Levitsky A. P., Pavlenko K. V., Selivanska I. O., Lapinska A. P. Enzymatic method for determining in vivo the mineralizing efficiency of osteoprotective drugs. Journal of Education, Health and Sport. 2026;87:68087. eISSN 2391-8306.
https://dx.doi.org/10.12775/JEHS.2026.87.68087
https://apcz.umk.pl/JEHS/article/view/68087
https://zenodo.org/records/18202641

The journal has had 40 points in Minister of Science and Higher Education of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of 05.01.2024 No. 32318. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical culture sciences (Field of medical and health sciences);
Health Sciences (Field of medical and health sciences). Punkty Ministerialne 40 punktów. Załącznik do komunikatu Ministra Nauki i Szkolnictwa Wyższego z dnia 05.01.2024 Lp. 32318. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe: Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).
The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 14.12.2025. Revised: 30.12.2025. Accepted: 30.12.2025. Published: 09.01.2026.

UDC 577.07.003+611.018.4

ENZYMATIC METHOD FOR DETERMINING IN VIVO THE MINERALIZING EFFICIENCY OF OSTEOPROTECTIVE DRUGS

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Abstract

For screening osteoprotectors, it is proposed to investigate the mineralizing activity of rat bone tissue (femur, jaws, teeth) in which experimental osteoporosis was reproduced (stress, inadequate nutrition, dysmetabolic syndrome). It is proposed to determine the mineralizing activity by the ratio of alkaline (ALP) and acid (AP) phosphatase activity in bone tissue.

It is proposed to determine in vivo the mineralizing efficiency of the drug by the percentage increase in mineralizing activity per dose of the drug in mg/kg of live weight.

Of the three osteoprotectors studied (EkSoVit, quercetin and ascorbic acid), quercetin was the most effective, and ascorbic acid was the least effective.

Keywords: osteogenesis, mineralization, osteoprotectors.

The main mineral component of bone tissue is hydroxyapatite, which is formed by the interaction of inorganic phosphate and calcium salts [1-3]. Inorganic phosphate is a product of hydrolysis of organic phosphorus-containing compounds (glucose phosphate, glycerophosphate,

phosphatidic acid) under the influence of phosphatase enzymes: alkaline (optimum action pH 10.5) and acidic (optimum action pH 4.8) [4].

Alkaline phosphatase (ALP) is synthesized in osteoblasts and osteocytes and secreted together with the protein procollagen. Acid phosphatase (AP) is synthesized K in osteoclasts and secreted together with acid [5, 6]. If ALP causes the formation of hydroxyapatite, then AP promotes its hydrolysis. The final result of the accumulation of hydroxyapatite in bone tissue depends on the optimal ALP/AP ratio [7]. In the first period of life (up to 50 years), this ratio increases, and after 50 years begins to decrease [8], and the level of the ALP/AP ratio is significantly higher in men than in women [8].

We have proposed to call the ratio of ALP/AP the mineralization index (MI) [7, 8]. The mineralization index decreases not only in old age, but also from taking corticosteroids [9, 10], omeprazole [11], in diabetes mellitus [8], when consuming fats with a high content of linoleic acid [12], in various intoxications [13] and, as a rule, in osteoporosis [13].

A large number of drugs (calcium salts, hormonal drugs, protein products, plant extracts) have been proposed for the prevention and treatment of osteoporosis [14, 15].

Recently, antioxidant drugs have attracted great interest [16].

The aim of our work was to develop a method for determining in vivo the mineralizing efficacy of various osteoprotective drugs, taking into account their effect on the mineralizing index MI.

From a practical perspective, the best biological object for solving this problem is rats, in which it is possible to study the effect of osteoprotective drugs on the level of MI in various bones: teeth, jaws, femurs, vertebrae.

To carry out the procedure for determining mineralizing efficiency (ME), it is necessary to conduct a study of the mineralizing index MI in at least 3 groups of rats using a certain pathogenic factor (stress, nutrition, intoxication, etc.).

One group of rats should be intact, the second group − experimental with pathology and the third − with pathology, but additionally with the use of osteoprotector. As a result, 3 MI indicators will be obtained: MIn (group I), MIe (group II) and MIp (group III). The calculation of mineralizing efficiency (ME) is carried out by the formula:

$$\mathrm{ME} = \frac{(MIp - \mathrm{MI}_e)\cdot 100}{(\mathrm{MI}_n - \mathrm{MI}_e)\cdot \mathrm{C}},$$

where C is the dose of the drug in mg/kg of live weight;

MI_n – indicator of the intact group of rats;

MI_e – indicator of the group with pathology but without the drug;

MI_p – group with stress + drug.

A specific example of the definition of ME.

This study used 20 white rats (males, live weight 220±10 g), divided into 5 groups: Group I – normal (intact), Group II – stress + oral applications of gel without osteoprotector; Group III – stress + gel with EkSoVit (dose 13.6 mg/kg); Group IV – stress + oral applications of gel with quercetin (dose 6.82 mg/kg) and Group V – stress + oral applications of gel with ascorbic acid (dose 6.82 mg/kg).

Stress was reproduced by exposing rats to a temperature of −20 °C for 30 minutes.

After euthanasia of the animals, the femur was isolated, in the homogenate of which the activity of ALP and AP [4] was determined and the mineralization index MI was calculated based on the ratio of ALP/AP [7].

The results of determining the mineralization index MI and mineralization efficiency ME are presented in the table.

Table. Mineralizing efficacy of osteoprotectors

№№	Group	Dose of drug mg/kg	MI	ME ΔMI/mg/kg
1	Norm (intact)	0	9,80±0,37	−
2	Stress (study group)	0	5,27±0,25 $p<0,01$	−
3	Stress + EkSoVit drug	13,6	9,83±0,70 $p=1$; $p_1<0,01$	7,35±0,56
4	Stress + Quercetin drug	6,82	8,31±0,74 $p>0,05$; $p_1<0,05$	9,86±0,60
5	Stress + Ascorbic acid drug	6,82	6,93±0,71 $p<0,05$; $p_1>0,05$	5,37±0,49

Notes: p – compared to group 1; p_1 – compared to group 2.

As can be seen from the presented data, the most effective osteoprotector of the three studied was quercetin, and the less effective was the ascorbic acid preparation.

Conclusions

1. A method for determining the mineralizing efficiency of an osteoprotector is proposed, taking into account the increase in MI in percent per 1 mg/kg of the drug.

2. Of the three drugs, quercetin was the most effective and the less effective was ascorbic acid.

Author Contributions

The authors agree to equal distribution of partial participation.

Funding

This study received external funding.

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.

Acknowledgments

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

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