April 30, 2019

PharmacologyOnLine

Archives • 2019 • vol.1 • 429-437

CHROMONE-3-ALDEHYDE DERIVATIVES IMPROVE MUSCLE FUNCTION BY SUPPRESSING THE FORMATION OF APOPTOSIS-INDUCING FACTOR

Voronkov AV.[1], Pozdnyakov DI.[1], Rukovitsyna VM.[1], Veselova OF.[2], Olokhova EA.[2]

[1]Pyatigorsk medical and pharmaceutical Institute-branch of Volgograd state medical University of the Ministry of health of the Russian Federation (Pyatigorsk, Russia (357532, Pyatigorsk, av. Kalinina 11).

[2]Krasnoyarsk state medical University Professor named after V. F. Voyno-Yasenetsky Ministry of health of the Russian Federation (Krasnoyarsk, Russia, St. Partizan Zheleznyak, 1)

Email address: pozdniackow.dmitry@yandex.ru

Abstract

In this study investigated the influence of 5 new derivatives of chromone-3-aldehyde on the development of muscle dysfunction in the conditions of electromyostimulation test was estimated. The acute toxicity of the studied compounds was preliminarily estimated with the calculation of LD50. Further, myoprotective activity of chromone-3-aldehyde derivatives on the background of muscle dysfunction during prophylactic 7-day oral administration at a dose of 1/100 LD50 was studied. The degree of muscle fatigue was evaluated in the «strength-grip» test. To assess the severity of muscle fatigue, a series of biochemical tests were performed to determine: serum LDH, CK activity, myoglobin and creatinine concentrations; muscle tissue concentrations of lactic acid, pyruvic acid, total protein and AIF.In the result of the study found that the derivatives of chromone-3-aldehyde have a pronounced myoprotective effect associated with low toxicity (5 class of toxicity according to the GHS classification), which was confirmed by the results of the test «strength-grip» and the data of biochemical studies. The study of the connections are prevented, as myodystrophy processes, as evidenced by the decrease in serum concentration of myoglobin, CK and LDH activity, and the demonstrated metabolic action expressed in the normalization processes of aerobic oxidation, protein and macroergic compounds synthesis. In the series of investigated compounds under the ciphers X3Al and X3A5 showed more pronounced activity than the compounds X3A2, X3A3 and X3A4. The study showed that the derivatives of chromone-3-aldehyde are promising objects for further study in order to create a drug with a high myoprotective effect and an optimal profile of safety.

Keywords: *chromone derivatives, muscle dysfunction, mioprotector, apoptosis, AIF*

Introduction

The formation, growth and regeneration of skeletal muscle is essential to maintain optimal functioning of the organism. During various stages of life, the striated muscle may be exposed to various types of adverse effects, such as injury, infections that cause muscle weakness, pain or paralysis, which in turn can lead to death [1]. This heterogeneous group of disorders leading to the loss of muscle tissue activity is defined as muscle dysfunction. In terms of muscle dysfunction skeletal muscles are not able to perform the required amount of functional tasks, while reducing the intensity and strength of the work performed. [2]. Without proper correction, muscle dysfunction can sufficiently worsen the patient's quality of life, provoke chronic fatigue and overtraining syndrome, endocrine disorders, immune system dysfunction, and organic diseases [3].

Among the features of the pathogenesis of muscle dysfunction is particularly marked decrease in the activity of energy-producing cell systems. It was found that in conditions of intensive muscle work and a significant increase in oxygen consumption by skeletal muscles, early inclusion of mechanisms of glycolytic cleavage of glucose, leads to the formation of underoxidized metabolic products (lactate, pyruvate), as a result of which, there is a shift in the pH of the intracellular medium to the acid side, which in turn reduces the sensitivity of troponin C to calcium ions, thereby reducing the force of contractions of skeletal muscles [4].

In addition to acidosis, increased anaerobic metabolism in muscle tissue leads to accelerated hydrolysis of creatine phosphate, which exacerbates energy deficiency [5]. In conditions of muscle dysfunction is also noted the development of oxidative stress that may also be associated with early activation of the mechanisms of anaerobic metabolism makroergov. Thus, the separation of mitochondrial function at the stages of oxygen inclusion in the mitochondrial respiratory chain (complex I and complex II) leads to enhanced ROS generation, which in combination with increased NOX activity enhances membrane lipoperoxidation [6]. In addition, mitochondrial damage and loss of structural integrity in the process of performing enhanced physical activity leads to the intensification of apoptosis, and as a consequence of accelerated death of striated myocytes. Together, the above-mentioned interrelated pathogenetic processes underlying muscle dysfunction worsen the course of metabolic processes of muscle tissue and worsen its contractility. Thus, correction of metabolic changes in muscle tissue may be one of the strategies of pharmacotherapy of muscle dysfunction [7].

4H-benzopyran-4-ones , 4H-chromen-4-ones or chromone are six-membered oxygen-containing heterocyclic compounds that are widespread in nature. The structural diversity of types, number and location of substituents in the main nucleus is mediated by physical, chemical and biological features of natural and synthetic derivatives of chromium [8]. In addition, the structure of chromium is currently used as an active pharmacophore in various fields of chemical synthesis [9]. The spectrum of pharmacological activity of chromone derivatives is extensive, and includes such types as: anti-inflammatory, antioxidant, anticytokine, antitumor activity, effect on the genome, which makes these compounds promising substances for studying the ability to correct the effects of muscle dysfunction [10].

Methods

Animals. Ethical approval

As a biological model, 220 male mice of the Balb/c line weighing 23-25 grams, obtained from vivarium of PMFI-a branch of FSEI HE «Volgograd state medical University», were used. The study was carried out in compliance with the norms of international experimental ethics and met the requirements of the European Convention for the protection of vertebrates used for experimental and other scientific purposes (Strasbourg, 22 June 1998) and was approved by the local ethical Committee (Protocol No. 25 of 25.09.2018).

Studied compounds. Introduction scheme

The studied compounds were obtained at the Department of organic chemistry of Pyatigorsk medical-pharmaceutical Institute and represented new substituted derivatives of chromone-3-aldehyde under conditional ciphers X3A1, X3A2, X3A3, X3A4 and X3A5.

The studied substances were obtained in the cyclization reaction of 2-hydroxyacetophenone with dimethylformamide by adding phosphorus chloroxide (Fig.1). The structures of the compounds were confirmed by NMR spectroscopy.

Determination of acute toxicity of the test substances

To assess the acute toxicity of the studied objects, 15 experimental groups of 10 individuals in each (30 animals per compound) were formed. The studied substances were administered in doses of 1000 mg/kg, 2500 mg/kg and 5000 mg/kg in fractions with a two-hour interval. Further, the assessment of mice survival and 14-day monitoring of the general condition of animals were carried out. LD50 was calculated using the Finney method [11].

Muscle dysfunction experimental model

Muscle dysfunction was reproduced by electromyostimulation: under chloral hydrate anesthesia (350 mg / kg) in experimental animals in m. biceps brachii implanted electrodes and then after 24 hours produced electromyostimulation mode: 100 Hz, 3 seconds. (3 contraction)→3 min fatiguing contraction (sub-maximum stimulation 40 HZ) →100 Hz, 3 seconds. (3 contraction) [12]. In the course of the study, 7 groups of animals were formed, 10 individuals in each: the first group of mice with positive control (PC) – without muscle dysfunction, the second group of animals with negative control (NC) – with reproduced muscle dysfunction, without pharmacological support. The remaining groups of mice were administered the studied compounds (per os prophylactically for 7 days at a dose of 1/100 of LD50 for each compound).

Evaluation of muscle strength

The muscle strength of the animals was evaluated in the test of the «grip- strength» («Panlab» device). The test procedure was performed three times: before muscle dysfunction (T1 point), immediately after myostimulation (T2 point) and 30 minutes after myostimulation (point T3). Muscle strength was expressed in kgf units. Then the animals were euthanized by dislocation of cervical vertebrae and biological material (blood and muscle tissue) was taken.

Sample preparation of biological material

To conduct a series of biochemical tests produced the preliminary preparation of the biomaterial: the citrate blood was centrifuged with obtaining a serum in the regime of 3500 RPM for 15 min. the Muscle tissue is homogenized in phosphate buffer (pH 7.4) in a ratio of 1:10 followed by obtaining the supernatant (6000 g, 10 min). Serum concentrations of myoglobin and creatinine, as well as lactate dehydrogenase and creatine phosphokinase activity were determined. Changes in the concentration of lactic and pyruvic acids, the level of total protein and AIF (apoptosis induced factor) were evaluated in muscle tissue supernatant.

Assessment of lactate dehydrogenase activity

The activity of lactate dehydrogenase (LDH) was determined by kinetic method by the ability to catalyze the reaction of lactate formation from pyruvate in the presence of NADH coenzyme. The incubation medium contained: Tris-phosphate buffer (pH of 7.15), (hydroxymethyl –aminomethane 50 mmol/l, NADH 0.9 mmol/L). The volume of the test sample 100 µl. (kit «OLVEX» (Sweden)). Extinction of samples was determined at 340 nm for 5 minutes with an interval of 1 minute. LDH activity was calculated by the formula: U/L = 16030*ΔE340/min.

Evaluation of activity of creatine kinase (CK)

The activity of CK was determined in the conjugated creatine kinase-hexokinase-glucose-6-phosphate dehydrogenase reaction in the presence of NADP. Incubation medium: imidazole buffer (pH 6,7), ADP 20 mmol/l, NADP 20 mmol/l, creatine phosphate 300 mmol/l, g6fdg 20000 U/L, hexokinase 30000 u/l. The volume of the test sample is 100 µl. (kit «OLVEX» (Sweden)). Extinction of samples was determined at 340 nm for 5 minutes with an interval of 1 minute. The activity of CK was calculated with the formula: U/L = 4127*ΔE340/min.

Determination of the creatinine concentration

The creatinine concentration was evaluated by the method of Jaffe. The principle of the method consists in the formation of a colored complex of creatinine with picric acid in an alkaline medium with a maximum absorption at 505 nm. A standard kit of «OLVEX» reagents (Sweden) was used in the work.

Calculation of the creatinine concentration was made using the formula: C = Ex / E0 * 177 µmol / L, where

Ex - absorbance of the test sample;
E0 - absorbance calibration sample.

Lactic acid concentration

The concentration of lactate was determined in the enzymatic reaction with the formation of quininomine, the concentration of which is proportional to the content of lactic acid in the sample. Incubation medium: phosphate buffer (pH 6.8), Pipes 50 mmol / L, 4-chlorophenol 6 mmol / L, 4-AAP 0.4 mmol / l, 2000 U / L lactoxydase, U / L peroxidase. The volume of the test sample is 10 µl. Sampling was carried out at 500 nm. Calculation of lactic acid was carried out according to the formula: C = Ex / E0 * 3.34 µmol / L, where

Ex - absorbance of the test sample;
E0 - absorbance calibration sample.

Total protein concentration

The protein content was determined by reaction with bromophenol blue. The method is based on the formation of a colored protein complex with bromophenol blue in an acidic medium, the intensity of which is proportional to the protein concentration in the sample. Incubation medium: buffer (pH7.4) 180 µl, chromogen 20 µl. The volume of the sample was 40 µl. Sampling was carried out at 613 nm. Calculation of the content of lactic acid was carried out according to the formula: C = Ex / E0 * 0.25 g / L, where

Ex - absorbance of the test sample;
E0 - absorbance calibration sample.

Pyruvic acid concentration

The content of pyruvic acid was determined by the decrease in NADH in the lactate dehydrogenase reaction. Incubation medium: Good's buffer 1000 µL, NADH 200 µL, LDH (2000 U / L) 20 µl. The volume of the sample was 600 µl. Samples were extruded at 340 nm. Calculation of the content of pyruvic acid was carried out according to the formula: C = Ex / E0 * 1.25 µmol / L.

Ex - absorbance of the test sample;
E0 - absorbance calibration sample.

Enzime-linked immunosorbent assay

The change in the concentration of myoglobin and AIF was determined by enzyme-linked immunosorbent assay. Myoglobin concentration was assessed in the blood serum of animals, the content of AIF in the supernatant of muscle tissue. We used species-specific (mouse) sets of reagents for ELISA analysis by Cloud lone corp. (USA) manufacturing. The progress of the analysis was in accordance with the instructions attached to the kit.

Statistical methods

Statistical processing of the data was carried out using the software package «STATISTICA 6.0» (StatSoft, USA). The results were presented as M (median value) ± SD. To compare the groups of means, one-factor variant of ANOVA with the Newman-Keuls post-test was used. Differences were considered statistically significant at $p < 0.05$.

Results

During the first series of experiments on the study of acute toxicity of the studied derivatives of chromone-3-aldehyde, it was found that LD50 for the compound X3A4 was 2005 mg/kg, for the substance X3A5 2224 mg/kg, for compounds X3A1, X3A2 and X3A3 - 3120 mg/kg; 3059 mg/kg and 2587 mg / kg, respectively, that according to the GHS classification (New York and Geneva, 2011) corresponds to class 5 toxicity. Thus, for the second phase of the experimental work on the impact of derivatives chromone-3-aldehyde, to development of muscle dysfunction in mice injected doses of substances X3A4, X3A5 X3A1, X3A2 and X3A3 was 20 mg/kg as; 22 mg/kg 31 mg/kg 31 mg/kg and 26 mg/kg, respectively.

In the course of the second stage of research it was found that the background muscle strength in all experimental groups of animals was comparable with each other (Fig.2). In the PC group of mice muscle tone did not significantly change at all points of registration (point T1-T3). In animals of NC group at point T2 there was a decrease in muscle strength relative to the background value of this group of mice by 134.6% ($p<0.05$). It should be noted that after 30 minutes. Muscle tone in the group of NC animals was not restored (no statistically significant differences between points T2 and T3 in the group of mice deprived of pharmacological support were found).

ISSN: 1827-8620

Prophylactic administration of the studied compounds under the ciphers X3A1, X3A2 and X3A5 contributed to an increase in the grip strength of mice at point T2 (Fig.2). So use of the compounds X3A1, X3A2 and X3A5 grip strength of animals, exceeded similar parameter of the NC group of mice 54.7% ($p<0.05$) and 60.6% ($p<0.05$), 51% ($p<0.05$), respectively. At point T3 the muscle power of animals treated with test compound under the ciphers X3A1, X3A2 and X3A5 statistically significant was higher than that of NC group of mice for 72.8% ($p<0.05$) of 108.1 % ($p<0.05$) and 97.3% ($p<0.05$), respectively. In addition, the use of substances X3A2 and X3A5 had a positive impact on the process of restoration of the activity of striated muscles, so the muscle tone in animals treated with compound X3A2 in T3 was superior to the force of the grasp of this group of mice at T2 to 17% ($p<0.05$), and for the object under the code X3A5 analyze the difference (grip strength in point T3 and T2) was 18% ($p<0.05$). At the same time, recovery of muscle function in animals, which were injected connection X3A2, proceeded much faster than in mice treated with the test compoumd X3A1. So at point T3 the grip strength of animals, which corrects muscle dysfunction produced by introducing the compound X3A2, exceeded the same parameterr groups of mice receiving the object X3A1 20.4% ($p<0.05$). It should be noted that the use of substances under the ciphers X3A3 and X3A4 had no significant effect on the functional activity of skeletal muscle under the conditions of electromyostimulation (Fig.2).

During biochemical tests (tab.1) it is established that muscle dysfunction in electromiostimulation test, accompanied by an increase in from NC group of mice the activity of LDH and CK in the serum by 146.3% ($p<0.05$) and of 114.5%, respectively, increases in serum concentrations of myoglobin in 4.6 times ($p<0.05$) and decrease in serum creatinine concentration by 174,2% ($p<0.05$). In addition, the NC group of animals relative to the PC group of mice was found to have an increase in the concentration of lactic and pyruvic acids, a decrease in the protein concentration in muscle tissue homogenate by 3.3 times ($p<0.05$), 5.2 times ($p<0.05$) and 141.3% ($p<0.05$), respectively.

In the injection of the studied substances X3A1 and X3A5 the concentration of lactate in the muscle homogenate of experimental animals decreased in comparison with NC group of mice by 35.8% ($p<0.05$) and 50% ($p<0.05$), the content of pyruvate was also decreased by 59.6% ($p<0.05$) and 66% ($p<0.05$), respectively. The total protein concentration of the muscle tissue in the conditions of application of compounds X3A1 and X3A5 relative to the NC group of mice increased by 51.2% ($p<0.05$) and 78.5% ($p<0.05$), respectively. Additionally, when administered to animals of the studied compounds X3A1, X3A2 and X3A5 LDH activity compared with the NC group of mice decreased by 45.1% ($p<0.05$), 72.3% ($p<0.05$) and 29.3% ($p<0.05$), respectively, the activity of CK also decreased by 27.5% ($p<0.05$); and 87.5% ($p<0.05$) and 35.2% ($p<0.05$), respectively. The concentration of myoglobin in the blood serum of mice on the background correction of muscle dysfunction compounds X3A1, X3A2 and X3A5 reduce by 25.8% ($p<0.05$) and 39.4% ($p<0.05$) and 30.3% ($p<0.05$), respectively, on the contrary, the content of creatinine increased by 112,1% ($p<0.05$), 118,6% ($p<0.05$) and 104,2% ($p<0.05$), respectively. Introduction connections X3A3 and X3A4 significant influence on the change of the studied biochemical parameters in conditions of muscle dysfunction are not provided (tab.1).

Assessing the change in the concentration of AIF, it was found that in the negative control group of rats the content of AIF was 2.4 times higher than in the PC group of animals ($p<0.05$). In the conditions of correction of muscle dysfunction by the studied compounds, the concentration of AIF significantly changed when using compounds X3A1 and X3A5, exactly, there was a decrease in the content of AIF in animals receiving these objects, relative to the NC group of rats by 47.7% ($p<0.05$) and 85.7% ($p<0.05$), respectively. The introduction of test substance under the ciphers X3A2, X3A3 and X3A4 had no significant effect on the content of AIF in terms of muscle dysfunction (Fig.3).

Discussion

Muscle dysfunction is one of the main factors limiting physical activity, which can adversely affect the work of organs and systems, especially cardiovascular and respiratory. It was found that the decrease in physical activity contributes to an increase in the number of cases of hypertension, coronary heart disease, asthma – diseases with a high risk of complications, up to death [13]. In addition,

muscle fatigue is the main result-a limiting factor in professional sports [14]. The above makes the restoration of muscle tone and associated physical activity to the optimal level of one of the urgent problems of modern pharmacology. The study showed that the course application of new derivatives of chromone-3-aldehyde contributed to a much smaller manifestation of muscle dysfunction, compared with animals that did not receive pharmacological support, in terms of muscle electrical stimulation test, which was confirmed by the data of biochemical studies.. It is important that along with the high pharmacological activity of the studied compounds have low toxicity (5 class of toxicity according to the GHS classification).

The decrease in the concentration of lactic and pyruvic acids, an increase in creatinine levels was noted against the background of the use of the studied compounds. Apparently, it may reflect the ability of the studied compounds to normalize the oxidative function of mitochondria, pushing the moment of inclusion of glycolysis and intensifying, thereby, the work of skeletal muscles. In addition, a decrease in the concentration of AIF on the background of the use of the studied substances may indicate the preservation of the optimal structure of mitochondria and suppression of apoptosis reactions. It is known that an increase in the concentration of AIF is observed in the conditions of mitochondrial destruction, resulting in the launch of caspase- independent pathway of apoptosis, thereby contributing to accelerated destruction of muscle tissue [15]. Thus, a statistically significant decrease in the concentration of AIF on the background of the use of studied objects X3A1 and X3A5 can contribute to the preservation of the optimal structure and function of mitochondria, and positively affect the activity of skeletal muscle in conditions of muscle dysfunction.

Conclusion

The study showed that the new derivatives of chromone-3-aldehyde in prophylactic administration contribute to the elimination of muscle dysfunction, normalize energy and plastic metabolism in muscle tissue, suppressing the formation of AIF. In this case, the studied compounds are low-toxic substances. Based on the findings, it can be assumed that further study of the derivatives of chromone-3-aldehyde is a promising direction of search for remedies for the correction of muscle dysfunction. In this connection ciphers X3Al and X3A5 in the series of studied substances was more pronounced mioprotective properties than objects X3A2, X3A3 and X3A4.

References

1. André LM, Ausems CRM, Wansink DG, Wieringa B. Abnormalities in Skeletal Muscle Myogenesis, Growth, and Regeneration in Myotonic Dystrophy. Front Neurol. 2018;9:368. Published 2018 May 28. doi:10.3389/fneur.2018.00368
2. Al-Mulla MR, Sepulveda F, Colley M. A review of non-invasive techniques to detect and predict localised muscle fatigue. Sensors (Basel). 2011;11(4):3545-94.
3. Wan JJ, Qin Z, Wang PY, Sun Y, Liu X. Muscle fatigue: general understanding and treatment. Exp Mol Med. 2017;49(10):e384. Published 2017 Oct 6. doi:10.1038/emm.2017.194
4. Stackhouse SK, Reisman DS, Binder-Macleod SA. Challenging the role of pH in skeletal muscle fatigue. Phys Ther 2001; 81: 1897–1903.
5. Allen DG, Trajanovska S. The multiple roles of phosphate in muscle fatigue. Front Physiol 2012; 3: 463.
6. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiol Rev. 2014;94(3):909-50
7. Pfeffer G, Chinnery PF. Diagnosis and treatment of mitochondrial myopathies. Ann Med. 2011;45(1):4-16.
8. Gaspar A., Matos M.J., Garrido J., Uriarte E., Borges F. Chromone: A valid scaffold in medicinal chemistry. Chem. Rev. 2014;114:4960–4992. doi: 10.1021/cr400265z.
9. Vazquez-Rodriguez S., Uriarte E., Santana L., Borges F. Synthesis and pharmacological activities of non-flavonoid chromones: A patent review (from 2005 to 2015) Expert Opin Ther Pat. 2015;25:1285–1304. doi: 10.1517/13543776.2015.1078790.
10. Csepanyi E, Szabados-Furjesi P, Kiss-Szikszai A, et al. Antioxidant Properties and Oxidative Transformation of Different Chromone Derivatives. Molecules. 2017;22(4):588. Published 2017 Apr 6. doi:10.3390/molecules22040588

11. Finney, D.J. *Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve*. Cambridge University Press, London and New York, 1947.

12. Gregory NS, Gibson-Corley K, Frey-Law L, Sluka KA. Fatigue-enhanced hyperalgesia in response to muscle insult: induction and development occur in a sex-dependent manner. Pain. 2013;154(12):2668-2676. doi:10.1016/j.pain.2013.07.047.

13. Rutherford G, Manning P, Newton JL. Understanding Muscle Dysfunction in Chronic Fatigue Syndrome. Journal of Aging Research. 2016;2016:2497348. doi:10.1155/2016/2497348.

14. Johannes F,Jan W, Lutz V ,, Winfried B., Preventive and Regenerative Foam Rolling are Equally Effective in Reducing Fatigue-Related Impairments of Muscle Function following Exercise. J Sports Sci Med. 2017; 16(4): 474–479.

15. Bano D, Prehn JHM. Apoptosis-Inducing Factor (AIF) in Physiology and Disease: The Tale of a Repented Natural Born Killer. EBioMedicine. 2018;30:29-37

***ISSN:** 1827-8620*

Figure 1. Schematic representation of the synthesis of the studied objects

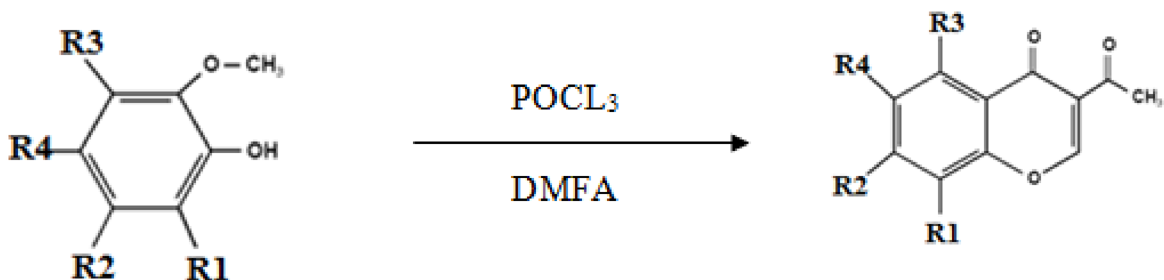
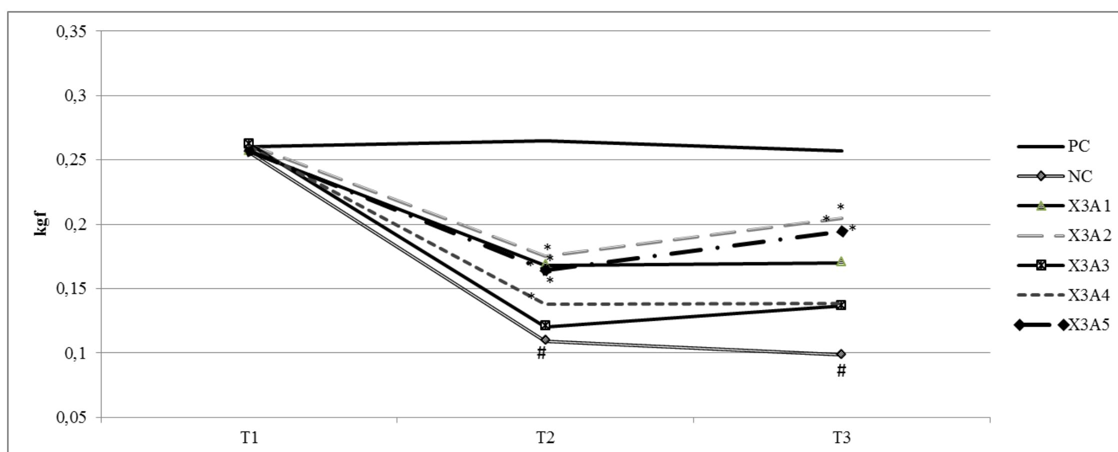


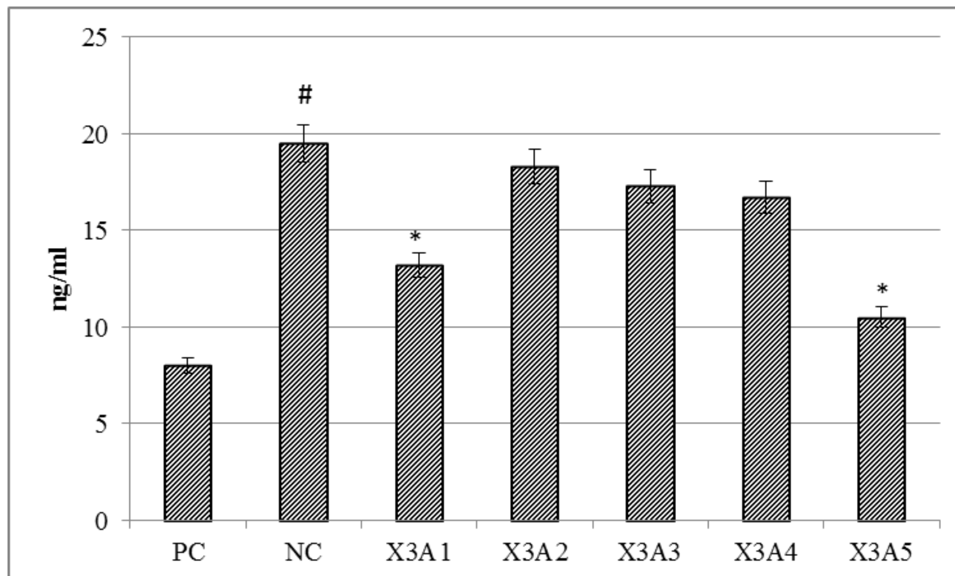
Figure 2. Influence derived chromone-3-aldehyde on the muscular strength of the animals in the test «grip- strength»



Note: PC – the group of animals positive control; NC – negative group of animals control;
* - statistically significant relative to the NC group of animals;
# - statistically significant relative to the PC group of animals.

ISSN: 1827-8620

Figure 3. Changes in the concentration of AIF in the conditions of correction of muscle dysfunction by chromone-3-aldehyde derivatives



Note: * - statistically significant relative to the NC group of animals;
- statistically significant relative to the PC group of animals

Table 1. Changes in biochemical parameters against the background of correction of muscle dysfunction by chromone-3-aldehyde derivatives

Group	PC	NC	X3A1	X3A2	X3A3	X3A4	X3A5
Lactic acid, mmol/g	0,22± 0,005	0,72± 0,08#	0,53± 0,02*	0,58± 0,023	0,65± 0,024	0,69± 0,045	0,48± 0,036*
Piruvic acid, mmol/g	0,016± 0,003	0,083± 0,041	0,052± 0,005*	0,068± 0,004	0,071± 0,001	0,08± 0,001	0,05± 0,007*
Total protein, g/l	16,12± 0,332	6,68± 0,442#	10,1± 0,235*	9,6± 0,993	7,41± 0,211	7,03± 0,314	11,93± 0,631*
Myoglobin, ng/ml	9,42± 0,273	43,02± 2,963#	34,19± 5,237*	30,85± 3,142*	39,44± 2,652	40,81± 3,033	33,01± 2,951*
LDH, U/L	996,78± 42,599	2454,61± 56,571#	1691,13± 31,012*	1424,23± 50,141*	2400,32± 20,106	2310,25± 48,231	1897± 56,447*
CK, U/L	558,85± 46,525	1198,89± 45,483#	940,12± 14,921*	639,25± 43,296*	1016,38± 63,175	960,33± 108,837	886,54± 41,136*
Creatinine, mmol/l	88,41± 3,574	32,15± 2,417#	68,18± 4,911*	70,27± 2,239*	37,06± 4,971	29,85± 5,104	65,64± 4,965*

Note: PC – the group of animals positive control; NC – negative group of animals control;
* - statistically significant relative to the NC group of animals;
# - statistically significant relative to the PC group of animals.

ISSN: 1827-8620