Levitsky A. P., Malinovskii V. O., Pavlenko K. V., Yuzkiv Ya. S., Selivanska I. O., Lapinska A. P. Method for determining in vivo antielastasy activity and effectiveness of treatmental and prophylaxis drugs. Journal of Education, Health and Sport. 2026;88:70001. eISSN 2391-8306.
https://dx.doi.org/10.12775/JEHS.2026.88.70001
https://apcz.umk.pl/JEHS/article/view/70001
https://zenodo.org/records/19117819

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: 20.01.2026. Revised: 27.01.2026. Accepted: 11.02.2026. Published: 26.02.2026.

UDC 577.07 + 611.018

METHOD FOR DETERMINING IN VIVO ANTIELASTASY ACTIVITY AND EFFECTIVENESS OF TREATMENTAL AND PROPHYLAXIS DRUGS

A. P. Levitsky[1], V. O. Malinovskii[1], K. V. Pavlenko[2], Ya. S. Yuzkiv[3], I. O. Selivanska[2], A. P. Lapinska[1]

[1]Odessa National Technological University, Odessa, Ukraine
[2]Odessa National Medical University, Odessa, Ukraine
[3]State Enterprise Ukrainian Research Institute for Medicine of Transport, Ministry of Health of Ukraine, Odessa, Ukraine

Abstract

Aim. A method for determining in vivo antielastase activity in rat bone tissue after stress has been proposed**.**

Methods. Stress was reproduced in rats by exposing the animals to −20 °C for 30 minutes. The dietary supplement “EkSoVit” (containing soy extract with proteolysis inhibitors), quercetin and ascorbic acid were used as anti-stressors. Anti-stressors were administered orally 3 days before stress and 3 days after stress. Animals were euthanized on day 4.

Results. An increase in elastase activity in the femur of rats after stress was established. All 3 anti-stressors reduce elastase activity: "EkSoVit" by 49 %, quercetin by 136

% and ascorbic acid by 91 %. In terms of anti-elastase efficacy, Quercetin exceeded "EkSoVit" by almost 5 times, and ascorbic acid by three times.

Conclusion. Stress causes activation of elastase activity in bone tissue. The main reason for the increase in elastase activity may be the translocation of the enzyme from leukocytes and the intestine. The most active anti-elastase agent was the flavonoid quercetin, which does not have a direct inhibitory effect on the elastase enzyme.

Keywords: elastase; protease inhibitors.

Activation of proteolysis plays an important role in the pathogenesis of various diseases [1]. Activation of proteolysis in various organs and tissues of the body occurs not only due to an increase in endogenous biosynthesis of proteolytic enzymes, but also as a result of a decrease in the level of endogenous inhibitors of proteolysis. Moreover, activation of proteolysis can occur due to proteolytic enzymes from other sources (leukocytes, pancreas, intestine) [2]. Moreover, these sources can cause a significantly greater increase in the level of proteolysis than endogenous activation of proteolysis.

Considering these circumstances, it can be assumed that various factors that influence the endogenous accumulation of proteolytic enzymes in a particular organ may not have a direct antiprotease effect.

Aim. A method for determining in vivo antielastase activity in rat bone tissue after stress has been proposed.

Material and methods

To assess the antielastase effect in vivo, at least 3 groups of rats (or other experimental animals) are required: group I − intact rats, group II − rats with experimental pathology (stress, intoxication) and group III − with pathology + the investigated therapeutic and prophylactic drug. If it is necessary to study several drugs, the number of groups can be increased.

In our work, we investigated the antielastase effect of three drugs: the dietary supplement "EkSoVit", which contains 75 % soy extract with proteolysis inhibitors (Kunitz inhibitor and Bauman-Birk inhibitor (TC U 10.8-37420386-010:2025), the dietary supplement "Antistress-Vitamin P (quercetin)" (TC U 10.8-37420386-010:2025), ascorbic acid (vitamin C).

The work used 25 white Vistar rats (males, 5 months, live weight 270-290 g), divided into 5 groups: I − intact, II − stress, III − stress + "EkSoVit ", IV − stress + quercetin, V − stress + ascorbic acid. The object of study was the proteolytic enzyme bone elastase.

Stress was reproduced by keeping rats in a chamber with a temperature of −20 ºC for 30 minutes (the state of proteolysis was assessed on the 4th day after stress. Therapeutic and prophylactic drugs were administered into the body as part of oral gels (3 % CMC-Na + 1 % Na benzoate) 3 days before stress and 3 days after stress.

The doses of the drugs are presented in Table 1. Elastase activity was determined spectrophotometrically using the substrate N-t-BOC l-alanine p-nitrophenyl [4]. The results of the activity were determined in microcatalogs per 1 kg of bone tissue.

Table 1. Drug doses, elastase activity (EA) in the femur of rats on the 4th day after stress (n = 5 in all groups)

№№	Groups	Doses, g/kg	Elastase activity, μκ-kat / kg
I	Intact	0	6,66 ± 0,40
II	Stress, day 4	0	9,61 ± 0,32 $p<0,01$
III	Stress, day 4 + "EkSoVit"	0,430	8,16 ± 0,56 $p<0,05$; $p_1<0,05$
IV	Stress, day 4 + quercetin	0,257	5,60 ± 0,28 $p<0,05$; $p_1<0,01$
V	Stress, day 4 + ascorbic acid	0,257	7,20 ± 0,47 $p>0,05$; $p_1<0,05$

p_1 – compared to group I; p_2 – compared to group II

Antielastase activity (AEA) was calculated using the formula:

$$AEA = \frac{(EA_2 - EA_3)}{(EA_2 - EA_1)} \cdot 100,$$

EA_1 – elastase activity in group I (intact);

EA_2 – elastase activity in group II (stress, day 4);

EA_3 – elastase activity in group III (stress+"EkSoVit")

AEA was calculated using a similar formula for group IV (stress + quercetin) and group V (stress + vitamin C).

The antielastase efficacy (AEE) of the drugs was calculated using the formula:

$$AEE = \frac{(EA_2 - EA_{3(4,5)})}{(EA_2 - EA_1)C} \cdot 100,$$

C − doses of drugs in g/kg of live weight of rats.

Research results

Table 1 presents data on drug doses and elastase activity. These data show that stress significantly increases elastase activity, and all the studied drugs reduce it.

Table 2 presents the results of calculating the antielastase activity and antielastase efficacy of the drugs.

Table 2. Antielastase activity (AEA) and antielastase efficacy (AEE) of antistressors in rat femur on day 4 after stress

№№	Group	AEA,%	AEE, %/g/kg live weight
III	Stress + "EkSoVit"	49,15±3,82	114,31±5,97
IV	Stress + quercetin	135,93±8,29	528,91±22,90
V	Stress + ascorbic acid	81,19±6,13	315,91±12,65

These data show that antielastase activity is exhibited by drugs that lack direct antielastase action, and their effectiveness in reducing the level of elastase activity in bone tissue significantly exceeds the effect of drugs containing protease inhibitors.

The most effective antielastase agent was quercetin, which exceeded the drug containing direct protease inhibitors by almost 5 times in its antielastase efficacy.

We believe that other sources of this enzyme, namely leukocytes and the intestine, play a crucial role in the activation of elastase activity in rat bone tissue. Quercetin is known to have anti-inflammatory properties, inhibiting leukocyte translocation and their secretion of pro-inflammatory cytokines and destructive enzymes.

Perhaps these protective effects of quercetin depend on its extremely high antioxidant activity, surpassing the protective effect of vitamin C by almost two times.

Conclusions

1. Stress causes activation of elastase activity in bone tissue.

2. The main reason for the increase in elastase activity may be the translocation of the enzyme from leukocytes and the intestine.

3. The most active anti-elastase agent was the flavonoid quercetin, which does not have a direct inhibitory effect on the elastase enzyme.

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.

References

1. Veremeenko KN. Proteolytic enzymes and their inhibitors. New areas of clinical application. Medical Affair. 1994;31:8-13. (in Russian)

2. Lundwall A, Band V, Blaber M, Clements JA, Courty Y, Diamandis EP, Yousef GM. A comprehensive nomenclature for serine proteases with homology to tissue kallikreins. Biological chemistry. 2006;6:637-641. doi: 10.1515/BC.2006.082.

3. Levitsky AP, Malinovskii VO, Yuzkiv YaS, Pavlenko KV, Selivanska IO, Lapinska AP. Four stages after stress reactions. Journal of Education, Health and Sport. 2025;86:67246. DOI:10.12775/JEHS.2025.86.67246

4. Levitsky AP, Makarenko OA, Demyanenko SA. Methods of experimental dentistry. Simferopol, Tarpan, 2018:78. (in Russian)