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THE EFFECT OF FLUORIDE ON THE SERUM LEVEL OF CALCIUM IN THE RAT (*RATTUS NORVEGICUS*)

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Abstract –The effect of fluoride on the calcium level in serum was analyzed in the laboratory rat *Rattus norvegicus*. The control group consisted of 10, and the experimental group of 15 animals. In the experimental group, fluoride at a concentration of 3 mg/100 g body weight of rats was intramuscularly injected into the *musculus gluteus maximus*. The concentration of calcium was measured by the CPC method. The average serum calcium concentration was 2.46 mmol/l, with female rats having higher values of serum calcium than male rats. Fluoride caused the reduction of calcium concentration in serum ($p<0.05$); the reduction was significantly expressed in female rats ($p<0.000$).

Key words: Serum calcium, sodium fluoride, laboratory rat, CPC method

INTRODUCTION

Calcium is a mineral whose daily intake should be ensured, especially after stress reactions in the body when calcium is excreted into the blood from the bones. A positive intake of calcium should be ensured for younger organisms as well, in order to facilitate the growth of bones. Calcium in the blood occurs in three forms: non-diffusible (bound to proteins), ionic and molecular (non-ionized). In research done by Mellanby et al. (1940), it was discovered that ionized calcium is very important because it provides its optimum concentration in the blood. Nordin (1976) states that this chemical element in one of the factors of coagulation, specifically the 4th clotting factor. The entry of calcium ions into the cell is the main cause of muscle contraction. The process of muscle contraction in the skeleton attracts calcium ions.

According to Guixueu (2009), calcium ions are essential for repolarization of cells. The concentration of total calcium in mammals ranges from 2.2 mmol/l to 2.6 mmol/l, while the concentration of ionized calcium is 1.1 to 1.4 mmol/l, which was determined in research by Boron et al. (2003). The concentration of total calcium depends on the amount of albumin, as well as proteins that bind to calcium ions.

Given that fluorine is not an essential nutrient, the question is what its importance to the organism is. To date, no disease associated with deficiency of fluorine has been detected. Strunecka et al. (1991) reported that fluoride acts as a poison that accumulates in organism. Approximately 50% of the fluoride that is introduced into the body daily is excreted through the kidneys, while the rest accumulates in the bones, pineal gland, teeth and other tissues in the body. Fluorine appears in several forms. The best-known forms are sodium monofluorophosphate, calcium fluoride, sodium fluoride and amine fluoride. Sodium fluoride is the most common form of fluoride. Its main function is to strengthen the bones and tooth enamel. Bones and teeth are made of calcium and phosphate crystals named calcium hydroxyapatite.

The fact is that fluorine has a high chemical reactivity and small radius of the atom, which allows the hydroxyl group of calcium hydroxyapatite to be replaced by the fluorine atoms, forming fluorapatite. Fluorapatite strengthens the tooth enamel and bones. Organic acids that originate from sugars in the oral cavity can lead to erosion of tooth enamel and demineralize it, as claimed by Jurić (2002).

Fluoride is absorbed in the duodenum by enterocytes (Allain, 1996). Once it gets into the blood it enters into the mineralized tissue (bone and teeth, especially during their development), and it does not accumulate in the soft tissues. Imanishi et al. (2009) found that fluoride, given orally, builds hydrofluoric acid with gastric acid. Fluoride interrupts oxidative phosphorylation, glycolysis, coagulation, and neurotransmission (calcium binding during signal transduction).

According to research of Julie et al. (1998), fluoride inhibits Na^+/K^+ ATP-synthetase, which can lead to hyperkalemia due to release of extracellular potassium. When bound to aluminum fluoride it prevents the proper functioning of G proteins and the mechanism of transmission pathways in the cell. Fluoride normally binds to calcium in all places where it is located, including teeth, blood, bones, ligaments, skeletal muscle, glands and brain, as demonstrated by the analysis of Luke (2001). Fluor has certain negative effects on the reproductive system of male rats, because it increases the level of sterility (Susheela et al., 1991).

Fluoride, when given in combination with tetracyclines, has a strong effect on the reduction of calcium and phosphate (Baker, 1974) However, the most important function related to the fluoride-calcium interaction is the mechanism of heart muscle contraction. Research by Kenny (1962) has shown that if there is a reduction of calcium in the blood, organs that are rich in calcium will precipitate calcium fluoride. This leads to secretion of the hormone calcitonin from the thyroid gland, which mobilizes calcium from calcified organs and leads to calcium concentration at an optimum level. The aim of this study was to determine the effect of fluoride on the concentration of serum calcium, with special emphasis on the effect of fluoride on the gender structure.

MATERIALS AND METHODS

The influence of intramuscularly applied sodium fluoride on the calcium concentration was studied in the laboratory rat *Rattus norvegicus* (Wistar strain) Berkenhout, 1769. The rats used in this study were bred on the premises of the Department of Biochemistry and Physiology, Department of Biology, Faculty of Science and Mathematics in Sarajevo. The rats were bred according to all standards for laboratory animals. The age of the rats was on average three months. There were two groups, control and experimental. The control group consisted of 10 specimens, of which five were males and five females. The experimental group consisted of 15 specimens, eight males and seven females. The average body weight of the control group was 223.50 grams, and 249.20 grams of the experimental one.

Laboratory and experimental techniques

Body weight

The body weight of rats was determined by precise digital scales to specify the amount of fluoride that should be injected into the body.

The application of fluoride

Fluoride was applied in tablet form of 2.2 mg sodium fluoride, which is equivalent to 1 mg of fluoride. Tablets of sodium fluoride, factory name NaF®, are produced by Bosnalijek Sarajevo. The chemical composition of the tablet is as follows: mannitol, cornstarch, talc, povidone, magnesium stearate, indigocarmine CI 73 015 E 132 and 2.2 mg sodium fluoride. After consultation with a pharmacist from Bosnalijek Sarajevo, it was found that minor components of the drug do not have any effect on the concentration of the electrolytes in the blood. Tablets were dissolved in water for injections. In such a prepared injection solution, the optimal concentration of NaF, depending on body weight, (3 mg per

100 g body weight or three tablets dissolved in 1 ml water for injections) was determined. After dissolving, the solution was sterilized in autoclave for 15 min at 110° C. The injection was applied in the *musculus gluteus maximus.*

Puncture of the heart

Control specimens were punctured in the heart after they had been slightly anesthetized by diethyl ether (according to IUPAC: ethoxyethane). Puncture of the heart in the experimental group was performed 30 min after the application of fluoride. The experimental group was also anesthetized by ethoxyethane.

Blood centrifugation

The obtained blood was left for about 45 min to coagulate spontaneously, and subsequently centrifuged at 3000 rpm for 10 min. Once we isolated the serum, the concentration of calcium was determined spectrophotometrically using the CPC method.

Spectrophotometric method

Calcium ions react with the o-krezolphtaleinic-complex in an alkaline medium, forming a purple-colored compound. Spectrophotometric determination of the calcium in serum was conducted by Spectronic 20 Genesys. Absorbance was determined at a 570 nm. Samples of 40 µl of serum were pipetted into tubes previously filled with 2000 µl of reagens (BUF + RTG, produced by Human®). The tubes were incubated for 30 min at room temperature in order to develop a purple-colored compound.

Statistical methods

Descriptive and analytical statistics were used during the analysis of the research findings. Data analysis and processing were conducted using the statistical program Microsoft Office-Microsoft Excel 2010. The arithmetic mean, standard deviation, coefficient of variation, minimum value, maximum value and the student t-test were used as mathematical statistical indicators.

RESULTS

Measurement and results of body weight of all tested animals are shown in Table 1.

Table 1. Values of body weight in control and experimental groups of rats

	Body weight (grams)	
	Control group	Experimental group
Arithmetic mean	223.50	249.20
Standard deviation	51.30	31.77
Minimum value	155.00	206.00
Maximum value	304.00	315.00
Coefficient of variation	23.01	12.74

By observing the obtained values of body mass, it can be concluded that the individuals in the experimental group, although both groups were the same age, had a slightly greater weight than the control group. This can be explained by the fact that some specimens differed in regards to body weight, and that the majority of analyzed specimens had fairly balanced values, which is demonstrated through the variation coefficient. Table 2 shows the values of calcium in the control and experimental groups.

Table 2. Serum calcium in control and experimental groups

	Values of calcium (mmol/l)	
	Control group	Experimental group
Arithmetic mean	2.46	1.86
Standard deviation	0.32	0.31
Minimum value	1.87	1.00
Maximum value	2.91	2.19
Coefficient of variation	12.68	1.24

By observing the obtained values of serum calcium in both analyzed groups, it was apparent that there was a pronounced decrease of calcium concentration in the experimental group. It is very important to note that the values of serum calcium showed individual differences in the control group. However, the low variation coefficient of the experi-

Table 3. Values of serum calcium of male and female rats

	Calcium (mmol/l)			
	Control group		Experimental group	
	♂♂	♀♀	♂♂	♀♀
Arithmetic mean	2.31	2.62	1.91	1.77
Standard deviation	0.32	0.23	0.37	0.21
Minimum value	2.82	2.91	2.19	1.91
Maximum value	1.87	2.35	1.00	1.29
Coefficient of variation	14.05	8.96	19.80	11.82

mental group indicates that this decrease in serum calcium concentration was equally present in all analyzed specimens without pronounced individual variations. Table 3 shows the value of serum calcium based on gender.

By analyzing the concentration of serum calcium based on the gender structure, the greater value of concentration in the serum of female rats was evident, but still more balanced than the calcium concentration in males. On the other hand, the values of serum calcium in the experimental group were higher in males; however, there was a pronounced reduction of these values in both sexes, with the lowering of serum calcium concentration slightly increased in the serum of females.

DISCUSSION

By comparing the obtained values of serum calcium in rats, it is evident that intramuscularly applied sodium fluoride (3 mg/100 g of body weight) leads to a decrease in the concentration of calcium in the rat serum after 30 min of application. These studies are consistent with the research of Kenny (1962). When observing the concentrations of calcium depending on gender, lower values in males compared to females could be observed, while the applied NaF^* led to a decrease in the concentration of serum calcium in both sexes. However, it was evident that NaF^* had a stronger effect on the reduction of serum calcium in females than in males.

After the statistical analysis and comparison of the results of measurements, it could be concluded that the NaF^* applied intramuscularly led to a statistically significant reduction in serum calcium concentration in the treated group. By studying the available literature, it was possible to note that all the literature data indicate that fluoride leads to a reduction in serum calcium.

The reason for the decline in the value of calcium in the blood is the following: the element fluorine is electronegative and acts as an antagonist of calcium. The calcium in the blood is transported to the intercellular fluid after which it is deposited in bones and teeth. Although there is a decrease in serum calcium concentration, the total amount of calcium in the body remains the same, because of its deposition in the bones and teeth. The reason for this is the transport of calcium from the blood into the tissues. In the research of Gibbs et al. (1995), it was shown that calcium from the teeth (where it is in the form of calcium hydroxyapatite) is transported to the blood.

Based on the research of Whitford (1991) and Baker (1974), it was established that in these cases the concentration of calcium returns to the previous value after approximately four hours of fluoride application. The reason for this is that Ca^{2+} ions are essential for the process of blood coagulation and hemostasis, so calcium from bones and teeth is released back into the blood in order to maintain optimal hemostasis. This rapid calcium homeostasis is understandable because of its multiple importances for blood.

The reasons can also be interpreted in the light of the different metabolic pathways of calcium between males and females.

Following the statistical analysis and the comparison of the results between the male control and experimental groups, the value of the t-test was on the verge of statistical significance. However, a high statistical significance (0.000) was evident in the females, suggesting that among them the level of the calcium concentration decline was higher than that in males.

CONCLUSION

After a precise statistical analysis of the obtained data and results, the following general conclusions can be derived: (i) the calcium levels in the serum of female rats is higher than in the males; (ii) intramuscularly injected NaF^{*} leads to a reduction of calcium concentration in the serum of laboratory rats; (iii) a decrease of serum calcium was found in both male and female animals, but the larger reduction occurs in female rats.

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