Yuzkiv Ya. S. Markers of pathology in serum of rats after “mild” stress. Journal of Education, Health and Sport. 2025;84:65126. eISSN 2391-8306. https://dx.doi.org/10.12775/JEHS.2025.84.65126
https://apcz.umk.pl/JEHS/article/view/65126
https://zenodo.org/records/16988616

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: 06.07.2025. Revised: 16.07.2025. Accepted: 11.08.2025. Published: 28.08.2025.

UDC 636.085+531.512+633.174

MARKERS OF PATHOLOGY IN SERUM OF RATS AFTER “MILD” STRESS

Ya. S. Yuzkiv

State Enterprise Ukrainian Research Institute for Medicine of Transport, Ministry of Health of Ukraine, Odessa, Ukraine

Abstract

Background. Stress occurs as a result of the action of a very large number of factors. "Mild" stress occurs with moderate action of a stress-inducing factor, however, repeated action of such a factor can cause a number of diseases: cardiovascular, neuro-endocrine, neurotrophic, metabolic.

The aim of our work was to study the state of pathology under the influence of "mild" stress by such biochemical markers as casein hydrolysis, BAEE hydrolysis (benzoyl-arginine ethyl ether) and ascorbic acid

Methods. “Mild” stress was induced in rats by holding the animals at −20 °C for 5 minutes. After 5 and 24 hours, blood serum was obtained and proteolysis activity (substrate casein, pH 7.6), BAEE-esterase activity (substrate benzoyl-arginine ethyl ether), and peroxidation activity (substrate ascorbic acid) were determined in it.

Results. An increase in proteolysis activity was found already 5 hours after stress and an almost twofold increase after 24 hours. On the contrary, peroxidation activity decreases after

5 hours and returns to normal after 24 hours. BAEE-esterase activity does not change after “mild” stress.

Conclusion. Activation of proteolysis in the blood serum of rats under conditions of "mild" stress was detected. The level of oxidative processes in the blood serum of rats under conditions of "mild" stress is briefly reduced.

Keywords: stress; pathology markers; proteolysis; peroxidation.

Introduction

Stress is caused by a wide range of factors, especially trauma and pain [1, 2]. Post-stress pathological processes include oxidative stress [3, 4], activation of hydrolytic processes, in particular proteolysis [5, 6], and disruption of the vascular and intestinal barrier function [2].

"Mild" stress occurs with moderate exposure to a stress-inducing factor, but repeated exposure to such a factor can cause a number of diseases: cardiovascular, neuro-endocrine, neurotrophic, and metabolic [7, 8].

Activation of proteolysis, in particular of the kallikrein-kinin system [6] and activation of peroxidation processes (so-called oxidative stress) [9] play a significant role in the pathogenesis of many diseases.

It is believed that oxidative stress plays the most important role in the pathogenesis of post-stress reactions [10].

The aim of our work was to study the state of pathology under the influence of "mild" stress using such biochemical markers as casein hydrolysis, BAEE hydrolysis (benzoyl-arginine ethyl ether) and ascorbic acid oxidation. The first marker makes it possible to assess the activity of proteolysis under the action of a number of proteolytic enzymes (trypsin, chymotrypsin, elastase, collagenase). The second marker indicates the level of an enzyme such as kallikrein. The third marker indicates the total activity of oxygenases and peroxidases, which determine the development of oxidative stress.

Materials and research methods

The experiments were conducted on 15 white Wistar rats (males, 180-220 g), divided into 3 equal groups: 1st − control (intact), 2nd − "mild" stress, 5 hours, 3rd − "mild" stress, 24 hours. "Mild" stress was induced in rats by keeping them at −20 °C for 5 minutes.

Rats were euthanized under thiopental anesthesia by total bleeding from the heart. Blood was collected and serum was obtained.

Proteolysis was assessed by the rate of casein hydrolysis at pH 7.6 according to our modification of the Kunitz method [11]. BAEE esterase activity was assessed by the

hydrolysis of benzoyl-arginine ethyl ester by the spectrophotometric method [6]. Oxidation status was assessed by the rate of ascorbic acid oxidation using $K_3[Fe(CN)_6]$ and $FeCl_3$ [12] Serum protein content was determined by the Lowry method [11].

The results of the experiments were subjected to standard statistical processing with the determination of the standard error.

Results and discussion

Table 1 presents the results of determining proteolysis activity, which show that "mild" stress causes an increase in the level of proteolysis in rats already 5 hours after stress and almost doubles after 24 hours.

Table 1. Effect of stress on proteolysis activity (substrate casein, pH 7,6)

№ groups	Group	Activity, ng/min·ml	Specific activity, ng/min·mg protein
1	Control	867±100	28±3,5
2	Stress, 5 hours	1233±145 p>0,05	40±5,0 p>0,05
3	Stress, 24 hours	1700±220 p<0,05	56±7,6 p<0,05

Table 2 shows that the level of BAEE-esterase activity in blood serum does not change under conditions of "mild" stress.

Table 2. Effect of stress on BAEE-esterase activity of rat blood serum

№ groups	Group	Activity, nmol/min·ml	Specific activity, nmol/min·mg protein
1	Control	213±31	7,02±1,28
2	Stress, 5 hours	214±30 p>0,5	7,02±1,44 p=1
3	Stress, 24 hours	198±46 p>0,3	6,93±2,12 p>0,5

Table 3 shows the data on the rate of oxidation of ascorbic acid by oxidative enzymes in rat serum. It can be seen that after 5 hours the level of ascorbic acid oxygenation decreases

significantly ($p<0.05$), but 24 hours after stress it normalizes.

Table 3. The effect of stress on oxidative activity
(rate of ascorbic acid oxidation) of rat blood serum

№ groups	Group	Activity, μg/min·ml	Specific activity, μg/min·mg protein
1	Control	21,60±1,10	0,71±0,02
2	Stress, 5 hours	15,11±1,32 p<0,05	0,50±0,10 p<0,05
3	Stress, 24 hours	20,41±1,71 p>0,5	0,70±0,05 p>0,5

The relative indicators (in percentages) of biochemical markers of pathology are presented in the figure.

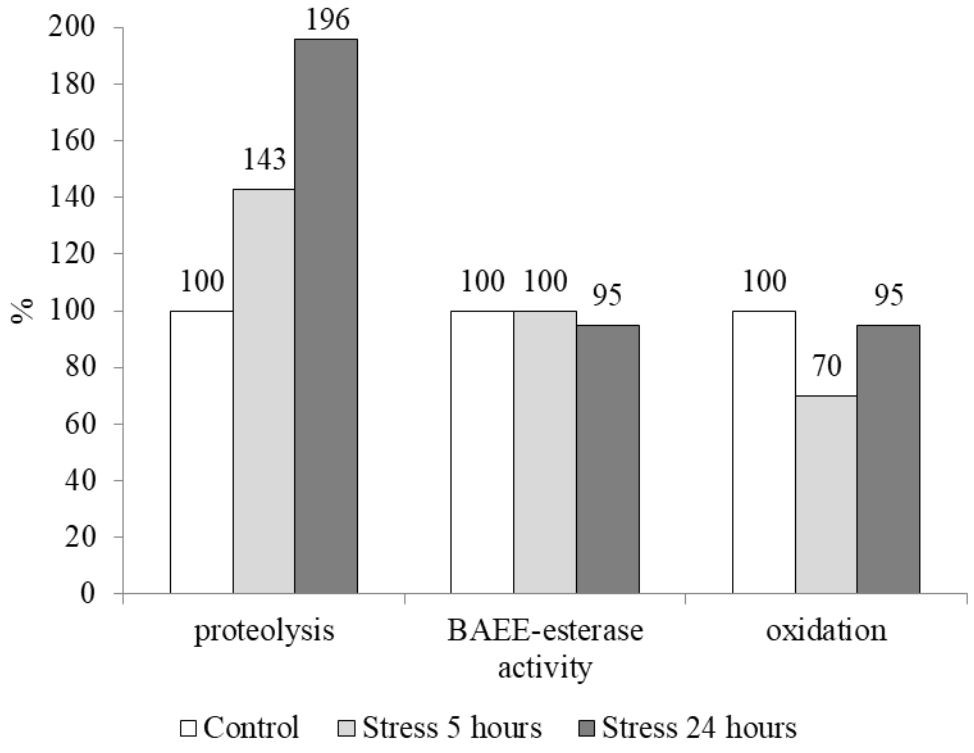

Fig. Relative level of markers of pathological processes in the blood serum of rats 5 and 24 hours after “mild” stress

Our results indicate that “mild” stress, unlike “acute” [13], does not activate

oxygenation processes, but, on the contrary, even reduces them for a short time.

In the case of “mild” stress, the activation of proteolysis comes to the fore, which makes it possible to increase the level of free amino acids in the blood serum, which are necessary for the biosynthesis of proteins in the body’s tissues. It is possible that the activation of proteolysis under conditions of “mild” stress occurs under the influence of adrenaline, which is the first mediator of the stress process. As is known, a similar process of activation of lipolysis to increase the level of free fatty acids occurs as a result of the action of adrenaline.

Thus, “mild” stress should be considered as a mobilizing factor for the body [14]. However, repeated “mild” stress, without effective utilization of free amino acids and free fatty acids, can negatively affect metabolic processes.

Conclusion

Activation of proteolysis in the blood serum of rats under conditions of "mild" stress was detected. On the contrary, the level of oxidative processes in the blood serum of rats under conditions of "mild" stress is briefly reduced.

Funding

This research received no external funding.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

All information is publicly available and data regarding this particular patient can be obtained upon request from corresponding senior author.

References

1. Levitsky AP, Malinovskii VA. Structural classification of vitamins P. Journal of Education, Health and Sport. 2025;83:64284. https://dx.doi.org/10.12775/JEHS.2025.83.64284.

2. Lambert GP. Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. J Anim Sci. 2009;87(14):E101-108. doi: 10.2527/jas.2008-1339.

3. Gorbenko NI, Ivanova OV, Borikov AJu. Oxidative stress as a pathophysiological mechanism of development of diabetic macroangiopathies and prospects for its correction with flavonoids (literature review and own results). Problems of endocrine pathology. 2016;3:91-99. https://doi.org/10.21856/j-PEP.2016.3.10. (in Russian).

4. Ndrepepa G. A bridge linking inflammation and oxidative stress with cardiovascular

disease. Clin Chim Acta. 2019;493:36-51. doi: 10.1016/j.cca.2019.02.022.

5. Rodney G, Swanson A. The effect of a series of flavonoids on hyaluronidase and some other related enzymes. J.Biol.Chem.1950;183:789. DOI:10.1016/S0021-9258(19)51199-1.

6. Middleton JrE, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev. 2000;52(4):673-751.

7. Bacchetti T, Turco I, Urbano A [and others]. Relationship of fruit and vegetable intake to dietary antioxidant capacity and markers of oxidative stress: A sex-related study. Nutrition. 2019:61:164-172. doi: 10.1016/j.nut.2018.10.034.

8. Gorchakova NO, Shumejko OV, Klimenko OV [and others]. Anti-stress and antioxidant properties of herbal medicines. Proceedings of the scientific congress "NATO: comprehensive complementary and alternative interventions for persons injured during military events, military personnel, their families, during the period of physical and medical rehabilitation", June 13-14, 2024, Kyiv: 141-142. (in Ukrainian).

9. Ray PD, Huang BW, Tsuji Y. Reactive Oxygen Species (ROS) Homeostasis and Redox Regulation in Cellular Signaling. Cellular Signalling. 2012;24:981-990. https://doi.org/10.1016/j.cellsig.2012.01.008.

10. Arkelius K, Wendt TS, Andersson H [and others]. LOX-1 and MMP-9 Inhibition Attenuates the Detrimental Effects of Delayed rt-PA Therapy and Improves Outcomes After Acute Ischemic Stroke. Circulation Research. 2024;134(8):954-969. https://doi.org/10.1161/CIRCRESAHA.123.323371.

11. Makarenko O, Levitsky A. Biochemical Mechanisms of Therapeutic and Prophylactic Effects of Bioflavonoids. Journal of Pharmacy and Pharmacology. 2016;4 (8):451-456. DOI:10.17265/2328-2150/2016.08.013.

12. Moharram HA, Youssef MM. Methods for Determining the Antioxidant Activity: A Review. Alex. J. Fd. Sci. & Technol. 2014;11(1):31-42. doi: 10.12816/0025348.

13. Maksjutina NP, Mojbenko AA, Mohort NA. [and others]. Bioflavonoids as organoprotectors (quercetin, corvitin, quertin). Kiyv: Naukova dumka, 2012: 274. (in Russian).

14. Gorchakova NO, Bjelenichev IF, Buhtijarova NV [and others]. Anti-aging effects of antioxidants from the bioflavonoid group. In the book "Synthesis of theory and practice in educational, methodological and clinical support of a healthy lifestyle." Materials of the scientific congress. Kyiv. 2022:141-142. (in Ukrainian).