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SUBSTANTIATION OF THE PECULIARITIES OF THE USE OF THE COMPLEX SUPPLEMENT LEVOBOL BY ATHLETES IN ORDER TO INCREASE PHYSICAL ENDURANCE

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Abstract

According to the literature, many plants have the ability to regulate metabolic processes. A promising plant with predicted anabolic activity is Lefzia safflower (Rhaponticum carthamoides), the chemical composition of which is rich in ecdysteroids, proteins, vitamins, micro- and macronutrients Thus, the previously analyzed composition of the dosage form with the main active components ecdysteroids Lefzia safflower and vitamin C became the basis for further preclinical studies.

According to the results of behavioral reactions, the total indicator of all types of activity of animals that received Levobol and Tribulus, significantly increased relative to the same control group by almost 30%, indicating a stimulating effect of drugs on locomotor activity, but not on emotional reactions (in particular the level of anxiety) of experimental animals. According to the results of tests for physical endurance (swimming with load) and the rate of fatigue (forced swimming for a distance), Lebobol revealed powerful anabolic and actoprotective effects, which significantly exceeded the similar properties of the comparison drug Tribulus. Serum biochemical studies confirmed a more pronounced effect of Levobol on protein synthesis: a significant increase in total protein levels by 1.3 times at decrease in urea by 30% relative to control animals compared with Tribulus, which was characterized by only a clear tendency to on these indicators. Preclinical studies of the new drug Levobol based on ecdysteroids Lefzia safflower and vitamin C confirmed the presence of distinct anabolic properties of this drug, which predominate in the comparison of Tribulus, which is the basis for further study of Levobol for clinical practice.

Keywords: *physical endurance*, *anabolic drugs, ecdysteroids*, vitamin C.

Introduction

The current problem in pharmacy and medicine is the search, creation, development and implementation in clinical practice of new safe anabolic drugs of plant origin [3, 26, 5]. This is primarily due to low toxicity, affinity of the chemical structure of biologically active substances that are part of phytopreparations and cells of the human body and the practical absence of side effects in herbal medicines in comparison with steroidal anabolic drugs [4, 1, 18, 17].

According to the literature, many plants have the ability to regulate metabolic processes [20, 22, 23]. A promising plant with predicted anabolic activity is Lefzia safflower (Rhaponticum carthamoides), the chemical composition of which is rich in ecdysteroids, proteins, vitamins, micro- and macronutrients [9, 2]. Ecdysteroids are chemicals of the cyclopentaneperhydro-phenanthrene structure that are able to increase anabolism in muscle tissue and organs, without androgenic effect, do not cause water retention in the body and do not inhibit the production of testosterone in contrast to synthetic and natural male steroids. Ecdysteroids contribute to a positive nitrogen balance and inhibit catabolic processes [19, 16].

The role of vitamin C in the regulation of vital metabolic reactions in the body is well known [12, 15, 8, 25], and its combination with ecdysteroids Lefzia safflower in one dosage form makes it possible to predict the receipt of an effective drug of natural origin with pronounced anabolic activity with minimal side effects.

Thus, the previously analyzed composition of the dosage form with the main active components ecdysteroids Lefzia safflower and vitamin C became the basis for further preclinical studies.

Material and Methods

Levobol, capsules for oral use. Comparison drug: Tribulus, capsules for oral use.

Aim: method of determining the anabolic and metabolitotropic properties of Levobol and comparing its activity with the drug Tribulus.

During the experimental study, the anabolic properties of drugs were determined in models of swimming with load to exhaustion, forced swimming, effects on coordination of movements, muscle tone, behavior by use of conventional tests, effects on total weight and muscle mass of animals, protein content in the calf muscle and heart, as well as protein, lipid and carbohydrate metabolism [6, 21].

The experimental work was performed on 18 white randomized male mice and 18 white nonlinear male rats. Animals were kept in standard conditions in accordance with sanitary and hygienic norms and principles of the European Convention for the Protection of Laboratory Animals (Strasbourg, 1986). During the experiment, animals were kept in the vivarium at t =19-24°C, humidity not exceeded 50%, with natural light regime "day and night" in plastic cages on a standard diet with free access to water and food. All experiments were performed in accordance with the «General ethical principles of animal experiments».

Euthanasia of rats was performed by dislocation of cervical vertebrae under light chloroform anesthesia.

The following methods and experimental models were used while conducting experimental research: pharmacological, biochemical and medical-statistical.

The main principle in choosing the methods was their maximum informativeness and relevance.

The design of the study is presented in Figure 1.

Aqueous solutions of Levobol and Tribulus capsules were administered intragastrically by gavage at a dose of 170 mg/kg (equivalent to a dose of 1000-1050 mg/day for humans) in a prophylactic mode for 10 days, the last time at 30 minutes before the experiments. Animals in the control group received intragastrically

purified water in a similar volume (0.1 ml per 10 g of body weight).

All experiments to detect the effects of a particular drug in behavioral tests were performed synchronously with the appropriate control, as the effects largely depend on the chronopharmacological factor [7].

The exercise swimming test was used to study the physical endurance of the animals that received the studied drugs. The load (10 or 20% of body weight) was fixed at the root of the tail. Animals were placed in a pool of water at room temperature (22-23 °C). The thickness of the water layer was 60 cm, the height of the sides 15 cm, which did not allow the animals to escape on the pool wall. Recorded the time of swimming to exhaustion, the criterion of which was the immersion of the animal's head under water with the inability to float to the surface for 10 s [14].

The development of fatigue in mice was studied in the test of forced swimming at 150 m for 10 consecutive attempts. The speed was determined by overcoming the distance from the 1st, 5th and 10th attempts [10].

The rotating rod test assessed coordination and skeletal muscle tone. The device consists of a wooden rod with a diameter of 2 cm and a length of 75 cm, divided into 6 sections by plastic discs and raised to a height of 50 cm above the table surface to prevent jumping of animals. Rotation of the mechanism with a constant speed of 10 revolutions per minute is provided by the electric motor which shaft fastens directly to a core. The retention time on the rod was chosen as the evaluation criterion.

Animal muscle tone was also studied under the horizontal bar test. In this experiment, mice were hung with their front paws on a metal wire with a diameter of 1 cm, raised to a height of 30 cm above the table surface. The length of the period during which the animal was able to kept on the crossbar was taken into account [10].

Locomotor activity, behavioral and emotional reactions of animals under the influence of drugs were determined under the conditions of the open field test. The "open field" test is a white square platform measuring 22 × 22 × 15 cm (length × width × height), the floor of which is divided into 16 identical squares (5 × 5 cm) with holes 1.5 cm in diameter in the center of each square. Evaluation criteria are the number of squares crossed, uprights, gaps, fecal bolus, urination and grooming episodes per 3 minutes of observation [10].

Electronic scales were used to determine body weight gain, and calf muscle mass was determined by use of torsion scales. The mass coefficient was determined by the formula:

$KM = (MM/MT) \times 100\%$,

KM – coefficient of mass, %; MM – muscle mass, g; MT – body weight of the animal, g. This allowed us to assess muscle growth under the influence of drugs.

Calf muscle and heart of euthanized rats were homogenized and the total protein content of the homogenate was measured by the Lowry method in the Miller modification [10].

To determine the total protein, urea, cholesterol and glucose were used standard kits produced by SPE "Philisit-Diagnostics" (Ukraine) and LLC "SpineLab" (Ukraine) [11].

Statistical processing of the obtained results was performed by methods of variation statistics using indicators: mean value, standard deviation, mean error, reliability of differences between comparison groups according to the Student's parametric criterion. (t) – in cases of normal distribution, nonparametric Mann-Whitney test (U), Fisher's angular transformation - when accounting in an alternative form, the confidence interval (p); differences were considered significant in $p<0,05$ [24].

Results and Discussion

According to the results of the test of swimming with a load before exhaustion (Table

1), it was found that Lebobol shows pronounced anabolic properties significantly relative to the control increases the physical endurance of animals when swimming with a load of 20% of body weight ($p<0,05$), and also unreliable ($p<0.17$) – when swimming with a load of 10% of body weight. Tribulus also slightly prolonged the swimming time of mice to complete exhaustion with a load of both 10 and 20% of the body weight of animals, but these figures probably did not differ from the control, so the effect of Tribulus is only tendentious.

The anabolic and actoprotective properties of Lebobol, which significantly exceed the similar effects of Tribulus, were also proved in the test of forced distance swimming (Fig. 2). Levobol significantly reduces the development of fatigue in mice from both the 5th and 10th attempts to overcome the distance, while the statistically significant effect of the comparison drug Tribulus develops only from the 10th attempt.

According to the results of tests for physical endurance (swimming with load) and the rate of fatigue (forced swimming for a distance), Lebobol revealed powerful anabolic and actoprotective effects, which significantly exceeded the similar properties of the comparison drug Tribulus.

The results of the test of the rotating rod are given in table. 2. Analysis of the table data shows that Lebobol and Tribulus do not have a negative effect on skeletal muscle tone and coordination of movements. In contrast, Levobol significantly reduced the percentage of mice that fell before 1 and 5 minutes by 50% relative to the control group. Tribulus also reduces the number of animals that did not stay on the rod until the 1st and 5th minutes, but in the scope of this sample, these deviations do not reach the level of statistical significance.

The effect of drugs on the skeletal muscle tone of animals was additionally evaluated in the horizontal bar test (Table 3).

As can be seen from the table, Lebobol significantly increases the retention time of animals on the wire by almost by 1.36 times relative to control. Although the Tribulus index exceeds the corresponding indicator of the control group by 1.27, this difference does not reach the level of statistical significance, but is manifested only in the form of an incredible trend ($p>0,05$).

The tests of the rotating rod and the horizontal bar showed a clear positive effect of Lebobol (but not Tribulus) on skeletal muscle tone, and coordination of movements of experimental animals.

The results of the open field test are shown in table 4.

The obtained data testify to a significant positive effect of Levobol on locomotor and orientation research activities, which was manifested in a statistically significant increase in the number of crossed squares, as well as racks (vertical research activity) and surveyed holes (horizontal research activity).

At the same time, at the background of Tribulus, there was an incredible increase in the number of crossed squares, as well as a significant increase in the total indicator of approximate research activity of animals (but for some components – vertical uprights and surveyed holes.

The vegetative support of emotional reactions was not affected by the studied drug and the comparison drug, which indicates the absence of an anxiogenic component in the anabolic properties of Levobol and Tribulus.

The total rate of all activity of animals receiving Lebobol and Tribulus increased significantly compared to the same indicator of the control group by almost 30%, indicating the stimulating effect of drugs on locomotor activity and orientation research, but not on emotional reactions (including anxiety) experimental animals.

As evidenced by the data of Fig. 2, the studied drugs significantly increase the total body

weight of rats in the experiment. However, the effect of Levobol ($p<0.01$) compared to Tribulus ($p<0.05$) is much more pronounced.

Levobol and Tribulus are also likely to increase the calf muscle mass ratio of rats (Fig. 3), which is an additional marker of the anabolic properties of drugs.

The effect of Levobol and Tribulus on the intensity of protein-synthesizing processes in muscle tissue was assessed by the content of total protein in the calf muscle and heart of rats (Table 5).

Data analysis table 5 shows that Levobol contributes to a probable increase in protein content in the calf muscle by 1.4 times ($p<0.001$), and in the heart – more than 1.2 times ($p<0.01$) compared with the control group. Under similar conditions, Tribulus significantly increased the protein content in the calf muscle by 1.3 times ($p <0.01$), and in the heart almost by 1.2 times ($p <0.05$), which is slightly inferior to Lebobol.

The study found that Levobol and Tribulus stimulate anabolic processes in the muscle tissue of experimental animals by the criterion of intensification of protein-synthesizing processes in the calf muscle and myocardium of rats.

The effect of Levobol and Tribulus on protein, lipid and carbohydrate metabolism was assessed by the level of total protein, urea, cholesterol, β-lipoproteins, glucose and serum creatinine (table 6).

In the experimental evaluation of the anabolic effect, an important indicator is the nitrogen balance: in the positive anabolic processes predominate and catabolic in the negative balance. For this purpose, the level of total protein and urea was diagnosed as an indicator of the breakdown of proteins in serum.

The results show that at the background of the administration of Levobol, the level of total protein increases significantly by 1.3 times, while the level of urea is reduced by 30% relative to control animals. Tribulus also contributed to a 1.3-fold increase in total protein and a 25% decrease in urea, but the data are not reliable, which indicates only the tendency of the anabolic effect of the comparison drug when administered at this dose and mode.

Determination of cholesterol as an indicator of lipid metabolism in the study groups indicates a clear tendency to reduce cholesterol levels under the activity of Lebobol and Tribulus by 16% and by 17%, respectively, which indicates a positive effect of the studied drugs on fat metabolism. Diagnosis of blood glucose levels in all study groups indicates that the value of this indicator does not change under the influence of drugs.

The results of biochemical studies of the serum of animals that recived Lebobol and Tribulus indicate a pronounced anabolic effect of Levobol, which is predominant in Tribulus; positive dynamics of Levobol and Tribulus to normalize lipid metabolism without changes in glucose levels under the action of the studied drugs.

Conclusions:

1. According to the results of behavioral reactions, the total indicator of all types of activity of animals that received Levobol and Tribulus, significantly increased relative to the same control group by almost 30%, indicating a stimulating effect of drugs on locomotor activity, but not on emotional reactions (in particular the level of anxiety) of experimental animals.
2. According to the results of tests for physical endurance (swimming with load) and the rate of fatigue (forced swimming for a distance), Lebobol revealed powerful anabolic and actoprotective effects, which significantly exceeded the similar properties of the comparison drug Tribulus.
3. Serum biochemical studies confirmed a more pronounced effect of Levobol on protein

synthesis: a significant increase in total protein levels by 1.3 times at decrease in urea by 30% relative to control animals compared with Tribulus, which was characterized by only a clear tendency to on these indicators.

4. Preclinical studies of the new drug Levobol based on ecdysteroids Lefzia safflower and vitamin C confirmed the presence of distinct anabolic properties of this drug, which predominate in the comparison of Tribulus, which is the basis for further study of Levobol for clinical practice.

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Table 1. Effect of Levobol and Tribulus on physical endurance in mice (M±m)

Group	Swimming time to exhaustion, s	
	Load – 10%	Load – 20%
Control, n=6	176,67±24,36	86,50±4,68
Levobol, n=6	228,17±24,58	112,17±7,72 *
Tribulus, n=6	193,50±14,46	103,33±8,55

Notes: * – relative differences with the control indicator (p<0,05).

Table 2. Effect of Levobol and Tribulus on muscle tone and coordination in mice under a rotating rod test

Group	Fell to 30 s	Fell to 1 m	Fell to 5 m
Control, n=6	1/16,7%	3/50%	4/66,7%
Levobol, n=6	0/0%	0/0% **	1/16,7% *
Tribulus, n=6	0/0%	1/16,7%	2/33,3%

Notes:
In the numerator the absolute number of animals in the denominator – %;
* – relative differences with the control indicator (p<0,05);
** – relative differences with the control indicator (p<0,01).

Table 3. Effect of Lebobol and Tribulus on skeletal muscle tone of mice in the horizontal bar test (M±m)

Group	Retention time, s
Control, n=6	181,00±17,57
Levobol, n=6	246,67±26,15 *
Tribulus, n=6	231,83±25,82

Notes:
* – relative differences with the control indicator (p<0,05).

Table 4. Influence of Lebobol and Tribulus on the behavior of mice in the open field test (M±m)

Indicators (in 3 minutes)	Control, n=6	Levobol, n=6	Tribulus, n=6
Locomotor activity (crossed squares)	28,50±3,84	45,17±5,64 *	43,50±6,45
Orientation research activity:			
- racks	6,83±0,95	11,17±1,74 *	8,17±1,22
- surveyed holes	25,33±4,54	37,00±1,91 *	36,33±3,22
- total	32,17±5,03	47,83±2,39 *	44,50±2,58 *
Vegetative support of emotional reactions:			
- bolus	2,00±0,45	1,83±0,65	2,50±0,72
- urination	0,33±0,21	0,67±0,21	0,83±0,31
- grooming	1,00±0,37	0,83±0,31	1,17±0,17
- total	3,33±0,70	3,17±0,88	4,50±0,62
The total sum of all activities	63,83±8,93	96,67±6,19 *	92,50±8,14 *

Notes:
* – relative differences with the control indicator (p<0,05).

Table 5. Influence of Levobol and Tribulus on the content of total protein in tissues (M±m)

Group	Protein content, mg/100 g of tissue	
	Calf muscle	Heart
Control, n=6	44,17±1,78	50,33±1,71
Levobol, n=6	61,50±1,95 ***	60,67±2,51 **
Tribulus, n=6	56,50±2,54 **	60,17±3,07 *

Notes:
* – relative differences with the control indicator (p<0,05);
** – relative differences with the control indicator (p<0,01);
*** – relative differences with the control indicator (p<0,001).

Table 6. Effect of Levobol and Tribulus on protein, lipid and carbohydrate metabolism (M±m)

Indicators	Control, n=6	Levobol, n=6	Tribulus, n=6
Total protein, g/l	70,23±2,67	99,05±4,73*	98,09±14,73
Urea, nmol/l	10,35±0,80	7,26±0,63*	7,63±1,69
Cholesterol, mmol/l	2,00±0,36	1,68±0,30	1,66±0,11
Glucose, µmol/l	6,94±0,61	7,26±0,51	7,11±0,40

Notes:
* – relative differences with the control indicator (p<0,05).

Figure 1. Design of study

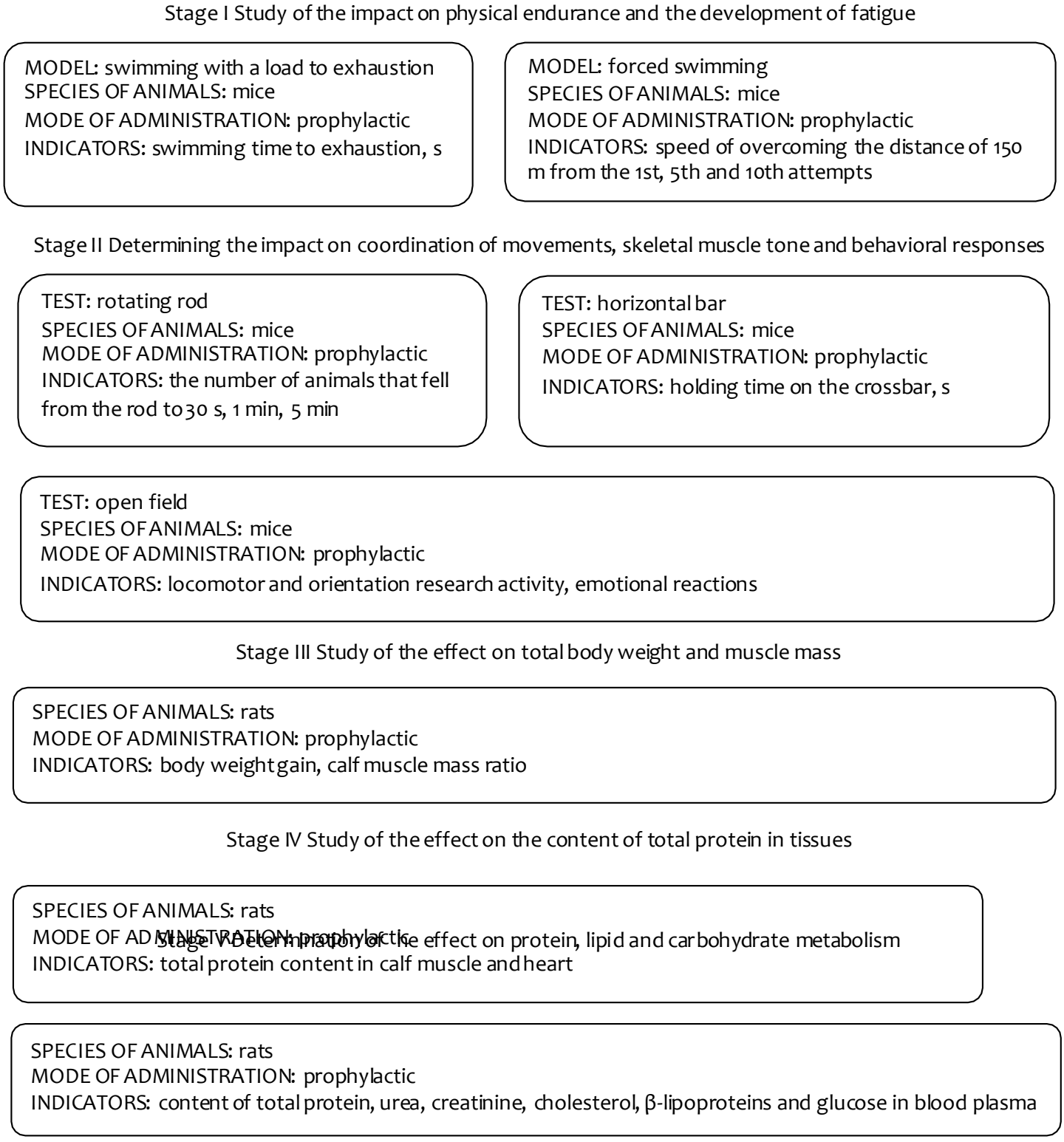
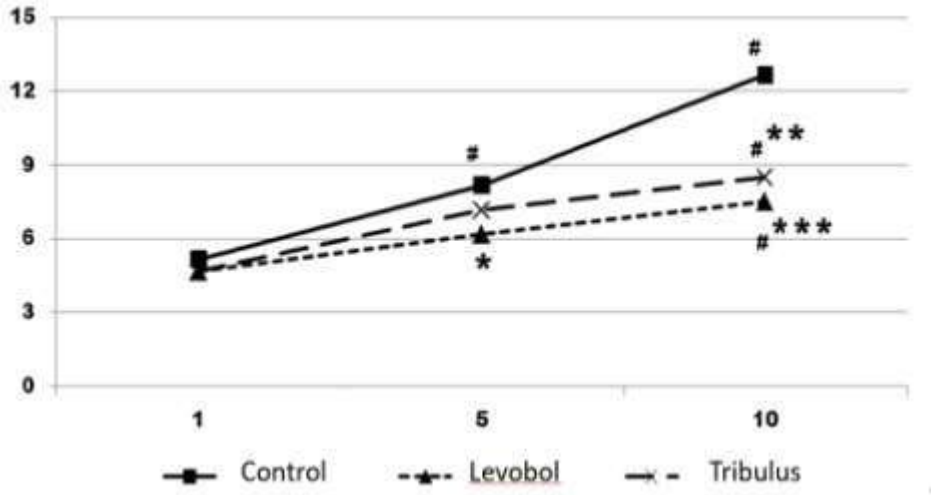


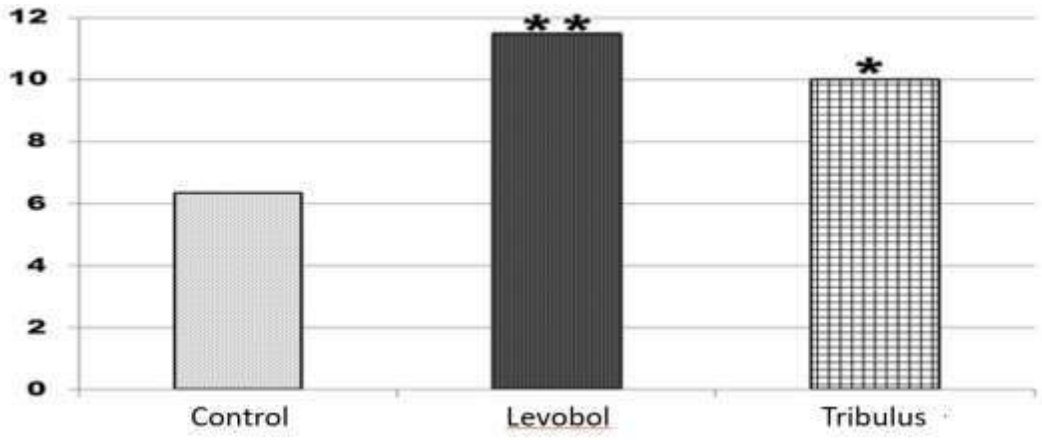
Figure 2. Influence of Levobol and Tribulus on the rate of fatigue development in mice



Notes:

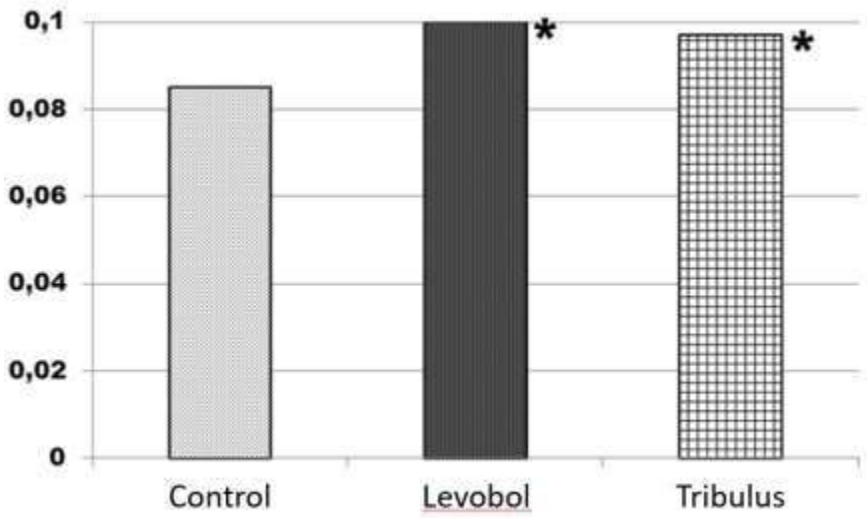
1. On the abscissa – the number of the attempt.
2. On the y-axis – the time of overcoming the distance, s.
3. * – relative differences with the control indicator (p<0,05);
   ** – relative differences with the control indicator (p<0,01);
   *** – relative differences with the control indicator (p<0,001);
4. # – relative differences with the initial state (p<0,05).

Figure 3. Effect of Levobol and Tribulus on total body weight gain in rats



Notes:
On the y-axis – weight gain, g (Δ);
* – relative differences with the control indicator (p<0,05);
** – relative differences with the control indicator (p<0,01).

Figure 4. Influence of Levobol and Tribulus on the calf muscle mass ratio of rats



Notes:
On the y-axis - the ratio of calf muscle mass (MM), %;
* – relative differences with the control indicator (p<0,05).