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**ACTOPROTECTIVE ACTIVITY OF THE KATEKHIN HYDRATE FLAVONOID ON THE BACKGROUND OF ANIMAL PHYSICAL OVERLOADS**© **Voronkov A.V.<sup>1</sup>, Gerashchenko A.D.<sup>1</sup>, Lysenko T.A.<sup>1</sup>, Shabanova N.B.<sup>1</sup>,  
Voronkova M.P.<sup>2</sup>**<sup>1</sup>*Pyatigorsk Medical Pharmaceutical Institute-branch of Volgograd State Medical University, 11, Kalilina Av., 357532, Pyatigorsk, Russia*<sup>2</sup>*Volgograd State Medical University, pl. Fallen Fighters, 1, 400131, Volgograd, Russia**Abstract***Objective.** To study the actoprotective activity of the flavonoid catechin hydrate against the background of physical overload of animals.**Methods.** The experiment was conducted on 60 male mice (weight 20-25 g.) divided into six equal experimental groups (n=10). Analyte-the substance catechin hydrate (100 mg/kg), the drugs comparison – Metaprot (25 mg/kg), Hypoxen (150 mg/kg) and Mexidol (50 mg/kg). Physical activity was reproduced on the model of running animals on the treadmill for 5 days. The studied substance and drugs of comparison were administered intragastric for 30 minutes until a reproducible load. On the 6th day were evaluated antioxidant activity, by sampling the skeletal muscles of animals (chloralhydrate anesthesia-350mg/kg).**Results.** When studying the actoprotective activity of the substance catechin hydrate, it was found that the course administration leads to an increase in physical endurance of animals. At the same time, there is a removal of oxidative stress, which is reflected in the reduction of Pro-oxidants, and an increase in antioxidant activity.**Conclusions.** In the course of the study, it was found that the studied substance catechin hydrate exhibits actoprotective activity, while not inferior in activity to the true actoprotector Metaprot.**Keywords:** actoprotector, catechin hydrate, physical overload, flavonoids, antioxidant activity**АКТОПРОТЕКТОРНАЯ АКТИВНОСТЬ ФЛАВОНОИДА КАТЕХИН ГИДРАТА НА ФОНЕ ФИЗИЧЕСКИХ ПЕРЕГРУЗОК ЖИВОТНЫХ****Воронков А.В.<sup>1</sup>, Геращенко А.Д.<sup>1</sup>, Лысенко Т.А.<sup>1</sup>, Шабанова Н.Б.<sup>1</sup>, Воронкова М.П.<sup>2</sup>**<sup>1</sup>*Пятигорский медико-фармацевтический институт – филиал ФГБОУ ВО ВолГМУ Минздрава России. 357532, Россия, Пятигорск, пр. Калинина, 11*<sup>2</sup>*Волгоградский государственный медицинский университет, Россия, 400131, Волгоград, пл. Павших Борцов, 1**Abstract***Цель.** Изучить актопротекторную активность флавоноида катехин гидрата на фоне физических перегрузок животных.**Методика.** Эксперимент проведен на 60 мышах-самцах (масса 20-25 гр.), разделенных на шесть равных экспериментальных групп (n=10). Исследуемое вещество-субстанция катехин гидрата (100 мг/кг), препараты сравнения – Метапрот (25 мг/кг), Гипоксен (150 мг/кг) и Мексидол (100 мг/кг). Физические нагрузки воспроизводили на модели бега животных на тредбане в течение 5 дней. Исследуемую субстанцию и препараты сравнения вводили интрагастрально за 30 мин. до воспроизводимой нагрузки. На 6-е сутки оценивали антиоксидантную активность, путем забора скелетной мышцы животных (хлорагидратный наркоз – 350 мг/кг).**Результаты.** При изучении актопротекторной активности субстанции катехин гидрат было установлено, что курсовое введение приводит к повышению физической выносливости животных. При этом, наблюдается устранение явления окислительного стресса, что нашло свое отражение в снижении прооксидантов, и повышение антиоксидантной активности.**Заключение.** В ходе проведенного исследования было установлено, что катехин гидрат проявляет актопротекторную активность, при этом данная субстанция не уступала по активности Метапроту.

*Ключевые слова:* актопротектор, катехин гидрат, физические перегрузки, флавоноиды, антиоксидантная активность

## Introduction

The modern rhythm of life dictates its own conditions, while directly affecting both professional and daily activities [1, 5, 7, 15]. These factors include almost any exogenous effect (noise, vibration, ionizing radiation, stressors) that provoke the development of certain negative shifts in homeostasis [13]. Today, the diagnosis of psycho-physical dysfunction (fatigue) is a global problem [12]. Recent studies by the WHO show that the proportion of chronic diseases associated with fatigue is 76.6% [18]. In modern highly urbanized society, a person is constantly exposed to the influence of these factors, which cause the development of new diseases, the transition of existing pathologies to an exacerbation phase, ultimately worsening the quality of life of the population.

There is no doubt that to ensure the proper living standard, a modern person must resort to various kinds of "corrective" measures aimed at limiting the action of the damaging factor, the progression of an acute pathological process and its transfer to the phase of convalescence [11].

In this regard, the problem of increasing the body's defenses, as well as increasing its physical and mental performance with the help of pharmacological agents belonging to the actoprotectors group, becomes urgent [6]. However, despite the promising use of this class of drugs, the only representative is Metaprot, which is currently deprived of registration for use in the Russian Federation [17]. It should be said that a promising direction is the study of plant objects that have a sufficiently high therapeutic efficacy and safety of use [14, 16]. Thus, it makes sense to search for substances – actoprotectors, mainly of natural origin.

The aim of the study was to study the actoprotective activity of the flavonoid catechin hydrate against the background of physical overload in animals.

## Methods

The experiment was carried out on 60 outbred male mice weighing 20-25 g. Animals were randomized according to running time and weight, and then divided into two equal groups of 10 mice each. The first group - the group of positive control animals (PC), the second experimental group (the group without pharmacological correction - the negative control group (NC)), received 0.9% sodium chloride solution, the third group (n = 10), received the investigated substance catechin hydrate at a dosage of 100 mg / kg [2]. Comparison drugs were Metaprot (25 mg/kg) [9], Mexidol (100 mg/kg) [1] and Hypoxen (150 mg/kg) [8]. The studied substances were injected into the animals intragastrically one hour before testing the animals on the treadmill.

After repeated physical activity, pro/antioxidant activity was assessed. Determination of the content of malondialdehyde (MDA), diene conjugates (DC), superoxide dismutase (SOD), catalase, and glutathione peroxidase (HP) was carried out in a muscle tissue homogenate by spectrophotometric method.

Determined indicators. In order to assess the actoprotective activity of the studied substance, the following indicators were assessed: the running time of the animals (test «Running on a tedbane»). In the post-nuclear fraction of muscle tissue, the content of diene conjugates (DC), TBA-active products was assessed in terms of malondialdehyde (MDA), and the activity of endogenous antioxidant defense enzymes: SOD, catalase, and HP was determined.

The data obtained were processed using the STATISTICA 6.0 software package (StatSoft, Inc., USA, for the Windows operating system) and Microsoft Excel 2010. The mean value and its standard error ( $M \pm m$ ) were determined. The distribution normality was assessed by the Shapiro-Wilk test. Parametric Student's t-test was used for normal distribution of data. In the case of an abnormal distribution, statistical processing was performed using the Mann-Whitney U-test. At a significance level of more than 95% ( $p < 0.05$ ), the differences were considered significant [3].

## Research results and discussion

A group of positive control mice were subjected to physical activity (FN) in groups of n=3, n = 3 and n=4 animals daily. Throughout the experiment, the running duration of the animals remained practically

unchanged. By the end of the study, no statistically significant differences were found between the initial running duration and the 5th final day.

According to the results of the study, it was found that the endurance of the animals of the negative control group, after five days, was lower (Fig. 1). At the same time, the minimum working time of the mice was recorded on the fifth day of the experiment, and amounted to  $74.4 \pm 8.79$  sec, which was significantly lower than the initial running time of the animals of this group by 40.1% ( $p < 0.05$ ).

The use of the catechin hydrate substance increased the running time of the animals during the five days of the experiment. A linear increase in animal endurance was noted, without sudden jumps and falls. The peak endurance of mice fell on the 5th day, which was by 122.3% higher ( $p < 0.05$ ) relative to the initial (first day) day of the experiment in this group, and by 289.4% higher ( $p < 0.05$ ) regarding the fifth day of the experiment of the group of mice NK. It should also be said that the peak endurance of the animals receiving catechin hydrate was by 109.3% higher ( $p < 0.05$ ) on the peak day (day 3) of the negative control group.

Similar changes in increased performance were observed when using the reference drug Metaprot. Positive dynamics was observed from the first day of Metaprot administration to animals, as evidenced by the fact that already on the second day of the experiment, the running duration of the animals was by 90.2% higher than the initial indicators of this group ( $p < 0.05$ ). At the same time, the peak of working capacity was observed on the 5th day of the experiment, which was by 212.8% higher ( $p < 0.05$ ) than the initial indicators of this group of mice, and by 213.2% higher ( $p < 0.05$ ) (Fig. 1).

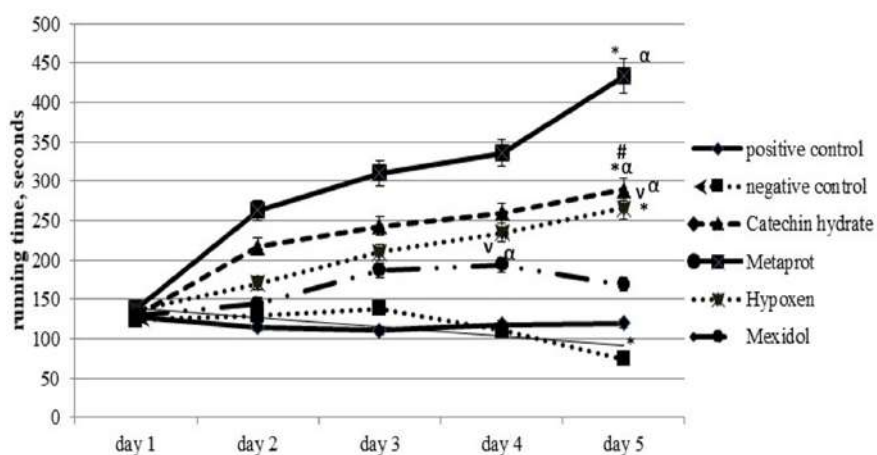


Fig. 1. Changes in the running time of male mice against the background of the study substance and comparison drugs on the «treadmill running» model.

\* – Statistically significant relative to the initial day of running in this group (Student's t-test, ( $p < 0.05$ )); # – statistically significant relative to the final running day of the NK group (Student's t-test, ( $p < 0.05$ ));  $\alpha$  – statistically significant relative to the peak running day of the NK group (Student's t-test, ( $p < 0.05$ ));  $\nu$  – statistically significant relative to the peak day of the group receiving Metaprot (Student's t-test, ( $p < 0.05$ ))

Against the background of the introduction of comparison drugs Mexidol and Hypoxen to the animals, changes similar to the previously described substances were observed. So, physical performance was higher, the peak of activity was recorded on the 5th day (the group receiving Hypoxen) and on the 4th day of the experiment (the group receiving Mexidol). It was found that the peak endurance in the group receiving Hypoxen and Mexidol was higher by 94.4% ( $p < 0.05$ ) and 50.3% ( $p < 0.05$ ), respectively, relative to the initial data of the groups. The indicator of maximum performance in the groups receiving the comparison drugs Hypoxen and Mexidol was higher than that of the NK group by 92% ( $p < 0.05$ ) and 40.2% ( $p < 0.05$ ), respectively.

There were no statistically significant differences in the «endurance» indicator of the peak day of the experiment between the groups receiving the catechin hydrate substance and the groups receiving Hypoxen and Mexidol. However, the comparison drugs considered above (Hypoxen and Mexidol) are inferior in physical activity to the group of mice treated with Metaprot, as evidenced by the decrease in working capacity recorded on the peak day by 38.7% ( $p < 0.05$ ) and 55.2% ( $p < 0.05$ ), respectively (Fig. 1).

During intense physical exertion, maintaining the prooxidant-antioxidant balance is decisive in the development of compensatory processes in the body.

Psycho-physical activity of animals in the negative control group leads to an increase in the concentration of formation of TBA-active products by 260.7% ( $p < 0.05$ ) and 43.2% ( $p < 0.05$ ), respectively, in comparison with the positive group of animals (Fig. 2).

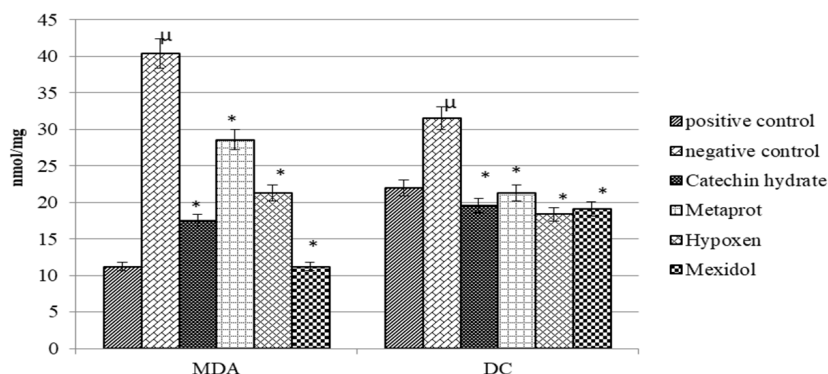


Fig. 2. The influence of the studied substance and comparison drugs on the level of MDA and DC in the muscle tissue of mice against the background of physical and psycho-emotional overloads.

$\mu$  – Statistically significant relative to the group of positive control mice  $p < 0.05$  (Mann-Whitney U-test); \* – statistically significant relative to the group of NC mice  $p < 0.05$  (Mann-Whitney U-test)

At the same time, in this group of animals (NC), the activity of the antioxidant defense of the body significantly decreased, which is reflected in a decrease in the concentration of SOD, catalase and HP: by 43.7% ( $p < 0.05$ ), 42.9% ( $p < 0.05$ ), 30.4% ( $p < 0.05$ ), relative to the positive control group of mice (Fig. 3, 4). Most likely, such changes in the balance of pro / antioxidants in the direction of excessive production of the former may indicate the development of the phenomenon of oxidative stress, which is consistent with the literature [4].

Daily use of the substance catechin hydrate, against the background of physical overload, leads to a noticeable decrease in peroxidation, thereby statistically significantly reducing the formation of TBA-active products (MDA) by 56.7% ( $p < 0.05$ ) and DC by 37.8 % ( $p < 0.05$ ), in comparison with the group of animals without pharmacological correction (NC).

The «defense» system was noted in an increase in the concentration of superoxide dismutase: 69.3% ( $p < 0.05$ ), glutathione peroxidase by 88.8% ( $p < 0.05$ ) and catalase by 163.2% ( $p < 0.05$ ), respectively, relative to the group of NK mice.

At the same time, there were no statistically significant differences between the groups of mice receiving the substance catechin hydrate and the groups of mice receiving reference drugs in terms of the «MDA level» and «DC level» indicators.

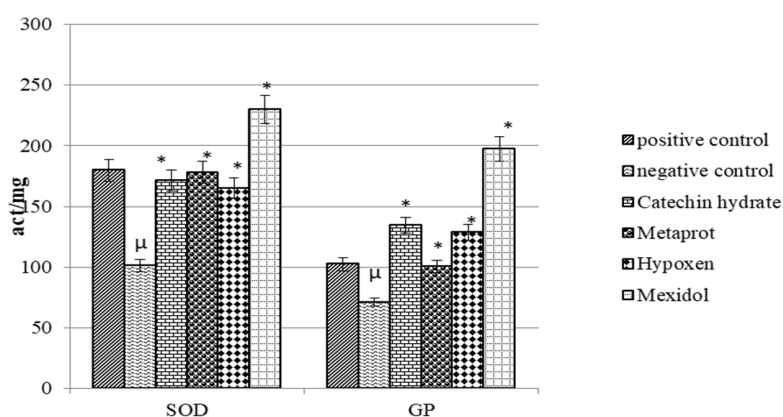


Fig. 3. The effect of the test substance and comparison drugs on the level of SOD and HP in the muscle tissue of mice against the background of physical and psycho-emotional overloads.

$\mu$  – Statistically significant relative to the group of positive control mice  $p < 0.05$  (Mann-Whitney U-test); \* – statistically significant relative to the group of mice of the NK group  $p < 0.05$  (Mann-Whitney U-test)

In the group of mice treated with Metaprot, relative to animals that did not undergo pharmacological correction (NC), there was a significant decrease in the concentration of TBA-active products and DC by

29.2% ( $p < 0.05$ ), and 37.8% ( $p < 0.05$ ) (Fig. 2). The activity of AOD enzymes, on the contrary, was higher: SOD - 76.1% ( $p < 0.05$ ), catalase by 114.5% ( $p < 0.05$ ), GP - 40.8% ( $p < 0.05$ ).

Against the background of the introduction of the drug Hypoxen to experimental animals, an increase in the activity of antioxidant protection was observed, which was reflected in a significant increase in SOD, catalase and HP, respectively, by 63.2% ( $p < 0.05$ ), 142.1% ( $p < 0.05$ ), 80.4% ( $p < 0.05$ ), in relation to the NK group. In turn, while reducing the level of MDA and DC by 47.3% ( $p < 0.05$ ) and 41.6% ( $p < 0.05$ ), respectively, in comparison with the group of NC mice.

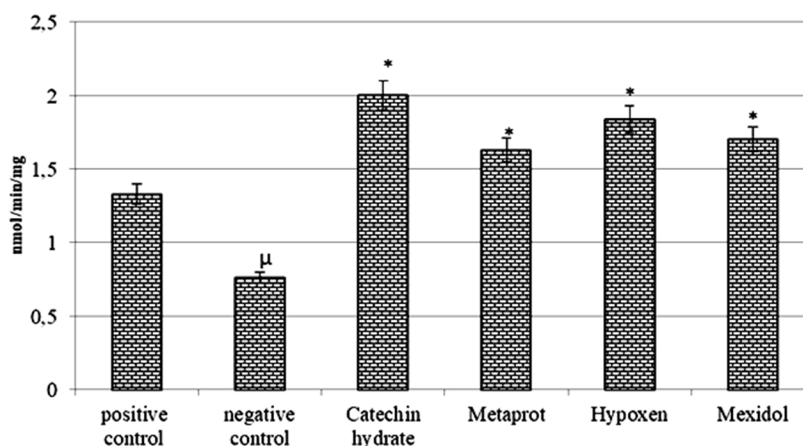


Fig. 4. The effect of the test substance and comparison drugs on the level of catalase in the muscle tissue of mice against the background of physical and psycho-emotional overloads.

μ – Statistically significant relative to the group of positive control mice  $p < 0.05$  (Mann-Whitney U-test); \* – statistically significant relative to the group of mice of the NK group  $p < 0.05$  (Mann-Whitney U-test)

The course use of Mexidol made it possible to correct the disturbance in the «pro / antioxidants» system that arose against the background of daily physical exertion. As a result of the experiment, it was found that the reference drug Mexidol had a positive effect on the state of endogenous enzymes AOD (SOD, catalase, GP), while increasing their content in relation to the group of negative control animals by 127.4% ( $p < 0.05$ ), 123.7% ( $p < 0.05$ ) and 176.9% ( $p < 0.05$ ), respectively. Compared with the NK group in animals treated with Mexidol, against the background of daily physical activity, there was a decrease in the concentration of malondialdehyde by 72.3% ( $p < 0.05$ ) and diene conjugates by 39.4% ( $p < 0.05$ ).

## Conclusion

Thus, repeated preventive use of the substance catechin hydrate at a dosage of 100 mg / kg showed that its administration helps to prevent muscle fatigue, which is accompanied by an increase in the physical endurance of mice. At the same time, this substance is able to eliminate the imbalance between pro / and antioxidants, increasing the activity of the latter.

It can be assumed that this substance has not only actoprotective, but also antioxidant activities and is of great interest for further research in certain fields of medicine.

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