

Velychko V. V. The influence of “mild” stress on biochemical markers of pathology in the liver of rats. *Journal of Education, Health and Sport*. 2025;84:65128. eISSN 2391-8306. <https://dx.doi.org/10.12775/JEHS.2025.84.65128>  
<https://apcz.umk.pl/JEHS/article/view/65128>  
<https://zenodo.org/records/16989209>

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 2025;  
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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

## THE INFLUENCE OF “MILD” STRESS ON BIOCHEMICAL MARKERS OF PATHOLOGY IN THE LIVER OF RATS

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### Abstract

**Background.** It is known that stress negatively affects the liver. Under the influence of stress factors, oxidative stress occurs in the liver, which is the main pathogenetic mechanism of hepatitis development. In the process of post-stress reactions, other pathogenetic processes are also activated, due to the activation of hydrolytic processes, increased permeability of histo-hematic and intestinal barriers.

**Aim.** To investigate the effect of stress on the condition of the liver.

**Methods.** Rats were stressed by holding the animals at  $-20^{\circ}\text{C}$  for 5 minutes (“mild” stress). 5 and 24 hours after stress, proteolysis activity (substrate casein, pH 7,6; substrate albumin, pH 5,5) was determined in the liver by the Kunitz method, BAEE esterase activity by the spectrophotometric method, and oxidative status by the rate of ascorbic acid oxidation.

**Results.** After 24 hours, a decrease in proteolysis activity is observed (substrate casein, pH 7,6) and after 5 hours the level of peroxidation decreases. Albumin proteolysis activity (pH 5,5) does not change. There is a tendency to increase BAEE-esterase activity.

Conclusion. A decrease in the level of proteolysis and oxidation may indicate a positive effect of “mild” stress on the condition of the liver.

**Keywords:** stress; liver; pathology markers; proteolysis; oxidative stress.

## **Introduction**

It is known that stress negatively affects the condition of the liver [1, 2]. It is believed that under the influence of stress factors, oxidative stress occurs in the liver [3], which is the main pathogenetic mechanism of hepatitis development [4].

It is also known that in the process of developing post-stress reactions, other pathogenetic processes are activated, caused by the activation of hydrolytic processes, an increase in the permeability of histo-hematological and intestinal barriers [4-6].

The goal of our work was to determine the impact of “mild”, one-time stress on such indicators of liver pathology as proteolysis activity, including kallikrein, as well as the level of oxidative systems.

## **Materials and research methods**

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

In rats after euthanasia under thiopental anesthesia, the liver was isolated and a homogenate was obtained at the rate of 50 mg per 1 ml of 0.9 % NaCl solution. In the homogenate, the proteolysis activity (substrate casein at pH 7.6) was determined by the Kunitz method in our modification [7], as well as the proteolysis activity (substrate albumin, pH 5.5) by the Kunitz method. To assess the state of the kallikrein-kinin system, BAEE-esterase activity (substrate benzoyl-arginine-ethyl ether) was determined by the spectrophotometric method [8]. The state of oxidative stress was determined by the rate of ascorbic acid oxidation [9]. The protein content in the homogenates was determined by the Lowry method [8].

Statistical processing of the experimental results was carried out using standard methods.

## **Results and discussion**

Table 1 presents the results of proteolytic activity determination (casein substrate, pH 7.6). It can be seen that the level of proteolysis in the liver begins to decrease 5 hours after stress, and after 24 hours it decreases by 3.7 times.

Table 1. The effect of stress on the activity of proteolysis  
(substrate casein, pH 7,6) in the liver of rats

№ groups	Group	Group activity, ng/min·h	Specific activity, ng/min·mg protein
1	Control	4957±720	64±9,5
2	Stress, 5 hours	4751±680 p>0,5	71±10,2 p>0,3
3	Stress, 24 hours	1351±195 p<0,01	25±4,0 p<0,01

At the same time, albumin proteolysis (pH 5,5) in the rat liver (Table 2) did not change after stress.

Table 2. The effect of stress on proteolysis activity  
(substrate albumin, pH 5,5) in rat liver

№ groups	Group	Group activity, ng/min·h	Specific activity, ng/min·mg protein
1	Control	7125±993	96±15
2	Stress, 5 hours	7207±1050 p>0,5	104±15,5 p>0,3
3	Stress, 24 hours	7125±1163 p=1	100±12,7 p>0,5

Table 3 presents the results of determining the kallikrein-kinin system by the rate of BAEE hydrolysis. It is seen that there is a tendency to increase BAEE esterase activity already 5 hours after stress.

Table 3. The effect of stress on BAEE-esterase activity in rat liver

№ groups	Group	Group Activity, ng/min·h	Specific activity, ng/min·mg protein
1	Control	27,5±4,3	0,37±0,05
2	Stress, 5 hours	38,7±5,6 p>0,05	0,54±0,07 p>0,05
3	Stress, 24 hours	37,1±5,0 p>0,05	0,51±0,07 p>0,05

It is known that kinins, which are formed under the action of kallikrein, significantly increase the permeability of histo-hematological barriers [6].

Table 4 presents the results of determining the state of liver oxidative systems by the rate of ascorbic acid oxidation. It can be seen that the rate of ascorbic acid oxidation is halved already 5 hours after stress.

Table 4. The effect of stress on oxidative activity  
(rate of ascorbic acid oxidation) of rat liver

№ groups	Group	Group activity, ng/min·h	Specific activity, ng/min·mg protein
1	Control	9,95±1,27	0,130±0,017
2	Stress, 5 hours	4,64±0,78 p<0,05	0,080±0,015 p<0,05
3	Stress, 24 hours	5,57±0,82 p<0,05	0,080±0,016 p<0,05

The results we obtained are more clearly presented in the figure.

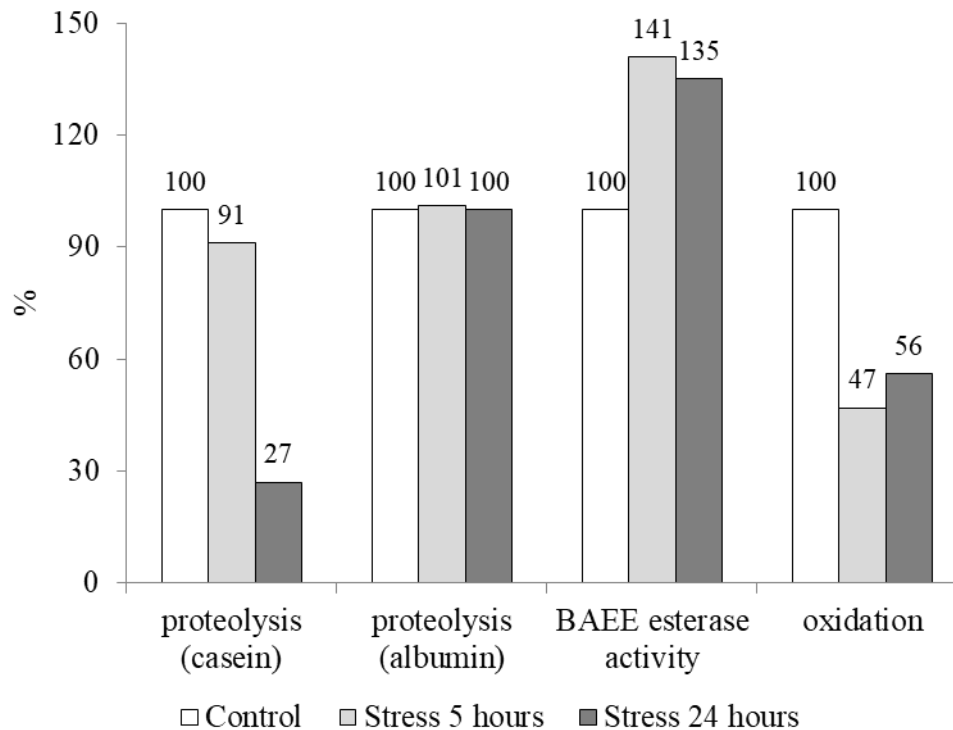


Fig. Relative levels of markers of pathological processes in the liver of rats 5 and 24 hours after "mild" stress

Thus, as a result of the experiments conducted, it can be assumed that a “mild” one-time stress does not cause the development of oxidative stress in the liver, but, on the contrary, significantly reduces it.

“Mild” single stress suppresses proteolysis in the liver.

The decrease in the level of two biochemical markers of pathology (proteolysis and peroxidation) can be considered as a protective response of the liver to stress.

### **Conclusion**

"Mild" one-time stress reduces the activity of proteolysis and peroxidation in the liver, which may indicate a positive (stimulating) effect of stress on this organ.

### **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.

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