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Research Article

Study of dose-dependent actoprotective effect of ATACL on physical performancend psychoemotional status of animals under exhausting exercise

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Abstract

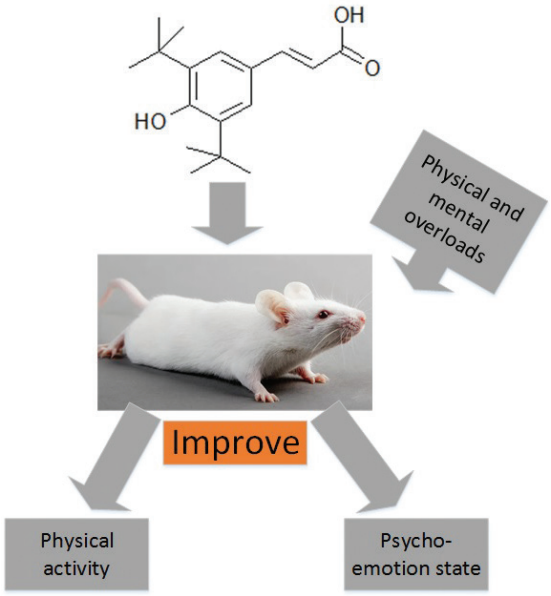
Introduction: The aim of the study was to investigate the dose-dependent actoprotective effect of ATACL on physical performance and psychoemotional status of animals under conditions of exhausting exercise.

Materials and methods: Outbred male mice (23–25 g) were used in the experiment. The test compound in various dosages, as well as the reference drug, were administered intragastrically 60 minutes before the forced swimming test for 10 days of the experiment. At the end of the physical activity, the psychoemotional status of the animals was assessed in the Open Field (OF) and Elevated plus maze (EPM) tests.

Results and discussion: In the course of the experiment, it was found that under conditions of exhausting physical execise, a smooth increase in performance was observed in the group that had received the test compound 4-hydroxy-3,5-di-tert butyl cinnamic acid (ATACL) at a dosage of 100 mg/kg for 10 days. The peak of performance was recorded on the 8th day, which was 47.3% ($p<0.05$) higher than the physical activity of the mice treated with the reference drug ethylthiobenzimidazole hydrobromide (EBH). When assessing changes in the Open Field test, it was found that the test compound ATACL at a dosage of 100 mg/kg is also a leader in stabilizing the psychoemotional status of the animals, which is reflected in the improvement of the motor activity (the number of sectors crossed by 4.7 times ($p< 0.05$)), exploratory activity (an increase in the number of «peeps» and rearings by 8.5 times ($p<0.05$) and 12.7 times ($p<0.05$), respectively) and changes in the level of anxiety (a 2.5-time decrease in the number of short-term grooming acts ($p<0.05$)) in comparison with the negative control (NC) group. The results obtained in the EPM test are completely consistent with the results of the OF test; the most pronounced activity was observed for the ATACL compound at a dosage of 100 mg/kg.

Conclusion: Based on the combination of reproducible methods, it can be concluded that the most pronounced actoprotective effect is exerted by the compound at a dosage of 100 mg/kg, not inferior, at the same time, to the reference drug EBH.

Graphical abstract:



Keywords

actoprotectors, fatigue, cinnamic acids, ethylthiobenzimidazole hydrobromide, mice, physical activity, psychoemotional stresspyrroloquinolones.

Introduction

Fatigue is a disabling, multifaceted symptom that is widespread and persists in all areas of life. In medical terminology, fatigue is an early onset of lassitude after the start of an activity; it is a feeling of exhaustion or difficulty in performing physical or intellectual activity without recovery after a period of rest (Avellaneda et al. 2009; Frone and Tidwell 2015; Hendrickse et al. 2020). Fatigue can manifest itself in a temporary decrease in performance, changes in the psychoemotional background, expressed in a decrease in cognitive and mnestic functions of the body, metabolic disorders, etc. (Voronkov et al. 2012). This symptom negatively affects many spheres of life, such as: sports, aviation, space, and extreme medicine (Yakovlev 2011; Kupko et al. 2014; Savilov 2014). Of course, a properly selected pharmacological support is of great importance in this aspect. In order to maintain an optimal level of physical and mental activities, medicines of various pharmacotherapeutic groups are used, including psychostimulants, adaptogens and nootropics. In this series, the medicines with an actoprotective effect, which have a complex effect on the human body, are particularly distinguished (Bolotova et al. 2020; Bugaeva et al. 2020; Belinskaia et al. 2021). According to the existing definition, actoprotectors are substances of various structures and origin (synthetic or natural) that increase physical and mental performance without increasing oxygen consumption. It follows from the definition that actoprotectors normalize energy production and energy consumption in favor of more economical consumption of macroergs, which distinguishes these compounds from typical psychostimulants. The type of the effect of actoprotectors also determines the time of the onset of the optimal therapeutic effect, which in most cases is significantly lower than that of adaptogens and nootropics (Bihdan 2020). Of undoubted scientific interest is the study of substances of natural origin that exhibit a high level of therapeutic efficacy with relatively low systemic toxicity. One of these groups of compounds are cinnamic acid derivatives, which can be potentially effective actoprotectors (Sathyapalan et al. 2010; Su et al. 2014).

Materials and methods

Laboratory animals

In the experiment, outbred male mice (23–25 g) were used, which were kept in a vivarium of Pyatigorsk Medical and Pharmaceutical Institute (PMPI), a branch of Volgograd State Medical University and obtained from Rappolovo Nursery (St. Petersburg, Russia). The manipulations carried out on animals were in accordance with the international ethical standards presented in ARRIVE 2.0 guideline. During the experiment, the mice were kept under standard vivarium conditions (humidity of 65±5%, temperature of + 22±2 °C).

Chemical substances

In this work, the actoprotective properties of 4-hydroxy-3,5-di-tert butyl cinnamic acid (laboratory code – ATACL) were studied in comparison with ethylthiobenzimidazole hydrobromide (Metaprot, capsule, ZAO Pharmproekt, Russia). The test compound (ATACL) was synthesized at the Department of Organic Chemistry on the basis of the PMPI-branch of Volgograd State Medical University under the leadership of Professor Eduard T. Oganesyan. The structure of ATACL compound was confirmed by NMR-spectroscopy.

Study design

Before the start of the experiment, the animals were randomized according to the indicator "swimming time" in the Forced-swimming-with-a-load test (Voronkov et al. 2015) and behavioral activity in the Open field (OF) tests (Polkovnikova et al. 2011) and the Elevated plus maze (EPM) test (Sestakova et al. 2013). Based on the results of randomization, six groups were formed. The first group – positive control (PC) (when swimming was carried out, there were days of rest, n=30). The second group is negative control (NC). The third group received the reference drug ethylthiobenzimidazole hydrobromide (EBH) (25 mg/kg, n=10) (Voronkov et al. 2015) in the suspension form. The fourth, fifth and sixth groups received the investigated cinnamic acid derivative under the laboratory code ATACL at dosages of 50 mg/kg, 100 mg/kg and 200 mg/kg, respectively. The test compound, as well as the reference drug, were administered intragastrically 60 minutes before the forced swimming test for 10 days of the experiment. At the end of the physical exertion to which the mice were subjected, the psychoemotional status of the animals was assessed in the OF («Open science», Russia) and EPM («Open science», Russia) tests.

Physical overloads model - forced swimming with a load

Forced swimming was carried out in an device consisting of an acrylic cylinder 30 cm high and 10 cm in diameter, with desaturated water at a temperature of 15 °C. A load equal to 20% of animal's body weight was attached to the mice tail, after which the animal was placed in the cylinder. Previously, all the animals were randomized by swimming time. The test extracts were administered daily 1 hour before the physical overloads modeling. Swimming was carried out until the animals got exhausted and gave up struggling for life (the animal was at the bottom of the pool for 7 seconds). The swimming time of the mice in seconds was recorded

Open field (OF) test (OF)

An open field is a round arena with a diameter of 97 cm. with a wall height of 42 cm. and 13 holes (diameter of each 2 cm). The animal was placed in the center of the arena; the testing time was 3 min, while the recorded parameters were the following: time spent in the central sector, the number of crossed sectors, the number of defecations, the number of urinations, the number of grooming episodes, the number of rearings, and the number of peeps.

Elevated plus maze test

The elevated plus maze labyrinth is a 4-sleeve system, where 2 sleeves are open, and 2 are closed with a wall height of 30 cm. The dimensions of the sleeves (50 cm × 14 cm) allow the animal to move freely. During testing, the animal was placed in the center of the installation with its tail to the closed sleeve; the testing time was 3 minutes. The recorded parameters were the following: time spent in closed and open sleeves, time spent in the central sector, the number of grooming episodes, the number of rearings and and the number of overhangs

Statistical analysis

The obtained experimental data were statistically processed using the STATISTICA 6.0 software (StatSoft, Inc., USA for the Windows operating system) and Microsoft Excel 2010. The mean and its standard error (M±m) were calculated. The results obtained were checked for normal distribution using the Shapiro-Wilk test. The Student's t-test was used to assess the mean values under normal distribution. Otherwise, when the distribution was abnormal, the nonparametric Mann-Whitney U-test was used. Multiple comparison methods were Newman-Keuls parametric test, and Kruskal-Wallis nonparametric test.

Results and discussion

The performance of the PC group did not change throughout the experiment, which confirms the preliminary randomization of the mice.

In the group of animals that had not received any pharmacological support, the duration of swimming increased from the first day of the experiment, but insignificantly. The peak of the performance fell on the 4th experimental day and reached 164.6 ± 26.4 sec. By the end of the experiment, the duration of mice swimming was 2.4 times lower ($p<0.05$) relative to the 1st swimming day of the mice in this group and 2.3 times lower ($p<0.05$) than on the final day of the experiment of the PC group. The data obtained can probably indicate the development of fatigue in the animals (Voronkov et al. 2015).

The introduction of the reference drug EBH promoted a smooth increase in the performance. High physical activity was observed in the animals on the 7th day of swimming with a load (peak); these indicators were higher both relative to the peak day of the NC group (127.8%, $p<0.05$)) and in comparison with the initial swimming time of this group (181.5%, $p<0.05$). The

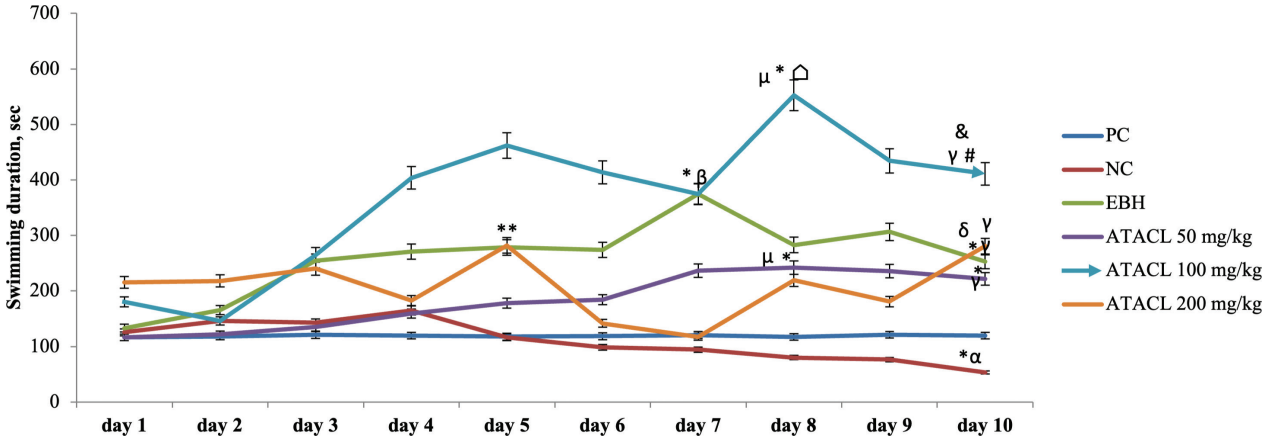


Figure 1. The effect of different doses of the test compound ATACL on the physical activity of experimental animals, reproduced in the Forced swimming test. **Note: PC –** positive control (PC); NC – negative control; EBH – ethylthiobenzimidazole hydrobromide; ATACL – 4-hydroxy-3,5-di-tert butyl cinnamic acid; * – statistically significant relative to the initial swimming time of this group ($p<0.05$, Newman-Keusl test); α – statistically significant relative to the final swimming day of animals of the PC group ($p<0.05$, Newman-Keusl test); β – statistically significant relative to the peak swimming day of the NC group ($p<0.05$, Newman-Keusl test); γ – statistically significant relative to the final swimming day of the animals of the NC group ($p<0.05$, Newman-Keusl test); μ – statistically significant relative to the peak swimming day of the group receiving EBH ($p<0.05$, Newman-Keusl test); ⌂ – statistically significant relative to the peak swimming day of the group receiving ATACL at a dosage of 50 mg/kg ($p<0.05$, Newman-Keusl test); # – statistically significant relative to the final swimming day of the group receiving EBH ($p<0.05$, Newman-Keusl test); & – statistically significant relative to the end day of the group receiving ATACL at a dosage of 50 mg/kg ($p<0.05$, Newman-Keusl test); ** – statistically significant relative to the peak swimming day of the group receiving ATACL at a dosage of 100 mg/kg ($p<0.05$, Newman-Keusl test); δ – statistically significant relative to the end day of the group receiving ATACL at a dosage of 100 mg/kg ($p<0.05$, Newman-Keusl test).

course administration of the reference drug led to the fact that by the 10th experimental day the performance of the animals of this group was 1.9 times higher compared with the initial swimming time ($p<0.05$) and 4.8 times higher ($p<0.05$) in comparison with the same day of the group of NC mice (Fig. 1).

Against the background of the introduction of the test compound under the laboratory code ATACL at a dosage of 50 mg/kg, a linear increase in performance was observed, while the peak of activity was observed on the 8th day, which was 90.5% higher ($p<0.05$) relative to the initial swimming time of this group. It is worth noting that the peak day performace of the group receiving ATACL was 35.5% lower ($p<0.05$) when compared to that of the group receiving the reference drug. The performance of the mice on the final day of the experiment was 1.9 times higher ($p<0.05$) in comparison with the initial swimming time of this group, and 4.2 times higher ($p<0.05$) relative to the indicator of the 10th day of the experiment in the NC animals , and was not inferior to the same indicators in the group of the mice treated with EBH.

Under the conditions of exhausting physical exercises, a gradual increase in performance was observed in the group that had been receiving the test compound ATACL at a dosage of 100 mg/kg for 10 days. The peak of the performace was recorded on the 8th day, which was 47.3% ($p<0.05$) higher than the physical activity of the mice that had been receiving the reference drug, 206.8% ($p<0.05$) higher than the initial indicator of this group, and 128.6% ($p<0.05$) higher than that on the peak day of the group that had been receiving ATACL at a dosage of 50 mg/kg. On the 10th day of the experiment, the physical activity of the animals that had received the test compound ATACL at a dose of 100 mg / kg was 7.7-time ($p<0.05$), 1.6-time ($p<0.05$) and 1.9-time ($p<0.05$), respectively, higher than that on the end day of the experiment of the NC groups, as well as of the groups which had been receiving EBH and ATACL at a dose of 50 mg/kg (Fig. 1).

In the mice that had been injected with ATACL at a dosage of 200 mg/kg, the maximum efficiency was observed on the 5th day of swimming with a load, which was 282±31.4 sec. It should be noted that the peak of activity was significantly lower than this indicator of the group that had received ATACL at a dosage of 100 mg/kg by 50% ($p<0.05$). Throughout the experiment, the swimming level of the mice did not undergo significant changes. The swimming time by the end of the experiment was 5.3 times higher ($p<0.05$) than the the indicator of the 10th day of swimming of the NC group, but 1.5 times ($p<0.05$) lower than in the group that had received ATACL at a dosage of 100 mg/kg, not inferior to the groups that had received EBH and ATACL at a dosage of 50 mg/kg.

It should be noted that the group of NC animals in the Open Field test, after exhausting physical exercise, showed a deterioration in the psychoemotional state, which was reflected in a decrease in the number of crossed sectors, the number of «peeps, rearings and an increase in the number of grooming acts in comparison with the PC group of animals.

The introduction of the reference drug to the group of animals led to an increase in motor activity (the number

of crossed sectors) and exploratory activity (the number of «peeps», the number of rearings) by 4.8 times ($p<0.05$), 3.7 times ($p<0.05$) and 11 times ($p<0.05$), respectively, relative to the group of NC mice (Fig. 2). EBH has a beneficial effect on the psychoemotional background under conditions of prolonged exhausting exercise, which is confirmed by a 2.1-time decrease ($p<0.05$) in acts of short-term grooming in relation to the indicators of the NC group.

An improvement in the locomotor activity was also observed with the course a of the test compound ATACL at a dosage of 50 mg/kg relative to the group which had not received any pharmacological support, which was reflected in a 4-time increase in the number of sectors crossed ($p<0.05$).

Attention is drawn to the fact that the number of «peepsing» and rearings was significantly higher – by 3.7 times ($p<0.05$) and 4.9 times ($p<0.05$), respectively – in relation to the NC group. There were no statistically significant differences in terms of grooming in the groups receiving ATACL at a dosage of 50 mg/kg and in the NC group.

In relation to the group of NC animals in the group of mice that had been injected with ATACL at a dosage of 100 mg/kg, an improvement in the locomotor activity was observed, as evidenced by a 4.7-time increase in the number of sectors crossed ($p<0.05$), an improvement in the exploratory activity, which was reflected in the increase in the number of «peeps» and in the number of rearings – by 8.5 times and 12.7 times ($p<0.05$), respectively. In the group that had received ATACL at a dosage of 100 mg/kg, there was a significant 2.5-time decrease ($p<0.05$) in the number of acts of short-term grooming, one of the indicators of anxiety, in comparison with the group of NC mice (Fig. 3). According to the indicators of the locomotor and exploratory activities, the studied compound at the administered dosage of 100 mg/kg was not inferior to the reference drug.

The use of the test compound at a dosage of 200 mg/kg promoted an increase in the number of sectors crossed (by 4.3 times ($p<0.05$)), the number of peeps (by 4.4 times ($p<0.05$)) and rearings (by 7.4 times ($p<0.05$)) (Fig. 2.) when compared to the group of mice that had not received any pharmacological support. The rest of the studied parameters did not change statistically significantly in relation to those in the group of NC mice.

A comparative evaluation of the ATACL compound in the studied dosages of 50 mg/kg, 100 mg/kg and 200 mg/kg showed that the most significant changes were observed with the course administration of ATACL at a dosage of 100 mg/kg. This fact is confirmed by an increase in the exploratory activity, which is reflected in an increase in the number of «peeps» relative to that in the groups which had received ATACL at dosages of 50 and 200 mg/kg – by 2.3 times ($p<0.05$) and 1.9 times ($p<0.05$), respectively; the number of rearings – by 2.6 times ($p<0.05$) and 1.7 times ($p<0.05$), respectively, as well as a decrease in the level of anxiety (acts of short-term grooming) by 2 times ($p<0.05$) and 1.8 times ($p<0.05$), respectively (Fig. 3).

Deterioration of the psychoemotional background in the group of NC animals was also observed in the Elevated plus maze test. In the group without any pharmacological correction in comparison with the PC group, there was a decrease in the number of rearings – by 3.4 times ($p<0.05$), the number of overhangs – by 3.1 times ($p<0.05$), the number of crossings – by 1.7 times ($p<0.05$), respectively. It is also worth noting that the level of anxiety in rodents was higher, which was confirmed by a more frequent (1.1 times ($p<0.05$)) presence of the mice in the dark arms of the plus maze more than in light ones (2 times ($p<0.05$)) in relation to that in the PC group. In addition, there was also a 14-time increase in the number of boluses, in comparison with the PC group($p<0.05$) and a 2.5-time increase in acts of short-term grooming($p<0.05$).

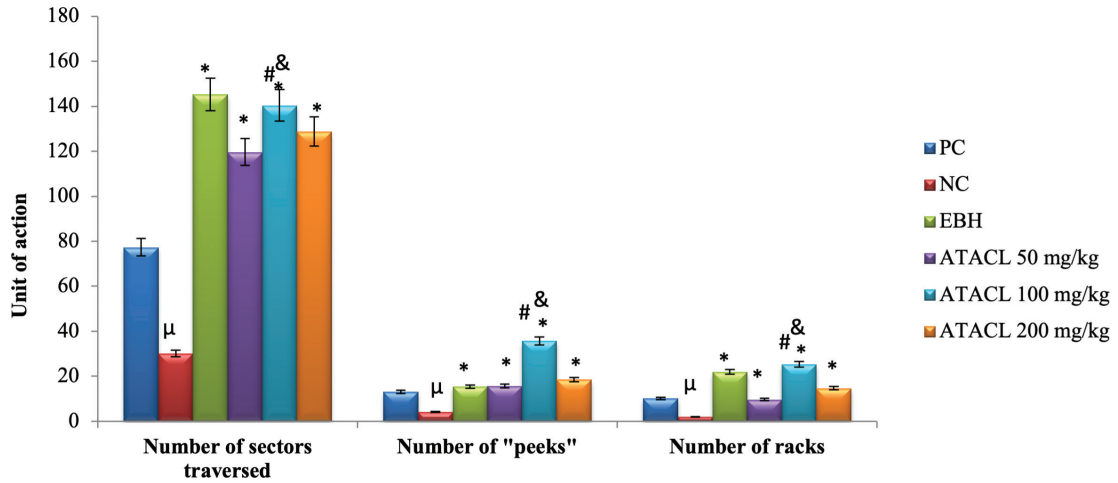


Figure 2. Influence of different doses of the test compound ATACL on the motor and orientation-exploratory activities of the animals in the Open field test. **Note: PC** – positive control (PC); NC – negative control; EBH – ethylthiobenzimidazole hydrobromide; ATACL – 4-hydroxy-3,5-di-tert butyl cinnamic acid; μ – statistically significant relative to the group of PC animals ($p<0.05$, Newman-Keusl test); * – statistically significant relative to the group of NC animals ($p<0.05$, Newman-Keusl test); & – statistically significant relative to the group receiving ATACL at a dosage of 50 mg/kg ($p<0.05$, Newman-Keusl test); # – statistically significant relative to the group receiving ATACL at a dosage of 200 mg/kg ($p<0.05$, Newman-Keusl test).

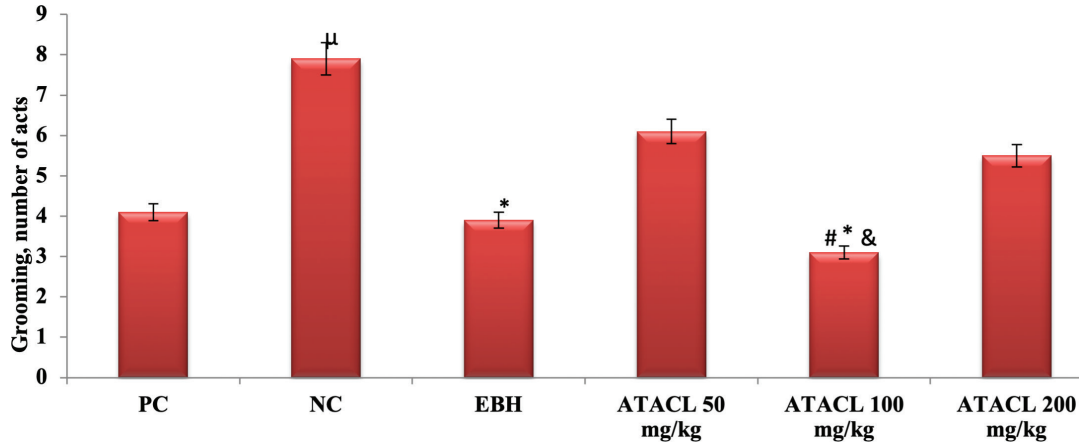


Figure 3. Influence of different doses of the test compound ATACL on the level of anxiety in animals in the Open field test. **Note: PC –** positive control (PC); NC – negative control; EBH – ethylthiobenzimidazole hydrobromide; ATACL – 4-hydroxy-3,5-di-tert butyl cinnamic acid; μ – statistically significant relative to the group of PC animals ($p<0.05$, Newman-Keusl test); * – statistically significant relative to the group of NC animals ($p<0.05$, Newman-Keusl test); & – statistically significant relative to the group receiving ATACL at a dosage of 50 mg/kg ($p<0.05$, Newman-Keusl test); # – statistically significant relative to the group receiving ATACL at a dosage of 200 mg/kg ($p<0.05$, Newman-Keusl test).

Against the background of intragastric use of EBH, there was observed an increase in exploratory activity – by 7.4 times ($p<0.05$) (rearings) and by 5.6 times ($p<0.05$) (overhangs); and an increase in the motor activity – by 2 times ($p<0.05$) (number of crossings). The administration of the reference drug contributed not only to an increase in the time spent by the animals in the open arms of the maze, which was 3.1 times higher than this indicator in the negative control group ($p<0.05$), but also to a decrease in the number of grooming acts by 1.4 times ($p<0.05$). In turn, the time of visiting the closed sleeves of the EPM was 1.7 times lower ($p<0.05$), which, together with the data obtained from the OP test, confirms the positive effect of the reference drug on the psychoemotional status of the animals subjected to physical exercise.

An improvement in the exploratory activity (rearings, overhangs) was observed against the background of the course administration of the test compound at a dosage of 50 mg/kg, relative to the negative control group – by 2.8 times ($p<0.05$)in both cases), while the time spent in the open arms of the EPM was 2.4 times higher ($p<0.05$) (Fig. 5). There were no statistically significant differences between the groups that had received ATACL at a dosage of 50 mg/kg and the NC group in terms of the locomotor activity (number of crossings), the time spent in closed arms, and grooming.

Against the background of exhausting physical exercise in the group that had received ATACL at a dosage of 100 mg/kg, the psychoemotional background of the experimental animals stabilized. This fact is reflected in an increase in the number of rearings, the number of overhangs, the number of crossings , as well as the time spent in open sleeves – by 9.4 times ($p<0.05$), by 3.8 times ($p<0.05$), by 1.7 times ($p<0.05$), and by 3 times ($p<0.05$) (Fig. 5), respectively, in comparison with those in the NC group. It is worth noting that all the parameters under consideration in the group that had received the test compound at a dosage of 100 mg/kg were not inferior to those in the group which had received EBH. Relative to the negative control group, there was a decrease in anxiety in the mice, which was expressed in a decrease in grooming acts by 2.1 times ($p<0.05$) (Fig. 6).

In the group of animals that had been injected with the test compound ATACL at a dosage of 200 mg/kg for 10 days of the experiment, there was only an increase in the number of rearings, in comparison with that in the NC group by 4.8 times ($p<0.05$) (Fig. 4), as well as the time spent by the animals in the open arms of the maze installation by 3 times ($p<0.05$).

It is interesting to note that the group of animals that had received ATACL at a dosage of 100 mg/kg was superior to the groups treated with the dosages of 50 mg/kg and 200 mg/kg, respectively, in terms of the exploratory activity: rearings – by 3.4 times ($p<0.05$) and by 2 times ($p<0.05$), respectively; overhangs – by 1.3 times ($p<0.05$) and by 2.1 times ($p<0.05$), respectively; in terms of the motor activity: crossings – by 1.9 times and 2 times ($p<0.05$), respectively (Fig. 4). At the same time, the time spent in the light sleeves of the maze was also 1.3 times higher ($p<0.05$) than that in the group which had received ATACL at a dosage of 50 mg/kg, and 1.2 times higher ($p<0.05$) than in the group, which had. received the test compound at a dosage of 200 mg/kg. The level of anxiety in mice, expressed in the number of acts of short-term grooming, was 2.1 ($p<0.05$) and 1.6 times ($p<0.05$) lower, than those in the groups which had received ATACL at dosages of 50 mg/kg and 200 mg/kg, respectively.

Discussion

The study showed that the use of 4-hydroxy-3,5-di-tert-butyl cinnamic acid, mainly at a dose of 100 mg/kg/per os, contributed to the restoration of physical activity in the animals, as well as stabilization of their psychoemotional status in conditions of long-term

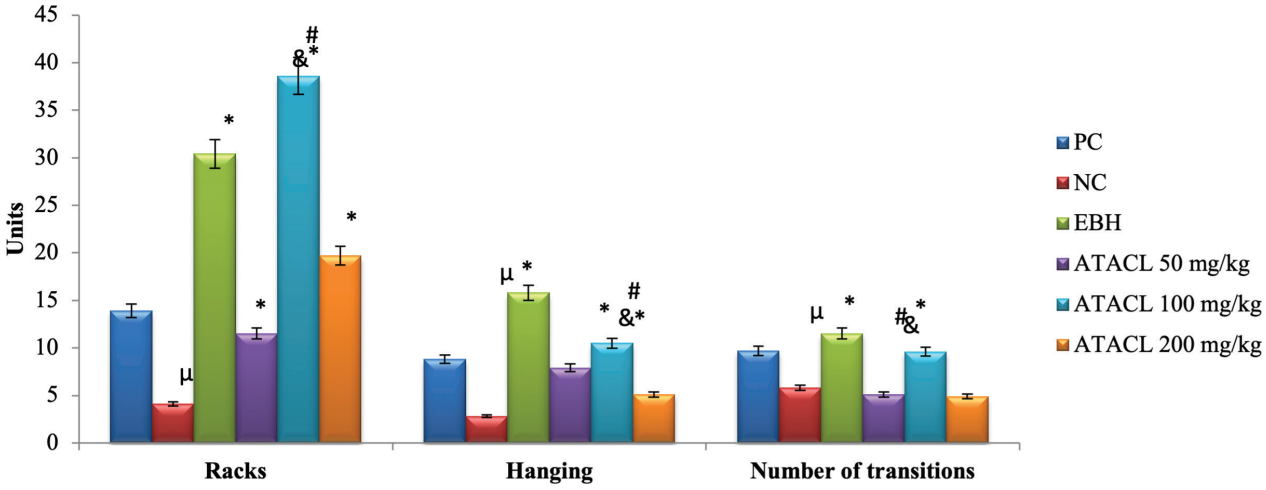


Figure 4. Influence of various doses of the test compound ATACL on the motor and exploratory activity of animals in the Elevated plus maze test. **Note**: **PC –** positive control (PC); NC – negative control; EBH – ethylthiobenzimidazole hydrobromide; ATACL – 4-hydroxy-3,5-di-tert butyl cinnamic acid; μ – statistically significant relative to the group of PC animals ($p<0.05$, Newman-Keusl test); * – statistically significant relative to the group of NC animals ($p<0.05$, Newman-Keusl test); & – statistically significant relative to the group receiving ATACL at a dosage of 50 mg/kg ($p<0.05$, Newman-Keusl test); # – statistically significant relative to the group receiving ATACL at a dosage of 200 mg/kg ($p<0.05$, Newman-Keusl test).

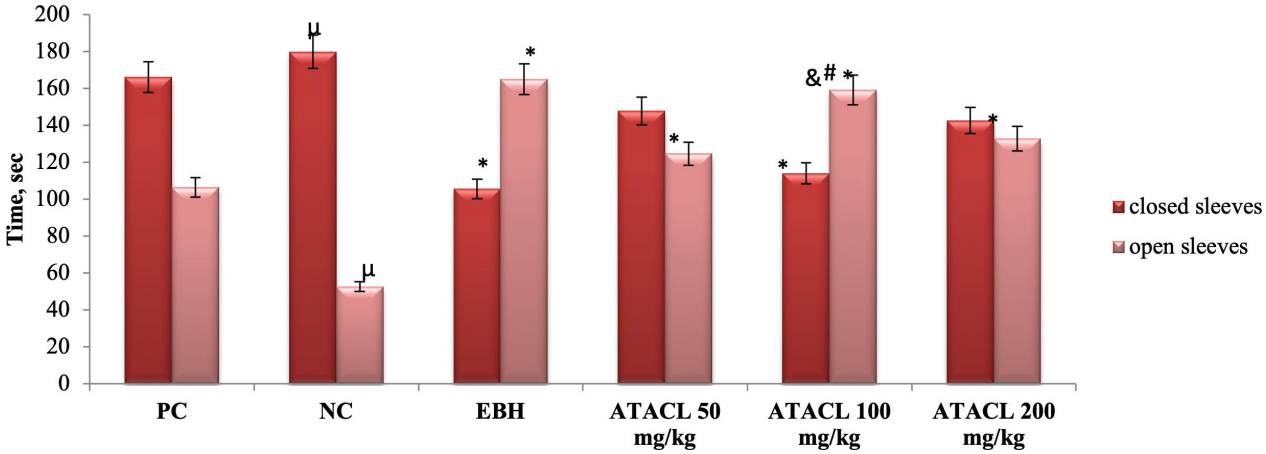


Figure 5. The effect of different doses of the test compound ATACL on the zones of interest of animals in the test Elevated plus maze. **Note**: **PC –** positive control (PC); NC – negative control; EBH – ethylthiobenzimidazole hydrobromide; ATACL – 4-hydroxy-3,5-di-tert butyl cinnamic acid; μ – statistically significant relative to the group of PC animals ($p<0.05$, Newman-Keusl test); * – statistically significant relative to the group of NC animals ($p<0.05$, Newman-Keusl test); & – statistically significant relative to the group receiving ATACL at a dosage of 50 mg/kg ($p<0.05$, Newman-Keusl test); # – statistically significant relative to the group receiving ATACL at a dosage of 200 mg/kg ($p<0.05$, Newman-Keusl test).

combined (causing physical and cognitive imbalance) extreme situation. Such a complex character of the pharmacological activity of 4-hydroxy-3,5-di-tert-butyl cinnamic acid makes it possible to classify this compound as an actoprotective agent (Voronkov et al. 2017). It is known that actoprotectors are compounds of various origins that normalize physical and mental performance under conditions of adverse effects of a destabilizing factor on the human body (Oliynyk and Oh 2012). The important aspects of the mechanism of action of actoprotectors is the normalization of energy production and the optimization of cellular metabolism. It was found that against the background of the use of agents with actoprotective activity, there was an intensification of the processes of glucose resynthesis – gluconeogenesis, which occurs, as a rule, in the liver, and partly in the cortical layer of the kidneys (Rennie and Tipton 2000). Gluconeogenesis is an important mechanism for the energy supply of cellular activity under conditions of deficient oxidation substrates (which is observed during exhaustion of the body), aimed at obtaining the necessary energy pool in the form of high-energy phosphates from the degradation products of carbohydrates and proteins (Fournier et al. 2002; Nisr et al. 2019; Miranda-Silva et al. 2020). At the same time, the enhancement of gluconeogenesis, observed with the use of actoprotectors, ensures the time of onset of the pharmacological effect and its duration (Zarubina and Mironova 2002; Lu et al. 2018). In addition to activating glucose resynthesis processes, the metabolic action of

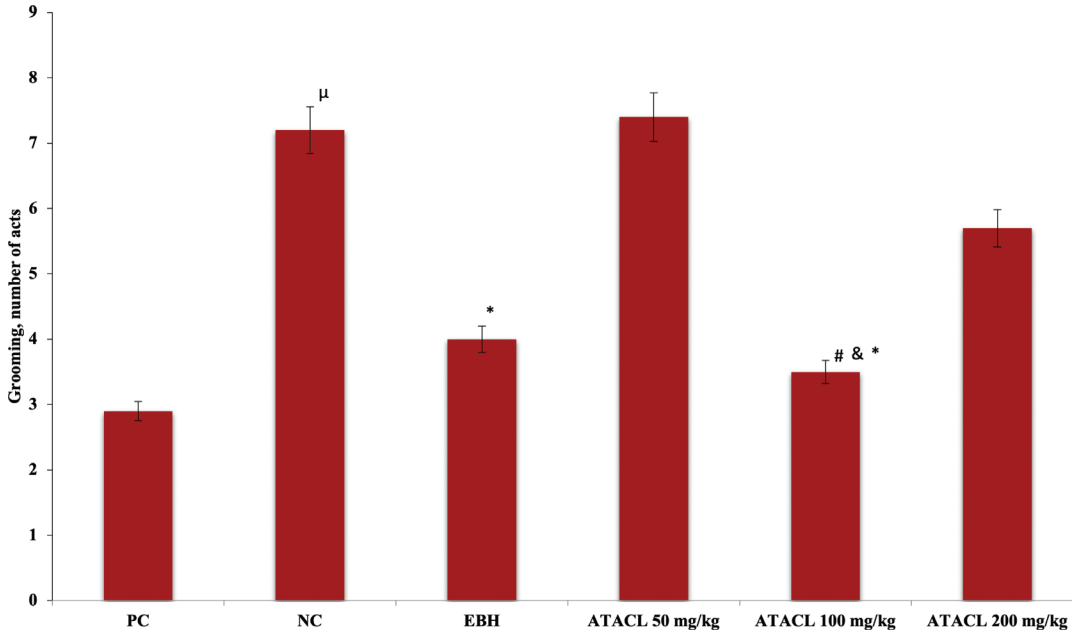


Figure 6. Influence of different doses of the test compound ATACL on the level of anxiety of animals in the test «Elevated plus maze». **Note: PC –** positive control (PC); NC – negative control; EBH – ethylthiobenzimidazole hydrobromide; ATACL – 4-hydroxy-3,5-di-tert butyl cinnamic acid; μ - statistically significant relative to the group of PC animals ($p<0.05$, Newman-Keusl test); * - statistically significant relative to the group of NC animals ($p<0.05$, Newman-Keusl test); & - statistically significant relative to the group receiving ATACL at a dosage of 50 mg/kg ($p<0.05$, Newman-Keusl test); # - statistically significant relative to the group receiving ATACL at a dosage of 200 mg/kg ($p<0.05$, Newman-Keusl test).

actoprotectors is also aimed at increasing the activity of mitochondrial enzymes and restoring mitochondrial protein synthesis, which is expressed in maintaining the energy production due to stabilization of oxidation and phosphorylation reactions, and provides the maximum yield of ATP under conditions of oxygen deficiency (Panossian and Wikman 2010; Nishida et al. 2020). In addition to the metabotropic action, another important aspect of the mechanism of action of actoprotectors is the presence of antioxidant activity (Bloomer and Smith 2009). It is known that the initiation of free-radical peroxide processes under conditions of maximum and submaximal physical activity may be associated with the activation of the sympathoadrenal system in response to an increase in the activity of skeletal muscles. In this case, reactive oxygen species (ROS) can be generated both in the synthesis reactions and in the decay of catecholamines (oxidation of adrenaline to adrenochrome) (Powers et al. 2020). Under conditions of ROS overproduction, intensive oxidation of cellular structures and activation of secondary effector systems (aldolase D, MEK kinase) are observed, which can lead to increased cell destruction and the development of psychophysical dysfunction (Gul et al. 2011; Hanai et al. 2020; Yang et al. 2020). At the same time, against the background of the use of actoprotectors, no inhibition of the formation of free radicals is observed, but their inactivation increases under the action of antioxidant defense enzymes (superoxide dismutase, catalase, glutathione series enzymes), i.e. actoprotectors have an indirect mechanism of antioxidant action aimed at increasing the activity of the endogenous anti-peroxide defense system (Rahal et al. 2014). Similar activities (metabolic and antioxidant effects) have been described for 4-hydroxy-3,5-di-tert-butyl cinnamic acid. For instance, Pozdnyakov et al. (2018) noted that under conditions of cerebral ischemia, the use of ATACL at a dosage of 100 mg/kg contributed to the normalization of pro/antioxidant equilibrium by increasing the activity of superoxide dismutase, catalase and glutathione peroxidase, as well as by suppressing free radical lipid oxidation reactions, which can indicate a decrease in the concentration of diene adducts and TBA-active compounds, when using 4-hydroxy-3,5-di-tert-butyl cinnamic acid. In this case, the metabolic action of the ATACL compound can be aimed at stabilizing the mitochondrial function, which is realized through the normalization of the activity of mitochondrial subcomplexes V (F_1F_0 ATP synthase) and IV (cytochrome c oxidase) (Voronkov et al. 2018).

Conclusion

Summarizing all of the above, it can be noted that the most pronounced effect on the level of performance in mice is exerted by the compound under study at a dosage of 100 mg/kg. It was found that physical performance increased linearly, while the peak activity of the mice was 47.3% higher ($p<0.05$) in comparison with that in the group of animals that had received EBH.

When assessing changes in the Open Field test, it was found that the test compound ATACL at a dosage of 100 mg/kg is also a leader in stabilizing the psychoemotional status of animals, which is reflected in the improvement

of the motor (a 4.7-time increase in the number of sectors crossed ($p<0.05$)), the exploratory activity (an increase in the number of «peeps» and rearings s by 8.5 times ($p<0.05$) and 12.7 times ($p<0.05$), respectively) and changes in the level of anxiety (a 2.5-time decrease in short-term grooming acts ($p<0.05$)) in comparison with those in the NC group.

The results obtained in the EPM test are completely consistent with the results of the OF test; the most pronounced activity was observed for the ATACL compound at a dosage of 100 mg/kg. It should be noted that this compound was not inferior to the reference drug EBH in terms of the exhibited actoprotective effect.

Conflict of interests

The authors declare no conflict of interests.

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