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Sodium Fluoride Induced Neurotoxicity and Possible Antioxidant Role of Selenium and Curcumin in Male Mice

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Selenium and *Curcumin longa L* are full of natural antioxidants. The aim of this study was to investigate the effect of sodium fluoride on antioxidant enzymes and the possible protective effects of selenium and Curcumin extract against the neurotoxicity and oxidative stress of sodium fluoride (NaF) that adversely affects antioxidant defense system especially in brain. Mature male mice (weighing 35-45 g and each group of ten animals) were given sodium fluoride (10.3 mg/Kg bw) and/or Selenium (0.5 mg/Kg) + Curcumin extract (60 mg/Kg) daily intraperitoneally (I.P) for 4 weeks. In the present study, Sodium fluoride induced oxidative stress by decreasing levels of SOD, CAT and increased MDA in the brain tissues which are ameliorated by Curcumin extract and/or selenium to great extent and thus improving the antioxidant defense capacities.

Key words: Sodium fluoride, Selenium, Curcumin extract, Brain, Neurotoxicity, Oxidative stress.

Fluorine is not freely found in nature. Fluorine in drinking water is totally in ionic form and hence it rapidly, totally and passively pass through the intestinal mucosa and interferes with major metabolic pathways of the living system. Fluoride in small doses has remarkable prophylactic influence by inhibiting dental caries while in higher doses it causes dental and skeletal fluorosis (Shanthakumari *et al*., 2004).

Fluoride enter the body through drinking water, food, toothpaste, mouth rinses, and other dental products; drugs and fluoride dust and fumes from industries using fluoride containing salt and or hydrofluoric acid (Shulman and Wells.,1997).

Millions of people are currently at risk all over the world because they drink water containing carcinogenic amounts of arsenic and fluoride (Chakraborti *et al.,* 2002). Permissible limit of arsenic in water is 10 µg/l and for fluoride it is 1 mg/l as per WHO guidelines. In West Bengal, the arsenic concentration in some tube wells is as high as 3400 µg/l (Guha *et al*., 1998) .Although several hypotheses have been proposed, the exact mechanism of arsenic and fluoride toxicity has not been clearly defined.

Exposure to fluoride results in generation of anion superoxide (O2-), increased O2 concentration and its downstream consequences such as hydrogen peroxide, peroxynitrite and hydroxyl radicals which are important in mediating the toxic effects of fluoride. Experimental evidence has indicated that exposure to fluoride results in oxidative stress both in vitro and in vivo in soft tissues such as liver, kidney, brain, lung and testes (Barbier *et al*., 2010). Fluoride inhibits the activities of antioxidant enzymes—superoxide dismutase, glutathione peroxidase and catalase—and reduces levels of glutathione. Glutathione reduction leads to overproduction of reactive oxygen species at the mitochondrial level, resulting in damage of cellular components. Besides, production of excessive reactive oxygen species results in oxidation

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of macromolecules, membrane phospholipid breakdown, lipid peroxidation, mitochondrial membrane depolarization and apoptosis (Barbier *et al*., 2010).

The naturally occurring element selenium (Se) is essential for a wide variety of biological processes in mammals (Schomburg *et al*., 2004). 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 (Driscoll and Copeland, 2003; Romero *et al*., 2005).

Selenium was recognized as an essential trace element within a relatively low concentration range (Schwartz and Foltz, 1957) and its physiological role was established when it was shown to be one of the glutathione peroxidase (GPx) components (Rotruck et al., 1973). This enzyme is termed a selenoprotein since it contains l-selenomethionine and l-selenocysteine residues (Low and Berry, 1996).

Curcumin, 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 (Cole *et al*., 2007).

Curcumin increasing expression of the xenobiotic detoxifying enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), glutathione reductase (GR), andNAD(P) H:quinone oxidoreductase (Subudhi *et al*., 2008), inhibiting hepatic stellate cells activation (Fu *et al*.,2008), and supporting the mitochondrial function (Subudhi *et al*., 2008).

MATERIAL AND METHODS:

Animals

This study was performed on 70 young male mice, weighing about 35–45 g b.wt. Animals were obtained from the animal house of the King Fahd Center for Medical Research, King Abdul-Aziz University in Jeddah. They were breeding in a well ventilated room with the temperature ranging between 22 and 25 ÚC and maintained under standardized conditions away from any stressful conditions with 12/12 light and dark cycle with free access to humidity and were fed dry balanced meal for experimental animals provided by the General Organization for Grain Silos and Flour Mills in Jeddah, with a constant source of water. All experimental procedures and animal maintenance were conducted in accordance with the accepted standards of animal care per cage (Council of Europe, European convention for the protection of vertebrate animals 2006). We have followed the European community Directive (86/609/EEC) and national rules on animal care. One group served as control. Animals were weighed and randomly allocated into 6 groups (7 rats each) as following:

Chemicals

Sodium Fluoride

Sodium fluoride (NaF) was purchased from Sigma Chemical Co., St. Louis, Mo., USA. The tested dose of NaF (10.3 mg/kg b.wt) was chosen based on the previous studies of Zabulyte *et al.,* (2007). A stock solution was prepared by dissolving of 100 g of NaF in 1000 ml of distilled water. The dose schedule was so adjusted that the amount of NaF administration per animal was as per their respective weight.

Selenium

Selenium was purchased from BDH Chemicals Ltd., England. The tested dose of selenium (0.5 mg/kg) was chosen based on the previous studies of Ibtissem *et al.* (2011).

Curcumin extract

Fresh Curcumin was obtained from local market (Cairo, Egypt), then washed and was soaked in water for 24 hours and after that it was dried then homogenized by using electrical mixer and then the dose was prepared (60 mg/Kg) and this dose was chosen according to Abdul-Hamid and Moustafa . (2013).

Experimental protocols

The study was performed on 70 mature male mice, divided into 7 main groups; each group was consisted of 10 rats. The 1st Control group: Animal's received 1ml of distilled water orally daily for 30 successive days. The 2nd Sodium Fluoride (NaF) treated group: Animals were daily received NaF (10.3 mg/Kg) for 30 successive days intraperitoneally (I.P).The 3rd Selenium treated group: Animals were received selenium (0.5 mg/Kg) for 30 successive days intraperitoneally (I.P). The 4th Curcumin extract treated group: Animals

were received Curcumin extract (60mg/kg) for 30 successive intraperitoneally (I.P).The 5th NaF + Selenium treated *group*: Animals were given sodium fluoride (NaF) (10.3 mg/Kg) for 30 successive days and then co-administered by selenium (0.5 mg/Kg) intraperitoneally (I.P). The 6th NaF + Curcumin treated group: Animals were given sodium fluoride (NaF) (10.3 mg/Kg) for 30 successive days and then co-administered Curcumin extract (60mg/Kg) for 30 successive days intraperitoneally (I.P). The 7th NaF+ Selenium+ Curcumin extract treated group*:* Animals were given NaF (10.3mg/Kg) and then co-administered with *selenium* (0.5mg/Kg) and then followed by Curcumin extract (60 mg/Kg) for 30 successive days (I.P).The substances were administered in the morning (between 09.30 and 10.30 h) to non fasted rats. The first day the animals were treated was considered experimental day 0.At the end of the 30 days of treatment, all animals were scarified and dissected. The testis tissues were quickly excised to light microscope investigations and biochemical examinations.

Preparation of Tissue Homogenates for measurement of antioxidant enzymes

The remainder tissues of brain (about 1.0 g) were used for the analysis of oxidative stress parameters. it was washed with physiological saline (0.9%) and distilled water for the removal of blood, and later the fatty parts were removed as not to record a reading on spectrophotometer for blood antioxidant enzymes, so to get more accurate reading for antioxidant enzymes in brain tissues homogenates only and then we blotted the brain tissues over a piece of filter paper. Prior to dissection, tissue was perfused with a 50 mM (sodium phosphate buffer saline (100 mM Na_2HPO_4 / NaH_2PO_4) (pH 7.4) in an Ice containing medium containing 0.16 mg/ml heparin or containing 0.1 mM ethylenediaminetetraacetic acid (EDTA) to remove any red blood cells and clots. Then tissues were homogenized in 5–10 mL cold buffer per gram tissue and centrifuged at 5000 r.p.m for ½ hours. The resulting supernatant was transferred into eppendorf tubes, and preserved in a deep freeze -80 oC until used for various biochemical Assays (Habig *et al*., 1974).

Antioxidant capacities determination

Measurement of superoxide dismutase (SOD)

SOD activity was measured according to the method described by Marklund and Marklund (1974) by assaying the auto oxidation of pyrogallol at 440 nm for 3 min. 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 Mm Tris, 10 mM EDTA, pH 8.2). The SOD activity is expressed as U/mg protein.

Measurement of catalase (CAT)

CAT activity was measured determined according to the method described by Aebi (1984) by assaying the hydrolysis of H_2O_2 and the resulting decrease in absorbance at 240 nm over a 3 min period at 25ÚC. 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.

Measurement of Malondialdhyde (MDA)

TBARS content was evaluated using the thiobarbituric acid (TBA) test as described by Ohkawa *et al*. (1979). After incubation of testis homogenate with TBA at 95 ÚC, 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 protein.

Statistical analysis

Data were collected, arranged and reported as mean ± standard error of mean (S.E.M) of nine 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 analyzes of variance ANOVA test (F-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.,1982) to estimate the effect of different treated groups.

RESULTS

Effect of Sodium Fluoride, selenium, Curcumin extract and their combinations on Catalase activity

Regarding the effect of Sodium Fluoride on catalase activity of normal rats, Sodium Fluoride afforded a marked decrease ($P<0.05$) in brain catalase after the end of the study when compared with control group, whereas, non significant changes in the enzyme activity was recorded in

Table 1. Effect of Sodium fluoride (10.3 mg/kg), Selenium (0.5 mg/ Kg), Curcumin extract (60 mg/Kg) and their combinations on antioxidant activities in brain tissues homogenates in male mice (mean ± SE). (N = 7)

Groups	Brain Catalase (U/g)	Brain SOD (U/g)	Brain MDA (nmol/g)
Control group	159.92±2.21^{ab}	133.50±0.63^{f}	108.06±3.70^{f}
Sodium fluoride	47.60±7.16^{g}	62.47±1.60^{a}	289.91±3.77^{a}
Selenium	157.07±0.99^{b}	135.30±1.64^{f}	100.07±2.26^{ef}
Curcumin extract	150.53±1.08^{c}	130.04±0.45^{ef}	103.80±3.92^{f}
Sodium fluoride +Selenium	106.93±1.45^{ef}	100.39±0.16^{c}	220.27±1.90^{b}
Sodium fluoride +Curcumin extract	105.37±2.47^{f}	96.52±0.67^{bc}	207.90±2.20^{c}
Sodium fluoride + Selenium+Curcumin extract	115.75±2.25^{d}	110.18±0.56^{d}	192.77±10.79^{d}

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

selenium treated group. Treatment of normal rats with selenium alone exhibited non significant changes in brain Catalase after the end of the experiment when compared with control group , Whereas , a significant decrease was reported in brain when administered Curcumin extract only compared with control group (Table1and Fig.1).While combinations of Sodium Fluoride with either Selenium or curcumin extract exhibited a significant decrease in brain catalase activity after the end of the study as compared with normal control group.

Effect of Sodium Fluoride, selenium, Curcumin extract and their combinations on Superoxide dismutase (SOD) activity

The results of the study revealed that treatment of normal rats with Sodium Fluoride elicited a highly significant decrease ($P<0.05$) in brain SOD level after the end of the study when compared with control group. Treatment of normal rats with either selenium or curcumin extract for 4 weeks elicited a non significant increase in SOD activity of brain tissues after the end of the study except 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 sodium fluoride afforded a slight decrease ($P<0.05$) in SOD activity of brain tissues compared with normal control group (Table 1 and Fig.1). Meanwhile combination of sodium fluoride with Curcumin extract and selenium afforded slight decrease in SOD activity but the effect was much better than group treated with sodium fluoride only and other treatment combinations.

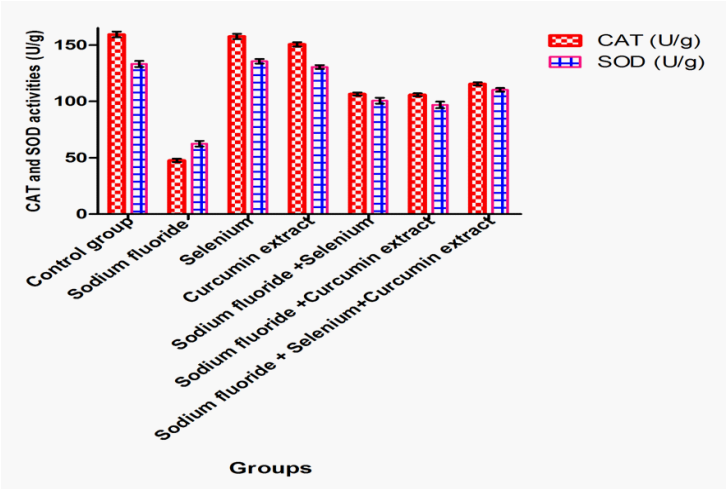

Fig. 1. Effect of Sodium fluoride (10.3 mg/kg), Selenium (0.5 mg/ Kg), Curcumin extract (60 mg/Kg) and their combinations on Catalase (CAT) and superoxidedismutase (SOD) activities (in brain tissues) in male mice

Effect of Sodium Fluoride, selenium, Curcumin extract and their combinations on Malondialdhyde (MDA) activity

The MDA content of the brain tissue was elevated ($P<0.05$) in response to treatment of normal male rats with Sodium Fluoride for 4 weeks compared with normal control group. The same previous response was reported with selenium, curcumin extract combinations with Sodium Fluoride compared with control group (Table1and Fig.2) but the effect was much less intense. Meanwhile, groups treated with either selenium or curcumin extract induced non significant changes in brain MDA level as compared to normal control group.

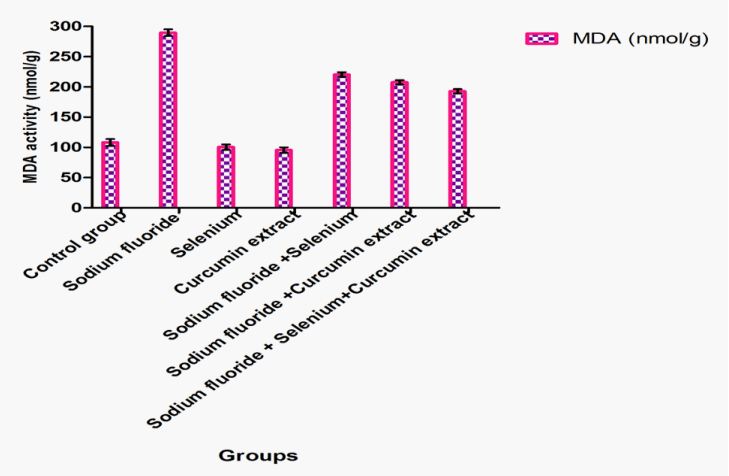

Fig. 2. Effect of Sodium fluoride (10.3 mg/kg), Selenium (0.5 mg/ Kg), Curcumin extract (60 mg/Kg) and their combinations on Malondialdhyde (MDA) (in brain tissues) in male mice

DISCUSSION

The present study was an attempt to evaluate the toxic effect of sodium fluoride on brain tissues in male mice 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, therefore, the present study aimed to elucidate the possible ameliorative role of Selenium and/or Curcumin extract in alleviating the toxicity of sodium fluoride when given to normal rats. The antioxidant parameters (SOD, CAT and MDA) parameters in brain homogenates.

Increased generation of reactive oxygen species (ROS) is implicated in the pathogenesis of many diseases and in the toxicity of a wide range of compounds (Halliwell and Gutteridge, 1985). Lipid peroxidation represents one of the most frequent reactions resulting from free radical's attack on biological structures (Stohs, 1995).

In harmony with the present results, a decrease in the activity of free radical scavenging enzymes, SOD and GSH-Px, was found in people living in areas of endemic fluorosis (Li and Ca, 1994). A similar inhibitory effect of fluoride on SOD in germinating mung-bean seedlings support the above findings and indicate the possibility of greater toxicity if fluoride can impair the free radical scavengers (Rzeuski *et al*., 1998).

In another study and in accordance with the present findings, A decreased GST, SOD and catalase activities in rat brain upon ingestion of sodium fluoride (20 mg/kg body weight/day, ip) for 14 days were observed (Vani and Reddy, 2000). Liu *et al*. (2003) suggested the mechanism of fluoride injuring soft tissues that it causes excessive production of lipid peroxidation (LPO) and oxygen free radicals, leads to the ability of scavenging free radicals and antioxidation being reduced. 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.

Selenium treated group has greatly ameliorated the antioxidant enzymes capacities and our findings are greatly in accordance with (Kipp et al.,2009) who reported that feeding a

moderate selenium-deficient diet to mice resulted in a consistent down-regulation of the plasma selenium level and GPx activity.

Results of Tirkey et al. (2005) indicated that the presence of curcumin with sodium arsenite alleviated its toxicity and ameliorated SOD and CAT levels, and this is in agreement with the results of Tirkey *et al*. (2005) who showed that treatment with curcumin improved the levels of renal. SOD and CAT to reach the control level. Furthermore, curcumin increase endogenous antioxidant defense enzymes (Thiyagarajan and Sharma, 2004). Dinkova-Kostova and Talalay (1999) reported that the protective effects of curcumin as an antioxidant are attributed to the presence of the hydroxyl groups at ortho-positions on the aromatic rings and the α-diketone functionality.

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