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Fluoride and Liver Toxicity: A Zebrafish Model

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Abstract **–** Fluoride, which is exposed by living organisms through food and water intake, causes many health problems. The liver is one of the organs most affected by sodium fluoride (NaF) toxicity. Studies have shown that NaF causes many pathological and metabolic changes in the liver. Many studies have shown that exposure to high fluoride concentrations in drinking water elevates the levels of kidney and liver function enzymes in serum and causes severe histological changes in the liver and kidneys. However, there are limited studies on the liver toxicity of chronic fluoride intake in amounts close to daily usage doses. Therefore, in our study, the effect of chronic fluoride (NaF) exposure at low doses on zebrafish liver toxicity was investigated. Liver tissues of zebrafish exposed to NaF at doses of 1.5 ppm, 5 ppm, and 100 ppm for 6 weeks were used in the study. GSH and MDA levels were measured in these tissues. In addition, specific activities of GST and GR enzymes were determined. When the data obtained were examined, it was observed that while GST, GR, and GSH levels decreased, MDA levels increased, especially in the groups treated with 5 and 100 ppm NaF. This suggests that chronic exposure to fluoride, albeit at low doses, may be a risk factor.

Keywords – Fluoride, Zebrafish, Toxicity, Liver

I. INTRODUCTION

Fluoride can enter the body from many sources frequently used in daily life, such as drinking water, baby food, carbonated beverages, instant soup and packaged ready-to-eat foods, anesthetic chemicals, drugs, cosmetics, and teeth cleaning products [1]. The daily amount of fluoride taken can vary between 0.5-5 mg depending on the sources of exposure [2]. The amount of fluorine allowed in drinking water has been recommended by the World Health Organization (WHO) as 1.5 ppm [3]. It has been determined that exposure to fluoride above this limit has immunotoxic and neurotoxic effects. [4]. Humans face fluoride toxicity when exposed to fluoride above the recommended limit [5]. Oxidative stress resulting from the accumulation of free radicals is one of the most important mechanisms of F-toxicity [6]. Many studies have found that exposure to F adversely affects the antioxidant system by triggering oxidative stress in the serum, liver and brain of animals [7]. Although fluoride exposure in adulthood has been associated with nephro- and hepatotoxicity in animals and humans [9][10] few studies have investigated the relationship between fluoride exposure and kidney or liver function. there is work [11].

Many studies have been conducted on the relationship of F- with oxidative stress. However, research on liver toxicity of NaF in zebrafish is limited. In this study, the effects of chronic exposure to low-dose fluoride that we can be exposed to in daily life on liver toxicity were tried to be clarified.

II. MATERIALS AND METHOD

Animal Exposure and Collection of Tissue Samples

AB genotype zebrafish used in the study were obtained from Atatürk University Fisheries Experimental Research Unit. Fish were kept in a system with a water temperature of 28 °C and fed a standard diet twice daily.

Experimental groups were formed as follows.

Group 1 (Control): No treatment was applied to the fish in this group.

Group 2 (Treatment 1): Fish in this group were exposed to 1.5 ppm fluoride (NaF).

Group 3 (Treatment 2): Fish in this group were exposed to 5 ppm fluoride (NaF).

Group 4 (Treatment 3): Fish in this group were exposed to 100 ppm fluoride (NaF).

Zebrafish were exposed to fluoride at doses of 1.5 ppm, 5 ppm and 100 ppm for 6 weeks. At the end of the exposure period, the fish were dissected and liver tissue samples were taken.

Measurement of Metabolite Levels Associated with the Antioxidant System

GSH and MDA levels were measured in liver samples from zebrafish chronically exposed to fluoride.

Measurement of Total Glutathione (GSH) Level

The amount of GSH in the liver tissues of fish exposed to F- was determined by the method proposed by Sedlak and Lindsay (1968). Absorbance was measured at 412 nm using a spectrophotometer. GSH level results are expressed as nmol/mg tissue [12][13].

Determination of Malondialdehyde (MDA) Levels

The amount of MDA in the liver tissues of fish exposed to F- was determined by the method proposed by Ohkawa (1979).

Absorbance was measured spectrophotometrically at 532 nm. Results are expressed as nanomoles of MDA per gram of wet tissue (nmol/g tissue) [14][15].

Enzyme Activity Analysis

Liver samples were weighed and then homogenized in 1/5 phosphate buffer (50 mM, pH: 7.4, containing 10 mM EDTA). The supernatant obtained by centrifugation at 10000 g for 30 minutes at 4°C was then used for activity measurement.

Bradford (Coomassie Blue) protein determination

Quantitative protein determination was performed spectrophotometrically at 595 nm according to the Bradford method [16].

Glutathione S-transferase (GST) Activity

Total GST activity of liver tissues was determined according to the method proposed by Habig and Jakoby (1981). Absorbance was measured spectrophotometrically at 340 nm [17] [18].

Glutathione Reductase (GR) Activity

GR activity was determined according to the method suggested by Carlberg and Mannervik (1985). Absorbance was measured spectrophotometrically at 340 nm [19][20].

Statistical analysis

Experimental results were statistically analyzed using GraphPad Prism Software version 8.0 (GraphPad Software, San Diego, CA). Analysis of the results of the control and experimental groups was performed with one-way ANOVA and Tukey post-hoc test. $p > 0.05$ (not significant, ns); $*p < 0.05$ (significant); $**p < 0.01$ (very significant); $***p < 0.001$ (very significant).

III. RESULTS

MDA and GSH levels were measured to evaluate the toxicity of chronic fluoride exposure in zebrafish liver tissue for 6 weeks. When the obtained results were analyzed, it was determined that the MDA level increased and the GSH level decreased depending on the dose.

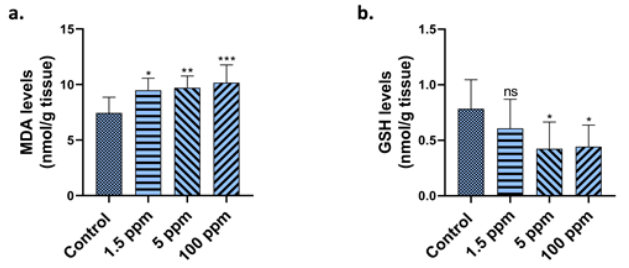

Figure 1. Measurement of MDA (a) and GSH (b) levels in fluoride-exposed zebrafish liver tissue.

The specific activities of GST and GR enzymes were measured to evaluate the toxicity of chronic fluoride exposure in zebrafish liver tissue for 6 weeks. When the results were analyzed, it was determined that the activity of GST and GR enzymes decreased depending on the dose.

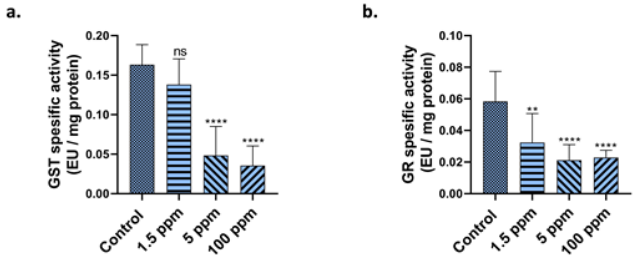

Figure 2. Measurement of specific activities of GST (a) and GR (b) enzymes in fluoride-exposed zebrafish liver tissue.

IV. DISCUSSION AND CONCLUSION

Since the liver is the main organ responsible for xenobiotic detoxification, it is one of the organs most affected by F-toxicity [21]. Studies have shown that kidney and liver functions are impaired in children exposed to fluoride concentrations [22] [23][24]. In a study, it was determined that the activities of antioxidant system enzymes were inhibited in rats exposed to 100 and 200 mg/kg NaF for 7 days [25]. In a similar study, it was determined that liver enzyme activities and kidney damage occurred in rats administered NaF at 0.5-5 and 20 ppm levels [24].

Fluoride can also inhibit the activities of antioxidant enzymes by increasing reactive oxygen species (ROS) and causing changes in cellular glutathione levels [25]. Oxidative stress is defined as the uncontrolled overproduction of free radicals that damage macromolecules such as DNA, proteins, and lipids [7]. Lipid peroxidation (LPO) as the main index of oxidative damage has been found to be involved in the toxicity of many xenobiotics [26]. Fluoride also induced oxidative stress by increasing reactive oxygen species (ROS) and malondialdehyde (MDA) levels and decreasing glutathione (GSH) content and glutathione peroxidase 4 (gpx4) levels in adults and larvae [27].

When the data obtained from the studies were evaluated, it was determined that when NaF is used in high doses, it impairs liver functions and causes toxicity and damage to the liver. However, there are not many studies on the effect of liver toxicity when chronic exposure to low doses of fluoride that we can be exposed to in daily life. In our study, the effect of fluoride used in low doses on liver toxicity was evaluated. When the data were evaluated together, it was determined that especially 5 and 100 ppm fluoride decreased GSH, GSH, and GR levels in liver tissue. In addition, it has been found to cause an increase in MDA levels. This showed that fluoride could be a risk factor by creating toxic effects on liver tissue, even when used at low doses.

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