

# The Investigation of Biochemical and Histological Effects of Ellagic Acid in Experimental Chronic Fluorosis Induced Mice

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## ABSTRACT

**Objective:** The present analysis deals with the effects of ellagic acid treatment and biochemical and histopathological alterations in mice with NaF-induced experimental chronic fluorosis.

**Materials and Methods:** Treatments were carried out on male 28 Swiss albino mice divided into 4 equal groups for 8 weeks. Group I (control) was not applied experimental operation. Group II received subcutaneously (sc) 10 mg/kg/day ellagic acid injection, Group III received per orally 25 mg/L NaF, and Group IV received ellagic acid plus NaF. Levels of lipid bound sialic acid (LSA), malondialdehyde (MDA), paraoxonase (PON) activity and reduced glutathion (GSH) in blood samples were analyzed by spectrophotometric method. The levels of calcium, glucose, total cholesterol, triglyceride, high density lipoprotein (HDL), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in plasma were measured by autoanalyzer. Following euthanasia, liver and kidney tissues obtained from subjects were evaluated as histopathological.

**Results:** MDA level was significantly higher when levels of GSH and PON activity of group III were lower as compared to other groups. Levels of PON activity and GSH of group IV increased as compared to group III. LSA levels of group III and IV were lower than control group.

**Conclusion:** As a result, in experimental chronic fluorosis was detected to decrease in levels of LSA, GSH, PON activity, calcium and glucose, and increase in MDA level and changes in lipid profiles and cellular degenerations in liver and kidney. Injection of ellagic acid was protective during chronic fluorosis.

**Keywords:** Fluorosis, ellagic acid, paraoxonase, sialic acid, oxidative stress.

## INTRODUCTION

Soluble inorganic fluorine tends to accumulate substituting with anions as carbonate or hydroxyl in calcified tissues of living. <sup>[1]</sup> Fluorosis occurs because of the excessive intake of fluoride, either through fluoride in the water supply, naturally occurring or through other sources including plants. <sup>[2]</sup> The World Health Organization restricted as 1.5 mg/L the highest amount of fluoride in drinking water and was reported that chronic fluorosis in organisms could occur if this level was more than 4 ppm. <sup>[3,4]</sup> In chronic fluorosis consists aplastic anemia

due to degeneration of bone marrow cells with the affected teeth and bones. Spinal cord due to deterioration in periosteal surface of bones exposures to mechanical stress and neurological symptoms in organism are seen. <sup>[5]</sup>

In organisms with fluorosis may also vary in the oxidant/antioxidant balance. <sup>[6]</sup> High levels of reactive oxygen species, which indicates the oxidative stress, are known to be associated with the etiopathogenesis of chronic diseases and induce lipid peroxidation that can be further shown by increased MDA levels. <sup>[7]</sup> GSH

including the thiol group prevents oxidative damage to specially red blood cells. [8] GSH and MDA concentrations have been used for analysis of effects on the oxidant/antioxidant balance. [6-8]

The levels of SA with negative electrical charge are important for physiological and pathological functions such as cellular transmission, embryogenesis, immune system regulations, metastasis of neoplastic cells, and carrying out membrane receptor functions. [9] The ratio of SA/glucosamine is recommended in the diagnosis of osteoflorosis. [10] It is suggested that levels of SA and  $Ca^{2+}$  along with levels of urine and bone fluoride are important. [11,12] VLDL, LDL, HDL and lipoprotein (a) are major lipoproteins which occurs fraction LSA. PON1 which depends on  $Ca^{2+}$  for enzymatic activity and circulates as part of HDL particles in the blood is an ester hydrolase and inhibits oxidative modification of LDL. [13]

Changes in blood and urine of fluorosis creating systemic disorders in the organism occur at an earlier period. Primary excretion place of orally taken fluoride are kidneys and its excretion increases in slow period of bone development. [14] Chronic fluorosis consists and its metabolic effects increase even if it is at low levels for that plasma half-life of fluorine take long depending on dysfunction of kidney. [3] Levels of unsaturated fatty acids with phospholipids of liver due to high doses of fluoride is stated to reduce. [14] In addition, findings associated with lipid metabolism such as serum total cholesterol and triglycerides of earlier studies are conflicting with each other during to fluorosis. [11,15-17]

Ellagic acid (2,3,7,8-tetrahydroxy [1] benzopyrano [5,4,3-cde] [1] benzopyran-5,10-dione) which is known as a plant polyphenolic acid molecule and hydroxybenzoic acid derivate has important biological activities such as antioxidant and anticarcinogenic. [18,19] It is claimed that EA in terms of free radical scavenger activity has an ideal chemical structure and may be a

more effective antioxidant property than vitamins C and E as in vitro. [20,21]

Chronic fluorosis are not well documented in a comprehensive manner as both biochemical and histological. Therefore, the present study was designed to evaluate both pathological changes and effects of ellagic acid in mice with experimental chronic fluorosis, in biochemical and histological terms.

## MATERIALS AND METHODS

### Experimental Design

The ethical approval of the study was confirmed by Kafkas University Animal Care and Use Committee (Registration Number: 2011/17). 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.

Experiments were carried out on 28 male Swiss albino mice each weighing 30-41g at 14-16 weeks of age. Animals were divided into 4 equal groups namely Group I through Group V and housed in a room maintained at  $18\pm 1^{\circ}C$  with an alternating 12 h light-dark cycle. Food and water were provided *ad libitum*. All experimental injections were carried out for 8 weeks and at the same hours of the light cycles of the study. Treatments were performed as follow; Group I: normal diet and drinking water, Group II: sc injection of 10 mg/kg/day ellagic acid, Group III: per orally 25 mg/L/day NaF, and Group IV: per orally 25 mg/L/day NaF plus sc injection of 10 mg/kg/day ellagic acid.

### Biochemical Analysis

#### Collection and processing of blood samples

At the end of the 8<sup>th</sup> week, blood samples were collected into heparin tubes from the hearts via cardiac puncture under ether anesthesia for biochemical measurements. For the GSH estimation was allocated a little of all blood samples. The remaining blood samples were centrifuged (1200 g, 4 °C) for 10 minutes, and the

plasma were obtained and then kept at -25°C until the analyzes were carried out.

#### *Estimation of lipid bound sialic acid*

Briefly, 44.7µl of plasma was extracted with chloroform-methanol (2:1 v/v) maintained at 4°C. The lipid extract was separated with 0.5 ml of distilled water. The aqueous layer was precipitated with phosphotungstic acid. The precipitates were resuspended in 1 ml of distilled water and lipid bound sialic acid in suspension was determined with resorcinol reagent. [22]

LSA contents were calculated using standard curves obtained for various concentration of N-acetyl neuraminic acid (Sigma, A- 0812).

#### *Glutathione estimation*

GSH level was estimated as spectrophotometric method. [23] A standard calibration curve was prepared using reduced GSH. Absorbance of all the samples was measured spectrophotometrically at 412 nm and the results were expressed as mg of GSH/g of tissue.

#### *Lipid peroxidation estimation*

The LPO level was estimated in terms of malondialdehyde (MDA). [24] 1,1,3,3-tetramethoxypropane was used as an external standard and the level of LPO was measured spectrophotometrically at 532 nm and expressed as nmol MDA/g of tissue.

#### *Estimation of Paraoxonase Activity*

Paraoxonase activity was measured in the absence (basal-activity) and presence of NaCl (salt-stimulated activity), using paraoxon substrate. The rate of paraoxon hydrolysis (diethyl p-nitrophenylphosphate) was measured by monitoring the increase of absorbance at 412 nm. The molar extinction coefficient of p-nitrophenol released at pH 8.0 was 18,290. One unit (U) of paraoxonase activity is defined as 1 µmol of p-nitrophenol formed per min, and activity was normally expressed as U/L of plasma. [25]

#### *Estimation of total cholesterol, triglycerides, HDL, LDL, VLDL, glucose and calcium in plasma*

The levels of total cholesterol, triglycerides, HDL, LDL, glucose and calcium in plasma were measured by an automatic analyzer (humast 600, Germany) using assay kit (Total Cholesterol, Triglycerides, HDL, LDL, Glucose and Calcium Liquicol is humast kits, Germany). VLDL level was calculated using Friedewald formula [ $\text{Triglycerid (mg/dL)} < 400 \text{ mg/dL VLDL (mg/dl)} = \text{Trigiserid (mg/dl)/5}$ ]. [26]

#### *Fluor estimation in urine*

The levels of fluor in 5 urine samples taken from each of control, NaF and ellagic acid plus NaF groups were measured by ion specific electrode (Orion 9609BN ionplus) (Suleyman Demirel University, Geothermal Energy, Groundwater and Mineral Resources Research and Application Center, Isparta/TURKEY).

#### **Histopathological Investigations**

Tissue sections of liver and kidney were sliced and fixed in 10% phosphate buffered formalin. After the specimens were dehydrated in serial ethanol and xylene, they were embedded in paraffin. Tissue sections were then cut and processed for hematoxylin and eosin (HE) staining. The sections were examined unbiased under a light microscope.

#### **Statistical Analysis**

For the statistical analysis, differences between the groups were tested by analysis of variance (ANOVA) and Tukey test using SPSS for Windows version 20.0 Data were presented as mean±standard deviation ( $X \pm \text{Std.D}$ ). The relations between the parameters were determined by Pearson correlation analysis.

## **RESULTS**

### **Biochemical Findings**

Levels of LSA, MDA, PON1 activity, total cholesterol, triglyceride, HDL, LDL, VLDL, glucose, and calcium in the plasma with GSH levels in the all blood samples of mice were shown in Table 1. The level of plasma MDA of group was significantly higher in mice received NaF

alone compared to other groups. The levels of LSA, PON1 activity, total cholesterol, glucose, calcium, and GSH was significantly lower in mice received NaF alone compared to other groups. Correlations between biochemical parameters were shown in Table 2. Fluoride

content in urine of control, NaF and ellagic acid plus NaF was determined as  $1.66 \pm 0.05$ ,  $60.40 \pm 0.55$  and  $25.00 \pm 0.26$  mg/L, respectively. According to these data in fluor ion levels of NaF and NaF plus ellagic acid groups were significantly increased compared to control group.

Table 1. The levels of LSA, MDA, PON, total cholesterol, triglyceride, HDL, LDL, VLDL, glucose and calcium in plasma with GSH in all blood of control and treatment groups

	Groups (n= 7)				P
	Control	Ellagic acid	NaF	Ellagic acid plus NaF	
LSA (mg/dl)	19.86±0.93 <sup>a</sup>	20.68±2.45 <sup>a</sup>	13.31±1.75 <sup>b</sup>	16.73±1.70 <sup>ab</sup>	< 0.001
MDA (µmol/L)	10.35±0.97 <sup>a</sup>	9.80±1.86 <sup>ab</sup>	12.08±1.61 <sup>b</sup>	10.04±1.32 <sup>a</sup>	< 0.05
GSH (mg/dl)	5.11±0.33 <sup>a</sup>	4.51±0.41 <sup>b</sup>	2.74±0.35 <sup>c</sup>	4.14±0.48 <sup>ab</sup>	< 0.001
PON (U/L)	130.40±6.14 <sup>a</sup>	137.29±18.27 <sup>a</sup>	95.88±11.06 <sup>b</sup>	122.72±6.69 <sup>a</sup>	< 0.001
Total cholesterol (mg/dl)	156.17±26.85 <sup>a</sup>	122.89±24.71 <sup>ab</sup>	114.09±24.84 <sup>b</sup>	138.16±16.40 <sup>ab</sup>	< 0.05
Triglyceride (mg/dl)	134.86±18.59 <sup>a</sup>	116.86±22.12 <sup>a</sup>	95.89±29.22 <sup>b</sup>	97.23±13.05 <sup>b</sup>	< 0.05
HDL (mg/dl)	57.69±14.18 <sup>a</sup>	40.73±7.49 <sup>b</sup>	41.09±8.42 <sup>b</sup>	40.47±7.51 <sup>b</sup>	< 0.05
VLDL (mg/dl)	26.91±3.84 <sup>a</sup>	23.37±4.42 <sup>ab</sup>	19.20±5.90 <sup>b</sup>	19.13±2.73 <sup>b</sup>	< 0.05
LDL (mg/dl)	73.74±18.30 <sup>a</sup>	75.97±18.01 <sup>a</sup>	53.34±16.59 <sup>a</sup>	79.23±15.26 <sup>a</sup>	> 0.05
Glucose (mg/dl)	190.37±29.65 <sup>a</sup>	100.49±16.77 <sup>bc</sup>	87.71±32.89 <sup>c</sup>	79.24±17.38 <sup>c</sup>	< 0.001
Calcium (mg/dl)	8.06±0.33 <sup>a</sup>	8.94±0.72 <sup>ab</sup>	7.48±0.23 <sup>b</sup>	8.34±0.98 <sup>ab</sup>	< 0.05

X ± Std.D<sup>a, b, c</sup>: Values with different letters within the same row indicates significant differences (P < 0.001 or < 0.05)

Table 2. The correlations between biochemical parameters in control and treatment groups

	Triglyceride	HDL	LDL	Glucose	LSA	GSH	PON	Calcium
VLDL	0.997 <sup>**</sup>	0.370 <sup>*</sup>		0.552 <sup>**</sup>		0.442 <sup>**</sup>		
Total cholesterol		0.632 <sup>**</sup>	0.631 <sup>**</sup>	0.389 <sup>*</sup>		0.353 <sup>*</sup>		
Triglyceride		0.376 <sup>*</sup>		0.550 <sup>**</sup>		0.451 <sup>**</sup>		
HDL				0.539 <sup>**</sup>		0.350 <sup>*</sup>		
LDL								
Glucose					0.399 <sup>*</sup>	0.522 <sup>**</sup>		
LSA							0.386 <sup>*</sup>	
MDA						-0.410 <sup>*</sup>	-0.468 <sup>**</sup>	
GSH							0.582 <sup>**</sup>	0.366 <sup>*</sup>
PON								0.390 <sup>*</sup>

\*\* : Correlation level is important (P<0.01), \* : Correlation level is important (P<0.05)

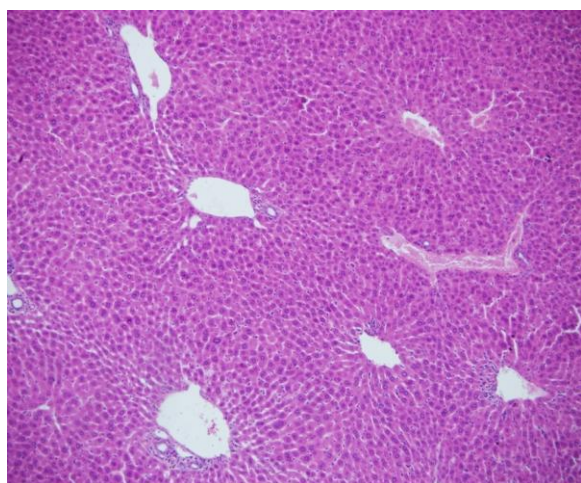


Figure 1. In the liver of control group, normal findings are observed (HE, X20)

### Histopathological Findings

The liver and kidney samples of excised from control group were either normal or showed minimal degenerative changes (Figure 1 and 6). In

histopathological examination of liver tissues in NaF alone given mice was usually observed hydropic degeneration of hepatocytes, dissociation of sinusoids, hiperchromasia, growth of hepatocyte nuclei, marked focal necrosis in midzonal and periacyinary zones (Figure 2 and 3). In NaF plus ellagic acid group was determined a reduction in severity and number of mentioned pathological changes (Figure 4 and 5).

Morphological structures of kidney tissues of control and ellagic acid groups were normal and similar quality (Figure 6). In kidney tissue of NaF group were observed hydropic degeneration in all tubules and dilatation of tubular lumen and necrosis in some places (Figure 7). In NaF plus ellagic acid group were detected a reduction in severity of degeneration

compared to NaF group and necrosis in this group never seen (Figure 8).

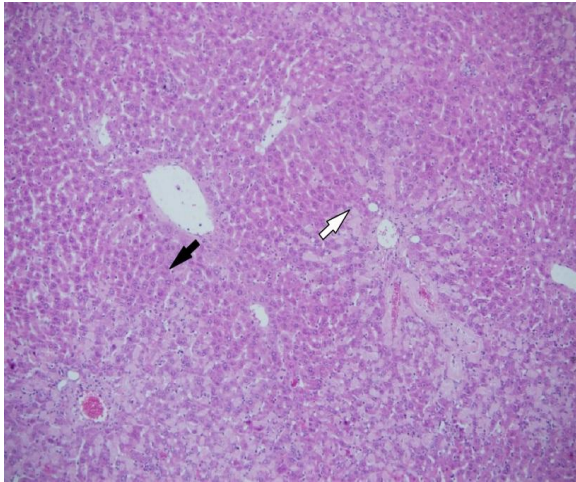


Figure 2. In the liver of a mouse given NaF alone is seen edema (white arrowhead) and dissociation (black arrowhead) (HE, X20)

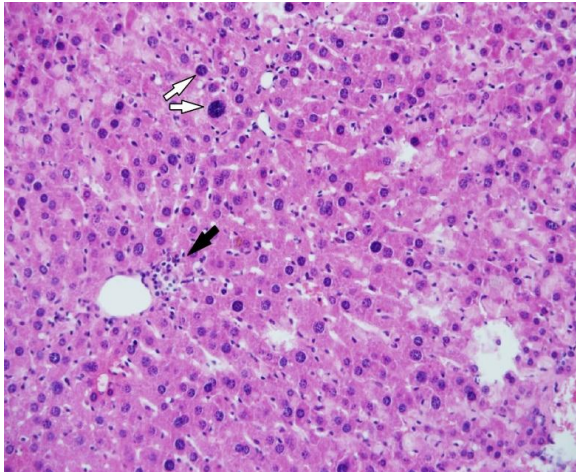


Figure 3. In the hepatocytes of a mouse given NaF alone are seen necrosis (black arrowhead), growth of nuclei and hyperchromasia (white arrowhead) (HE, X40)

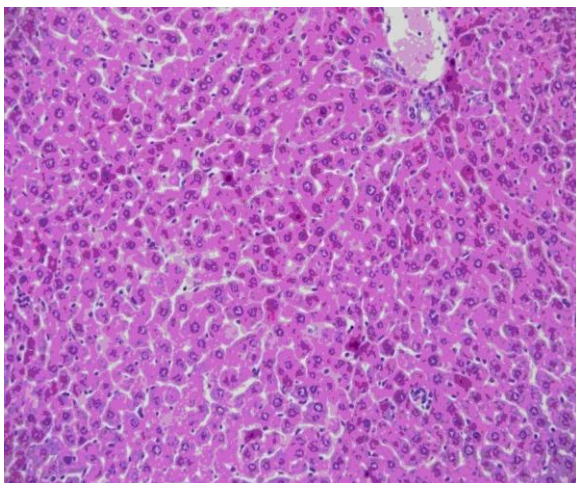


Figure 4. In the liver of a mouse given ellagic acid alone is seen slightly degeneration (HE, X40)

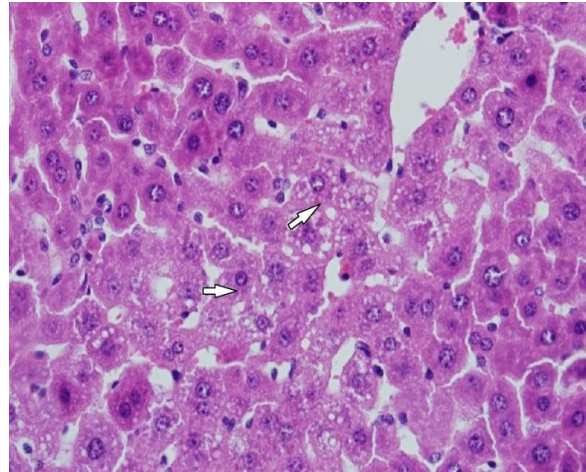


Figure 5. The fatty degeneration in the liver of a mouse given ellagic acid alone (white arrowheads) (HE, X40)

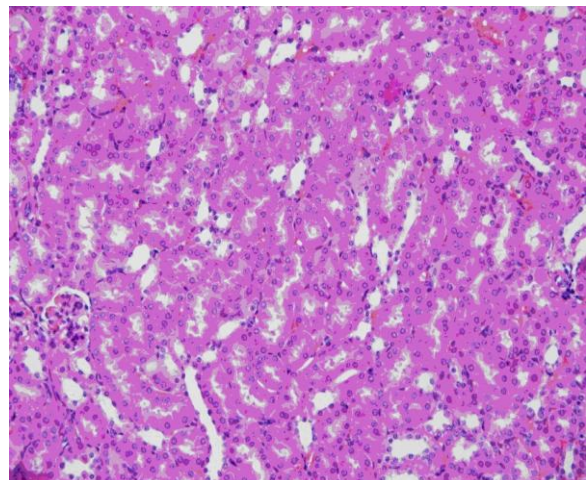


Figure 6. In the kidney of control group, normal findings are observed (HE, X20)

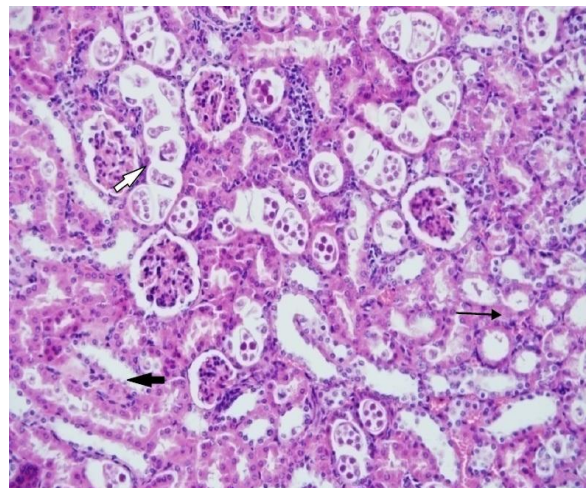


Figure 7. In the kidney of a mouse given NaF alone is seen hydropic degeneration of the proximal tubule epithelium (black arrowhead), the necrosis (thin black arrowhead) and dilation of tubular lumen (white arrowhead) (HE, X20)

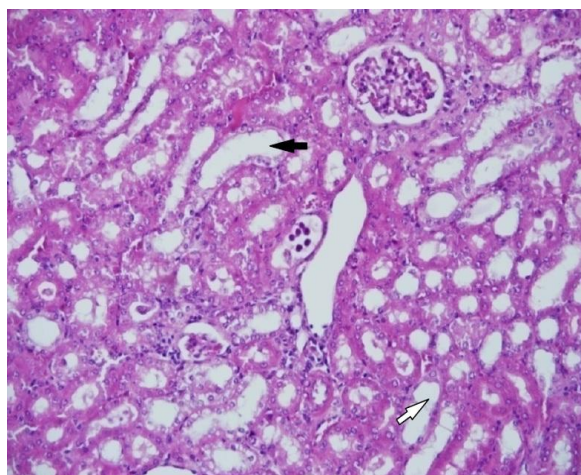


Figure 8. In the kidney of a mouse given ellagic acid plus NaF is seen mild hydropic degeneration of proximal tubule epithelium (black arrowhead), the dilatation of tubular lumen (white arrowhead) with few necrotic epithelial cells (HE, X20)

## DISCUSSION

Excessive amounts of fluoride are recorded that in soft tissues such as liver, kidney and blood of organisms created disorders due to its inhibitory effects on vary enzyme systems binding calcium. [27,28] It is emphasized that addition of vitamin (A, C, E and D) with precipitators such as aluminum sulfate, calcium hydroxide and magnesium into diet and drink water of organisms in areas with intensive fluoride to prevent pathological effects of fluoride is need. It is declared that use of clean water sources in areas with intensive fluoride reserve is a more effective method. [29] In the present study, administration of ellagic acid was tested effects on changes of biochemical and histopathological in experimental chronic fluorosis.

Recent data from several reports about levels of low GSH and high MDA during to excessive intake of fluoride indicate that fluorosis increase production of free. [6,30] In this study, in mice with NaF-induced chronic fluorosis, decrease in blood GSH level and increase MDA level were determined compared to control group. These levels were concluded to be able to be normalized by ellagic acid injection. Decreased GSH and increased MDA levels in chronic fluorosis may be associated with elevated levels of reactive oxygen products ( $H_2O_2$ ,  $O_2^{\cdot-}$ ,  $OH^{\cdot}$  etc.) due to respiratory burst occurring by phagocytic cells during

immune response including the NADPH oxidase. [31] It is indicated that ellagic acid and phenolic compounds in urine and plasma can conjugate with methyl, glucuronyl and sulfate groups. Decrease of cystine concentrations contributing for GSH synthesis due to metabolical sulfate conjugation of ellagic acid can also effect indirectly GSH level. [32]

A negative correlation relationship between fluorosis with levels of antioxidant molecules such as GSH and PON1 that confers antioxidant properties to HDL is reported to be possible due to excessive production of free radicals. [6,13,33] Free sulphhydryl group of cystein-284 of PON1 is required for ability of PON1 to protect LDL against oxidation. [33] It is asserted that amount of free thiol group with antioxidative effect of PON1 is proportional. [34] Furthermore, it is reported that PON1 in plasma is show a significant peroxidase like activity hydrolyzing  $H_2O_2$  as dependent on HDL against molecule or atoms having strong oxidative characteristics. [35] In fluorosis period, it is concluded that activity of PON1 known as an antioxidant in plasma can decrease by oxidative stress because of low levels. [6,30,35] In our study was determined that PON1 activity increased significantly in NaF plus ellagic acid group as compared to NaF group with chronic fluorosis. The present study therefore reveals that ellagic acid may be an important compound for PON1 activity in fluoride toxication.

Glucose-6-phosphate dehydrogenase enzyme in the pentose phosphate metabolic pathway are important for the production of NADPH which will used in detoxification reactions, synthesis of cholesterol and reduction of glutathione. [36] It is recorded that fluoride has an inhibitory effect on activity of glucose-6-phosphate dehydrogenase enzymes. [37] In the present study, while total cholesterol and GSH levels in NaF group as compared to the control group decreased, these levels were ameliorated in NaF plus ellagic acid group. These findings suggest that ellagic acid

injection may be therapeutic for inhibition of glucose-6-phosphate dehydrogenase in the pentose phosphate metabolic pathway as related with synthesis of cholesterol and GSH.

In various studies carried out on rat and rabbits were demonstrated that main effect of fluoride on plasma  $\text{Ca}^{2+}$  levels could be originated from calcifications accelerating of solid  $\text{Ca}_{10}\text{F}_2(\text{PO}_4)_6$  or  $\text{CaF}_2$  formation. [10] The applications of ellagic acid revealed stimulating effects on the  $\text{Ca}^{2+}$  pumps of sarcoplasmic reticulum and cardiac and skeletal muscle cells. [38,39] Another study indicated that  $\text{Ca}^{2+}$  in the cardiac muscle cells could be more maintained preventing by ellagic acid of the inhibitory effect of phospholamban, which is an ATPase. [38] In our study was determined that plasma  $\text{Ca}^{2+}$  levels of NaF group as compared to the control and other groups were significantly decreased and these levels in ellagic acid group was higher than other groups. Based on previous reports and our findings, it is suggested that ellagic acid on decreasing plasma  $\text{Ca}^{2+}$  levels in chronic fluorosis period may be protective by means of wherein said mechanisms.

It was recorded that serum SAs in the early diagnosis of fluorosis was important and serum SA levels could be decreased about 50% in individuals with fluorosis as compared to healthy individuals. [12] These levels in fluorosis also change in opposite direction, even though levels of SA in several diseases increase. [27,40] In a study on male mice which received orally 10 mg/kg/day fluoride for 30 days was reported that free SA levels decreased in group with fluoride toxication as compared to control group and 2  $\mu\text{g}/\text{mouse}/\text{day}$  vitamin E application against toxic effects of fluoride increased significantly SA levels. [27] Reason for reduction in SA levels in same study was associated with enzyme activities in energy metabolism such as phosphorylase, ATPase and glycolysis enzymes requiring to  $\text{Mg}^{2+}$  or  $\text{Ca}^{2+}$  ions as cofactor. Together with

utilization of glucose and amino group donor in biosynthesis of SA is necessary grades catalyzed reactions of active enolase and condensation with pyruvate of mannosamin-6-phosphate. [41] In earlier studies were shown that excessive amount of fluoride inhibited enolase which catalyzed formation of phosphoenol pyruvate from 2-phosphoglycerate in glycolysis. [42] The decrease in plasma LSA level can be originated from interruptions in biosynthesis of SA and its conjugates. [43] Decreased levels of LSA may also be originated from detoxification mechanisms like reaction that aldehyde reductase detoxifies 3-deoxyglucosone. [42] The present study reveals to be lower than NaF plus ellagic acid group of plasma LSA levels of NaF group. The low levels of LSA and glucose in mice with NaF-induced chronic fluorosis and positive correlation between these levels may be associated with suppressed enolase activity and decreased biosynthesis reactions of LSA. ellagic acid application during fluorosis is also suggested as an important protective phenolic acid for biosynthesis of LSA.

Findings including parameters of lipid metabolism such as HDL, LDL, total cholesterol and triglycerides in studies related with fluorosis are different from each other. [11,16,17,44,45] It is particularly noted that chronic exposure of fluoride cause changes on levels of serum cholesterol and triglyceride by affecting enzyme activity of triglyceride lipase and certain non-specific esterases. [44-46] It is claimed that LDL because of the increased serum cholesterol levels may be more rapidly oxidize and activity of PON1 closely associated with HDL decrease. [47] It is stated that reorganization of energy balance and glucose utilization is also necessary because of blocked glycolysis and citric acid cycle with inhibition of enolase and pyruvate decarboxylase enzymes by fluoride. [42] In a study was claimed that lipid parameters such as total cholesterol, triglyceride, HDL and LDL of serum in fluoride treated rats could be normalized by

pretreated of epigallocatechin gallate known as derivate of a phenolic acid. [46] In our study was determined high positive correlation between levels of HDL, VLDL and triglyceride with glucose levels in plasma. Therefore, we share the idea that decreases of glucose and lipid parameters in chronic fluorosis may occur as a result of the metabolic changes associated with utilization of glucose and energy resources. [14,48]

Fluorine ions have been reported to cause dysfunction on soft tissues such as liver, kidney and brain passing through easily cell membranes. [30,49] In a study on female mice given 500 ppm NaF in their drinking water from the 15th day of pregnancy until day 14 after delivery is reported that biochemical modifications including serum transaminase activities which well known as markers of liver function in NaF-treated mice correspond histologically with extensive ballooning, hepatocellular necrosis and infiltration of mononuclear cells. [49] It was showed areas of necrosis within individual lobules and infiltrations of mononuclear cells in liver tissues of rats received 20 mg/kg/day NaF for 3 months. [50] It was reported markedly necrotic zones in liver of rabbits given orally NaF. [51] It is also recorded protective effects of epigallocatechin gallate supplementation against renal injury induced by fluoride intoxication in rat. [52] In the present study was determined that in hepatocytes of NaF group were hydropic degenerations and balloning, evident focal necrosis in periacinar and midzonal areas, dissosiation in sinusoids, growth in hepatocyte nuclei, dual nucleus formation, proliferation in Kupffer cells of parenchyma, picnosis and infiltrations of mononuclear cells (Fig 2 and 3). It was detected slightly degeneration in livers of mice given ellagic acid alone (Fig 4 and 5). It was determined that number and severity of pathological changes markedly decreased in NaF plus ellagic acid group as compared to NaF group.

Glomerular and tubular dysfunction in renal tissue of organisms in earlier studies related with chronic fluorosis has been reported to occur. [53,54] In pigs with 100 and 250 mg/kg of NaF treatment through diet for 50 days were shown to cause increased lactate dehydrogenase activity and histopathological changes such as necrosis and tubular dilatation in kidney. [54] In a study applied on rats with two generations was created chronic fluorosis by giving drinking water with 100 and 150 ppm fluoride to generations throughout study. [55] It was reported that histopathological examination kidney and liver sections showed clear differences between controls and experimental animals and renal histological changes consisted of degenerative changes in renal tubule cells, irregularly shaped nuclei with marginated chromatin. On the other hand, it was stated that fibrosis in the portal area of liver tissue and proliferation in bile ducts, abnormal focal cellular infiltration in the periphery of lobe, dilatation in sinusoidal capillaries and heterochromatisme in the nuclei of hepatocytes which occurred much more severe proportional to higher dose was shown. In the present study was observed hyaline cylinders and dilatation in tubul lumens with necrosis in some places, hydropic degeneration in epithelial cells of all tubules and particularly proximal tubule in group received NaF alone (Fig 7). In NaF plus ellagic acid group as compared to NaF group was detected a decrease in severity of degeneration, and in mice of this group was not encountered with necrosis (Fig 7 and 8). The histological findings also strongly support our biochemical findings that ellagic acid protects the NaF-induced oxidative stress mediated renal and hepatic injury.

In view of the results obtained in the present study, NaF-induced experimentally chronic fluorosis causes important changes in parameters including oxidative stress, lipid profile and histopathological structure of liver and kidney, and ellagic acid can be potentially a protective phenolic compound



on these changes. Further investigation is required to explore the exact role of energy balance and biosynthesis reactions in the protective mechanism of ellagic acid during chronic fluorosis.

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