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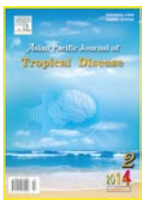

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Sodium fluoride induced antioxidant defense impairment and impaired renal biomarkers and the ameliorative role of selenium and curcumin in male mice

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ABSTRACT

Objective: To evaluate the sodium fluoride (NaF) intoxicated kidney function parameters and also assess the ameliorating effects of selenium and curcumin extract.
Methods: Mature male mice (weighing 35-45 g) were given intraperitoneally NaF (10.3 mg/kg) and/or selenium (0.5 mg/kg)+curcumin extract (60 mg/kg) daily for 4 weeks.
Results: In our study, NaF exposure resulted in an increase in the urea, creatinine, triglycerides, total cholesterol, high density lipoprotein, low density lipoprotein and very low density lipoprotein levels with respect to the control. As a result, NaF induced antioxidant defense system impairment and renal impairment which was reduced by curcumin extract and/or selenium to great extent by the entire restoration of the histological structures.
Conclusions: Our study showed that selenium and curcumin extract treatment alleviated NaF intoxication-induced oxidative damage of mice.

1. Introduction

Fluoride is a trace element which is widely distributed in the environment[1]. The wide spread allocation of fluoride in nature and chronic exposure of millions of people worldwide is an endemic problem in a number of countries[2]. Food of the sea and dark green vegetables as the plant of tea are very rich in fluoride[3]. Moreover, food additives, insecticides, anti-carcinogenic drugs and some anaesthetic inhalation such as methoxiflurane are other sources of fluoride exposure[4]. Also, cooking in cookware coated with teflon lining may be the cause for increasing the fluoride concentration in prepared food inside them[5].

Fluorides are distributed to great extent in the environment in different forms and their compounds are extensively used. Fluoride anions are present essentially in water sources in nature and drinking water as they are released from the run off of fluoride-containing rocks and soils and leach into groundwater[3]. In some areas, drinking water is fluoridated artificially. Therefore, the daily fluoride intake may be due to the water consumption. Furthermore, fluoride anions are incorporated in various insecticide formulations, fluoridated foodstuffs, dentifrices, drugs, vapors emitted from industries using fluoride containing compounds[6].

Upon the fatty acids peroxidation, non-enzymatic antioxidant malondialdehyde (MDA) is generated. Although MDA is not an essential indicator of the fatty acids peroxidation, it is considered as the most important marker of membrane lipid peroxidation (LPO) arising

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from the interaction of reactive oxygen types with cellular membranes. Among enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), SOD is considered as an antioxidant enzyme. In the presence of metal ions, SOD enzyme converts its superoxide anions into H_2O_2 (copper and iron). CAT and GSH-Px are enzymes which are essentially involved in the breakdown of H_2O_2[7].

Natural herbal constituents are extensively studied for their ability to protect cells from miscellaneous damages. Currently, the use of phytochemicals as a therapy in diseases related to the impairment of the antioxidant defense system and thus these natural herbs have gained immense interest for their ability to quench free radicals by electron or proton donation and their capability to protect body tissues against oxidative stress[8].

Selenium is an essential micronutrient for humans and animals, and plays a central role in a number of enzyme mediated pathways that form part of the antioxidant defense system[9]. An inadequate intake of selenium may result in suboptimal concentrations of selenoproteins and, therefore, diminished protection against oxidative stress[10].

Selenium, a human body essential trace element, displaying an antioxidant effective oxygen free radical scavenging, protect the organs and tissues from oxidative damage and improve the body's immune system[11].

The naturally occurring element selenium is essential for a wide variety of biological processes in mammals[12]. Its beneficial role in human health is due to low molecular weight selenium compounds, as well as to its presence within at least 25 proteins, named selenoproteins, in the form of the amino acid selenocysteine, that is incorporated during translation and is directly involved in redox catalysis[13].

Some studies also showed that selenium stimulated antioxidative enzymes in immature kidney of cadmium (Cd)-exposed rats and could protect against oxidative damage[14].

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene 3,5-dione) which is the main portion of turmeric, has been shown to have antioxidant potent factor[15]. Curcumin is a potent scavenger for LPO and nitrogen species such as hydroxyl radicals and nitrogen dioxide radicals[16].

Curcumin, a yellow pigment included in turmeric or the powder of curry, is a polyphenol natural product isolated from rhizome of plant *Curcuma longa*[17]. Curcumin is considered to be anti-inflammatory, antioxidant, chemopreventive and chemotherapeutic effects by modulating various signalling molecules such as transcription factors, protein kinases, adhesion molecules, growth factors and receptors[17].

Curcumin which is the active compound in turmeric because of its antioxidant and anti-inflammatory properties, has been demonstrated in the prevention and treatment of neurodegenerative disorders such as Alzheimer disease and multiple sclerosis[18].

2. Materials and methods

2.1. Experimental animals

This study was performed on 70 mature male mice, weighing about 35-45 g body weight. We have obtained animals from the animal house of the King Fahad Center for Medical Research, King Abdul-Aziz University of Jeddah. They were breeding in a well-ventilated room with the room temperature ranging between 22 and 25 °C and maintained under united conditions away from any stressful conditions with free access to water and were fed with dry balanced meal for experimental animals provided by the General Organization for Grain Silos and Flour Mills in Jeddah. All experimental procedures and animal maintenance were conducted in accordance with the accepted standards of animal care per cage (Council of Europe, the protection of vertebrate animals by the European convention for 2006). We have followed the Directive of the European community (86/609/EEC).

2.2. Chemicals

2.2.1. Sodium fluoride (NaF)

NaF was purchased from Sigma Chemical Co. The dose of NaF (10.3 mg/kg body weight) was chosen based on the previous studies of Zabulyte *et al*[19]. The dose schedule was adjusted so that the amount of NaF administration per animal was as per their respective weight.

2.2.2. Selenium

Selenium was purchased from BDH Chemicals Ltd., England. The tested dose of selenium (0.5 mg/kg) was chosen according to the previous studies of Ben Amara *et al*[20].

2.2.3. Curcumin extract

Fresh curcumin was obtained from local market (Cairo, Egypt), then washed. After that it was dried and homogenized by using electrical mixer and soaked in water for 24 h. The water extract was prepared (60 mg/kg) and this dose was chosen according to Abdul-Hamid and Moustafa[21].

2.3. Experimental protocols

The study was performed on 70 mature male mice, divided into 7 main groups; each group was consisted of 10 mice. The 1st control group: Animals received 1 mL of distilled water intraperitoneally daily for 30 successive days. The 2nd NaF treated group: Animals were daily received NaF (10.3 mg/kg) (1 mL of prepared solution) for 30 successive days intraperitoneally. The 3rd selenium treated group: Animals were received selenium (0.5 mg/kg) for 30 successive days intraperitoneally. The 4th curcumin extract treated group: Animals were received curcumin extract (60 mg/kg) for 30 successive days intraperitoneally. The 5th NaF+selenium treated group: Animals were given NaF (10.3 mg/kg) for 30

successive days and then co-administered by selenium (0.5 mg/kg) intraperitoneally. The 6th NaF+curcumin treated group: Animals were given NaF (10.3 mg/kg) for 30 successive days and then co-administered curcumin extract (60 mg/kg) for 30 successive days intraperitoneally. The 7th NaF+selenium+curcumin extract treated group: Animals were given NaF (10.3 mg/kg) and then co-administered with selenium (0.5 mg/kg) and then followed by curcumin extract (60 mg/kg) for 30 successive days intraperitoneally. The substances were given to animals in the morning (between 09.30 and 10.30 h) to non fasted mice. At the end of the experiment animals were scarified, the testis tissues were dissected; the testis tissues were quickly divided into two parts, one was fixed in buffered formalin (10%) for histopathological examination and the other part was used for preparation of tissue homogenates for biochemical estimation.

2.4. Blood samples collection

At the end of the experiment, blood samples were collected from the retro-orbital vein, which is considered as a simple, prosperous and effective procedure that allows the bleeding of the same animal more than one time[22]. At the end of 4th week, individual blood samples were drawn by orbital puncture (from eye plexus) using microhematocrit capillary tubes (Lancer, Athy, County-Kildare, Republic of Ireland). Serum was prepared from blood without ethylene diamine tetraacetic acid (EDTA).

2.5. Kidney function parameters

Serum urea was determined calorimetrically using Diamond kit[23], according to the following reaction:

$$\text{Urea}+HO_2 \xrightarrow{\text{Urease}} 2NH_3+CO_2$$

In an alkaline medium, ammonium ions react with the salicylate and hypochlorite to form a green colored indophenol (2.2 dicarboxyl indophenol). Creatinine form colored complex when react with picrate in alkaline solution[24]. The rate of the formation of complex was measured.

2.6. Preparation of kidney homogenate

One gram of the kidney tissues were washed in ice-cold isotonic saline containing 1 mmol/L EDTA. The tissues were then homogenized separately in 10 volumes of potassium phosphate buffer tissues that were perfused with a 50 mmol/L sodium phosphate buffer saline (100 mmol/L Na_2HPO_4/NaH_2PO_4) (pH 7.4) containing 1 mmol/L EDTA using a homogenizer at 4 °C. The tissue homogenates were then centrifuged at 8000 r/min for ½ h at 4 °C and the resulting supernatant was transferred into eppendorf tubes, and preserved at -20 °C in a deep freezer until used for various biochemical assays[25]. The supernatant was collected for the estimation of MDA, reduced glutathione (GSH) as well as the activity of SOD, CAT and GSH-Px.

2.7. Antioxidant enzyme determination

Lipid peroxides contents were evaluated by using the thiobarbituric acid test as described by Ohkawa *et al*[26]. After incubation of testis homogenate with thiobarbituric acid at 95 °C, thiobarbituric acid reactive substance (TBARS) reacts to form a colored complex. Absorbance was measured spectrophotometrically at 532 nm to determine the TBARS content. The specific activity is expressed as nmol/mg protein.

2.7.1. Measurement of SOD

SOD activity was measured according to the method described by Marklund by assaying the auto oxidation of pyrogallol at 440 nm for 3 min[27]. One unit of SOD activity was calculated as the amount of protein that caused 50% pyrogallol autooxidation inhibition. A blank without homogenate was used as a control for non-enzymatic oxidation of pyrogallol in Tris-EDTA buffer (50 mmol/L Tris, 10 mmol/L EDTA, pH 8.2). The SOD activity is expressed as IU/mg protein.

2.7.2. Measurement of CAT

CAT activity was measured according to the method described by Aebi by assaying the hydrolysis of H_2O_2 and the resulting decrease in absorbance at 240 nm over a 3 min period at 25 °C[28]. Before determination of the CAT activity, samples were diluted 1:9 with 1% (v/v) Triton X-100. CAT activity is expressed as mmol/mg protein.

2.7.3. Measurement of GSH-Px

GSH-Px activity was measured using H_2O_2 as substrate according to the method described by Paglia and Valentine[29]. The reaction was monitored indirectly as the oxidation rate of nicotinamide adenine dinucleotide phosphate at 240 nm for 3 min. A blank without homogenate was used as a control for non-enzymatic oxidation of nicotinamide adenine dinucleotide phosphate upon addition of hydrogen peroxide in 0.1 mol/L Tris buffer, pH 8.0. Enzyme activity was expressed as nmol/mg protein.

2.8. Statistical analysis

Data were collected, arranged and reported as mean± SEM of seven groups (each group was considered as one experimental unit), summarized and then analyzed using the computer program SPSS version 15.0. The statistical method was One way ANOVA test, and if significant differences between means were found, Duncan's multiple range test (whose significant level was defined as $P<0.05$) was used

according to Snedecor and Cochran to estimate the effect of different treated groups[30].

3. Results

3.1. Evaluation of biochemical parameters (lipid parameters)

3.1.1. Effect of NaF, selenium, and curcumin extract and their combinations on serum urea

Table 1 demonstrates that treatments of mice with NaF exhibit significant increase (P<0.05) in serum urea when compared with control group. The same previous effect was obtained in response to treatment with either selenium and/or curcumin extract and their combinations with NaF as compared with control group but the effect was much less intense. A non significant increase was recorded in group treated with selenium when compared also with control group.

Table 1

Effect of NaF (10.3 mg/kg), selenium (0.5 mg/kg), curcumin extract (60 mg/kg) and their combinations on kidney function in male mice (mean±SE, n=7).

Groups	Urea (mg/dL)	Creatinine (mg/dL)
Control group	22.50±1.20^{g}	0.58±0.01^{f}
NaF	51.00±4.08^{a}	0.97±0.02^{a}
Selenium	24.20±1.88^{fg}	0.59±0.02^{ef}
Curcumin extract	29.10±1.51^{d}	0.62±0.02^{c}
NaF+Selenium	32.54±0.70^{bc}	0.61±0.01^{c}
NaF+Curcumin extract	31.10±2.77^{c}	0.65±0.01^{bc}
NaF+Selenium+Curcumin extract	27.08±2.10^{e}	0.60±0.01^{d}

Values within the same column in each category carrying different letters are significant (P≤0.05) using Duncan's multiple range test, where the highest mean value has symbol ^{a} and decreasing in value was assigned alphabetically.

3.1.2. Effect of NaF, selenium, curcumin extract and their combinations on serum creatinine

Table 1 illustrates the effect of various treatments on serum creatinine of normal mice when given daily for successive 30 days. Regarding the effect of selenium on normal mice, selenium afforded non-significant increasing in serum creatinine at the end of the study when compared with control group. Whereas, treatments with NaF exhibited significant increase in serum creatinine at the end of the experiment compared to normal control groups. The same response was obtained in response to treatment of mice with the combination of the NaF used with either selenium or curcumin extract but the effect was less intense.

3.2. Antioxidant activities

3.2.1. Effect of NaF, selenium, curcumin extract and their combinations on CAT activity

Regarding the effect of NaF on CAT activity of normal mice, NaF afforded a marked decrease (P<0.05) in kidney CAT at the end of the study when compared with control group, whereas, non significant changes in the activity of CAT was recorded in selenium treated group. Treatment of normal mice with selenium alone exhibited non significant changes in CAT of kidney at the end of the experiment when compared with control group. Whereas, a significant decrease was reported in kidney tissues respectively when administered curcumin extract alone as compared with control group (Table 2). While combinations of NaF with either selenium or curcumin extract exhibited a significant decrease in CAT activity of kidney at the end of the study as compared with normal control group.

3.2.2. Effect of NaF, selenium, curcumin extract and their combinations on SOD activity

This study revealed that treatment of normal mice with NaF afforded a highly significant decrease in SOD activity of kidney when compared with control group. Treatment of normal mice with either selenium or curcumin extract for 4 weeks elicited a non significant decrease in kidney SOD activity except those treated with curcumin extract which showed a slight significant decrease in SOD activity compared with control group. Whereas, the combinations of the curcumin extract and/or selenium with NaF afforded a slight decrease (P<0.05) in kidney SOD activity as compared with normal control group (Table 2). Meanwhile, combination of NaF with curcumin extract and selenium afforded slight decrease in SOD activity but the effect was much better than group treated with NaF only and other treatment combinations.

3.2.3. Effect of NaF, selenium, curcumin extract and their combinations on MDA activity

The MDA content of the kidney was increased significantly (P<0.05) in response to treatment of normal male mice with NaF for 4 weeks as compared with normal control group. The same previous response was reported with selenium, curcumin extract combinations with NaF compared with control group (Table 2) but the effect was much less intense. Meanwhile, groups treated with either selenium or curcumin extract induced non significant changes in serum MDA level as compared to normal control group while selenium treated group afforded non significant changes in MDA activity in kidney homogenates as compared with normal control group. At the same time, curcumin extract afforded slight increase in MDA activities in kidney homogenates as compared to normal control group.

3.2.4. Effect of NaF, selenium, curcumin extract and their combinations on GSH reductase activity

Table 2 shows that treatment of mice with NaF alone afforded a significant decrease (P<0.05) in kidney reduced GSH at the end of the study when compared with normal control group. On the other hand, the results revealed that selenium exhibited a non significant change in reduced GSH content of the kidney as compared to normal control group with slight increase in the reduced GSH content of kidney homogenates in curcumin extract treated group compared

Table 2

Effect of NaF (10.3 mg/kg), selenium (0.5 mg/kg), curcumin extract (60 mg/kg) and their combinations on antioxidant activities in male mice (mean±SE, n=7).

Groups	Kidney CAT (IU/g)	Kidney SOD (IU/g)	Kidney MDA (nmol/g)	Kidney GSH reductase (IU/g)	Kidney GSH-Px (IU/g)
Control group	5.49±0.05^{ab}	12.41±1.79^{b}	8.67±0.02^{f}	6.41±0.50^{a}	5.13±0.36^{b}
NaF	1.41±0.04^{f}	5.59±1.22^{g}	28.68±0.42^{a}	1.62±0.30^{e}	1.47±0.36^{f}
Selenium	5.28±0.07^{b}	12.76±1.51^{ab}	8.60±0.18^{f}	6.50±0.43^{a}	5.32±0.40^{ab}
Curcumin extract	5.11±0.01^{c}	11.66±1.18^{c}	9.42±0.01^{e}	5.31±0.38^{b}	4.27±0.43^{c}
NaF+Selenium	4.01±0.07^{e}	9.62±1.22^{e}	14.79±0.03^{c}	3.25±0.92^{d}	2.25±0.92^{e}
NaF+Curcumin extract	4.07±0.07^{e}	9.05±1.24^{f}	19.27±0.15^{b}	3.41±0.29^{d}	2.41±0.29^{e}
NaF+Selenium+Curcumin extract	4.74±0.08^{d}	10.25±1.03^{d}	10.59±0.05^{d}	4.20±0.53^{c}	3.20±0.53^{d}

Valuess within the same column in each category carrying different letters are significant ($P\leq0.05$) using Duncan's multiple range test, where the highest mean value has symbol [a] and decreasing in value was assigned alphabetically.

with control group.

3.2.5. Effect of NaF, selenium, curcumin extract and their combinations on GSH-Px activity

The plasma GSH-Px level was significantly reduced (P<0.05) in all groups treated with NaF alone and in combination with selenium, curcumin extract for 30 d when compared with normal control group. Whereas, non significant change was recorded in the enzyme activity of the kidney homogenates of group treated with selenium with respect to control group, together with a significant decrease in the GSH-Px enzyme activity in kidney homogenates in response to treatment with curcumin extract as compared with normal control group. Whereas, a significant decrease was reported in response to treatments with all combinations used except combination of NaF with selenium and curcumin which showed a slight decrease compared with normal control group (Table 2).

4. Discussion

The present study was an attempt to evaluate the toxicity of NaF and possible ameliorative role of selenium or curcumin extract as it is well known that selenium and curcumin extract have been reported to be effective antioxidant.

The increment of generation of reactive oxygen species (ROS) is implicated in the pathogenesis of many diseases and in the toxicity of a wide range of compounds[31]. LPO represents one of the most frequent reactions resulting from free radical's attack on biological structures[32].

The present study revealed increased levels of TBARS, the marker of extent of LPO, in the liver of NaF-treated mice. And our findings are greatly reinforced by other studies that have recorded increased free radicals levels in the erythrocytes of fluorotic humans[33], and in the erythrocytes, liver, kidney, brain and ovary of experimental animals[31,33,34].

Reduced GSH level and total antioxidant capacity as well as the activities of SOD and CAT decreased with NaF testament. These results are in accordance with Shanthakumari *et al.* who found decrease in the levels of GSH with an increase in GSH-Px activity in the brain, erythrocytes and liver of rats exposed to fluoride[35]. They reported that the decrease in GSH level with an increase in MDA level and GSH-Px activity indicates utilization of GSH for GSH-Px catalyzed scavenging of H_2O_2 or lipid hydroperoxides generated. Thus, this degree of toxicity of fluoride resulted in reduction in the level of the free radical scavenger GSH.

The decreased GSH along with an increased activity of GSH-Px in the fluoride-treated group suggests increased conversion of GSH to oxidized GSH to combat lipid hydroperoxides or H_2O_2. Previous studies in this field reported decreased GSH and GSH-Px in various tissues of experimental animals subjected to chronic fluoride toxicity[34].

In children with endemic skeletal fluorosis, Shivarajashankara *et al.* observed decrease in GSH levels and increase in GSH-Px activity in erythrocytes[36]. Since ROS are implicated as important pathologic mediators in many disorders, various studies have investigated whether oxidative stress and LPO are involved in the pathogenesis of chronic fluorosis.

A decrease in the activity of free radical scavenging enzymes, SOD and GSH-Px was found in people living in areas of endemic fluorosis[37]. A similar inhibitory effect of fluoride on SOD in germinating mung-bean seedlings supports the above findings and indicates the possibility of greater toxicity if fluoride can impair the free radical scavengers[38].

In another study, in accordance with our findings, the decreased levels of glutathione S-transferase, SOD and CAT activities in rat brain upon ingestion of NaF (20 mg/kg body weight/day, intraperitoneally) for 14 d were observed[39]. Romero *et al.* suggested the mechanism of fluoride injuring soft tissues that caused excessive production of the level of LPO and oxygen free radicals, led to the ability of scavenging free radicals and antioxidation being reduced[13]. These radicals can seriously damage biological membrane structure, functions of cells, and biomacromolecules, such as proteins and nucleic acids, furthermore, damage the entire soft tissues.

Some studies indicated that increased generation of free

radicals and enhanced LPO had been shown to play an important role in fluorosis[40]. Meanwhile, the manner in which the whole body effects are produced is still unclear, and efforts to prevent and treatment fluorosis by therapeutic measures have had only limited success.

The relation between fluoride toxicity and elevated oxidative stress has been widely reported in humans and experimental animals[41]. In our study, fluoride treatment increased LPO while the estimated anti-oxidant parameters were diminished in all examined tissues of fluoride intoxicated mice.

Our results come in harmony with Guo *et al.* who clarified that the antioxidant defense system impairment informed imbalance in free radicals production and endogenous anti-oxidant system[42]. Our findings are in accordance with earlier observations which indicate that the excessive fluoride exposure can induce LPO, specifically polyunsaturated fatty acids. In previous studies, decreased level of GSH, as well as GSH-Px and SOD activity were estimated in animals treated with fluoride enhancing increased heavy accumulation of free radicals in erythrocytes[42,43], kidney[44], testis[45] and liver[41].

All these results indicate that free radicals may play an important role in the pathogenesis of fluorosis. Erythrocytes are more commonly employed in the evaluation of oxidative stress, since they are prone to be oxidative reactions because of relatively high oxygen tension and the presence of polyunsaturated lipid-rich plasma membranes[46].

Accumulation of fluoride in the brain and muscle of mice causes stress and inhibits auto-oxidation mechanism resulting in oxidative damage of tissues[47]. Furthermore, it was suggested that the use of anti-oxidants and anti-oxidant-rich foods for the beneficial effects of anti-oxidants acted as antidotes for fluoride toxicity[48].

Impairment of antioxidant defense system is believed to be an important mediating factor in the causation of detrimental effects of chronic fluoride toxicity. In addition to well-known effects on the skeleton and teeth, fluorosis can adversely affect many tissues and organs as exhibited by a broad array of symptoms and various pathological changes[39].

Endemic fluorosis is a severe hazard to human health and often is a serious health problem in a number of developing countries. Epidemiological investigations and animal experiments indicated that long-term high-level fluoride intake can lead to severe damage of the metabolism of many systems and organs in animals and humans[39,49].

It was reported that GPx activity was also strongly reduced in the liver and colon of these animals. In addition, the activity of GPx was lower in splenic leukocytes[50]. Accordingly, our results revealed that NaF had reduced the GPx activities. The present results come in harmony with Perottoni *et al.* who reported that the selenium was a component of several enzymes including GSH-Px and thioredoxin[51], which play a major role in the cellular oxidative defense and have been shown to be induced by oxidative stress[52]. In recent years, there have been a great deal of studies carried out on selenium metabolism[53].

The products of natural source are a promising source for the discovery of new pharmaceuticals. In the last decades, several works dealing with propolis' composition and biological properties have been published, revealing the interest of researchers on this bee product and its potential for the development of new drugs[54]. The cost of traditional medicinal plants is very low and raises significant interest to prevent morbidity and mortality from chronic diseases in some countries where low or middle income populations are primary.

Increased utilization of medicinal plants became a World Health Organization policy in 1970. Plants and herbs are chemical factories that directly provide about 25% of currently used drugs and another 25% of drugs comprise chemically altered natural products[55].

In accordance with our results, Wang *et al.* have reported that selenium which is an essential trace element, shows the significant protective effects against kidney damage induced by some heavy metals[11]. They have found protection effects of selenium on ROS mediated-apoptosis by mitochondria dysfunction in Cd-induced Lilly laboratories cell porcine kidney cells.

In harmony with our results, some studies also showed that selenium stimulated antioxidative enzymes in immature kidney of Cd-exposed rats and could protect against oxidative damage[56].

Our results go hand in hand with exposure to aluminum leading to the decrease in the total GSH and oxidized GSH. Viezeliene *et al.* reported redox ratio to about 50% of the control[57]. Upon co-exposure to selenium+aluminum, the concentration of total GSH and the redox ratio was restored to the control values.

Curcumin which is a phenolic compound, exhibits protective effects against oxidative damage and it is considered to be a potent cancer chemopreventive agent[58].

In agreement with Kalpana and Menon[59], curcumin alone significantly decreased the levels of TBARS. Manikandana *et al.* suggested that curcumin exerted its protective effect by modulating the biochemical marker enzymes, LPO and augmenting antioxidant defense system[60]. More specifically, curcumin significantly decreased the levels of free radicals and this protective effect of curcumin attributed to its free radical scavenging activity and induction of detoxification enzymes, and provided protection against degenerative diseases[60].

Furthermore, Halliwell and Gutteridge suggested that treatment with curcumin reduced oxidative damage, probably through its capacity to quickly and efficiently scavenge lipid peroxyl radicals before they attacked membrane lipids[61]. The protection mechanism of curcumin could be through its activity as free radical scavenging, particularly against oxygen radical, which would inhibit SH-group oxidation[59].

SOD and CAT enzyme activities have reached the control level. Furthermore, curcumin increase endogenous antioxidant defense enzymes. Dinkova-Kostova and Talalay reported that the protective effects of curcumin as an antioxidant were attributed to the presence of the hydroxyl groups at ortho-positions on the aromatic rings[62].

LPO is supposed to cause the destruction and damage to cell membranes, leading to changes in membrane permeability and fluidity and enhancing the protein degradation in mice[63]. In the present study, the levels of LPO were increased, indicating an increase in the generation of free radicals in the kidney tissues of NaF treated group and this level was decreased in other groups treated with NaF with co-administration of curcumin and/or selenium.

In conclusion, our study showed that selenium and curcumin extract treatment mitigated NaF intoxication-induced oxidative damage of mice, which could be due to its antioxidant nature that combines free radical scavenging and metal chelating properties.

Conflict of interest statement

We declare that we have no conflict of interest.

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