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Research Article

The Impact of Physical Load and Adaptogens on the Animal Work Capacity

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Abstract | This paper describes experimental studies aimed to restore the physiological functions of model animals after a heavy physical load using biologically active substances in their diet. The findings of this study may be applied in power training. The paper suggests using biologically active substances in the diet of animals to maintain their internal environment after maximum physical exertion. The conducted experiments showed that bioactive supplements of different nature optimized the homeostasis of the laboratory animals. It positively affected their skeletal and heart muscles, lung tissue, liver and kidneys, where immunity and blood cells develop. Although the swimming test at the beginning of the experiment showed that mice and hamsters had approximately the same indicators of physical endurance, by the end of the experiment, the swimming time of the experimental group animals was significantly longer. The animals receiving pantocrine turned to be stronger. Their interval time increased by 267 seconds ($P \leq 0.05$) while the swim time of animals consuming may chang rose by 96 seconds ($P \leq 0.05$) compared to the control. Male hamsters consuming lemongrass tincture showed the maximum efficiency, exceeding the control by 21.3 minutes ($P \leq 0.001$), animals receiving drone brood overran by 0.5 minutes ($P \leq 0.01$). Heart muscle of animals fed with pantocrine and lemongrass seed tincture at physical load had fewer dystrophic changes in muscle cells. The wall structure of most vessels did not have obvious pathological changes. Consequently, greater physical endurance in mice administered pantocrine and hamsters given lemongrass tincture is mainly due to better coordination of motor and vegetative functions and the functional stability of the body systems that ensure oxygen delivery to the working muscles.

Keywords | Adaptation, Adsorbed drone brood, Lemongrass seed tincture, Male hamsters, May chang tincture, Mice, Pantocrine tincture, Physical load

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INTRODUCTION

Technologies have made our life much easier compared to previous decades. The physical activity of people has sharply decreased, resulting in health problems. Reduced energy expenditure disorders the functioning of individual systems (muscle, bone, respiratory, cardiovascular) and the body as a whole worsens immunity and deteriorates metabolism. On the other hand, excessive physical activity is also very harmful (Brancaccio et al., 2010; Isaev et al., 2012).

Excessive physical load can negatively affect the body of experimental animals, resulting in abnormal physiological processes followed by decreased functioning. These can be observed when performing physical work, during training and sports competitions (Foss and Keteyian 1998; Kereselidze, 2002).

Prolonged and maximum loads lead to changes in the physiological processes associated with higher energy expenditure, and subsequently a reduced ability to perform physical actions. It is accompanied by changes in biochemical processes and morphogenesis in biological objects. Laboratory animals demonstrate changes in skeletal and heart muscles, lung, liver and kidney tissues and biochemical processes (Krivoshchekov, 2004; Mikashinovich et al., 2017).

A body with developed physical load tolerance has reserves to restore physiological functions and adapt to overload. However, when internal reserves are exhausted, and the body is at the breaking point, there are changes in physiological processes (Avdeeva et al., 2016; Khabibullin et al., 2020a, b).

Subsequently, the body requires more time to replenish and normalize its activity. Sometimes the recovery process is impossible due to destructive changes in the body or organs (Welsman and Armstrong, 1998; Wyatt and Mitchell, 1978).

The above indicates that maximum and prolonged physical exertion are stress factors that lead to the decreased adaptive properties of the body (Vøllestad and Blom, 1985; Kimura and Sumiyoshi, 2004; Nikolaidis et al., 2008).

Some scientists recommend taking biologically active substances to restore the adaptive properties of the body after overload and prolonged physical exertion (Lapaev, 1974; Lazaryan et al., 1999; Panossian and Wagner, 2005).

The conducted experiments showed that biologically active substances in the diet of laboratory animals could neutralize an adverse effect of excessive physical load on performance and morphogenesis in some internal organs (Porsolt et al., 1977a, 1978; Khabibullin, 2017; Semenova et al., 2020).

This indicates a need to conduct a comprehensive study of physiological processes in the body after maximum physical exertion and taking biologically active substances (Dinan et al., 2001; Benchaar et al., 2007; Kreider et al., 2010; Mironova et al., 2020).

It can also provide a timely transition from maximum physical load to the process of "sparing" activity or rehabilitation (Tesch and Lindeberg, 1984; Ulrikh et al., 2018).

Many researchers analyze issues of animal adaptation to solve the problem of preventing the negative impact on the physiological processes of biological objects, particularly animals and humans. Nevertheless, in most cases, they focus on individual physiological processes without taking into account their influence on the processes of morphogenesis (Porsolt et al., 1977b, 1978; Lazaryan et al., 1999).

Therefore, the study of the effect of biologically active substances on the morphogenesis processes in internal organs and the physical performance of laboratory animals after maximum physical exertion in a comparative aspect is one of the most relevant. Much less is known how biologically active substances affect the processes of morphogenesis in internal organs and the performance of laboratory animals after the highest physical load. This paper provides fresh insight into the problem.

The research aimed to study the effect of biologically active substances on physiological processes in the body of laboratory animals after maximum physical exertion.

The conducted experiment involved solving the following tasks: to estimate the dynamics of developing the physical performance of laboratory animals when using biologically active substances; to study the effect of adaptogens on the morphology of the internal organs of experimental animals at the administered swim test.

Condition, materials and methods

The object of the study. The experimental studies were carried on laboratory animals, namely 60 white mice, divided into three groups according to the pair principle. The weight of male mice at the beginning of the experiment was 22-24 g; at the end -18.5-22.2 g. The first test group was given an adaptogen, may chang tincture. The second trial group received a pantocrine tincture. The third control group of mice got distilled water.

The study involved the analysis of model hamster animals as well. There were 75 hamsters divided into three groups of twenty-five heads of laboratory animals, each by the matched pairs principle. Male hamsters weighed 21.5-21.9 g at the beginning of the experiment; 22.0-25.7 g at the end. All the experimental hamsters were of the same age. The first group hamsters were given lemongrass tincture; the second group got a biologically active beekeeping product - drone homogenate. The adaptogen dose was calculated by Clark's rule that requires the knowledge of the animal weight. The data mentioned above (1.25 ml and 22.0 g) was used to determine the dose. The preparation dose = 1.25 x (22 g/ 70,000 g) = 0.000392 ml or 3.92 μl, rounded up to 4 μl. 70,000 g is the average adult weight presented in g. According to the experiment scheme, the third control group of hamsters received distilled water. After the highest physical load, adaptation preparations were given to hamsters of experimental groups.

The experiment scheme: The research scheme and adaptogen preparation dose are shown in Figure 1.

All the preparations used are made in the Russian Federation: Leuzeae extract fluid (manufactured by CAMELLIA LLC, Lobnya), Pantocrine (Pantocrinum) (manufactured by St. Petersburg Pharmaceutical Factory OJSC), lemongrass seed tincture (Tinctura Schizandrae chinensis) (manufactured by Tula Pharmaceutical Factory LLC), native drone homogenate (LLC "MelMur", Sochi).

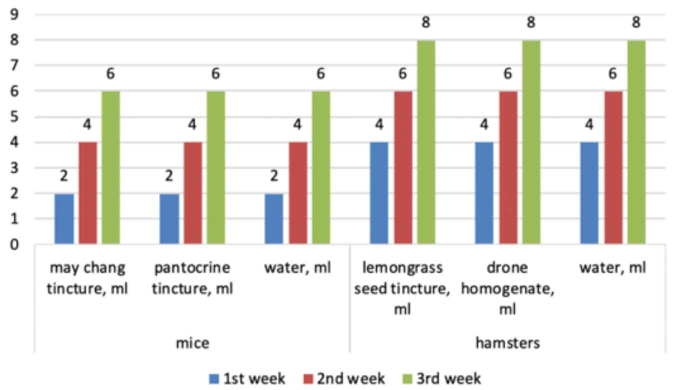

Figure 1: The experiment scheme.

The study periods: A series of experimental studies of maximum physical exertion by "forced swimming" consisted of several repetitions and was carried out in the following terms: on mice: 29.11.2013, 06.12.2013, 13.12.2013, 20.12.2013, 26.12.2013, on hamsters: 04.07.2016, 11.07.2016, 18.07.2016, 25.07.2016.

Research site: Experimental studies were conducted in the conditions of the Department of Private Animal Husbandry and Animal Breeding and vivarium of the Faculty of Biotechnology and Veterinary Medicine of the Bashkir State Agrarian University.

Diagnostic methods. All mice and hamsters were exerted the highest load by the Porsalt forced swim test once a week (1978). The endurance was developed during four swim tests. To conduct experimental studies, it is necessary to fill a glass cylinder of 120 mm in diameter and 300 mm in height with warm water (36.50 °C).

An experimental animal was put into a cylinder with water. The swimming start and finish times were determined by a stopwatch. The moment when the animal began to sink signalled the end of swimming. The animal was picked up with a lift net, put on a dry towel made of natural fibres heated to 38 degrees Celsius in a thermostat. The animal was dried and placed in a cage. All experimental animals received the maximum physical load by "forced swimming" once a week.

Histological examination of internal organs was performed with sterile instruments maintaining the necessary measures. The sample taken for histological studies was fixed in a 10% neutral formalin solution. Preparations for histological studies were prepared according to the method proposed by. After experimental studies, animals are autopsied. Morphometric parameters of internal organs are assessed, and their pieces for histological studies are selected. To this end, the animal's corpse was placed belly up in a tray filled with paraffin and stretched with tweezers. Paws were fixed with dissecting needles. The chest and abdomen were wiped with cotton soaked with 30% alcohol. Samples were taken from the thoracic and abdominal cavities. The material for histological studies was labelled and placed in a 10% formalin solution. Histological sections were fixed in increased concentrations of alcohol solutions and then embedded in paraffin to make paraffin sections. Histological sections were cut 7m thick with a sledge microtome. Sections were stained after the paraffin removal.

The section was taken from the water film and placed on a slide. 1-2 drops of hematoxylin were applied to the sample, and then in 3 minutes, they were washed with water. Then the histological section was kept in the hydrochloric acid alcohol solution for 3 minutes and then washed with running water for 30 minutes. Next, the histocision was stained with eosin; the colour exposure was 4 minutes. Eosin was washed off with running water for 5 minutes. After washing off the stain, the sample was dried with filter paper and fixed with 80% and 95% ethyl alcohol. After fixation, the histological section was cleared by fixing carbolxylene and then xylene (for 2 minutes). Xylene was removed with filter paper, a drop of cedar balm was applied, and the section was covered by glass. The slide with a stained histological section was dried for 28-32 hours, and then the studied section was described.

Methods of study

The basis was the search for preparations for rapid rehabilitation after maximum physical exertion. The conducted studies involved observation of the animal behaviour and experimentation addressing the research objectives. The research used up-to-date physiological and histological examination methods.

Statistical processing

Statistical criteria for the result difference reliability were determined by the Student's criteria.

RESULTS AND DISCUSSION

The dynamics of swimming activity was studied in four stages. At the beginning of the experiment, the mice of the control and experimental groups showed no significant differences. Swimming activity in model animals after seven days of experimental studies in all experimental groups was slightly lower than before, and this indicator significantly increased fourteen days after the experiment began (Figure 2).

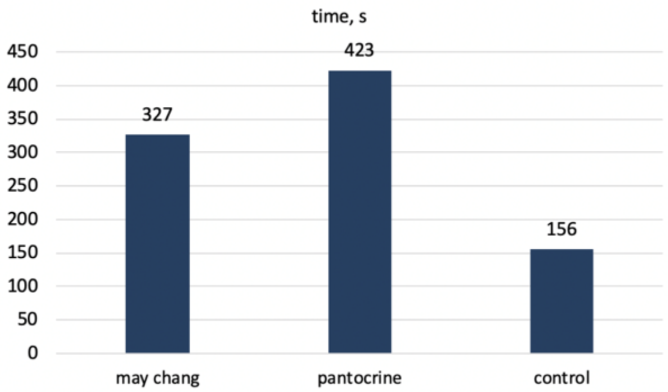

Figure 2: Swimming activity of experimental mice after the experiment.

The data analysis indicates that the swimming activity length of experimental animals is higher by 97.0 and 93.60 seconds, respectively, compared to the control group animals.

Hamsters of the first experimental group receiving an adaptogen in the form of lemongrass tincture increased physical endurance by the second, third and fourth reference periods of the trial (Figure 3).

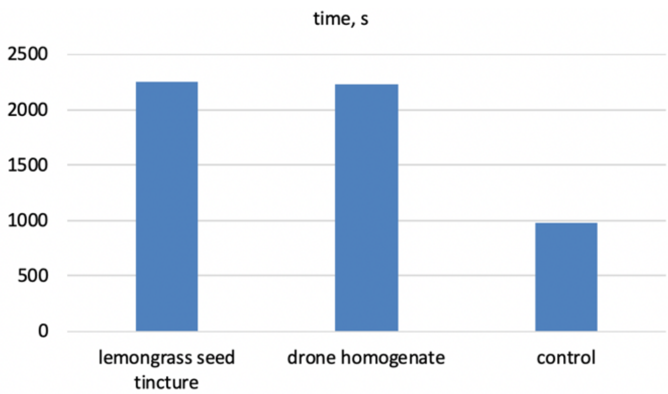

Figure 3: Swimming activity of hamsters.

By the fourth reference period, the indicators of swimming activity increased to 2259.0 seconds, or by 56.67%, respectively. In the second experimental group, where the hamsters were given drone homogenate as an adaptogen, the swimming activity was 2228.7s, exceeding the indicators of the control group animals by 56.06%.

After four experimental studies, the physical performance of experimental hamsters given lemongrass tincture increased by 58.7%, drone homogenate by 58.13%.

Excessive physical load have a negative effect on the body of laboratory animals, putting them under stress. This led to physiological disorders, affecting the cardiovascular system of the model animals.

Experimental studies conducted on mice using biologically active substances after physical exertion marked heart muscle changes (Figure 4).

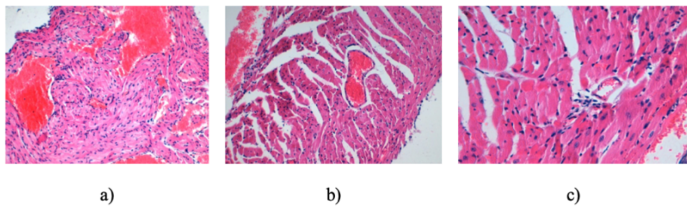

Figure 4: a) Cardiac myocytes of the control group animal H and E staining. 10Xoc.lens, 10Xobj.lens; b) Cardiac myocytes of the animal from the experimental group 1. H and E staining. 10Xoc.lens, 4Xobj.lens; c) Heart muscle tissue of animals given pantocrine. H and E staining. 10Xoc.lens, 20Xobj.lens.

There were apparent morphological changes in the heart muscle of control group animals that did not receive adaptogen preparations under heavy load. Blood channels were notably extended and filled with blood, and some areas of the heart muscle tissue were hemorrhagically impregnated. Heart muscle cells had dystrophic changes. There were assemblies of cellular elements in all layers of the heart and its outer surface. These were numerous lymphocytes, macrophages, and fibroblasts. The heart muscle of the control animals had edematous zones with nests of cellular elements.

The heart muscle of animals receiving may chang at high physical load differed from the control group. There were fewer dystrophic changes in muscular tissues, but they did not disappear. Some vessels had the standard structure of the wall without pathological changes. Most of the vessels were full-blooded, though the nearby cardiomyocytes had pronounced dystrophic changes.

Pathological changes in the heart sac did not wholly disappear; there were small inflammatory cellular infiltrates, the vessels were dilated and blood-filled. Myocardium in the group of animals, getting pantocrine under load did not completely recover. Some cardiomyocytes had signs of dystrophic changes. There were clusters of erythrocytes and

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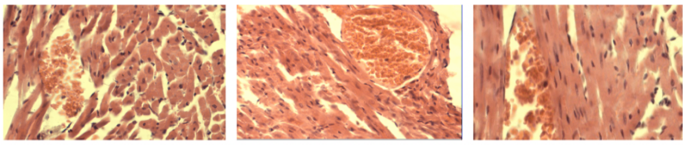
single ones. Some larger blood vessels were overfilled and edematous, while other vessels were of the usual structure, though there were signs of mild oedema around.

Similar changes are observed in the heart of hamsters (Figure 5).

Heart blood vessels in hamsters are characterized by blood filling and further migration of blood cell elements through the walls of the microcirculatory vessel into the pericapillary zone. The venous vessels have the stagnated myocardium areas closer to the outer layer of the heart when the blood vessel is significantly dilated. Dilation of blood vessels can lead to rupture of blood vessels, followed by a heart attack.



a) b) c)

Figure 5: a) Venous myocardial hyperemia of an experimental animal using lemongrass. H and E staining. 10Xoc.lens, 40Xobj.lens; b) Venous myocardial hyperemia when using drone brood. H and E staining. 10Xoc. lens, 40Xobj.lens; c) Marked venous hyperemia of the myocardium of the control group of animals. H and E staining. 10Xoc.lens, 40Xobj.lens.

Full capacity physical load lasting twenty-two days harmed the body of experimental animals, which is confirmed by our histological studies.

The use of biologically active substances after high physical exertion has a positive effect on the physiological processes in the body of animals and internal organs of skeletal muscles (Figure 6), kidneys (Figure 7), liver (Figure 8) and lung tissue (Figure 9) of hamsters.

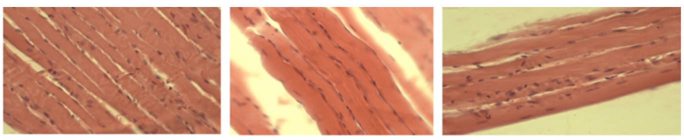


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Figure 6: a) Somatic musculature of control group animals. H and E staining. 10Xoc.lens, 40Xobj.lens; b) Skeletal muscle tissue of an animal after high physical exertion and the use of lemongrass as an adaptogen. H and E staining. 10Xoc.lens, 40Xobj.lens; c) Hypertrophied skeletal muscle tissue of a hamster after physical exertion and combined use of a drone brood preparation. H and E staining. 10Xoc. lens, 40Xobj.lens.

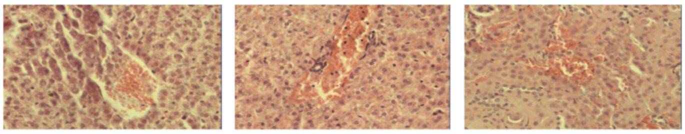

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Figure 7: a) Venous hyperemia of the kidney cortex in the control group animals. H and E staining. 10Xoc.lens, 40Xobj.lens; b) The hyperemia zone of the kidney cortex blood vessel in animals after physical exertion and the lemongrass use. H and E staining. 10Xoc.lens, 40Xobj. lens; c) Moderate stagnation in the kidney cortex blood vessels in animals using the drone brood after physical exertion. H and E staining. 10Xoc.lens, 40Xobj.lens.

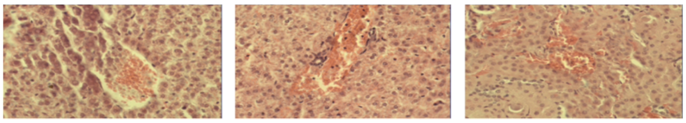

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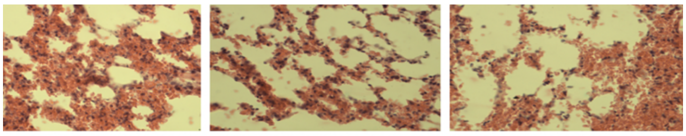
Figure 8: a) Fullness of the venous vessel of the liver in an animal of the control group after physical exertion. H and E staining. 10Xoc.lens, 40Xobj.lens; b) Venous hyperemia of the liver triad of animals when using lemongrass after physical exertion. H and E staining. 10Xoc.lens, 40Xobj. lens; c) Full blood vessels of the liver of animals when using the drone brood after physical exertion. H and E staining. 10Xoc.lens, 40Xobj.lens.



a) b) c)

Figure 9: a) Marked lung alveolitis in an animal from the control group. H and E staining. 10Xoc.lens, 40Xobj.lens; b) Lung alveoli in hamsters after physical exertion and the use of lemongrass. H and E staining. 10Xoc.lens, 40Xobj. lens; c) Focal alveolitis in the lung of animals after ultra-high physical exertion and the use of an adsorbed drone brood preparation. H and E staining. 10Xoc.lens, 40Xobj. lens.

Biologically active substances of animal origin are among the most effective drugs, as they contain many biologically active and energetic substances with high physiological activity. At maximum loads, they are necessary for the body of experimental animals for everyday physical activity. They contain physiologically active substances, amino acids, a complex of vitamins, hormone-like substances necessary to increase physical endurance and normalize physiological processes. Adaptogens of plant origin also positively affect the body of model animals, performance indicators, and adaptation processes to increased physical exertion.

When developing physical performance, biologically active substances have a different effect on morphogenesis in some internal organs of laboratory animals.

Higher physical endurance of the animal body can be achieved by using lemongrass tincture, may chang tincture, pantocrine and drone homogenate. One of the most effective is a composite form of adaptogens that increased the efficiency of hamsters by 2.97 times.

Maximum physical exertion leads to morphological changes in the body of laboratory animals, namely in the cardiac muscles of mice and hamsters and skeletal muscles, kidneys, and liver of hamsters. Biologically active substances reduce the damage level in internal organs.

CONCLUSIONS AND RECOMMENDATIONS

Physical exertion leads to morphological disorders in the organs. Control animals show blood supply disturbance accompanied by haemorrhagic tissue impregnation, dystrophic changes in myocardial fibers, cytoplasm of cardio myocytes, and inflammatory cell infiltration in the pericardium, parenchymal cells of the pericardium organs: lungs, kidneys and liver of varying degrees. The skeletal muscle tissue gets the most significant dystrophic changes. There was a pronounced reaction from the vascular bed, dystrophic changes in muscle fibres with partial fragmentation. Animals treated with adaptogens had fewer morphological changes in organs. However, the skeletal muscle tissue does not restore completely. Adaptogens contributed to better immunogenesis and hematopoiesis organs, expressed in the increased number of lymph nodes with active germinal centres in the spleen and new not large islands of the lymphoid tissue in the liver and kidneys.

In order to enhance the physiological function and adapt the animal body to physical exertion, it is necessary to use tinctures of may chang, lemongrass, pantocrine and biologically active bee product drone homogenate in the doses recommended by the conducted study.

The highest physical exertion requires a set of measures to prevent the effect of overtraining, even if adaptogens were included in animals' diets.

To restore physiological processes in the body of laboratory animals after maximum physical load, adaptogens of plant and animal origin are recommended.

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Not applicable.

NOVELTY STATEMENT

This paper provides fresh insight into the problem of biologically active substances affect the processes of morphogenesis in internal organs and the performance of laboratory animals after the highest physical load.

AUTHOR'S CONTRIBUTION

Ilvir Khabibullin- author of the research organization. Ruzel Khabibullin, Irina Mironova and Elmira Akhmadullina carried out the processing and assessment of results. Lyalya Musina and Victoria Morozova took part in preparation of an article for publication.

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Data availability
Data will be available on request.

Ethical approval
All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest
The authors have declared no conflict of interest.

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