.00	0.24 ± 0.00 *	0.20 ± 0.0 ***	0.35 ± 0.01 **	0.38 ± 0.02 **
Total protein (g/dl)	6.04 ± 0.24	5.01 ± 0.21 ***	5.80 ± 0.23 ***	5.74 ± 0.17 ***	4.55 ± 0.18 *
Albumin (g/dl)	3.11 ± 0.13	3.08 ± 0.10 ***	3.15 ± 0.12 ***	3.00 ± 0.09 ***	2.84 ± 0.11 *

*Values are mean ± SE (n = 10). Data for normal animals are considered as base-line data; there was no significant base-line difference between the groups. * P<0.01 versus Group I, ** P<0.001 versus Group I, *** P>0.05 versus Group I, **** P<0.05 versus Group I.*

Curcumin is well known to mitigate hepatoxicity induced by toxic agents like carbon tetrachloride (36), lindane (37), Freund's complete adjuvant (38), lipopolysaccharide-galactosamine (39) and iron (39).

In this study we put forward the hypothesis that curcumin administration in rats can effectively protect the liver, by its antioxidant effect, from fluoride toxicity. Protective activity of curcumin may be due to its potent antioxidant activity and ability to eliminate free radicals and oxidative stress as previously reported in literature. In particular, Barzegar and Moosavi-Movahedi (40) reported that curcumin showed a powerful capacity for scavenging intracellular free radicals such as $HO^{\cdot}$, $ROO^{\cdot}$. In fact curcumin was able to penetrate the cells and to protect them against the highly toxic and lethal effects of cumene hydroperoxide (30). Curcumin also showed good electron-transfer capability, with greater activity than trolox in aqueous solution. Curcumin can readily transfer an electron or easily donate an H-atom from two phenolic sites to scavenge free radicals. The excellent electron-transfer capability of curcumin is because of its unique structure and different functional groups, including a β-diketone and several π electrons that have the capacity to conjugate between two phenyl rings. Therefore, although curcumin is inherently a lipophilic compound, because of its superb intracellular ROS scavenging activity, it can be used as an effective antioxidant for ROS protection (30).

The present study showed that one-week administration of curcumin has a protective effect against fluoride-induced liver oxidative damage in the rat, and supports the hypothesis that superoxide radicals are involved in fluoride-induced pathogenesis.

Decreased levels of reduced glutathione, catalase and superoxide dismutase activities in different tissues have been observed in experimental animals (1, 28). Lipid peroxidation caused by free radicals has an important role in oxidative injury. Antioxidant enzymes such as catalase and superoxide dismutase and non-enzymatic antioxidants such as reduced glutathione can reduce free radicals and decrease lipid peroxidation. Shanthakumari et al. (1) reported that fluoride intoxication increased levels of lipid peroxides and decreased reduced glutathione and activities of catalase and superoxide dismutase. In addition, Shivarajashankara et al. (11) have suggested that increased oxidative stress may be one of the mediating factors in the pathogenesis of fluoride-induced toxicity.

In this study, we have shown that fluoride increases the production of reactive oxygen species, intensifies oxidative stress, and induces hepatotoxicity. We also have found that the pretreatment with polyphenolic compound curcumin ameliorates oxidative damage to liver tissues in the male rat and normalizes hepatic markers. Especially, reactive oxygen species may contribute to the pathophysiological change of fluoride-induced hepatotoxicity and liver

dysfunctions. Trichloroacetic acid reactive substance (TBARS) level (an index of lipid peroxidation in rat liver) was used as reliable parameter of oxidative stress-induced hepatotoxicity and for evaluation the protective role of curcumin. The level of reduced glutathione and activities of catalase and superoxide dismutase in liver homogenates were examined to evaluate the changes in antioxidant-oxidant balance. Antioxidants play an important role in protecting liver against free radical-induced hepatotoxicity by directly scavenging and/or inhibiting them. Curcumin is a natural antioxidant, which contains Michael-acceptor functionalities and hydroxyl groups, which can inhibit reactive oxygen species and induce antioxidative enzymes like glutathione-*S*-transferase generation, which expedite the elimination of toxic substances from the body (41). It is believed that antioxidant activity of curcumin is result of β-diketone moiety and C-H bond cleavage at active methylene carbon of the β-diketone moiety (42). Also, metal chelation activity of curcumin and its ability to suppress NF-κB activity may be the main mechanisms of curcumin antioxidant activity (43, 44).

Conclusion

Present study showed significant hepatoprotective effect of curcumin against hepatotoxicity induced by sodium fluoride that might be considered for clinical trials. Curcumin significantly reduced oxidative stress and normalized serum biochemical parameter levels of fluoride-intoxicated rats. The hepatoprotective activity of curcumin may be due to its antioxidant properties, and curcumin can be used as potent antioxidant natural product in order to limit fluoride induced damages.

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