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FEATURES OF THE MINERALIZING EFFICACY OF WATER-SOLUBLE ANTISTRESSANTS IN BONE TISSUE OF MALE AND FEMALE RATS AFTER STRESS

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Abstract

Stress ends with the development of dysmetabolic syndrome, which leads to disruption of mineralization processes in bone tissue.

Aim. To investigate the effect of anti-stress drugs on the mineralizing activity of bone tissue in rats after stress, taking into account the sexual characteristics of the mineralizing index.

Methods. Water-soluble preparations of vitamins P were chosen as anti-stressants: “EkSoVit” (soy extract containing isoflavones and protease inhibitors), quercetin (dietary supplement “Anti-stressant – Vitamins P (quercetin)” and for comparison – a preparation of ascorbic acid (vitamin C). Stress was induced in rats by keeping them at −20 °C for 30 minutes. Antistressants in the form of oral gels were administered 3 days before stress and 3 days after stress. The condition of the tissue (femur) was examined on the 4th day after stress.

The activity of alkaline phosphatase (ALP), acid phosphatase (AP) was determined, and the mineralization index (MI) and mineralization efficiency (ME) were calculated from the ALP/AP ratio.

Results. Mineralizing activity (MA) was the highest in the preparation "Quercetin", and the lowest − in ascorbic acid. Mineralizing efficiency was the highest in the preparation "Quercetin", and the lowest − in ascorbic acid. Indicators of mineralizing efficiency were higher in males.

Conclusion. Stress reduces the mineralization index in bone tissue. Antistressants stimulate mineralization processes by increasing the activity of alkaline phosphatase and reducing the level of acid phosphatase. In terms of mineralization efficiency, the most active drug was “Antistressant – Vitamins P (quercetin)”, and the efficiency of all three antistressants was 5-9 times higher in males.

Keywords: stress; bone tissue; mineralizing activity; anti-stressants; gender specificity.

Recently, the problem of metabolic disorders in bone tissue, which lead to the development of osteoporosis and an increased risk of bone fractures, has become one of the most urgent problems of modern medicine [1-3]. As is known, the structure of bone tissue in humans and animals consists of 4 types of cells: osteocytes, osteoblasts, osteoclasts and accessory cells, which together form an osteocellular structural unit [4, 5]. The main function of osteoblasts is the formation of a protein base and a mineral substance (hydroxyapatite), while osteoclasts carry out osteolysis. Among the numerous markers of osteoblasts and osteoclasts [6, 7], the most specific biochemical markers of osteoblasts are alkaline phosphatase (ALP), and for osteoclasts - acid phosphatase (AP) [8]. We proposed to use the ALP/AP ratio to assess the mineralizing activity of bone tissue [9]. The ALP/AP ratio, which was called the mineralizing index (MI), increases with the age of the animals and is very dependent on sex.

Among the causes of impaired bone mineralization are inadequate nutrition (deficiency of Ca, Zn, vitamin D) [11, 12], decreased levels of sex hormones [8, 13, 14], as well as dysmetabolic disorders that occur after stress [15, 16].

The aim of our work was to determine the effect of anti-stress drugs on the mineralization activity of bone tissue in rats after stress, taking into account the gender characteristics of the mineralization index.

Material and methods. Three drugs were used as anti-stressants: dietary supplement «EkSoVit» (TC U 10.8-37420386-010:2025), manufactured by «Biohimtech» LLC (Odesa, Ukraine); dietary supplement «Anti-stressants − Vitamin P (quercetin)» (TC U 10.8-37420386-011:2025), manufactured by «Biohimtech» LLC (Odesa, Ukraine) and ascorbic acid (pharm), manufactured in China. Antistressants preparations were used in the form of mucosal gels containing 3 % sodium carboxymethylcellulose and 1 % sodium benzoate. Gels with antistressants were applied to the oral cavity of rats in an amount of 0.5 ml per rat. The rats of the experimental group "Stress without antistressants" received the base gel (without antistressants).

The experiments were conducted on 29 white Wistar rats (males, 3-4 months, 210-225 g) and 25 white Wistar rats (females, 4-5 months, 260-280 g), divided into 5 groups. Group I consisted of intact rats (6 males and 5 females); Group II consisted of rats (5 males and 5 females) that had undergone stress, these rats received oral applications of the base gel (without anti-stressants); Group III of stressed rats (6 males and 5 females) received oral applications of the gels containing the anti-stressant "EkSoVit" 3 days before stress and 3 days after stress; Group IV (6 males and 5 females) received oral applications of gel containing the drug «Antistressant − Vitamin P (quercetin)» in the same way as group III; group V (6 males and 5 females) received oral applications of gel containing ascorbic acid (vitamin C) in the same way as rats in group III.

Stress in rats was reproduced by exposing the rats to −20 °C for 30 minutes (duration of after stress 3 days).

After euthanasia of rats on the 4th day after stress under thiopental anesthesia by total bleeding from the heart, femurs were isolated, cleaned of other tissues, and homogenates were made with the addition of crushed glass at the rate of 500 mg bone per 7 ml of 0.1M citrate buffer pH 6.1.

After centrifugation at 2500 rpm for 15 minutes at +4 °C, the supernatant was used to determine the activity of alkaline phosphatase (pH 10.5) and acid phosphatase (pH 4.8) [9].

The mineralization index (MI) was calculated from the ratio of ALP/AP. In accordance with the method for determining mineralization activity (MA) in vivo, the mineralization efficiency (ME) of antistressants was calculated [17].

Experimental studies were conducted in accordance with generally accepted bioethical standards. The animals were maintained under standard vivarium conditions in compliance with the principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Statistical analysis of the results was performed using the SPSS software package for Windows (SPSS Inc., version 21.0, Chicago, USA). As the data followed a normal distribution, they are presented as M±SD (where M is the arithmetic mean and SD is the standard deviation). The significance of differences was determined using Student's t-test. Differences were considered significant at $p<0.05$.

Research results and discussion

Table 1 presents the results of determining the activity of phosphatases in the femur of rats of all 5 groups.

Table 1. Gender-specific effects of antistressants on phosphatase activity in the femur of rats after stress (11-12 rats per group)

Group	Antistressant dose, g/kg	Alkaline phosphatase (ALP), μ-cat/kg	Acid phosphatase (AP), μ-cat/kg
I. Intact			
Males	0	17.45±0.75	1.78±0.15
Females	0	14.85±0.11 P<0,05	1.98±0.12 P>0.1
II. Stress			
Males	0	13.30±1,0 P_1<0.05	2.52±0.09 P_1<0.05
Females	0	11.13±0.09 P_1<0.05 P<0.05	2.84±0.15 P_1<0.05 P>0.05
III. Stress + EkSoVit			
Males	0,0816	16.90±0.58 P>0,1	1.72±0.13 P_1>0.1
Females	0,430	14.52±0.12 P_1>0,05 P<0,05	2.42±0.11 P_1<0.05 P<0.05
IV. Stress + Quercetin			
Males	0,04092	16.22±0.73 P_1>0.1	1.95±0.20 P_1>0.05
Females	0,257	14.17±0.12 P_1<0.05 P<0.05	2.22±0.13 P>0.1
V. Stress + Ascorbic Acid			
Males	0,04092	15.40±0.94 P_1>0,05	2.22±0.13 P_1<0.05
Females	0,257	12.87±0.10 P_1<0.05 P<0.05	2.38±0.18 P_1>0.05 P>0.1

Notes: P – compared to the group of males; P_1 – compared to the group I “Intact”

As can be seen from these data, the activity of ALP in females is significantly lower compared to the indicators in males in all groups. The activity of AP in females, on the contrary, is even higher than in males.

Table 2 shows the nature of changes in the activity of phosphatases and the mineralization index in rats after stress. It can be seen that the activity of ALP in rats after stress decreases by 23.8-25.0 %, while the activity of AP increases by 41.6-43.0 %. As a result, the mineralization index (MI) after stress decreases by 46-48 %.

Table 2. The influence of stress on the nature of changes in the activity of phosphatases and the mineralization index in the femur of rats (4th day after stress), %

Indicator	Stress	
	males	females
1.Alkaline phosphatase (ALP)	−23.8	−25.05
2. Acid phosphatase (AP)	+41.6	+43.4
3.Mineralization index (MI) ALP/AP	−46.1	−47.7

Fig. 1 presents the results of determining the mineralization index of the femur in rats after stress, which received antistressants. These data show that females in all 5 groups have significantly lower MI compared to males. The use of antistressants increases the level of MI in rats after stress, and in females it remains lower compared to males.

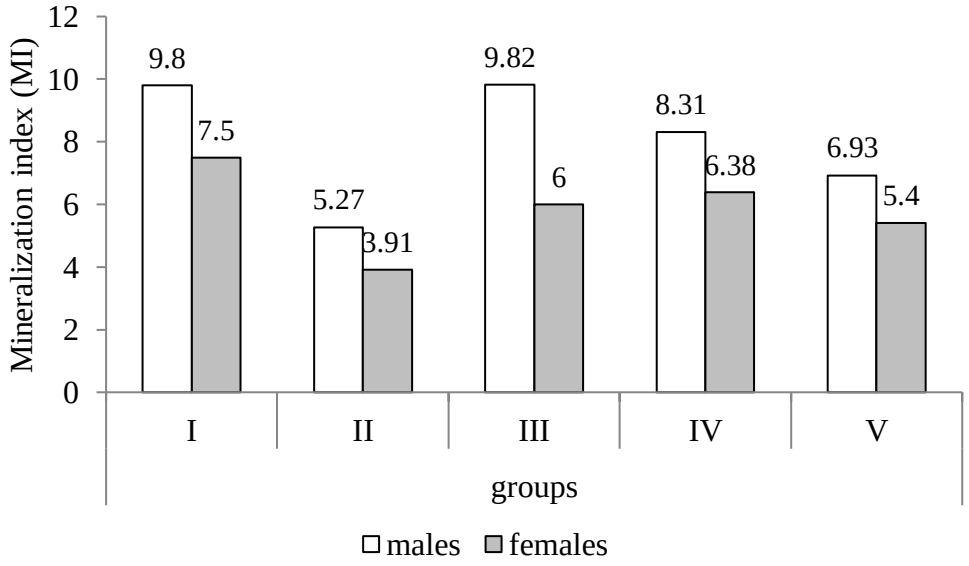


Fig. 1. The effect of antistressants on the mineralization index (MI) of the femur of rats after stress

(I – intact; II – stress; III – stress + EkSoVit; IV – stress + Quercetin;

V – stress + Ascorbic acid). MI =ALP/AP

Fig. 2 shows the calculated mineralizing activity (MA) of antistressants. The highest MA was found in the drug "Quercetin", and in males it was significantly higher than in females. Less MA was found in the drug "EkSoVit" and this indicator was even lower in the drug "Ascorbic acid".

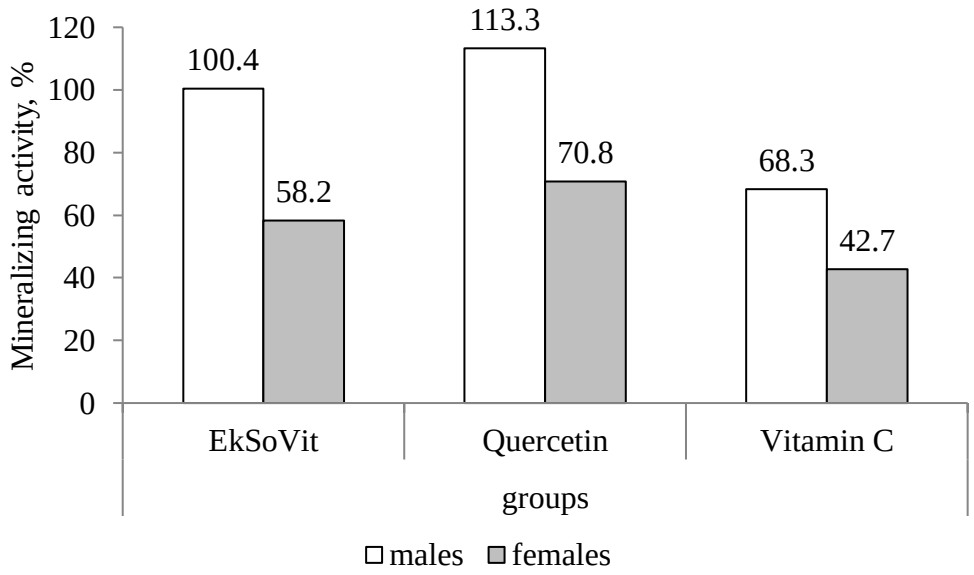


Fig. 2. Effect of antistressants on mineralizing activity (MA) in the femur of rats after stress.
MA = percentage increase in MI

Fig. 3 presents the results of determining the mineralizing efficiency of antistressants, calculated for 1g/kg of live weight of rats. From these data it is clear that the highest mineralizing efficiency was demonstrated by the drug “Antistressant – Vitamin P (quercetin)”, and the lowest by the ascorbic acid drug. Mineralazing efficiency of antistressants in males was significantly higher than in females.

The decrease in mineralizing activity of bone tissue of rats, caused by stress, we have established, coincides with the data of other authors [18-20]. We believe that bone tissue is very sensitive to manifestations of dysmetabolic syndrome, which are the result of post-stress intoxications [21]. The stimulation of osteogenesis by anti-stress drugs we have established coincides with the data of other authors who used fruits and vegetables rich in flavonoids [18, 22, 23].

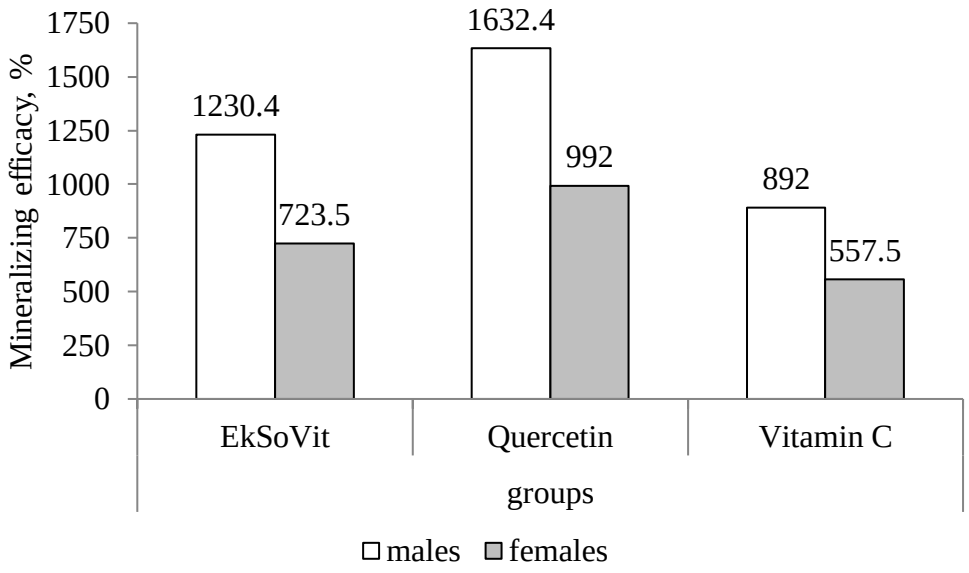


Fig. 3. Mineralizing efficacy (ME) of antistressants in the femur of rats after stress.
ME – MA per 1 g/kg dose of the drug

The sex-specific effect of antistressants on bone mineralization processes under stress is fundamentally new. The very high efficiency of the antistressants we used in males is additional confirmation of the peculiarities of male bone tissue compared to female bone tissue [10].

The most effective stimulator of bone mineralization processes was the flavanoid quercetin, which is part of the vitamins P group.

Conclusions

1. Stress reduces the mineralization index in bone tissue.

2. Anti-stressants stimulate mineralization processes by increasing alkaline phosphatase activity and decreasing acid phosphatase level.

3. In terms of mineralizing effectiveness, the drug “Antistressant – Vitamins P (quercetin)” was the most active, with the effectiveness of all three antistressors being 1.5-1.6 times higher in males.

Author Contributions

The authors agree to equal distribution of partial participation.

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Informed Consent Statement

Informed consent was obtained from all subjects who participated in the study.

Data Availability Statement

All information is in the public domain and specific graphic data can be obtained upon request from the corresponding senior author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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The study was carried out by the authors themselves without any outside assistance.

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