Deneysel Florozis Oluştulmuş Ratlarda Kitosan ve Kitosan Oligosakkaridinin Serum ve Doku Sıyalık Asit Düzeyleri Üzerindeki Etkileri

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ÖZET: Bu çalışmada deneyesel florozisli ratlarda, kitosan (CH) ve kitosan oligosakkaritin (COS), serum ve dokularda (karaciğer, böbrek, beyin ve testis) toplam sıyalık asit (TSA) düzeyine etkisi araştırılmıştır. Gruplar kontrol, sodyum florür (NaF), NaF + kitosan (NaF + CH), NaF + kitosan oligosakkarit (NaF + COS), kitosan (CH) ve kitosan oligosakkarit (COS) olarak oluşturuldu. NaF gruplarının içme suyu 100 ppm sodyum florür kontrasyonuyla hazırlandı. Deney gruplarında, doksan gün süren kitosan ve kitosan oligosakkarit 250 mg/kg dozunda oral yolla uygulandı. Çalışma sonunda serum ve karaciğer, beyin ve testis dokusu homojenizatlarının TSA düzeyi spektrofotometrik yöntemle belirlendi. Florür uygulanan grupta (NaF), kontrol grubu ile karşılaştırıldığında, serum, karaciğer, böbrek, beyin ve testis dokularında TSA düzeyin arttığı görüldü (p<0.05). NaF grubuna göre, NaF+CH grubunda serum seviyelerinde, NaF+COS grubunda ise serum, karaciğer ve böbrek dokularında belirgin düşüş vardır. Beyin dokusu sıyalık asit düzeyi açısından kontrol ve deney grupları arasında fark olmadığı belirlendi (p>0.05). Sonuç olarak, flor tokskısyonunun, serum ve dokularda hücre hasarına neden olarak, TSA düzeylerinde artışa neden olduğu düşünülebilir. Sunulan çalışmada, CH ve COS'un TSA seviyelerini düşürdüğü gösterilmiştir. Ayrıca bu çalışmada, COS'un TSA seviyesini azaltmada daha etkili olduğu görüldü.

Anahtar Kelimeler: Florozis, Sodyum florüd, Total sıyalık asit, Serum, Doku

Effects of Chitosan and Chitosan Oligosaccharide on Serum and Tissue Sialic Acid Levels in Experimental Fluorosis

ABSTRACT: In this study, the effect of chitosan (CH) and chitosan oligosaccharide (COS) on serum and tissue (liver, kidney, brain and testis) total sialic acid (TSA) level was investigated in rats with experimental fluorosis. The groups were formed as control, sodium fluoride (NaF), NaF+chitosan (NaF+CH), NaF+chitosan oligosaccharide (NaF+COS), chitosan (CH) and chitosan oligosaccharide (COS). Drinking water of NaF groups was prepared at a concentration of 100 ppm sodium fluoride. Chitosan and chitosan oligosaccharide were given to Experimental groups as 250 mg/kg dose by gastric gavage for ninety days. At the end of the study, TSA level was determined in serum, liver, kidney, brain and testicular tissues. Compared with the control group, it was found that TSA levels increased in serum, liver, kidney, brain and testis tissues in the group treated with sodium fluoride (p<0.05). According to the NaF group, there was a significant decrease in serum levels in the NaF+CH group and in the serum, liver and kidney tissues in the NaF+COS group. It was determined that there was no difference between the control and experimental groups in terms of brain tissue sialic acid level (p>0.05). In conclusion, it can be thought that fluoride intoxication causes an increase in TSA levels by causing cell damage in serum and tissues. In the study presented, CH and COS have been shown to reduce TSA levels. Also, in this study, COS was found to be more effective in reducing the TSA level.

Keywords: Fluorosis, Sodium fluoride, Total sialic acid, Serum, Tissue

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INTRODUCTION

Fluoride is one of the potent anions exposed both orally and by inhalation. Any form of exposure to excess fluoride causes fluorine toxicity (Yüksek et al., 2017). This is called fluorosis. In the damage caused by fluorosis, fluorine is known to increase the production of free radicals, causing changes in antioxidant enzyme activities and lipid peroxidation (Varol and Varol, 2010; Yur et al., 2013; Kurtdede et al., 2018).

Chitosan (CH) is obtained by deacetylation of chitin, a natural polymer commonly found in the exoskeletal structure of crustaceans and insects and in the cell walls of some fungi. Because of its many biological activities, the chitosan oligosaccharide (COS) derivative, which has a higher solubility and low molecular weight form of oligosaccharide, has been developed. Due to its widespread nature and non-toxic nature, interest to antioxidant activity of chitosan and its derivatives has increased in recent years. Chitosan and its derivatives show their antioxidant activities depending on the active hydroxyl and amino groups in the polymer chains (Yildiz and Yangilar, 2014). Besides the beneficial effects of CH and COS on immunity and performance, it also has antimicrobial, antioxidant, anticancer, antidiabetic effects, and lipid and cholesterol lowering effects (Keser and Bilal, 2010; Toz and Değer, 2018).

Sialic acids, acetylated derivatives of nine carbon sugar neuraminic acids, are structural components of the soluble and insoluble components of tissues and cells. In serum and body fluids, sialic acids are bound to proteins (protein-bound sialic acid (PSA)) and lipid molecules (lipid-bound sialic acid (LSA)). PSA and LSA fractions is form total sialic acid (TSA) (Kazezoğlu et al., 2009). Increasing or decreasing sialic acid amounts or changing the properties of sialic acids reveals damage to cells or tissues. Studies have indicated that sialic acid levels, which are indicators of the acute-phase reaction, increase in many diseases such as cancer, diabetes, cardiovascular diseases, bacterial infections, rheumatoid arthritis and chronic liver diseases (Özcelik et al., 2014).

In this study, we aimed to investigate the effect of chitosan and chitosan oligosaccharide in serum and tissue (liver, kidney, brain and testis) TSA level in rats with experimental fluorosis.

MATERIALS AND METHODS

In the study, 42 male Wistar albino rats, 2-3 months old, weighing 200-250 g, were obtained from the Animal Experiment Unit of Van Yuzuncu Yil University. Rats were kept at 22 ± 2 °C, 12 hours dark, 12 hours bright cycle and cages where feed and water were given as ad libitum. For this study, the approval of the Animal Experiments Local Ethics Committee of Van Yuzuncu Yil University was received with the decision number 2020/2 dated 27.02.2020. The animals were divided into six groups in 7 rats each.

Control group (K); drinking water was given, fluorine group (NaF); As drinking water, water containing 100 ppm sodium fluoride was given (Guan et al., 1998), fluorine and chitosan group (NaF+CH); As drinking water, it was given with 100 ppm sodium fluoride water and 250 mg/kg/day chitosan gastric gavage (Ito et al., 2000), fluorine and chitosan oligosaccharide group (NaF+COS); As drinking water, water containing 100 ppm sodium fluoride and 250 mg/kg/day chitosan oligosaccharide were given by gastric gavage (Zong et al., 2012), chitosan group (CH); 250 mg/kg/day chitosan were given by gastric gavage and chitosan oligosaccharide group (COS); 250 mg/kg/day chitosan oligosaccharide was given by gastric gavage.

At the end of the 90-day trial period, rats (75 mg/kg) were anesthetized with ketamine and blood samples were taken into the anticoagulant tubes by cannulating the heart. The liver, kidney, brain and
testicular tissues of rats killed by anemic release were collected quickly and carefully and washed with saline for cleaning.

Blood samples were centrifuged at 3500 rpm for 10 minutes and serum was obtained. Tissues were homogenized with 1/10 cold phosphate buffer (pH: 7.4 0.1 M) using a homogenizer. Supernatants were obtained by centrifuging tissue homogenates 20 minutes at 4000xg and +4 °C.

The method developed by Sydow was used to determine TSA levels in serum and supernatants (Sydow, 1985). Sialic acid level was calculated using the standard graph prepared from N-acetyl neuraminic acid at different concentrations prepared using phosphate buffer (pH 7.4, 0.1 M).

**Statistical analysis:**

In the study, one-way analysis of variance (ANOVA) was used to compare group averages in terms of features (variables), and Duncan test was used to determine different groups after variance analysis. Statistical significance level was taken as P<0.05 in calculations. All analyzes were done using SPSS (20.0) package program.

**RESULTS AND DISCUSSION**

| Groups   | Serum (mg/mL) | Liver (mg/gr) | Kidney (mg/gr) | Brain (mg/gr) | Testis (mg/gr) |
|----------|---------------|---------------|----------------|---------------|----------------|
| K        | 2.68±0.01d    | 2.46±0.04c    | 2.25±0.04c     | 0.31±0.01b    | 2.15±0.03b     |
| NaF      | 4.01±0.02a    | 3.66±0.10a    | 3.33±0.09a     | 0.33±0.01a    | 2.66±0.10a     |
| NaF+CH   | 3.69±0.04b    | 3.61±0.16c    | 3.28±0.14a     | 0.31±0.02ab   | 2.53±0.19a     |
| NaF+COS  | 3.28±0.47c    | 3.38±0.09b    | 3.07±0.08b     | 0.31±0.03ab   | 2.52±0.30a     |
| CH       | 2.63±0.04d    | 2.45±0.07c    | 2.22±0.07c     | 0.31±0.01b    | 2.16±0.10b     |
| COS      | 2.59±0.11d    | 2.42±0.14c    | 2.20±0.13c     | 0.30±0.02b    | 2.11±0.13b     |

Values are expressed as Median ± SD (n = 7), different letters in the same column show statistical significance (P<0.05).

The fluorine poisoning can be seen in two forms as acute and chronic fluorosis depending on fluorine intake. Acute fluorosis is formed in a short time and as a result of very high fluoride uptake. High doses and long-term intake of fluorine causes chronic fluorosis (Dobaradaran et al., 2008). Chronic fluorine toxicity is mainly observed in dental and bone tissues. However, in many experimental and epidemiological studies conducted in recent years, metabolic, functional and structural damages caused by chronic fluorosis in soft tissues and organs including kidney, liver, muscle, testicle and nervous tissue have been reported (Kaya et al., 2015; Wei et al., 2019).

Excessive intake of fluorine accelerates oxygen metabolism, causing more 'O₂ to be produced. O₂ does not directly harm, but it is harmful for living creatures as it is a source of H₂O₂. H₂O₂ causes lipid peroxidation in membrane lipids, and also causes enzyme inactivation and DNA damage (Koçak et al., 2020a). In fluoride-induced toxicity, oxidative stress causes greater damage to cells and tissues (Kurtdede et al., 2018).

Chitosan and its derivatives prevent lipid peroxidation, prevent disruption of the cell membrane structure and strengthen the body's antioxidant defense mechanism. It occurs by various mechanisms such as antioxidant activity, radical scavenging activity, chelating ability and preventing radical formation. Regardless of the mechanism, antioxidant capacity is closely related to the reactivity of the active hydroxyl and amino groups in the polymer chains (Yıldız and Yangilar, 2014; Toz and Değer, 2018).

It has been reported that by preventing lipid peroxidation of chitosan and chitosan derivatives in diabetes (Yuan et al., 2009), gastric ulcer (Anandan et al., 2004) and hepatic toxicity (Jeon et al., 2003;
Ozcelik et al., 2014; Ramasamy et al., 2014; Subhapradha et al., 2014), it prevents disruption of the structure of the cell membrane and strengthens the body's antioxidant defense mechanism. In addition, it has been reported that chitosan and its water-soluble derivatives are effective in preventing oxidative damage against lead (Wang et al., 2016; Toz and Değer, 2018) and zinc (Ma et al., 2014) toxicity.

Sialic acids are an important component of the terminal oligosaccharide chains of glycolipids and glycoproteins in serum and tissues. Increased sialic acid level in the tissue may be related with lipid peroxidation that occurs in the cell membrane after oxidative stress and DNA damage (Ozcelik et al., 2014; Oto et al., 2016). There is a positive correlation between serum SA levels and cellular damage. SA is an important biomarker of the diagnosis of inflammation, myocardial infarction, cancer and some other diseases. The sialic acid forms in diseases is the as a result of lipid peroxidation formed by free radicals and destruction of cell membranes (Oto et al., 2016).

Sialic acid is an important parameter for the diagnosis of fluorosis in both humans and animals (Kaya et al., 2015). Oto et al. (2016), in the study in which they investigated the effect of resveratrol on serum TSA and LSA in experimental fluorosis, determination that TSA and LSA levels were significantly increased in the fluorosis group and resveratrol was not effective in decreasing this increase. In another study, serum LSA level was increased in sheep with fluorosis (Doğan et al., 2016). In our study, the serum, liver, kidney, brain and testicular tissue TSA levels in the NaF group were found to be statistically (p<0.05) higher than the control group, and it is consistent with previous studies (Tablo 1). The increase in TSA levels may be due to the release of sialic acids in the membrane of cells damaged by oxidative stress developing due to fluorosis (Ozcelik et al., 2014; Koçak et al., 2020b). There are studies indicating that serum TSA (Jha et al., 1982; Ciftci et al., 2010; Doğan et al., 2016), PSA (Sharma, 1983) and testicular TSA (Kaya et al., 2015) levels decrease in fluorosis patients.

In the literature searches, we did not find any study investigating the effect of chitosan and chitosan derivatives on serum and tissue sialic acid levels in fluorosis. In the study investigating the effect of chitosan against liver toxicity created by acetaminophen in the liver, it was reported that increased serum and liver tissue TSA and LSA levels decreased significantly with the administration of chitosan. It has been stated that the decrease in sialic acid levels in serum and liver may be related with the antioxidant properties of chitosan (Ozcelik et al., 2014).

In our study, compared to the NaF group, it was determined that the serum TSA level decreased significantly (p<0.05) in the NaF+CH and NaF+COS groups, and this decrease was higher in the COS group, but was higher than the control group (Tablo 1). It was determined that there was no difference between the control and experimental groups in terms of brain tissue sialic acid level (p>0.05).

The significant or non-significant decreases in serum and tissue TSA levels in NaF+CH and NaF+COS groups may have resulted from CH and COS inhibiting lipid peroxidation by enhancing the endogenous antioxidant defense system. It was determined that COS has more efficacy on serum and tissue total sialic acid level compared to CH in fluorosis (Sun et al., 2008).

CONCLUSION

As a result, it can be said that fluorosis causes cell damage, which leads to an increase in TSA levels in serum and tissue. It can be said that CH and COS reduce the TSA level increasing after fluoride toxicity and COS is more effective. Further studies can be made on the ability of CH and COS to reduce TSA levels.
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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author’s Contributions

The authors declare that they have contributed equally to the article.

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