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Effect of chitosan application on lung tissue in rats with experimental fluorine toxicity

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ABSTRACT

Aim: The aim of this study is to investigate the effects of chitosan application on lung tissue in rats with experimental fluorine toxicity.

Material and Method: In the study, 21 healthy male wistar albino rats were used. Prior to the trial, the acclimation of the rats was provided. 3 groups were randomly generated in a way that there were 7 rats in each group. These were determined as the control group (C), the fluorosis group (NaF) and the fluorosis + chitosan (NaF+CS) group.

Results: In the NaF group, CAT, SOD and GSH values were found to be low compared to other groups and MDA values were found to be high. It was found that the chitosan application reduced the CAT, SOD and GSH values, and increased the MDA value.

Conclusion: It has been predicted that chitosan application may be beneficial in preventing cellular damage that may occur with fluorine exposure.

Keywords: Chitosan, sodium fluoride, antioxidant, lung

INTRODUCTION

Fluorine is an important substance that must be taken from the outside in terms of human health. Fluorine, which is naturally found on earth, is taken into the body as a result of consuming water, inhaling industrial gases, eating plant and animal foods. Approximately 80% of the fluorine taken into the body passes into the blood by a simple diffusion route. A small part of the fluorine is excreted from the kidneys, while a large part is stored in bone tissue. Therefore, we see the beneficial and harmful effects of fluoride mostly in the skeletal system. Fluorosis occurs if fluorine is taken too much (1-3). Fluorosis especially damages bone tissue, as well as heart, muscle, nerve, kidney, and lung tissues (4,5). In a study conducted, it was reported that the application of 4,5 and 9 mg fluorine/kg/day sodium fluorine caused necrosis in the rabbit lung parenchyma (6).

Chitosan is an environmentally friendly biopolymer that has no toxic effect, has an antimicrobial effect, accelerates wound and bone healing, and also reduces pain (7). It is known that chitosan and its derivatives have antioxidant, antidiabetic, and anticancer properties (8).

The aim of this study is to investigate the effects of chitosan application on lung tissue in rats with fluorosis.

MATERIAL AND METHOD

Animals and Experimental Design

In the study, 21 healthy male Wistar Albino rats provided from Van Yüzüncü Yıl University Experimental Animal Unit were used. Prior to the trial, the acclimation of the rats was provided. Experimental applications in the study were carried out in accordance with the conditions of care of laboratory animals (12 hours of light: 12 hours of dark and 22±1°C and 60% humidity). During the experimental applications, rats were given standard commercial rat feed (pellet feed) and drinking water ad libitum. 3 groups were randomly generated in a way that there were 7 rats in each group. The experiment lasted 12 weeks. The study was conducted with the approval of Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee (Date: 31/03/2022, Decision No: 2022/03-08). All procedures were performed adhered to the ethical rule.

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1. **Control group (C):** The control group was fed with drinking water and standard pellet feed.
2. **Fluorosis Group (NaF):** 100 ppm fluorine was applied to rats in the form of NaF by adding to their drinking water.
3. **Fluorosis and the Chitosan Group (NaF+CS):** 100 ppm fluorine was applied to rats in the form of NaF by adding to their drinking water. At the same time, 250 mg/kg/day of chitosan was administered daily by oral gavage.

Application

At the end of 12 weeks, all rats were anaesthetized with a combination of ketamine and xylazine through intramuscular (i.m) route and dissected. Later, lung tissues were taken to determine the levels of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and malondialdehyde (MDA) and washed using sodium phosphate buffer (pH 7.2). The tissues were stored at -80°C until the time of the study. In the homogenization buffer (pH 7.4), the tissues were homogenized using a homogenizer. The obtained homogenates were centrifuged and prepared to determine the amount of SOD, CAT, GSH and MDA. In the spectrophotometer, the antioxidant enzyme activities, and the amount of MDA of the samples were measured and their absorbance was determined in accordance with the literature (9-12).

Statistical Analysis

In the study, one-way variance analysis (ANOVA) was used to compare the group averages from various angles. After analyzing the characteristics and variance, the Duncan test was used to determine the different groups. The statistical significance level was taken as P<0,05 in the calculations. All analyses were done using the SPSS package program (Ver. 22).

RESULTS

The difference between the groups of chitosan application in rats with fluorine toxicity is shown in **Figure 1**.

The CAT value in the NaF group was found to be statistically different compared to the control and NaF+CS group. It was found that the decreasing CAT value in the NaF group increased in the Naf+CS group and was parallel to the control group. SOD values were similar in the control and NaF+CS groups, while they were found to be low in the NaF group. When the groups were examined in terms of GSH value, it was found that the value was low in the NaF group compared to the control group, and the GSH value in the NaF+CS group was similar compared to the control group. It was observed that the GSH value decreased in rats with fluorosis increased with the application of chitosan.

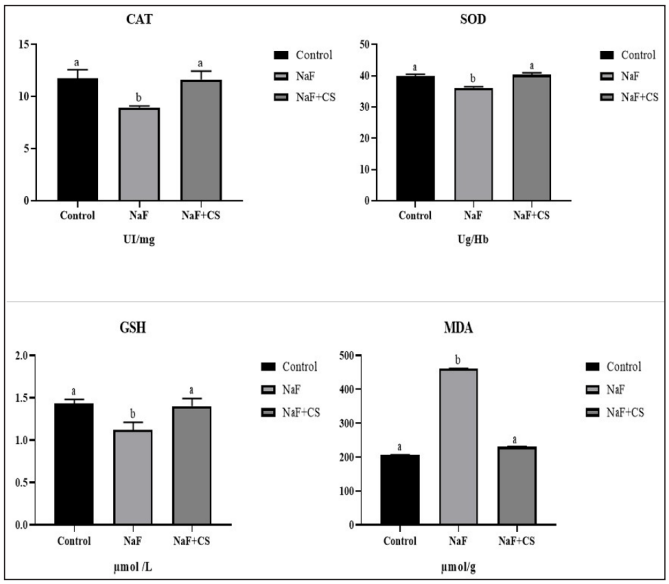

Figure 1. CAT, SOD, GSH and MDA values in the lung tissue of rats with fluorosis

When the MDA value was analyzed, the NaF group was found to be higher than the control group. The NaF+CS group was found to be similar compared to the control group and low compared to the NaF group.

DISCUSSION

It is known that excessive fluorine exposure causes cellular damage in the organism (13). With the decrease of Ca+2 levels in organisms with fluorosis, cellular oxygen use also decreases and hence causes various disorders (14). Aydın et al. (15) conducted a study in which they applied 10, 50 and 100mg/l fluoride to drinking water of Wistar albino male rats for 6 months. At the end of the study, they reported that necrosis, desquamation, and alveolar obstruction were observed when the lung tissue was examined. They also observed that CAT activity decreased. In the same study, it was revealed that the degree of lung damage also varied depending on the dose of fluorine. Nabavi et al. (16) showed that there was a decrease in CAT and SOD values in rats administered 600 ppm fluoride intraperitoneally. In another study, they reported that CAT and SOD values decreased significantly in rats with fluorosis (17). In a study conducted on people living in areas with an excessive fluorine concentration, it was found that the SOD value was low (18). It was revealed that there was a significant decrease in GSH levels in mice with chronic fluorine toxicity (19). In a study conducted on rats applied with 25 ppm fluorine daily by mixing it into their drinking water for 16 weeks, it was reported that GSH and other antioxidant activities decreased, and lipid peroxidation increased (20). CAT, SOD and GSH are among important antioxidants. A decrease in their

level indicates the formation of free radicals (21). It was found that antioxidant values are decreased in people living in endemic areas and in studies on experimental fluorine toxicity (22,23). In our study, we also found that the NaF application reduced the values of CAT, SOD and GSH, which are antioxidants. It was assumed that this was caused by oxidative stress caused by flora-related intoxication. It was believed that CAT, SOD and GSH enzymes decreased due to their use in the defense system against cellular damage by free radicals. It was reported in previous studies that chitosan application prevented oxidative damage by strengthening the antioxidant defense mechanism (24). Chitosan has antioxidant properties by affecting free radicals (25). It was found that chitosan application had a protective effect against oxidative stress in rats (26). In this study, it was observed that chitosan application affected the oxidative stress caused by fluorine. It was found that the application of fluorine alone reduced the CAT, SOD and GSH values, but the application of chitosan together with fluorine increased these antioxidant enzymes. It is thought that chitosan protects lung tissue by preventing oxidative stress.

When we looked at the MDA values, it was found to be quite high in the fluorine-applied group. It is known that MDA is a lipid peroxidation marker, it causes intracellular ion imbalance, and disruption of enzyme activities and leads to changes in the structure of DNA (27,28). In a study conducted on rabbits, it was reported that fluorine poisoning caused kidney damage, a decrease in the levels of SOD, GSH-Px, CAT, GSH-Rd, and an increase in the level of MDA (29). In another study conducted on rats, it was shown that fluorosis caused a fairly significant increase in the level of MDA (30). In this study, we found that fluorine application caused an increase in the level of MDA. We found that the application of chitosan reduced the MDA level, which increased with fluorine. In a study conducted on mice, it was found that chitosan oligosaccharide and its derivatives had a protective effect against liver damage caused by carbon tetrachloride. It was stated that the antioxidant ability of the organ was regained with chitosan (31). It is believed that the application of chitosan suppresses lipid peroxidation, thereby reducing MDA values.

CONCLUSION

It was found that experimental fluorine toxicity in rats reduced antioxidant enzyme values, increased MDA value, chitosan application regulated these values and may reduce cellular damage caused.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was conducted with the approval of Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee (Date: 31/03/2022, Decision No: 2022/03-08).

Referee Evaluation Process: Externally peer reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Author Contributions: The author has participated in the design, execution, and analysis of the paper, and approved the final version.

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