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Research Article

The Effects of Quercetin on the Fluorosis Toxicity in Kidney of Mice

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Abstract

There are many uses of fluorine compounds which we are exposed to in daily life unaware, have toxic effects on tissues. Demonstrating anti-oxidative effect is one of the most important biological properties of phenolic substances. It has been identified in vitro studies of antioxidant flavonoids such as quercetin that it reduces the oxidation of low-density lipoproteins and protects cells from toxicity. In this study we aimed to determine the effect of quercetin on the fluorosis toxicity in kidney. In the study, 40 Swiss albino male mice were used. The kidney tissues and blood samples were taken from the mice in each group. Total Oxidant Status and Total Antioxidant Status were measured by the commercially available kits. Ceruloplasmin and MDA levels are measured by chlorometric methods. Kidney tissues stained with Hematoxylin-Eosin dye and investigated under the light microscope. Histopathological changes were observed in the kidneys. Antioxidant status no statistically differences but increase with Quercetin, decreases in Fluorosis groups. In light of this study, we think that the environmental toxicities will be alleviated by taking the antioxidant molecules such as quercetin into the body.

Keywords: Quercetin; Fluorosis; Kidney; Toxicity; Mice

Introduction

Fluoride is a compound that abundant in the environment and it can be exists only in combination with other elements [1]. The plants absorb the fluoride in the gas form in the air and the amount of fluorine absorbed by the plants varies depending on the soil structure, plant type and amount of fluoride in the soil [2]. There are many uses of fluorine compounds. Fluorine element is used to enrich uranium in nuclear weapons production and in the production of many commercial chemicals. Fluoride is used in toothpaste production. Chlorofluorocarbons (CFCs) are used in products such as cooling and ventilation, but due to the harmful effects of these gases on the ozone layer, their production and use have been tried to be limited. In addition, as chlorofluorocarbons have damaged the ozone layer, their use and production are prohibited in many countries [3].

The effect of fluorine used in dentistry since 1940 in preventing and reducing caries has been clinically proven. During tooth development, it increases the resistance of tooth mines with fluorine apatite formation. Due to the presence of flour in plaque and saliva, remineralization is provided on the tooth surface. Antibacterial activity occurs with fluoride in plaque and inhibits the enolase on the glycolytic pathway by entering the bacterial cell, especially at low pH, thus acid production in plaque is reduced [4]. The main source of the element of fluorine is water. The plant with the most fluorine is tea. A cup of tea contains 0.12-0.10 mg of fluorine. There is fluorine in meat, eggs, offal (liver, kidney), chicken, rice, oats, spinach, and apple. Moreover, there is a total of 2-3 g of fluorine in tooth and bone structure and 0.3 mg in 100 ml blood [5]. The main ejection pathway for fluorine is the kidneys, so the soft tissue with the highest fluoride values is the kidney. Because of renal dysfunction, the half-life of the fluorine in plasma is prolonged

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and clinical toxicity can be formed even if fluoride is taken at low concentrations. Renal impairment may affect the distribution and levels of serum proteins and may cause significant changes in the distribution of serum protein in relation to the degree of impairment [6]. Demonstrating the anti-oxidative effect is one of the most important biological properties of phenolic substances. It has been identified in vitro studies of antioxidant flavonoids such as quercetin that it reduces the oxidation of low-density lipoproteins and cell toxicity [7]. In addition, quercetin provides protection against lipid peroxidation and prevents the formation of free oxygen radicals in cells. Also, quercetin has antibacterial, antiviral, antioxidant, anti-inflammatory, anti-carcinogenic effects [8]. Quercetin increases membrane fluidity, inhibits phospholipase A2 and protein kinases, inhibits tumor growth, induces apoptosis. In a study conducted, it was shown that quercetin protects erythrocyte membranes against oxidative damage in mice [9]. Quercetin and flavonoids among flavonoids inhibit Low-Density Lipoprotein (LDL) oxidation and increase the level of Nitric Oxide (NO) that causes vasodilatation following Nitric Oxide Synthase (NOS) activity [10]. Quercetin is often found in many plants with different flavonoids. Quercetin is present as a flavonoid compound in the leaves, flowers, and stalks of plants in nature. Therefore, quercetin is found in herbal teas. These herbs and herbal teas are used in various fields of pharmacy, biochemistry, food chemistry, medicine and pharmaceutical products [11]. Since quercetin is a potent antioxidant, also protects the cells in the body from the harms of free radicals. In this study, we aimed to determine the effect of quercetin on the fluorosis toxicity in the kidney.

Material and Methods

For the study, ethics committee approval numbered 2015-77 was taken from ondokuz mayıs university animal experiments local ethics committee. Mice were obtained from ondokuz mayıs university experimental animal research center (DEHAM) and housed there during the research. The mice subjects were taken 1 week before starting the trial plan envisaged in the project and the health status of the mice was monitored. In the study, 8 week-old 40 Swiss albino male mice weighing approximately 20-25 g. were used. Animals were maintained at 20°C-24°C and 55%-60% humidified environment. Forty mice were divided into 4 equal groups (n=10). The trial was arranged as described below. Group 1 control: normal drinking water (0.8 ppm fluorine), Group 2: 12 mg/kg/day NaF oral (Sigma Adlrich, Germany) [12] Group 3:40 mg/kg/day quercetin (Sigma Adlrich, Germany), Group 4: 12 mg/kg / day NaF+40 mg/kg/day quercetin was orally administered.

In addition, mice were fed ad libitum with standard mouse pellet diet throughout the day. Based on the previous study model, the trial lasted 30 days [12]. At the end of this study, individual blood samples were taken from the mice in each group, followed by euthanasia with xylazine (30 mg/kg i.p.) and ketamine (300 mg/kg i.p.). The kidney tissues were removed. The tissue required for biochemical assays was homogenized for 3 min at 1500 rpm with 50 ml of phosphate buffer (pH 7.5). The supernatants were then centrifuged at 1550 g, 10 min at +4°C and maintained at -80°C till the analysis. The tissue required for histopathological analysis was detected with buffered formaldehyde solution. Anticoagulated blood samples were centrifuged at +4°C for 10 min at 1550 g. Plasma was removed and transferred to Eppendorf tubes and they were kept at -80°C until used for analysis. The measurement of total oxidant capacity in serum is based on the principle that iron ion forms a colored complex with chromogen in acidic medium. The intensity of the color varies depending on the amount of oxidants in the sample. The commercially available kit (Total Oxidant Status Assay kit, Rel Assay Diagnostics, RL0024, TURKEY), in which the color density can be determined spectrophotometrically. The measurement of total antioxidant capacity in serum is based on the measurement of the change in absorbance of the antioxidants in the serum sample resulting from the dark blue-green color reduction resulting from ABTS. The commercially available kit (Total Antioxidant Status Assay kit, Rel Assay Diagnostics, RL0017, Turkey) was used. Serum CP analysis was conducted by a spectrophotometric method, which included P-phenyldiamine dichloride (PPD) use [13] serum MDA levels were measured by Uchiyama and Mihara's method [14].

Statistical methodology

Because of the clinical variables are not normally distributed within groups, non-parametric statistics were used.

Table 1: Biochemical parameters evaluated in the study. There were no significant differences between the groups in the biochemical parameters evaluated in the study.

Biochemical Variables	K			F			Q			F+Q			P
	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.	
TAS	2.75	1.82	3.57	2.75	1.61	3.26	2.72	1.52	2.92	2.57	1.99	2.82	0.424
TOS	2.53	1.74	2.98	2.75	2.28	2.94	2.6	2.33	3.22	2.61	2.04	2.97	0.401
Ceruloplasmin	15.05	3.79	19.91	11.41	6.4	21.33	16.59	11.38	28.68	12.92	8.3	35.31	0.108
MDA	28.37	5.86	79.45	44.32	7.66	116.4	34.67	19.1	54.52	63.79	28.85	119.8	0.089

Descriptive statistics were calculated by a median, minimum and maximum (Table 1).

Group comparisons according to TAS, TOS, Ceruloplasmin, and MDA were evaluated by using Kruskal-Wallis Variance Analysis. The Type-i error rate was taken as α=0.05 for statistically significance. SPSS 21 software was used for statistical analyses (IBM Corp. Released in 2012. IBM SPSS Statistics for Windows, Version 21.0 Armonk, NY: IBM Corp).

Results

When the kidney sections were examined, no histopathological changes were observed in the kidneys of the mice in the control group. Glomerular and tubular changes were observed in varying grades in the mouse kidneys of the experimental groups. These changes were glomerular lobulation in the glomerulus, mesangial cell enlargement, glomerular shrinkage and thickening in the Bowman's capsule, while in tubules it was like hydropic degeneration in the tubular epithelium (Figure 1).

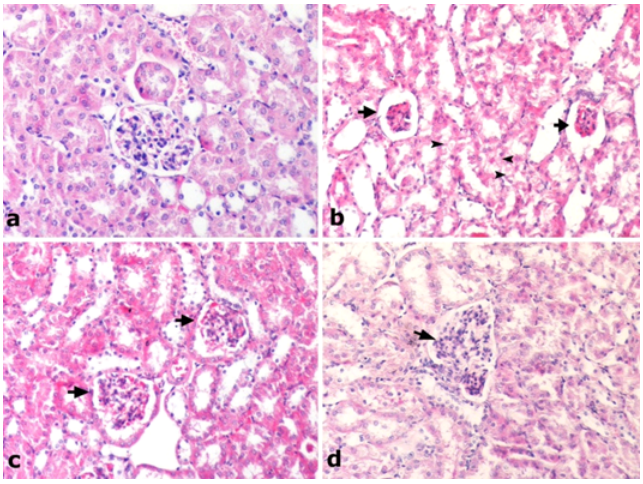

Figure 1: Microscopic views of the kidneys of the control and experimental groups. a: normal glomeruli and tubules structure in the control group mice's kidney. b: Glomerular shrinkage (arrows) in the kidney of the fluorine (F) group mice and hydropic degeneration (arrow heads) in the tubules epithelium, c: thickening in the Bowman capsule (arrows) in the kidney of the fluorine (F) group mice, d: increase in mesangial cells in the glomerulus (arrows) in the kidney of the fluorine quercetin group mice. Hematoxylin-Eosin x 260.

Discussion

Oxidative and antioxidant destabilization causes oxidative stress and deoxyribonucleic acid leads to oxidative damage in lipids and proteins [15]. Antioxidants clear free radicals in the environment. For this purpose, enzymes such as superoxide dismutase, catalase and glutathione are in anti-oxidative defense [16]. It has also been reported that quercetin is a good antioxidant besides its many biological and pharmacological effects [17]. In our study, total oxidant and total antioxidant capacity of kidney tissue and erythrocytes were investigated in order to investigate the anti-oxidative effects of quercetin in fluoride toxicity. There were no statistically significant changes in kidney TOC and TAC levels. However, when compared to the control group, the erythrocyte TAC level increased in fluorine and fluorine+quercetin groups ($p<0,01$). There was no statistical difference between the control group and the quercetin group; similarly, there was no difference between the quercetin group and the fluorine and fluorine+quercetin groups as well. Compared with the control group, the erythrocyte TOC level was found to increase in all groups ($p<0.001$). Increased superoxide anions can suppress enzyme activities such as superoxide dismutase [18,19]. In an in vivo study, they reported that they used 10 mg/kg/day and 20 mg/kg/day quercetin as a protective effect for fluorine toxicity for 7 days and decreased oxidative stress in liver tissue by increasing possible anti-oxidative activity [19]. In a study conducted by adding 15 ppm and 50 ppm fluoride to drinking water, experimental animals were exposed to fluoride water for 40 days and kidney injury specific gene expressions such as kidney injury molecule 1, clusterin, osteopontin were investigated. As a result of the study, it was observed that in the group drinking high fluoride water, renal damage increased and serum creatinine level went up [20]. It has been reported that in the experimental animals who were exposed to fluoridated water at concentrations of 0 mg/l, 5 mg/l, 10 mg/l and 15 mg/l for 60 days, as the amount of fluoride increased, the level of renal SOD, GPx, GSH, and antioxidant levels decreased; as the amount of fluoride increased, lipid peroxidation products increased [21]. In the kidneys of the mice drinking water with high fluoride for 120 days, it was observed that tissue damage increased and expression of apoptotic markers went up histologically [22]. In another study examining the effects of water containing 50 mg/l, 100 mg/l and 150 mg/l of fluoride on the kidney on 70th and 140th days, it was observed that there was no significant change in SOD activity despite the increase in renal tissue ROS production and CAT activity at the end of both periods [23]. It is obvious in related studies that a high amount of fluoride induces kidney damage. A significant change in the oxidative stress parameters may not be observed due to the fact that the dose used in our study is rather low and fluoride administration is kept short. In addition, the application of quercetin in combination with fluoride in our study may have prevented the occurrence of kidney damage by activating the antioxidant system. Blaszczyk studied the effects of methionine on fluoride-induced kidney damage and observed that GPx, GR, and GST values of methionine-receiving groups were lower [24]. In another study in which sodium selenite was given in combination with fluoride at different doses to experimental animals for 6 months, selenium was indicated to reduce apoptotic gene expressions such as Bax and Bcl-2 [23]. As a result, it is known that elements such as fluoride, which we are exposed to in daily life unaware, have toxic effects on tissues. These exposures last for a long time and result in serious damage. In light of the study we conducted, we think that the environmental toxicities will be alleviated by taking the antioxidant molecules such as quercetin into the body.

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