The Effect of Ellagic Acid and Sodium Fluoride Intake on Total Sialic Acid Levels and Total Oxidant/Antioxidant Status in Mouse Testicular Tissue

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Authors’ contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In the present study was aimed to investigate the levels of Total Oxidant Status (TOS), Total Antioxidant Status (TAS) and Total Sialic Acid (TSA) in the testis of mice treated with sodium fluoride (NaF) and ellagic acid (EA).

Methodology: Forty Swiss albino mice were randomized into 5 equal groups for 4 weeks as follows: Group I (control) received standard chow diet and drinking water. Group II, III, IV and V treated with subcutaneously 0.02% dimethylsulfoxide (DMSO) and 10 mg/kg/day EA in DMSO solution and 150 ppm/mouse/day NaF in drinking water and NaF plus EA, respectively. The levels of TOS, TAS and TSA were analyzed in the testis tissue by using spectrophotometric methods.

Results: EA treatment decreased levels of TOS and TSA during NaF uptake significantly. It was
1. INTRODUCTION

Fluorine is an univalent poisonous gaseous halogen with pale yellow-green colour and the most chemically reactive and electronegative of all the elements. Excessive amounts of fluorine, release from natural sources such as soil, water and plants, cause fluoride poisoning or fluorosis. The normal range of fluorine level is 0.7-1.5 mg/L in organism that is needed for prevention of dental caries formation and accelerates the maturation of firm bone tissue [1]. Fluorine in aquatic environment is commonly in fluoride form. High intakes of fluoride have mainly destructive effects on teeth and skeletal system formation. However, chronic and cumulative overexposure may also cause disorders in other organ and systems. The toxic effect of fluoride, including reproductive defects, has been put forward by numerous studies. For instance, it was demonstrated an opposite correlation between fluoride amount of drinking water with human fertility [2]. The data connected with fluoride toxicity have also showed that fluoride may adversely affect the reproductive systems of men living in endemic areas due to fluoride toxicity [3,4].

Oxidative degradation plays an important role in the immune response, it is a crucial reaction that occurs in phagocytic cells. This reaction may be cause to an increase in hydrogen peroxide ($H_2O_2$), superoxide ($O_2^-$) and hydroxyl radicals ($OH^-$) production as a consequence of excessive fluoride [5]. NADPH oxidase system is located in neutrophil membranes for this process. The NADPH molecule reacts increasing production of $O_2^-$ against to rise rapidly oxygen consumption. $O_2^-$ anions can be harmful as a $H_2O_2$ source that correlate with neutrophil chemotaxis. Although they do not direct damage on living organism [6, 7]. Oxidative process initiate with several reasons such as excessive intake of flour ion, high $H_2O_2$ and other free radicals levels. That reaction induce peroxidation of membran lipids and inactivation of antioxidant enzymes like as superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and DNA damage [6-8].

Total Oxidant Status (TOS) and Total Antioxidant Status (TAS) assays are used for the determination of total oxidant/antioxidant balance rather than measurement individual oxidative stress parameters as malondialdehyde (MDA) or antioxidants such as reduced glutathione (GSH), SOD and GSHPx [9,10].

Some researches have shown that sialic acid level is the key component for early diagnostic blood marker of fluorosis in both human and animals [11]. $H_2O_2$ and other free radicals cause oxidation in membrane lipids during fluorosis [12]. However some protective agents can reduce oxidative damage. EA, the phenolic compound found in numerous plants, is accepted as an ideal chemical structure for free radical scavenging activity and shown to be more potent antioxidant than vitamin E and C [13,14]. In this study, we aimed to investigate effects of EA on TSA, TAS and TOS levels of testicular tissue of NaF treated mice.

2. MATERIALS AND METHODS

In the present study we investigate the effects of concurrent administration of sodium flouride and EA in mice. In experimental period, 40 Swiss albino mice approximately 35±2 g weighing were used for 4 weeks period. All procedures were conducted in accordance with the “Guide for Care and Use of Laboratory Animals”, published by the National Institutes of Health and the ethical guidelines of the International Association for the Study of Pain. Mice were randomized into 5 groups and kept in cages in terms of adaptation for 10 days period. The animals were kept in an air conditioned room with controlled temperature (18±2°C), humidity (60±5%), and day/night cycle (12 h light, 12 h dark cycle) for all application period.

Group I (control ) were untreated which fed with normal diet and drinking water. Group II received subcutaneously (s.c.) 0.02% DMSO. Group III were treated with 10 mg/kg/day s.c. EA (Ellagic acid, Sigma Aldrich, UK) dissolved in 0.02% DMSO. At the same time, 150 ppm of NaF/mouse/day diluted in drinking water were given to group IV. Group V received NaF in

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Conclusions: It was concluded that EA may be protective on testicular oxidative stress caused of fluoride.

Keywords: Ellagic acid; oxidative stress; sialic acid; sodium fluoride.
drinking water and with combined EA subcutaneously. All injections were performed daily at the same time for 28 days period. The animals were killed by cervical dislocation 10 months after the beginning of the experiment. Then testicular tissue samples were taken for biochemical analysis, after diluted 5 times with phosphate buffer solution (PBS). They were homogenized at 12000 g for 2 minutes on ice. Homogenates were centrifuged at 15000 g at 4°C in 10 min. A part of supernatant which obtained from tissue was stored at -50°C till biochemical analysis.

TAS and TOS levels were analysed by spectrophotometric method using commercial kits (Rel assay diagnostic kits®, Gaziantep, Turkey) [9,10]. The assay of TAS has excellent precision values lower than 3% and data were expressed as mmol Trolox Eq/L. The assay of TOS was calibrated with H2O2 and the results were expressed as μmol H2O2 equivalent per liter (μmol H2O2 Eq/L). TSA analysis was assayed with spectrophotometric method at 525 nm optical density [15]. All optical densities obtained from samples were evaluated with the standart curve.

Statistical analysis of biochemical parameters were determined by statistical package programme in PC (IBM SPSS version 20.0 for Windows). Whether the significant differences of between groups were spesified by one way variance analysis (ANOVA) followed by Tukey’s post-hoc test. An alpha value of P<0.05 was accepted as significant. Results were shown as mean ± standard deviation.

3. RESULTS

TAS, TOS and TSA levels were measured on mice groups which were given 150 ppm of NaF during 4 weeks, and have been investigated the effect of these parameters on 10 mg/kg EA dosed by subcutaneous injection. The results obtained from the preliminary analysis of testicular tissue are presented in Figs. 1-3, respectively. Testicular TAS and TSA levels were decreased (P<0.05) when TOS levels were increased (P<0.05) in group IV (NaF) and group V (EA + NaF) compared to control group.

4. DISCUSSION

Excessive amount of fluorine ions accumulate in some disorders which affect Ca2+ binding. Thus, irritate soft tissues such as liver, kidney, testis and impairs enzymatic reactions such as glycolysis [16,17]. In this case, researchers mentioned that suplementations of vitamin A, C, E and D or precipitant substances such as aluminum sulfate, calcium hydroxide and magnesium on diets or drinking water to avoid the pathological effects of fluorine ion on ecological environments with high fluor reserves is necessary [18,19]. However, consuming of clean water sources are reported to be more effective method [18]. In the present study, it was aimed to investigate effects of fluorine on testicular tissue TAS, TOS and TSA levels in NaF treated mice by EA injection what is known as a powerful free radical scavenging activity. Fluorosis leads to oxidative stress and tissue damage especially by causing changes on the lipid components of cell membrane [20,21]. There are several active enzymes (GSHPx, SOD, Catalase, MPO, NADPHOx etc.) that maintain the oxidant/antioxidant balance in the body [6-8]. These enzymes control the amount of free radicals produced or scavange them and prevent their binding to macromolecules. Intracellular free radical scavenger enzymes provide main antioxidant defense [22]. The most important feature of antioxidant defense system is that all synergistic components assign against reactive oxygen species for homeostasis. Combined oxidants/antioxidants are more effective than existing alone in the blood [9,10]. Therefore, TAS and TOS measurement contribute in a manner may be more useful than individual antioxidant measurement to define total oxidant/antioxidant balance [9,10,23]. In a study related to endemic fluorosis, plasma TOS levels were statistically higher than healthy group while total oxidant capacity (TAC) were lower and researchers claimed that oxidative stress has important role for fluorosis pathogenesis [24]. Redox reactions resulted from respiratory explosion during immune response of phagocytic cells to the high amount of fluorine ion uptake may increase levels of reactive oxygen substances (H2O2, O2−, OH· etc.) [5].

In our study, the enhancing effect on the free radical production and reactive effect on the antioxidant defense system of testicular tissue were determined concomitantly. Our findings demonstrate that excessive amount of fluorine as related with decreased TAS and high TOS levels were detected in mice testicular tissue when excessive amount of fluorine was added to their drinking water (Figs.1 and 2). These findings are compatible with previous studies [3,7,17,24,25]. There is limited knowledge about EA’s protective effects on excessive fluoride exposure.
studies showed that it may be useful in experimental fluorosis characterized by low lipid peroxidation and high antioxidant levels [26,27]. It is well-known that phenolic compounds like EA are metabolized to methyl, glucuronyl and sulfate conjugates and these metabolites found in high concentrations in blood and urine [12,28]. Metabolic pathway of EA as sulfate conjugate can be indirectly affected by cystine concentration and synthesis of molecules containing thiol (-SH) [12]. Under this circumstance, it is possible to claim the idea that antioxidant molecule levels with -SH group such as GSH and GSHPx can directly affect TAS levels [9].

Fig. 1. Testicular TAS levels in mice. Group I: control. Group II: DMSO. Group III: EA. Group IV: NaF. Group V: NaF plus EA. Results with different superscripts (a,b,c) on the columns are significantly different (P<0.05)

Fig. 2. Testicular TOS levels in mice. Group I: control. Group II: DMSO. Group III: EA. Group IV: NaF. Group V: NaF plus EA. Results with different superscripts (a,b,c) on the columns are significantly different (P<0.05)
It has been recorded that sialic acid (SA) has antioxidative function for removal of $O_2^.$ from vascular system. Also, oxidative stress is initiates the release of SA from cell surface oligosaccharides without sialidase activation or induction [29,30]. There is a parallel relationship SA levels and severity of cellular damage. SA is an important biomarker to diagnose inflammation, myocardial infarction, cancer and other diseases [23,31]. Several studies reported that was levels of high SA during many diseases when the level of SA was low in period of flourosis this condition [16,32]. For example, in fluoric regions, it was demonstrated that levels of serum SA of individuals exposed 2.4, 5.6, and 6.13 ppm of fluoride ion were lower compared to exposed flouride ion under 1.5 ppm [33]. Measurement of serum SA levels is important for early diagnosis of flourosis in both animals and humans. Moreover, serum SA levels during disease condition could be decreased by 50% when compared with healthy subjects [11]. In a study, SA levels on reproductive system of oral fluoride (10 mg/kg/day) toxicity in male rats was observed at 30 days. It showed that free SA levels were significantly reduced when compared with the control group. Furthermore, it was reported that vitamin E treatment 2 µg/day/rat dose through 30 days maintained to increase SA levels. The reason for SA depletion during over exposure of flouride ions were possible depends on inhibition activities of enzymes (phosphorylase, ATPase, some glycolysis enzymes etc.) which require $Mg^{2+}$ or $Ca^{2+}$ ions as a cofactor [16]. It has been claimed that enolase inhibition can be provided with 2.28 mg/L flouride ion [34,35]. According to the findings, it is suggestive that TSA biosynthesis defects formed by suppression enzymes such as enolase in NaF group. In the present study on mice, testicular TSA levels in NaF group were determined lower (P<0.05) than EA plus NaF group (Fig. 3). Our findings about TSA levels decreased in NaF group compared to control group is consistent with other studies related to flourosis and levels of SA [11,16,32,33].

5. CONCLUSION

In conclusion, excessive amount of fluoride ions on mice testicular tissue were demonstrated that TSA and TAS levels decreased while TOS levels increased. In addition, it was concluded that EA treatment could provide oxidant/antioxidant balance and protective properties on testicular oxidative stress level.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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