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# STUDY OF POSSIBLE MECHANISMS OF ACTOPROTECTIVE ACTION OF CATECHIN HYDRATE

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**ABSTRACT** — A study was carried out to study the possible mechanisms of actoprotective action of catechin hydrate administered at a dosage of 100 mg/kg. The animals were subjected to daily exhausting loads in the forced swimming test with a load of 10% of the animal's weight. After the end of the experiment, the rats were decapitated under chloral hydrate anesthesia (350 mg/kg) and the skeletal muscle was taken to obtain the supernatant. Using the ELISA method, the concentrations of nitric oxide isoforms (eNOS, iNOS, nNOS), PPAR (peroxisome proliferator-activated receptors) and JNK were estimated.

It was found that against the background of the introduction of the test substance, an increase in eNOS activity was observed by 48.7% ( $p < 0.05$ ) relative to the group of negative control rats, as well as a decrease in iNOS and JNK — 1.9 times ( $p < 0.05$ ). In comparison with the group receiving Metaprot<sup>®</sup>, the concentration of endothelial synthase in the group receiving catechin hydrate was 1.3 times higher ( $p < 0.05$ ).

The experiment performed suggests that the actoprotective effect of catechin hydrate is likely to be associated with inhibition of pathways mediated by JNK, activation of PPAR receptors, and the effect of these compounds on NO isoforms.

**KEYWORDS** — actoprotectors, mechanism of action, rats, catechin hydrate.

## INTRODUCTION

Catechin is flavan-3-ol, a flavan derivative with four phenolic hydroxyl groups, which exhibits a wide range of pharmacological activities, including: antioxidant, antihypoxic, antibacterial [1, 2]. It is also worth noting that this compound is capable of increasing physical performance against the background of exhausting physical exertion [3]. In view of its poly-functional action, this compound can be classified as a group of actoprotectors.

Physical activity in natural conditions, and sometimes even exhausting physical activity, which athletes

are often exposed to, have an effect on all systems of the body, including skeletal muscles. It is known that the symptomatology of musculoskeletal fatigue combines factors that limit the activity of skeletal muscles, first of all: a decrease in the level of blood flow in skeletal muscles and the intensity of metabolic processes in muscle tissue. In this case, the leading role in maintaining the proper level of muscle blood flow is assigned to the vascular endothelium [4]. It has been established that endothelial dysfunction developing during physical exertion is associated with a decrease in the catalytic activity of endothelial nitric oxide synthase (eNOS), one of the key endothelial enzymes that provides the physiological secretion of nitric oxide (NO). In addition, it is worth noting that optimal blood flow in skeletal muscle is influenced by compounds that affect PPARs (Peroxisome Proliferator Activated). This type of receptor belongs to the superfamily nuclear glycoproteins activated by ligand transcription factors, of synthetic or endogenous origin.

Along with oxidative stress, apoptosis is also one of the main pathogenetic mechanisms of destruction of skeletal muscle myocytes under conditions of intense muscle work, and its modulation is a new promising direction for restoring the functional activity of striated muscles. According to recent studies, c-Jun-terminal kinase (JNK) may be a potential target for regulating apoptotic processes [5].

Thus, based on the available literature data, it can be assumed that by acting on specific pathological targets, it will be possible to reduce the manifestations of muscle dysfunction.

### Objective:

to study the possible mechanisms of the actoprotective action of catechin hydrate.

## MATERIALS AND METHODS

The experiment was carried out in accordance with the "Guidelines for the conduct of preclinical studies of drugs, ed. A.N.Mironov (2012 ed.) [6]. The animals were kept in the vivarium of the PMFI-branch of the Volgograd State Medical University (Volgograd, Russia). The study was carried out on male Wistar rats weighing 220–230 g. All animals were divided into 4 experimental groups. During the experiment, the rats

were kept in standard vivarium conditions (natural mode of light change, temperature, relative humidity, standard diet of laboratory animals, weekly change of bedding and cages, fixed time of feeding and drinking) in compliance with the International Recommendations of the European Convention for the Protection of Vertebrate Animals used in experimental studies. The animals were subjected to daily exhausting loads in the Forced Swimming test with a load of 10% of the animal's weight [6]. Positive control (PC), the first group of animals (rats swam with days of rest), the second — negative control (NC) group of untreated animals (0.9% sodium chloride solution in an equivalent volume), the third — received the studied substance catechin hydrate at a dosage of 100 mg/kg [7], the fourth group received actoprotector Metaprot® at a dosage of 25 mg/kg [8].

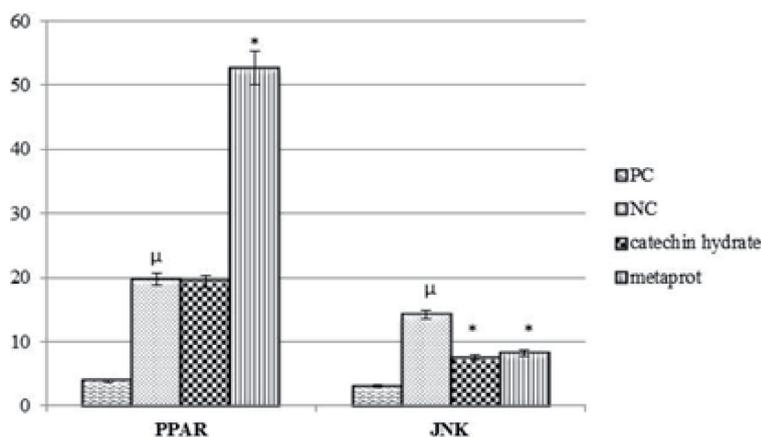
The substance catechin hydrate, as well as the reference drug, were administered per os for 10 days 60 minutes before the expected load. After the end of the experiment, the animals were decapitated under chloral hydrate anesthesia (350 mg/kg) and the skeletal muscle was taken to obtain the supernatant. In the obtained biomaterial, using the ELISA method on a microplate reader (Tecan Infinite F50, Austria), the concentrations of nitric oxide isoforms (eNOS, iNOS, nNOS), PPAR (peroxisome proliferator-activated receptors) and JNK (c-Jun-terminal kinase) were estimated. The results were processed using the STATISTICA 6.0 software.

## RESULTS

During the experiment, it was found that in the group of rats without pharmacological support (NC), after prolonged depleting loads, there was a decrease in the concentration of (eNOS) — endothelial synthase relative to the PK group by 53% ( $p < 0,05$ ), while the concentration of inducible NOS was 2,7 times higher in comparison with the same group ( $p < 0,05$ ). Relative to the PK group, a significant increase in the concentration of PPAR and JNK was found 4,6 and 5,1 times (Fig. 1).

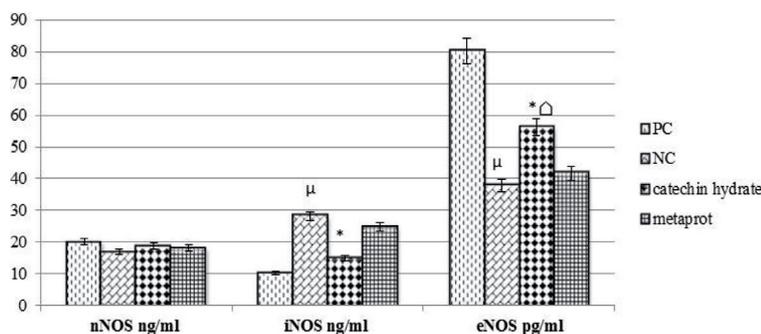
The course use of catechin hydrate led to an increase in the concentration of eNOS — by 48,7% ( $p < 0,05$ ) and a decrease in iNOS, as well as JNK — by 1,9 times ( $p < 0,05$ ) (Fig. 2). However, it should be noted that the concentration of endothelial NOS was 1,3 times higher than that of the group receiving Metaprot® at a dosage of 25 mg/kg ( $p < 0,05$ ). The PPAR concentration index did not differ significantly from the group of NC rats.

The introduction of Metaprot® promoted an increase in PPAR concentration by 2,7 times ( $p < 0,05$ ) and a decrease in JNK by 1,7 times ( $p < 0,05$ ) in relation to the group of NK animals. Against the back-



**Fig. 1.** Changes in the concentration of PPAR and JNK against the background of the introduction of the studied substance catechin hydrate in the supernatant of the skeletal muscle of rats exposed to exhausting physical exertion

*Note:*  $\mu$  — statistically significant relative to the group of animals PK (Student's *t*-test,  $p < 0,05$ ); \* — statistically significant relative to the group of NC animals (Student's *t*-test,  $p < 0,05$ )



**Fig. 2.** Changes in the concentration of NOS isoforms against the background of the introduction of the studied substance catechin hydrate in the supernatant of the skeletal muscle of rats subjected to exhausting physical exertion

*Note:*  $\mu$  — statistically significant relative to the group of animals PK (Student's *t*-test,  $p < 0,05$ ); \* — statistically significant relative to the group of NC animals (Student's *t*-test,  $p < 0,05$ );  $\hat{\mu}$  — statistically significant relative to the group receiving Metaprot®

ground of administration of the drug Metaprot®, there was no significant effect on the content of nitrogen monoxide isoenzymes in comparison with the group of NK rats.

It should be noted that neither the use of catechin hydrate nor Metaprot® led to a significant change in the expression of the isoenzyme of neuronal synthase (nNOS).

## CONCLUSION

The use of catechin hydrate increased the catalytic activity of eNOS by 48,7% ( $p < 0,05$ ) and 1,3 times ( $p < 0,05$ ), relative to the group of NK rats and the group receiving Metaprot, respectively. Against the background of oral intake of the test substance, a decrease in the concentration of JNK of 64,3% ( $p < 0,05$ ) was noted relative to the group of NK animals. As a result of the experiment, it can be assumed that the actoprotective effect of catechin hydrate may possibly be associated with inhibition of pathways mediated by JNK, activation of PPAR receptors, and the effect of these compounds on NO isoforms.

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