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THE EFFECT OF RESVERATROL ON SERUM PROTEIN FRACTIONS IN RATS EXPOSED TO EXPERIMENTAL CHRONIC FLUOROSIS

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ABSTRACT: Chronic fluorosis results from long-term fluoride intake at more than the normal doses. The aim of this study was to demonstrate the effects of resveratrol (Res) on the serum protein fractions in rats, in which experimental chronic fluorosis was induced. After an adaptation period, the rats were randomly divided into 4 groups of 10; namely the (i) control, (ii) sodium fluoride (NaF), (iii) Res, and (iv) NaF+Res groups. Serum protein fractions in the rat blood samples were determined by cellulose-acetate electrophoresis. While the NaF group had statistically reduced concentrations of total protein, albumin, and α-1 and α-2 globulin compared to the control group ($p<0.05$), these values were significantly increased ($p<0.05$) in the NaF+Res group, compared to the NaF group, and close to those of the control group. The β- and γ-globulin concentrations were the lowest in the NaF group statistically ($p<0.05$). Despite a significant increase ($p<0.05$) in these values in the NaF+Res group, compared to the NaF group, they were still lower compared to the control group. The examination of the percentage of serum protein fractions revealed a reduced albumin in the NaF group compared to the control group but the finding was not statistically significant ($p>0.05$). The albumin of the NaF+Res group was statistically higher than that of the control group ($p<0.05$). No statistical differences were observed in α-1 and α-2 globulin across the groups. The β-globulin of the NaF group was the highest but not statistically higher than that of the control group. The γ-globulin percentages in all the groups were found to be lower than the levels in the control group. The albumin/globulin (A/G) ratio decreased in the NaF group but was not significantly different than that of the control group. In conclusion, the alterations in the serum protein fractions due to NaF-induced toxicity, especially the alterations in their concentrations, approached values closer to those of the control group with the administration of resveratrol. We concluded that these results are of potential importance in indicating a favorable role for resveratrol use in preventing and treating fluoride toxicity.

Keywords: Fluorosis; NaF; Resveratrol; Serum proteins.

INTRODUCTION

Chronic fluorosis results from long-term fluoride ingestion at more than the normal doses. In chronic fluorosis, the natural exposure to fluoride via the soil, water, and plants should be taken into account, as well as the fluoride exposure resulting from certain industrial actions.[1-2]

It is reported that endemic fluorosis most commonly occurs in volcanic regions because the high temperature in volcanic regions increases the fluoride concentrations and acid rains containing 5,000–6,000 ppm fluoride may occur in these regions.[3]

Excessive fluoride levels can lead to various disorders such as dental and skeletal fluorosis, endocrine system disorders, reproductive decline, immunopathies,

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decreased intelligence quotient (IQ) scores, and hypertension. Several studies have investigated fluoride in relation to health and the environment. Fluoride intake by humans occurs most commonly via food and water since the air usually contains very low levels of fluoride.[4-6]

Resveratrol (Res) is a polyphenolic phytoalexin with a flavonoid structure; which is synthesized by plants against traumatic injury or fungal attacks. Phytoalexin is an antibiotic which is produced by plants in response to external stress or pathogenic (fungal) attacks. As a major element of a molecular family of glycosides and polymers, resveratrol is abundant in red wine and most significantly in grapes, as well as in some vegetables and other fruits including peanuts, raspberries, plums, mulberries, and blackberries.[7-8] Recent studies have shown that resveratrol has many beneficial effects on the organism via its antioxidant properties. In a variety of organisms ranging from yeast to vertebrates it has been shown that resveratrol increases the lifespan and prevents or slows down the development of several diseases, including cancer and cardiovascular diseases, with its antihyperlipidemic, anti-inflammatory, antiaggregant, antioxidant, and estrogen agonist or antagonist properties.[9-12]

Serum albumin is important in regulating blood volume by maintaining the oncotic pressure of the blood compartment. It also serves as a carrier for molecules of low water solubility and in this way isolating their hydrophobic nature, including lipid-soluble hormones, bile salts, unconjugated bilirubin, free fatty acids, calcium, ions, and some drugs. Globulins are a heterogeneous group of proteins that includes antibodies and other inflammatory molecules, haemostatic and fibrinolytic proteins, and carriers of lipids, vitamins, and hormones.[13-14]

The aim of the study was to examine the effects of resveratrol on the serum protein fractions in rats subjected to experimental chronic fluorosis.

MATERIALS AND METHODS

Experiment animals: All experiments were performed on 40 adult male Sprague-Dawley rats weighing 200–250 g from the animal experiment center of Van Yuzuncu Yil University. The serum samples used as the experimental material were obtained from a previous study.[15] This study was approved by Van Yuzuncu Yil University Experimental Animals Local Ethics Committee (YUHADYEK 28.01.2016/ Decision no: 2016/01).[15]

Experimental groups: After an adaptation period, the rats were randomly divided into 4 groups of 10. The rats were administered sodium fluoride and resveratrol in drinking water for 12 weeks at the doses presented in Table 1.

Table 1. Drinking water given, for 12 weeks, to the experimental groups (NaF=sodium fluoride, Res=resveratrol)

Experimental group	Drinking water given for 12 weeks
Control	Normal drinking water
NaF	Drinking water with 10 mg/L NaF
Res	Drinking water with 50 mg/L Res
NaF + Res	Drinking water with 10 mg/L NaF+50 mg/L Res

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Sample collection: At the end of the experiment, the blood samples were collected from the hearts of the rats under 90 mg/kg ketamine anesthesia by using the proper technique. Blood samples were placed into gel biochemistry tubes. After waiting for 30 minutes, they were centrifuged at 3,000 rpm to separate the serum. Serum samples were stored at –80ºC. All samples were analyzed simultaneously.

Serum fluoride analysis: While measuring the fluoride ion activity, a total ionic strength adjustment buffer (TISAB) was used in order to keep the total ionic strength at a constant level, to adjust the pH, and to disintegrate any complexes of fluoride and metal cations such as aluminum, iron, and magnesium. A volume of 1 mL of the obtained serum was transferred into polyethylene tubes and 1 mL of TISAB II solution was added. Firstly, a combination pH electrode and a combination ion-selective electrode were plunged into the mixture to check whether the pH was between 5.0 and 5.5. The measurement was completed when the reading was stable. The values were read in ppm units by using a fluoride electrode.[16]

Total protein analysis: Total protein concentrations were analyzed using the biuret method.[17]

Serum protein electrophoresis: Serum proteins fractions were separated by using a Helena Lab-Titan III® serum protein electrophoresis device (Cat. No. 3023), Helena Lab-Titan III cellulose acetate cards, and Electra HR buffer (Cat. No. 5805) solutions (Helena, Bioscience). The samples were stained with the Ponceau S stain solution. The Platinum 3.0 software was used for determining the protein concentrations of the serum protein fractions in the bands obtained by electrophoresis[17].

Statistical analysis: The level of statistical significance was accepted as 5%. Data were analyzed using the SPSS package program (version 22). ANOVA was used for group comparisons.

RESULTS

Serum fluoride analysis: The results of the fluoride analysis in the serum samples taken from the rats are shown in Table 2.

Table 2. The levels of fluoride (ppm) in the serum of the rats. (NaF = sodium fluoride, Res = resveratrol, SD= standard deviation, Min = minimum, Max = maximum)

Group	Serum fluoride (ppm)		
	Mean±SD	Min	Max
Control	0.0080±0.0009[a]	0.007	0.009
NaF	0.0127±0.0010[b]	0.011	0.014
Res	0.0083±0.0008[a]	0.007	0.009
Res+NaF	0.0108±0.0010[c]	0.009	0.0013

[a,b,c]: At the same measurement time, the difference between the group averages of groups with different lower case letters is significant ($p<0.05$).

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The fluoride levels of the NaF group were significantly higher than those of the control group ($p<0.05$). Only the serum fluoride levels of the Res group were similar to those of the control group ($p>0.05$). The serum fluoride levels of the NaF+Res group was lower compared to the levels found in the NaF group and this difference was significant ($p<0.05$).

Table 3 presents the serum protein concentrations and Table 4 presents the percentages of the serum protein concentrations in the total serum proteins of the rats in which chronic fluorosis was induced experimentally.

Table 3. Concentrations of total serum protein and serum protein fractions (g/dL) in the rats. (NaF=sodium fluoride, Res=resveratrol)

Group	Total protein (g/dL)	Albumin (g/dL)	α1-globulin (g/dL)	α2-globulin (g/dL)	β-globulin (g/dL)	γ-globulin (g/dL)
Control	10.557 ±1.509^{c}	3.100 ±0.739^{b}	1.890 ±0.425^{bc}	1.642 ±0.450^{b}	2.831 ±0.653^{c}	1.415 ±0.3611^{c}
NaF	1.509 ±0.927^{a}	0.450 ±0.139^{a}	0.294 ±0.140^{a}	0.361 ±0.254^{a}	0.668 ±0.443^{a}	0.2071 ±0.079^{a}
Res	12.117 ±2.849^{c}	4.147 ±0.991^{c}	2.077 ±0.399^{c}	2.035 ±0.781^{bc}	2.567 ±0.688^{c}	1.291 ±0.294^{c}
NaF+Res	8.232 ±1.133^{b}	3.187 ±0.507^{b}	1.525 ±0.470^{b}	1.147 ±0.625^{b}	1.740 ±0.283^{b}	0.630 ±0.630^{b}

^{abc}At the same measurement time, the difference between the group averages of groups with different lower case letters is significant ($p<0.05$).

Table 4. Percentage of serum protein fractions (%) and the albumin globulin ratio in the rats. (NaF=sodium fluoride, Res=resveratrol, A/G=albumin gobulin ratio)

Group	Albumin (%)	α1-globulin (%)	α2-globulin (%)	β-globulin (%)	γ-globulin (%)	A/G
Control	29.590 ±5.821^{ab}	17.828 ±2.550	15.512 ±2.935	26.608 ±3.215^{ab}	13.4071 ±2.579^{c}	0.4271 ±0.117^{a}
NaF	25.215 ±9.887^{a}	14.972 ±14.972	17.580 ±6.009	31.457 ±7.479^{b}	10.760 ±2.547^{b}	0.360 ±0.208^{a}
Res	34.421 ±5.01528^{b}	17.367 ±1.658	16.301 ±3.101	21.218 ±21.218^{a}	10.692 ±0.880^{b}	0.5329 ±0.124^{ab}
NaF+Res	38.862 ±5.116^{bc}	18.397 ±3.763	13.605 ±5.901	21.491 ±4.390^{a}	7.644 ±2.304^{a}	0.645 ±0.135^{b}

^{abc}At the same measurement time, the difference between the group averages of groups with different lower case letters is significant ($p<0.05$).

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While the NaF group had statistically significant ($p<0.05$) reductions in the concentrations of total protein, albumin, and α1- and α2- globulin compared to the control group, the corresponding values in the NaF+Res group were significantly increased ($p<0.05$), compared to the NaF group, and close to those of the control group. The β- and γ-globulin concentrations were significantly lower ($p<0.05$) in the NaF group compared to the other groups. Despite a significant increase ($p<0.05$) in the β- and γ-globulin concentrations in the NaF+Res group, compared to the NaF group, these values were still significantly lower ($p<0.05$) than those of the control group.

The examination of the percentage of serum protein fractions revealed a reduced albumin in the NaF group, compared to the control group, but this finding was not statistically significant ($p>0.05$). The albumin of the NaF+Res group was statistically higher than that of the control group ($p<0.05$). No statistical differences were observed in α1- and α2-globulin across the groups. The β-globulin of the NaF group was the highest but not statistically higher than that of the control group. The γ-globulin percentages in the NaF, Res, and NaF+Res groups were found to be significantly lower ($p<0.05$) than the level in the control group. The albumen/globulin ratio (A/G) was decreased in the NaF group, compared to the control group, but not to a significant extent ($p>0.050$.

DISCUSSION

Serum proteins are among the major biochemical parameters used for diagnosing, analyzing, and investigating the pathogenesis of various diseases. Different methods are used to determine serum protein fractions. One of these methods is cellulose acetate electrophoresis. Studies show that the cellulose acetate electrophoresis method can significantly contribute to conducting assessments in veterinary clinics in a manner which is reliable, less costly, and fast.[18-19]

All organisms are exposed to fluoride compounds from various sources, primarily via water and soil with toxic fluoride levels and followed by industrial waste areas, food and beverages processed with fluoridated water, fluoridated dental products, and several types of insecticides. Comprehensive studies are available in the literature, demonstrating how fluoride can act on critical biological processes. Prolonged fluoride exposure causes toxicity, known as fluorosis, which is characterized by disorders in the physiology and architecture of teeth (dental fluorosis), bones (skeletal fluorosis), and various soft tissues and organs (non-skeletal fluorosis).[20-22]

A Turkish study by Altıntaş et al. investigated the total protein levels and serum A/G ratios of animals that lived in the Caldiran district of Van province and the Kızılcaören district of Eskisehir province, which were districts with fluorosis due to naturally occurring fluoride, and in the Yatagan district of Mugla province, which was an area with industrial fluorosis. The study did not find any significant alterations in the total protein levels of animals living in these regions with fluorosis due to natural or industrial sources of fluoride. However, the serum A/G ratio was significantly higher ($p<0.01$) in the animals from the natural fluorosis regions but not significantly changed in the animals from the regions with industrial fluorosis.[23]

A study by Cenesiz et al.[24] found statistically lower levels of total protein concentrations in Tuj sheep in which chronic fibrosis was induced experimentally

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($p<0.01$). Also, the levels of albumin were found to be lower but that finding was not statistically significant. Ciftci et al.[25]conducted a study on rabbits with fluorosis and reported that the total serum protein concentration and albumin levels were significantly reduced ($p<0.05$). Our results are consistent with these reports in the literature.

A study by Bouaziz et al. investigated serum protein values in an induced liver toxicity model with NaF administration in adult female pregnant rats 15 days after conception and in their offspring within 14 days after the delivery. The authors reported that while the total protein and albumin were significantly decreased in both the mothers and the offspring ($p<0.01$), the α1-globulin, α2-globulin, β-globulin, and γ-globulin values remained unaffected. Fluoride poisoning primarily acts on protein synthesis by disrupting polypeptide chains and by impairing the strength of the bonds between amino acids. Excess fluoride has been reported to cause decreased protein synthesis.[26]

Kessabi et al. assessed the serum biochemical parameters in blood samples of 50 animals with chronic fluorosis from a Darmous area of Morocco and reported a significant reduction in total protein and albumin levels ($p<0.05$).[27] Another study by Kumar and Aravindakshan analyzed serum biochemistry in the blood samples of cattle from Kerala, India, a region with industrial fluorosis. They reported that the total serum protein and albumin levels were significantly lower compared to controls ($p<0.01$).[28] Our results are parallel to and consistent with the results of these studies in the literature.

Shivashankara et al. investigated the total serum protein and albumin values in the blood samples of children with clinical and radiological diagnoses of dental (89%) and skeletal (39%) fluorosis from Karnataka, India. The study reported a small but significant decrease in the total serum protein and albumin levels ($p<0.01$).[29]

In a study by Deng et al., the investigators fed broiler chickens with a high-fluoride diet and conducted serum biochemistry tests in the blood samples collected on the days 14, 28, and 42. They found that the total serum protein and albumin levels were significantly decreased on the days 28 and 42 ($p<0.01$).[30]

Agha et al.[31] induced fluorosis in rats by administering NaF. The serum protein levels were then investigated after vitamin E was administered in combination with L-carnosine and methionine to the rats to alleviate the fibrosis-inducing effects. They found that the total protein and albumin values decreased in the group which received NaF, while the total protein and albumin values were found to be significantly increased in the group that was administered vitamin E in combination with L-carnosine and methionine before the administration of NaF.

In another study, Kanbur et al.[32] similarly induced fluorosis in rats by administering NaF. The investigators also administered royal jelly to prevent the NaF-induced fluorosis. They reported that the total serum protein and albumin values decreased in the blood samples of the NaF group and that the administration of royal jelly significantly increased these values ($p<0.01$).

Liang et al.[33] induced fluorosis in rabbits by administering NaF and also administered calcium and protein to prevent the NaF-induced hepatotoxicity. They reported that the total protein and albumin values decreased significantly in the group

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that received the high doses of NaF. The values of the total protein were increased in the group that received the calcium and the albumin values were increased in the group that received the protein. In our study, we found that the reduced levels of total protein and albumin with NaF toxicity increased significantly after the administration of resveratrol. Our results are consistent with these literature reports.

In the present study, the rats were exposed to high doses of fluoride daily for 90 days. The analysis of the serum fluoride levels at the end of day 90 revealed that the serum fluoride levels increased only in the groups that received NaF or NaF+Res. The serum proteins decreased significantly ($p<0.05$) due to the NaF toxicity but the serum protein levels increased with the administration of resveratrol. This shows that resveratrol can act on fluoride levels in an organism with fluorosis.

It has been reported that the exposure to high doses of fluoride increases oxidative stress. Resveratrol treatment reduces fluoride-induced oxidative stress and tissue damage. Several studies have reported that oxidative stress causes hypertension[34] and that vascular oxidative stress causes vascular stiffness.[35-36]

In a study by Qujed et al., rats received commercial cellulose powder and the rats in the treatment group were administered 10–30 mg/kg of NaF orally for 90 days. The study found an increased food intake and body weight along with a 38.71% reduction in the total serum protein values in the animals treated with NaF ($p<0.05$). Sodium fluoride treatment appears to reduce protein concentrations by inhibiting protein synthesis because fluoride prevents the binding of amino acids to proteins by inhibiting the Na^+/K^+-activated ATPase, which is essential for the tissue uptake of amino acids. When protein synthesis is inhibited or when protein degradation is promoted, total protein concentrations are likely to decrease.[37]

The total serum protein and associated serum fractions may vary due to differences in the physiology, diet, gender, living environment, and genetic factors. Malnutrition and severe protein loss result in hypoproteinemia, leading to reductions in all the bands. However, this decrease is observed most prominently in the albumin values. The levels of albumin, α1-, β- and γ-globulin decrease in protein-losing enteropathies and nephropathies. Although plasma albumin level decreases in chronic hepatocellular diseases, little or no decrease occurs in the level of plasma albumin in acute liver disease.[16]

CONCLUSIONS

This study aimed to determine the effects of resveratrol on the serum protein fractions in rats which underwent NaF-induced chronic fluorosis experimentally. We concluded that the administration of resveratrol ameliorated the NaF-induced toxicity and caused the serum protein levels to be detected at levels close to those of the control group, suggesting that this could be a significant parameter for evaluating the effects of the resveratrol use in preventing or treating fluoride toxicity. However, considering the specific proteins of a variety of altered serum protein fractions, further studies are warranted to precisely assess the role of fluoride exposure on specific protein profiles and to evaluate the role and utility of the use of protein electrophoresis in the course of fluorosis.

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