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STUDY OF PHARMACOLOGICAL ACTIVITY OF NIGELLA DAMASCENA FIXED OIL IN EXPERIMENT

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Abstract

Introduction: Literature data, rich chemical macro-microelement composition set the stage for experimental pharmacological study of adaptogene and lipid protective activity of Nigella damascena essential oil.

Objectives: study of pharmacological activity of Nigella damascena essential oil while simulating pathology on laboratory animals.

Methods: irritant action – HET-CAM test and DRAIZE-test, acute toxicity, open field test, simulated hypoxia method, swimming with the load, No-way-out and Rotating-rod tests, acute lipid pathology under Tween 80 (tween lipid pathology), vitamin D_2 hyperlipidemia, simulated chronic heart failure (CHF).

Results and Discussion: Nigella damascena essential oil have low irritant effect along with almost non-toxicity. Nigella oil increase life duration with the presence of hypoxia and endurance while swimming-with-the-load test. Course administration of Nigella oil normalize hormone-mediatore metabolism on the background of acute stress. Course administration of Nigella damascena essential oil normalize lipids on the background of CHF, acute tween and subchronic vitamin D_2 hyperlipidemias.

Conclusion: stress protective, actoprotective and adaptogene properties of Nigella damascena essential oil was pharmacologically proved. It was substantiated experimentally that Nigella damascena essential oil has an impact on lipid metabolism on the background of chronic heart failure (CHF), acute tween, subchronic vitamin D_2 lipid pathologies and blood serum lipoprotein lipase (LPL) activity.

Keywords: Nigella damascena essential oil, actoprotector, adaptogene, hyperlipidemia.

Introduction

According to variety of literature data Nigella damascena seed essential oil contain: essential oil (31-42%); essential oil with nigellon and thymoquinone as main components (0.37 – 0.5%); sum of alkaloids (damascene and damascenine 0.1-0.3% respectively); flavonoids; sesquiterpene compounds; tocopherols; steroids; triterpenoid saponins; coumarins; quinones (thymoquinone); lipase enzyme; macro- (potassium, calcium, magnesium, ferrum) and microelements (manganese, magnesium, zinc, copper, molybdenum, strontium, selenium, chrome, lead, boron, iodine) [1, 2, 3, 4, 5].

Literature data, rich chemical, macro- and microelement composition set a base for experimental pharmacological research of Nigella damascena essential oil adaptogene and lipid protective activity [6, 7].

Objectives: study of pharmacological activity of Nigella damascena essential oil while simulating pathology on laboratory animals.

Methods

The research was conducted in accordance with the policy of Declaration of Helsinki. Experiments were carried out with 246 white male Wistar rats 180-200 g weight, 12 white mice 30-33 g weight, 4 guinea pigs 600-700 g weight and 12 Leggron chicken embryo 9-10 days.

Experiment started with oil irritant effect on chicken embryo – HET-CAM test. Fixed oil was applied on chorioallantoic membrane at a dose of 0.3 ml and monitored for 120 sec. DRAIZE-test was conducted on guinea pigs during 2 min. Sunflower oil GOST R 52465-2005 was used as a control (solvent).

Mice were administered once by Nigella damascena essential oil at a dose of 8.5 mg/kg as the rats at a dose of 30 mg/kg intragastrically to determine acute toxicity. Animals were monitored for 14 days.

Open field test was conducted 24 hours since mice were administrated with 0.17 ml of Nigella damascena essential oil to examine motion behavior features and emotional state. The experiment lasted for 3 min. Amount of center and squares crossing, postures, grooming (washing), defecation, diuresis served as indexes of behavior [8]. Experimental dose was defined during active rat swimming. Mature male rats were divided into 7 groups of 6 animals which were selected on the base of bodyweight and swimming ability. Oil had been administered intragastrically once a day before the experiment for 14 days at the doses of 1, 5, 10, 15, 20, 25 mg/kg in accordance with groups. Control animals received distilled water [9, 10].

The aim of the second stage was to determine adaptogene activity of Nigella damascena essential oil. The aim was gained by a series of experiments. Antihypoxic effect was modeling by normobaric normocapnic hypoxia. Rats were placed into hermetic chamber 1.5 l vol. Carbon dioxide was eliminated by soda lime. Animals life duration indicated the hypoxia impact resistance. Nigella damascena essential oil was administered intragastrically at the doses of 1mg/kg and 10mg/kg one time 40 min before experiment or as a course during 2 weeks. Control animals received distilled water [11, 12].

For examination of actoprotective activity of Nigella damascena essential oil were used: forced swimming in 33-35^{o}C water, with load – 7.5% of rat bodyweight to refusal and rotating rod tests with mice (animals were placed on horizontal rod, rotating at a rate of 10 rpm) [13, 14].

Stress has been evoked by fixation rats on its back for 6 hours resulting in development of pain-emotional stress type of No-way-out situation. Stress resistance was determined by following values: thymus and adrenal glands weight, gastric mucosal ulceration and ulceration rate. Biochemical values were studied by spectrofluorometric (spectrofluorometer "Hitachi" MPF-4) and colorimetric (photoelectric colorimeter КФК-2 – УХЛ4,2) methods. Rat's blood, hearts, cerebral cortex were analyzed and homogenized. Concentration of adrenalin (AD), noradrenaline (NAD), dopamine (DA), 11-oxycorticosteroid, serotonin (S) and histamine (H) [15, 16, 17, 18, 19].

Pharmacological effect of Nigella damascena essential oil was studied on the background of simulated hyperlipidemia by setting acute tween lipid pathology. Were studied two types of Nigella damascena essential oil administration schedule at the background of "tween" model of hyperlipidemia: one-time administration and a course administration. In a course oil was administered oral using tube for 14 days. Untreated animals and animals who got physiological solution at a dose of 1mg/100g were used as a control. After 14 days Tween-80 was administered intraperitoneally and 12 hours after that decapitation was performed [20]. Subchronic vitamin model was simulated by course administration of vitamin D_2 along with everyday administration of cholesterol alimentary and mercazolilum for metabolism inhibition. Vitamin model of hyperlipidemia was induced using Vsilenko's method [21]. Experimental animals' blood serum and liver were examined for biochemical values to make

a judgment about lipid metabolism. Those values are: rat's blood serum cholesterol, blood serum triglyceride, blood serum beta- and pre-beta-lipoproteins, blood serum lipoprotein lipase, liver cholesterol, liver triglyceride [22, 23].

Right ventricular chronic heart failure was simulated by intermittent admission of silicone oil into each pleural cavity at a dose of 1.5ml/100g rat weight according with the method of N. Pyatnitskiy and Y. Blinkov. Oil administration was performed under hexenalum anesthesia. After 30 days oil administrations were repeated at a dose of mg/100g of rat weight. Course of Nigella damascena essential oil has been administered for 14 days 24 hours after second administration [24, 25].

Results and Discussion

First step was to determine irritant effect. Chicken embryo coat was intact, clear, thin, with blood vessel and capillaries system functioning properly. Next was local irritating action. DRAIZE-test showed hyperemia and chemosis score <2. Results obtained allows for the conclusion that Nigella damascena essential oil has low irritant effect.

Animals' survival ability after injection of Nigella damascena essential oil for acute toxicity determination was 100% in 2 weeks monitoring. According with K. Sidorov's classification essential oil refers to 5 class as non-toxic compound.

Thereby while valuing using safety low irritating effect was determined along with practically non-toxic features.

Open field test administration showed 42.9% mood increase after Nigella damascena administration resulting in defecation, diuresis and posturing. Physical activity had a tendency to increase up to 9.5% resulting in center, squares crossing and grooming amount (Figure 1).

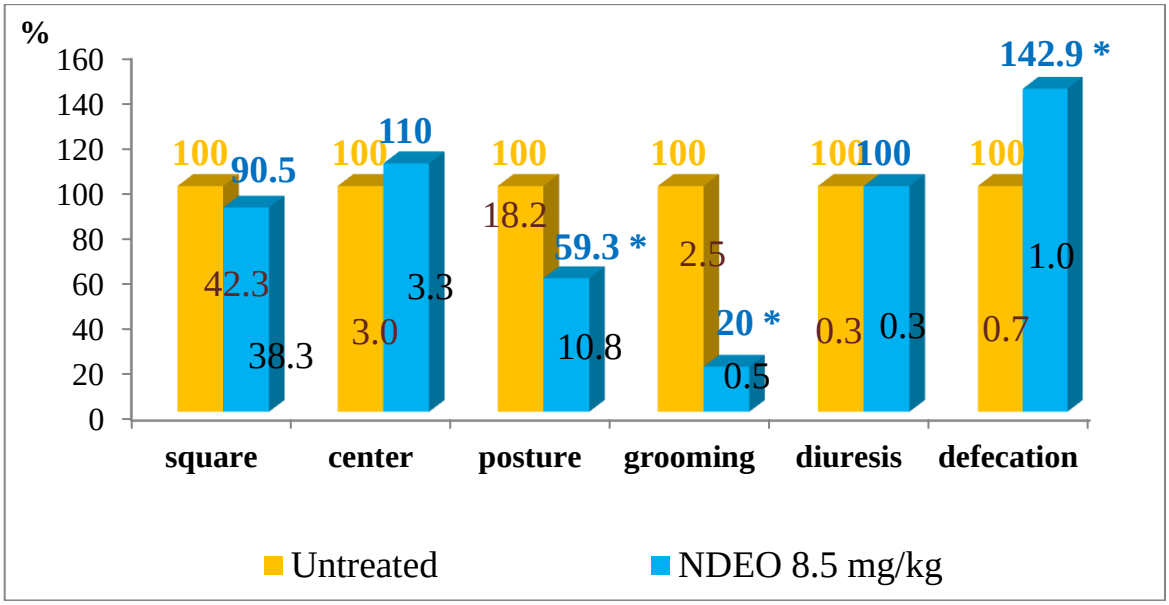

Fig. 1. The influence of Nigella damascena essential oil on nervous system.
Note: NDEO – Nigella damascena essential oil, * – p>0.05 reliable relatively to untreated animals

Experimental dose of Nigella damascena essential oil was chosen by the results of the research on dose addiction depended on dynamic performance rising. As the result was determined that in a 10mg/kg dose of Nigella damascena essential oil swimming time increased in comparison with swimming time of control group. What is more, increase had linear character from 1 to 15 mg/kg. Following dose escalation had no valued dynamic performance rising (Figure 2) [8].

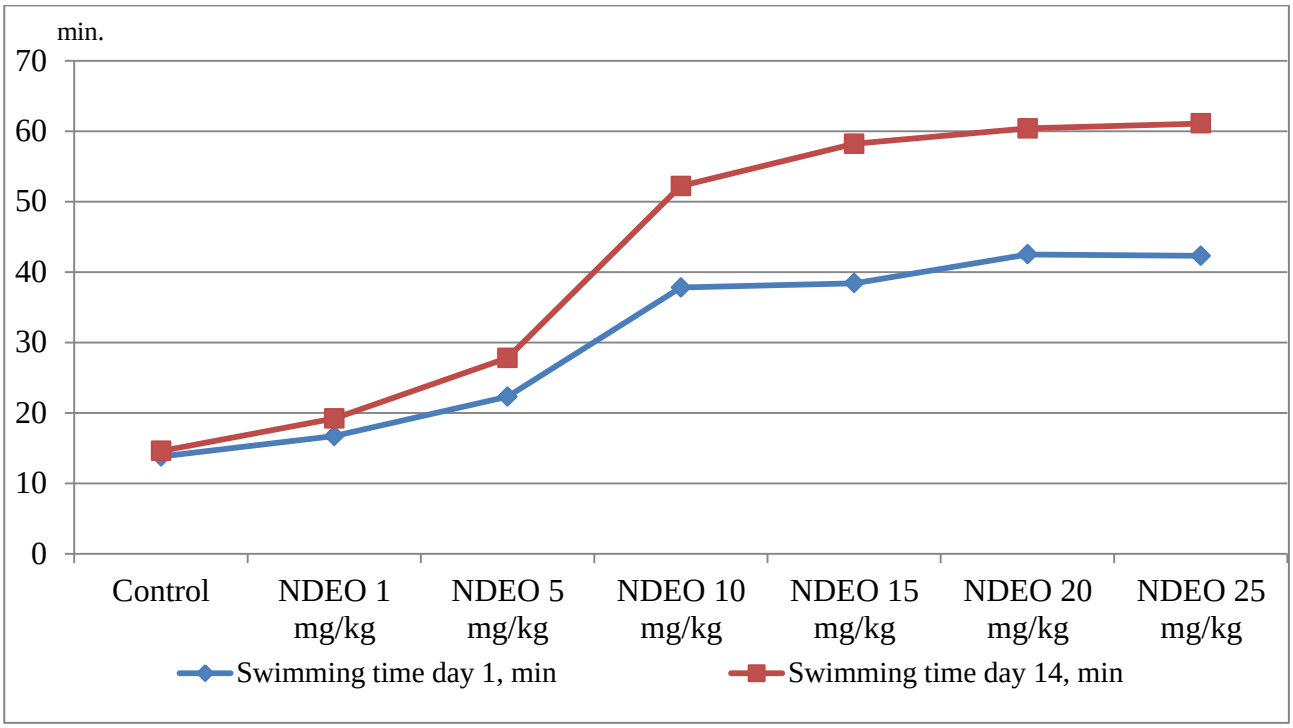

Fig. 2. Ergotropic effect escalation after Nigella damascena essential oil administration at the doses from 1 to 25 mg/kg.
Note: NDEO – Nigella damascena essential oil, min – minutes

Thereby according to results of 2 weeks' oil administration research there were chosen experimental groups with 1 mg/kg and 10 mg/kg one-time and course administration.

For the second stage of the research the aim was to determine adaptogene Nigella damascena essential oil activity. The adaptogene medications tend to combine at least three types of activity – actoprotective, antihypoxic and stress protective [26].

Antihypoxic effect showed in rat's life duration increase up to 63.5 ± 3 min with both types of administration – one-time and course in comparison to control group. However reliable data became available for Nigella damascena essential oil at a dose of 10 mg/kg, which increased rat's life duration on the background of hypoxia up to 35% in acute experiment and up to 26% after course administration (Fig. 3).

Actoprotective activity of Nigella damascena essential oil resulted in forced swimming with the load and Rotating rod test. Experimental data gathered while swimming shows that one-time administration of Nigella damascena essential oil at a dose of 10 mg/kg 2 times increases rat's performance in the comparison to control group and 3 times increases performance after course administration. Rotating rod test showed increase in mice' muscular load tolerance up to 81.1% after one-time administration and up to 209.1% after course administration (Figure 3).

Stress tolerance experiment showed that Nigella oil at a dose of 10 mg/kg prevents thymus involution, decreasing its presence up to 35% after one-time administration and normalizing this value after course administration. Adrenal gland examination showed prevention of gland hypertrophy accumulation after one-time and course Nigella oil administration up to 28.78% and 38.85% respectively. Gastric mucosa erosion reduced 2.6 times after one-time administration and 7.8 times after course administration in comparison with control (Figure 4).

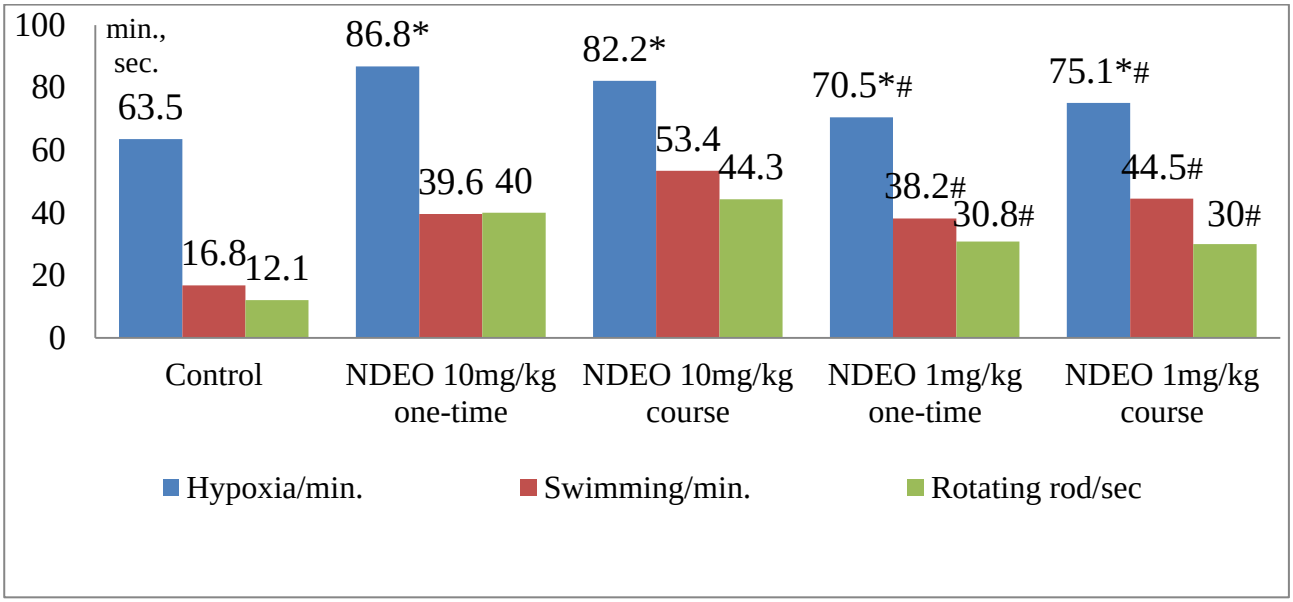

Fig. 3. The influence of Nigella damascena essential oil 10 mg/kg on muscular load and hypoxia.
Note: NDEO – Nigella damascena essential oil; min. – minutes; sec. – seconds; * – p>0.05 reliable relatively to control animals; # – p>0.05 reliable relatively to animals who got NDEO 10mg/kg one-time

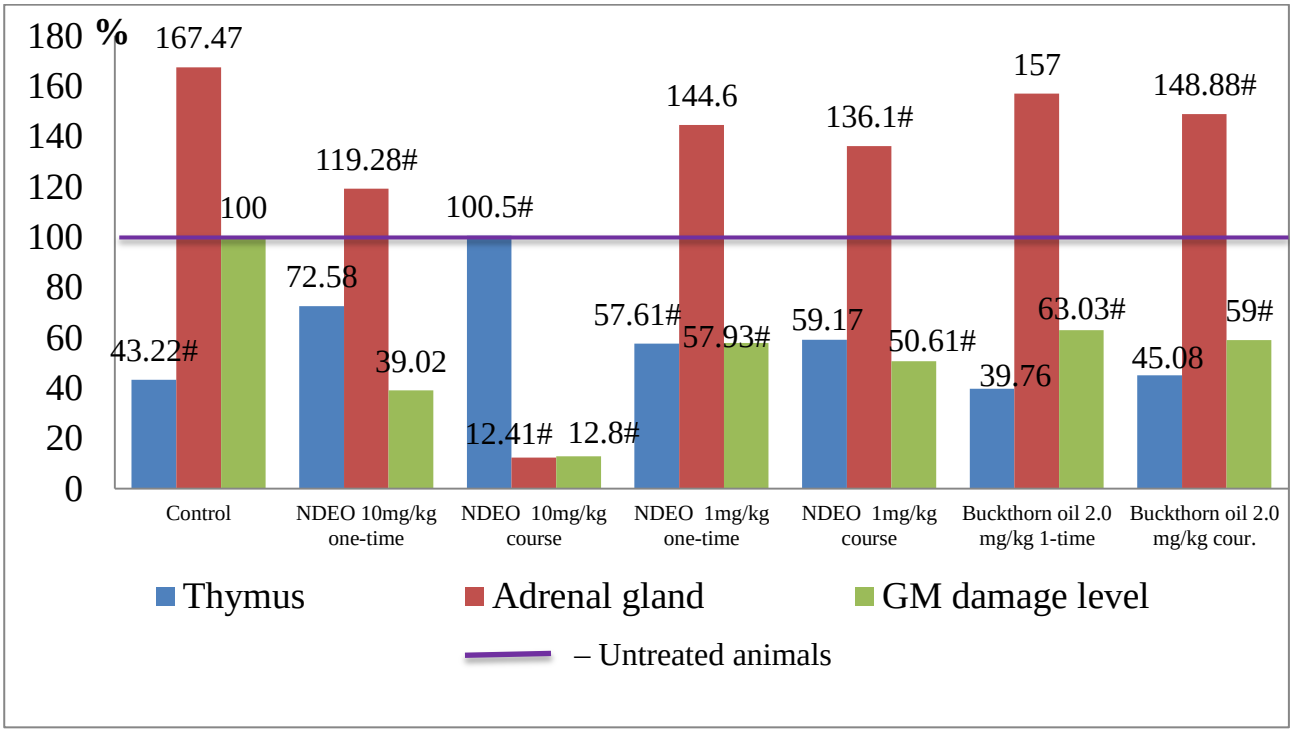

Fig. 4. Stress protective activity of Nigella damascena essential oil at a dose of 10 mg/kg and 1 mg/kg.
Note: NDEO – Nigella damascena essential oil; cour – course administration; GM – gastric mucosa; # – p>0.05 reliable relatively to control animals

Stress resistance reflected in changings of biochemical values: adrenalin (AD), noradrenaline (NAD), dopamine (DA), serotonin (S), histamine (H) and protective effect index (PEI) in blood, heart, cerebral cortex.

Blood plasma showed 2.23 times reducing concentration of DA mediator at the background of increasing AD 1.74 times and NAD 2.24 times. There was an increasing of concentration of NAD and DA in heart muscle

of stressed animals 2.03 and 1.62 times respectively. Adrenalin level in heart truly 1.77 times reduced in comparison to untreated rats. In cerebral cortex was seen truly increasing of adrenalin concentration – 1.87 times, noradrenaline – 6.1 times and dopamine – 1.56 times compared with the normal range.

For objective interpretation of gathered data were used protective effect index (PEI), which shows the ability of preparation to increase organism resistance against various interferences and negative effectiveness indicator (NEI). Preparation can be counted as adaptogene if its PEI>0.2 (Figure 5).

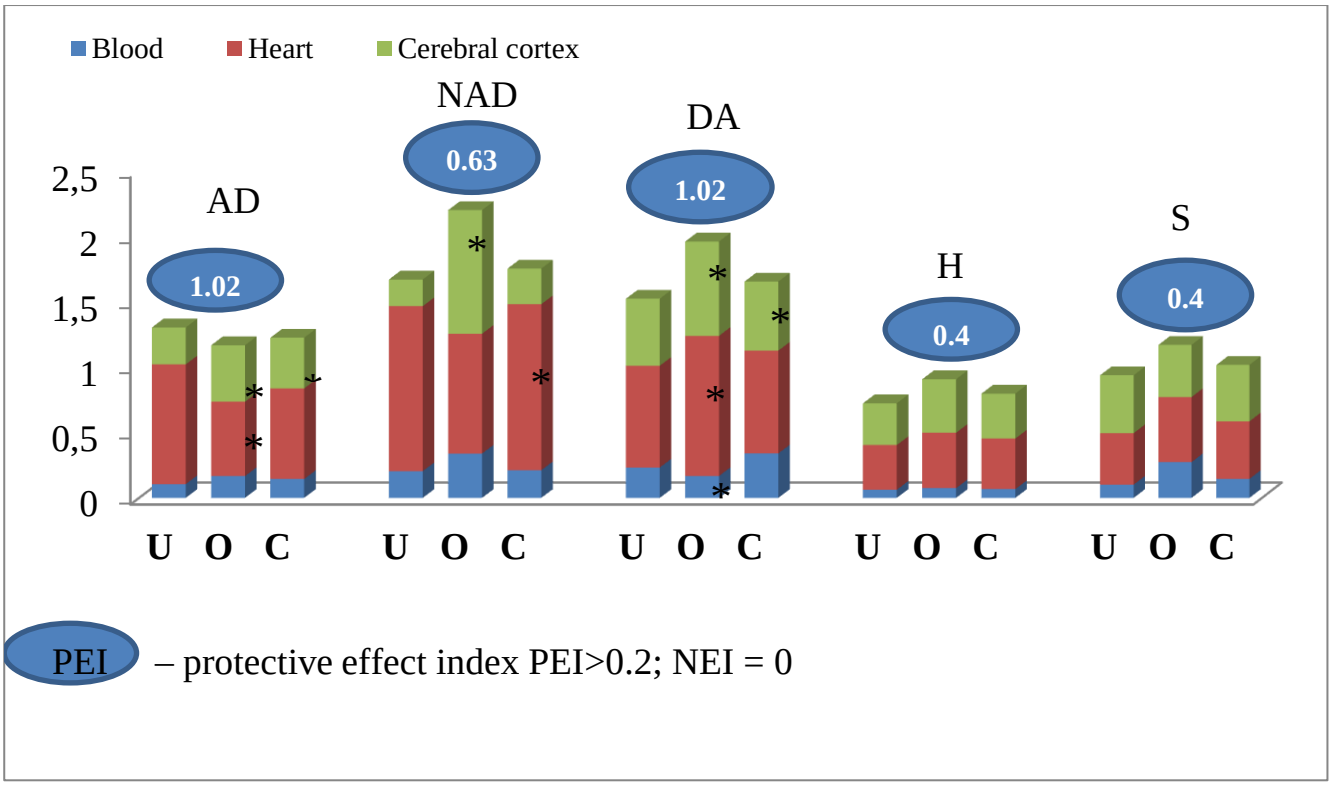

Fig. 5. The influence of Nigella damascena essential oil on hormone-mediator metabolism under simulated acute stress.
Note: U – untreated; O – one-time administration; C – course administration; AD – adrenaline; NAD – noradrenaline; DA – dopamine; H – histamine; S – serotonin;
* – p>0.5 reliable relatively untreated animals

Stress protective research would be incomplete without research of adrenal gland activity marker. During the experiment 1.9 times increase of 11-oxycorticosteroids (11-OXY) concentration in blood was determined in comparison to untreated group. Course administration of Nigella oil at a dose of 10 mg/kg led to complete normalizing of hormone level in blood.

Table 1

The influence of Nigella damascena essential oil 10 mg/kg on 11-OXY metabolism in rat's blood under acute stress

Value	Untreated	Control (stress)	NDEO 10 ml/kg one-time	NDEO 10 ml/kg course.
11-OXY, mkg/ml	0.575±0.041	1.108±0.062*	0.851±0.114*	0.592±0.056#

Note: NDFO – Nigella damascena essential oil, 11-OXY – 11-oxycorticosteroid; * – p>0.05 – reliable relatively untreated animals; # – p>0.05 – reliable relatively control animals.

Thereby Nigella oil 10 mg/kg one-time and course administration promoted normalization of catecholamine in all examined tissues. What is more, corrective oil effect became more evident after course administration.

Studying the influence of Nigella damascena essential oil on rat's lipid profile under simulated chronic heart failure (CHF), acute tween and subchronic vitamin D_2 lipid pathologies was the next stage of the research.

At the background of simulated CHF pathology Nigella damascena essential oil administration at a dose of 10 mg/kg prevented lipid metabolism failure and reflected on lipid profile. In experimental rat group normalization of lipid profile was related to reliable reducing of low-density lipoprotein concentration by 68.38% herewith high-density lipoprotein concentration was increased by 22.3% relatively control. At the background of Nigella damascena essential oil administration also total cholesterol reducing by 13.2% was observed (Figure 6).

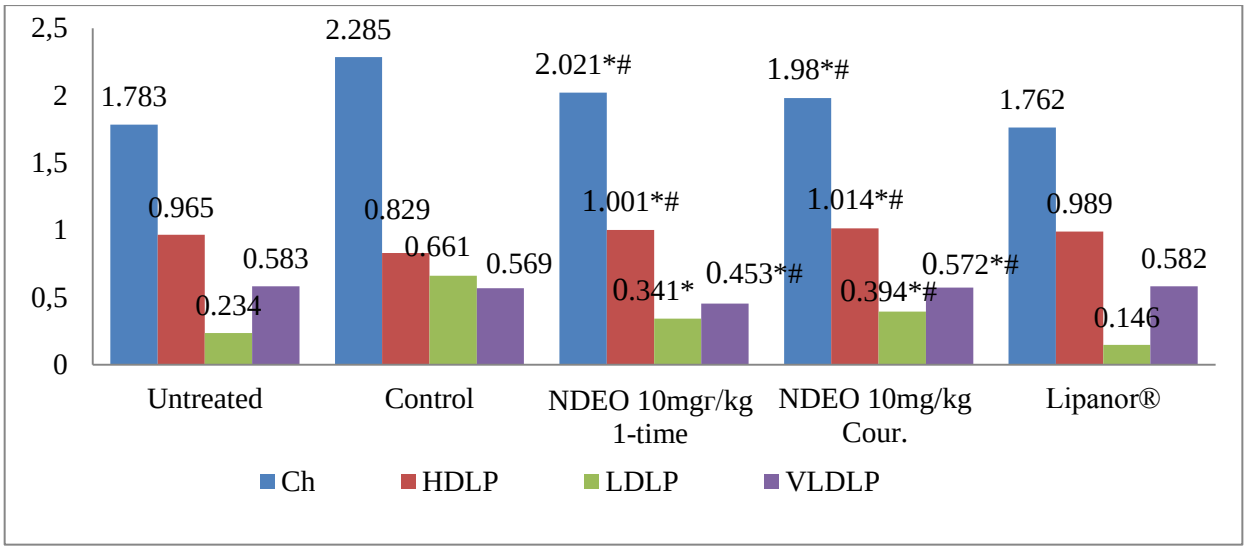

Fig. 6. The influence of Nigella damascena essential oil on rat's lipid metabolism under chronic heart failure.

Note: NDEO – Nigella damascena essential oil; Ch – cholesterol; HDLP – high-density lipoprotein; LDLP – low-density lipoprotein; VLDLP – very low-density lipoprotein; 1-time – one-time administration; Cour. – course administration; * – $p>0.05$ reliable relatively untreated animals; # – $p>0.05$ – reliable relatively control animals

The cholesterol level reducing was detected in blood serum by 39.6% and by 50.6% after one-time and course administrations respectively as in liver – by 94.1% and 96.2% after one-time and course administrations respectively under acute tween hyperlipidemia. Triglyceride content tended to reduce in blood serum by 36% and 78.5% after one-time and course administration respectively and in liver – by 56.9% (one-time) and 71.5% (course) (Figure 7).

Same lipid metabolism values changes happened under vitamin D_2 hyperlipidemia. Cholesterol in rat's blood serum and liver reduced by 10.43% and 24.63% respectively after one-time administration and by 22.44% and 22% respectively after course administration. Triglyceride level reduced in blood serum by 25.73% and by 36.73% in liver after course Nigella oil administration (Figure 8).

To improve gathered data lipoprotein lipase (LPL) activity was examined by separate set of experiments. LPL activity increased after Nigella damascena fixed oil administration to rats with acute tween and D_2 subchronical intoxication up to 1.321±0.20 lipase unit which in relative units would be 36% relatively control (Figure 9).

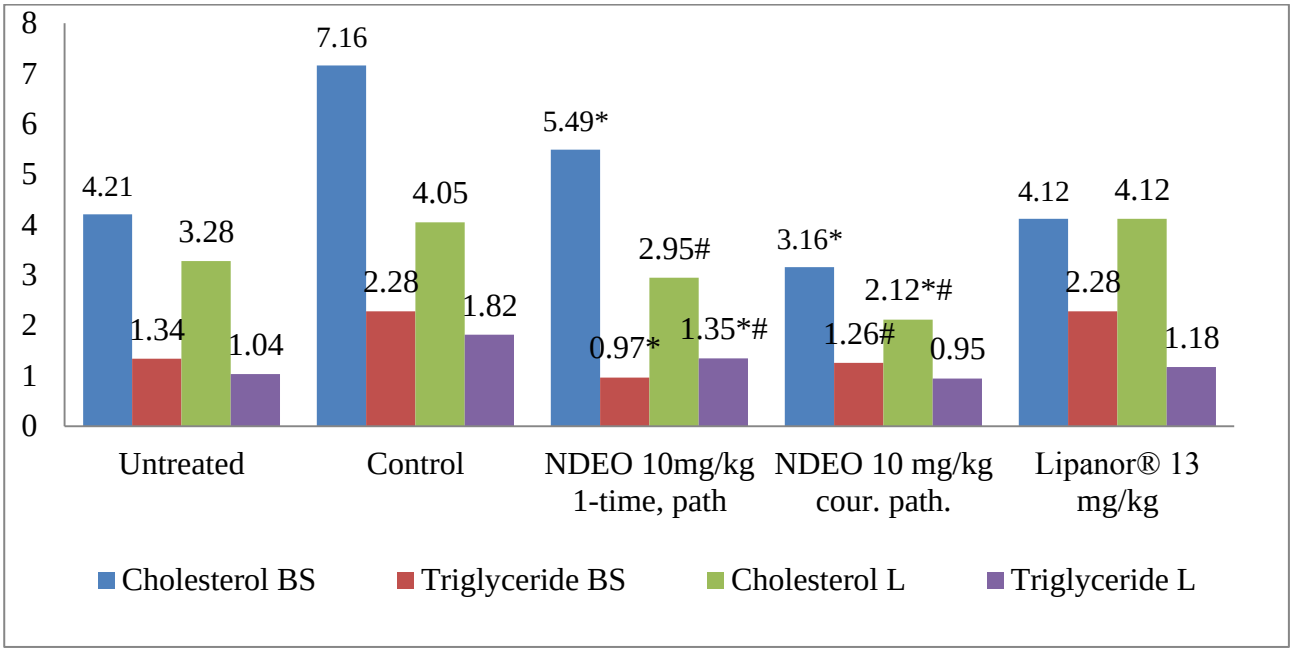

Fig. 7. Lipid metabolism values changing in blood serum and liver after Nigella damascena essential oil administration under acute tween lipid pathology.
Note: NDEO – Nigella damascena essential oil; 1-time – one time administration; cour. – course administration; path. – pathology; BS – blood serum; L – liver; * – p>0.05 reliable relatively untreated animals; # – p>0.05 reliable relatively control animals.

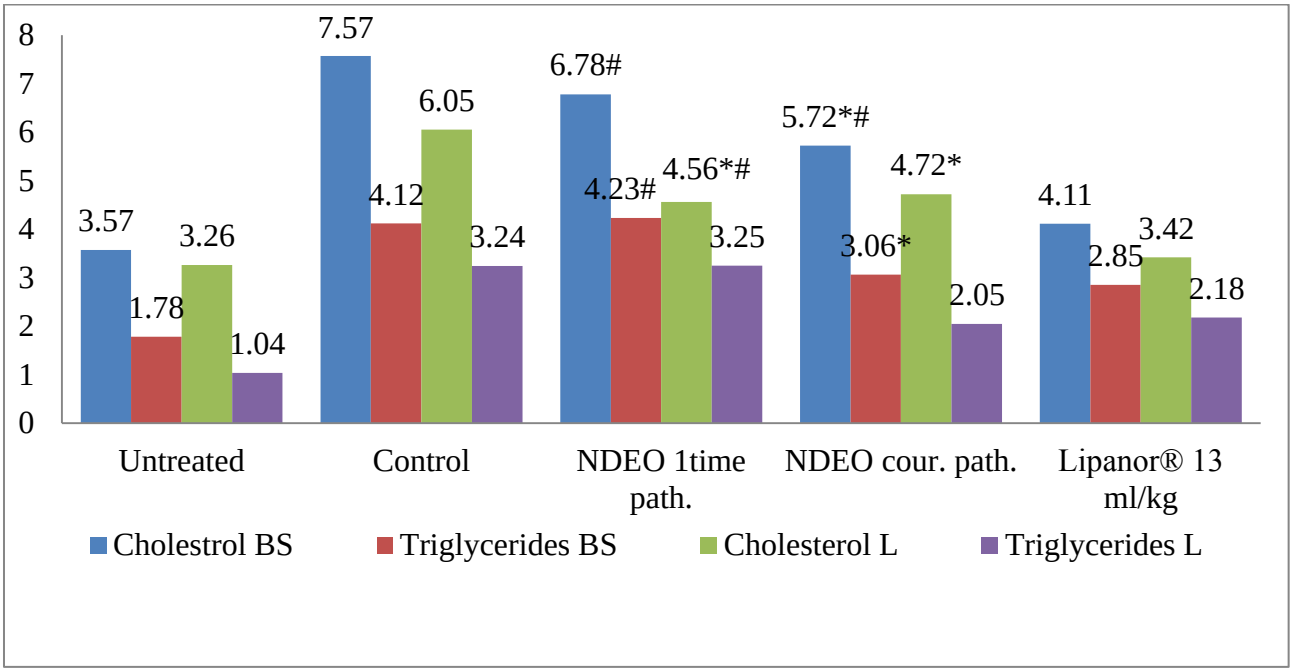

Fig. 8. Lipid metabolism values changing in blood serum (BS) and liver (L) after Nigella damascena essential oil administration at a dose of 10 mg/kg under subchronic vitamin D_2 lipid pathology.
Note: NDEO – Nigella damascena essential oil; 1time – one-time administration; cour. – course administration; path. – pathology; BS – blood serum; L – liver; * – p>0.001 difference reliability relatively untreated animals; # – p>0.001 difference reliability relatively control animals

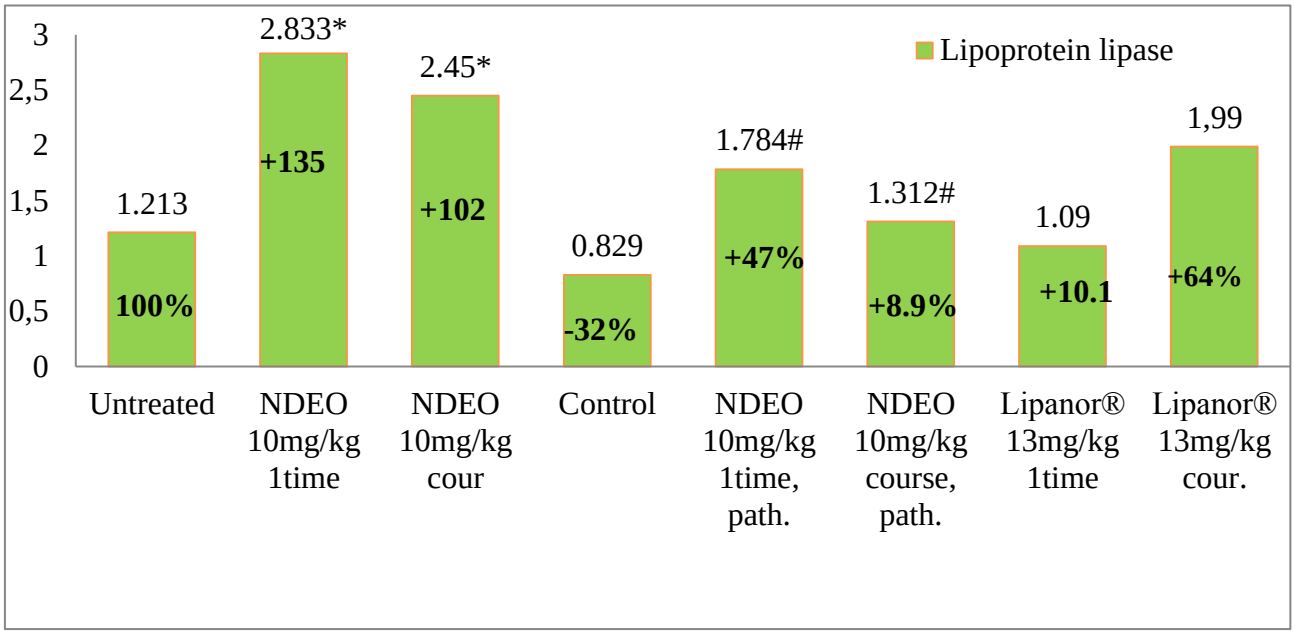

Fig. 9. The influence of Nigella damascena essential oil at a dose of 10 mg/kg on blood serum lipoprotein lipase activity.
Note: NDEO – Nigella damascena essential oil; 1time – one-time administration; cour. – course administration; path. – pathology; * – p>0.05 reliable relatively untreated animals; # – p>0.05 reliable relatively control animals

Conclusion

The experimental research resulted in conclusion that Nigella damascena essential oil has high activity while being safe for oral administration by mammal.

During the research adaptogene activity of Nigella damascena essential oil was determined by size of antihypoxic, actoprotective and stress protective effects whose intensity increases after course oil administration.

High stress protective effect of Nigella damascena essential oil was achieved at an experimental dose of 10 mg/kg.

Course administration of Nigella damascena essential oil normalized catecholamine level in blood, heart and brain under simulated stress. Changes of hormone-mediatore balance have positive compensatory character. Nigella damascena essential oil practically non-toxic and has no irritant effect. It was determined experimentally that Nigella damascena essential oil at a dose of 10 mg/kg has positive impact on lipid profile by normalizing it under simulated CHF. Also, it was determined that after lipid metabolism values were changed under acute tween and subchronical vitamin D_2 lipid pathology Nigella damascena essential oil' therapeutic effect at a dose of 10 mg/kg stays equal to comparator drugs.

The active impact on blood serum lipoprotein lipase was determined, after one-time administration and course administration alike.

Conflicts of Interest

The authors have no conflict of interest to declare.

References

1. Al-Kayssi AW, Shihab RM, Mustafa SH. Impact of soil water stress on Nigellone oil content of black cumin seeds grown in calcareous-gypsifereous soils. *Agricultural Water Management.* 2011;(100):46-57. [Fulltext]

2. Kokoska J, Havlik I, Valterova et al Comparison of chemical composition and antibacterial activity of Nigella sativa seed essential oils obtained by different extraction methods. *Journal of Food Protection.* 2008;71(12):2475-2480. [Fulltext]

3. Patudin AV, Mishchenko VS, Ilyenko LI, Kosmodemyanskiy LV. *Homeopathic medicinal products, allowed in Russian Federation for Healthcare Service and Veterinary usage*. Moscow; «Znak»: 2011: 352 p. (In Russian) [Fulltext]

4. Mashirova SU, Orlovskaya TV, Component composition analysis of Nigella Sativa and Nigella Damascena lipids. *Belgorod State University scientific journal. Series. Medicine. Pharmacy. [Nauchnye vedomosti Belgorodskogo gosudarstvennogo universisteta. Seriya. Meditsina. Farmatsiya]*. 2012;17(4):223-227. (In Russian) [eLIBRARY]

5. Rud' NK, Sampiev AM, Davitavyan NA. Core results of Nigella Sativa phytochemical and pharmacological study (review). *Belgorod State University scientific journal. [Nauchnye vedomosti Belgorodskogo gosudarstvennogo universiteta]*. 2013;25(168):207-212. (In Russian) [eLIBRARY]

6. Enikeev A, Zamotaev U, Kolomoets N. Adaptogenes in hypertonic disease complex therapy among people who have profession related to stressor impact. *Doctor. [Vrach]*. 2008;6:70-71. (In Russian) [eLIBRARY]

7. Studentsov EP, Ramsh SM, Kazurova NG, Neporozhneva OV, Garabadzhiu AV, Kochina TA, Voronkov MG, Kuznetsov VA, Krivorotov DV. Adaptogenes and relative medicinal drug groups – 50 years of research. *Clinical pharmacology and drug therapy reviews. [Obzory po klinicheskoi farmacologii i lekarstvennoi terapii]*. 2013;11(4):3-43. (In Russian) [eLIBRARY]

8. Mironov AN. *Medicinal product preclinical research execution guidance.* Part one. Moscow; Grif i K: 2012: 944 p. (In Russian) [eLIBRARY]

9. GOST 12.1.007-76 Occupational safety standards system. Noxious substances. Classification and general safety requirements. (As modified 1, 2). Moscow; Standartinform: 2007. (In Russian) [Abstract]

10. Prozorovskyi VB. Statistical pharmacological research results processing. *Psychopharmacology and biological narcology. [Psikhofarmakologiya i biologicheskaya narkologiya]*. 2007;7(3-4):2090-2120. (In Russian) [Fulltext]

11. Pavlov VA. Amino acids adaptogenes in the increasing of organism resistance to muscle loading and other stressors. *Health – is the basic of human potential: problems and ways of solution. [Zdorov'ye – osnova chelovecheskogo potentsiala: problemy i puti ikh resheniya]*. 2008;3(1):219-220. (In Russian) [eLIBRARY]

12. Khabriev RU. *Guidance for experimental (pre-clinical) study of new pharmaceutical substances.* 2-pub., updated and revised. – Moscow; OAO Izdatelstvo Medicina (a publicity held company under the laws of the Russian Federation): 2005: 832 p. (In Russian) [eLIBRARY]

13. Datieva FS, Borukaev BA, Khetaguriva LG. The impact of vegetable and animal adaptogenes on lipid metabolism in the experiment. *Health And Education in XXI century scientific articles magazine. [Zhurnal nauchnykh statey Zdorov'ye i obrazovanie v XXI veke]*. 2011;13(2):220-221. (In Russian) [eLIBRARY]

14. Shantanova LN, Nikolaev SM, Gulyaev SM, Tatarinova NK, Muruev BA, Matkhanov IA, Banzaraksheev VG. Fornicium Uniflorum L extract stress-protective activity. *Yakut Medical Journal. [Yakutskiy meditsinskiy zhurnal]*.2017;3(59):80-82. (In Russian) [Fulltext]

15. Sampieva KT, Vrubel' ME, Ramazanov MA, Tsikolia AM. The contribution of polyunsaturated fatty acids to Siberia pine oil activity. *International journal of experimental education. [Mezhdunarodnyy zhurnal eksperimental'nogo obrazovaniya.]*. 2016;3-1:59-61. (In Russian) [Fulltext]

16. Zatsepina EE, Usmanskii UV, Savenko IA, Sergienko AV, Ivashev MN, Bogdanov AN, Alieva MU, Yurkova AA. The fatty oil impact on experiment inflammation phases. *Present-day problems of education and science and. [Sovremennye problemy nauki i obrazovaniya]*. 2012;4:310 (In Russian) [eLIBRARY]

17. Suyarov AA, Komarin AS. The impact of flavonoid fraction isolated from Bidens tripartita in different doses on histamine content under experimental allergy. M*agazine*

of theoretical and clinical medicine. [Zhurnal teoreticheskoy i klinicheskoy meditsiny]. 2016;1:27-29. (In Russian) [eLIBRARY]

18. Kuznetsov VA, Shebeko PV, Enina TN, Soldatova AM, Melnikov NN, Rychkov AU. Adrenaline and noradrenaline in moderate heart failure. *Heart failure magazine. [Zhurnal serdechnaya nedostatochnost']*. 2013;14(5):252-255. (In Russian) [eLIBRARY]

19. Efremova MP. The impact of Nigella Damascena essential oil on animal hormone-mediator metabolism. *Successes of modern Natural Science. [Uspekhi sovremennogo estestvoznaniya]*. 2014;11-1:134. (In Russian) [Fulltext]

20. Ivanov VV, Rat'kin AV, Ppharger UA. Hyperlipidemic action of grossmizin along in vivo and in vitro experiments. *Siberian medical review. [Sibirskoe meditsinskoe obozrenie]*. 2015;5(89):43-48. (In Russian) [Fulltext]

21. Sergienko AV, Efremova MP, Ivashev MN. Rat's blood plasma lipid spectrum examine under simulated D2 lipid pathology after Nigella Damascena essential oil administration. *International applied and fundamental research magazine. [Mezhdunarodnyy zhurnal prikladnykh i fundamental'nykh issledovaniy]*. 2012;11:15-16. (In Russian) [eLIBRARY]

22. Kamyshnikov VS. *Clinical laboratory research methods.* Moscow: MEDpress-inform: 2013: 736 p. (In Russian)

23. Stroev EA, Makarova VG, Matveeva IV. *Biological chemistry manual.* Moscow: Meditsinskoe informatsionnoe agenstvo; 2012% 384 p. (In Russian)

24. Sergienko AV, Efremova MP, Savenko IA, Ivashev MN. The impact of Nigella Damascena essential oil on rat's blood plasma lipid spectrum under simulated chronic heart failure. *Modern knowledge-intensive technologies. [Sovremennye naukoemkie tekhnologii]*. 2012;8:42-43. (In Russian) [eLIBRARY]

25. Fedorov VN, Nozdrachev AD, Salnikov EV. Dynamic model of rat's total chronic heart failure. *Reporter of Saint Petersburg University. [Vestnik St. Peterburgskogo universiteta]*. 2006;2(3):103-107. (In Russian) [Fulltext]

26. Seregina EK, Fatihov IM, Nevolin YuS. Evaluation of new pharmacological activity of a pharmaceutical composition comprising an adaptogene, in the experiment. *Biomedecine*. *[Biomeditsina]*. 2011;1(4):126-128 (In Russian) [Fulltext]

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