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CORRECTION OF METABOLIC AND MORPHOLOGICAL DISORDERS IN EXPERIMENTAL ATHEROCALCINOSIS

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Abstract

This study examined the efficacy of a combination of plant extracts and the reference drug Rosuvastatin Agio in a rabbit model of experimental atherosclerosis. The condition was induced by administering atherogenic substances (cholesterol and vitamin D2) for three months, resulting in severe hypercholesterolemia and pronounced aortic arch atherocalcinosis. The results showed that 30-day use of the plant extract resulted in more pronounced lipid profile normalization than Rosuvastatin: total cholesterol decreased by 89%, and the atherogenicity index decreased from 23.0 to 2.7. Histological analysis confirmed the therapeutic efficacy, manifested in the regression of calcification foci and restoration of the structural order of vascular wall layers. Both therapies promoted normalization of blood glucose levels. These data demonstrate the high hypolipidemic and antiatherosclerotic potential of the studied extract.

Keywords: *experimental atherosclerosis, atherocalcinosis, plant extract, rosuvastatin, lipid profile, total cholesterol*

Introduction

Atherosclerosis remains one of the most serious medical and social problems of the 21st century, being the primary pathogenetic factor in the development of cardiovascular diseases such as coronary heart disease and myocardial infarction. The disease is characterized by chronic inflammation of the vascular wall, dyslipidemia, and the formation of atherosclerotic plaques, which leads to gradual occlusion of the arterial lumen and hemodynamic impairment.

Despite significant advances in modern pharmacology in lipid profile correction (in particular, the use of statins), the search for alternative and complementary treatments remains urgent. This is due to the side effects of synthetic drugs, as well as the need for a comprehensive approach to address various pathogenesis factors:

oxidative stress, inflammation, and endothelial dysfunction.

In this context, phytoextracts with high bioavailability and a polyvalent mechanism of action are of particular interest. Alfalfa (*Medicago sativa*): known for its high content of saponins and flavonoids, which can bind cholesterol in the intestine and reduce its levels in the blood. Hibiscus (*Hibiscus sabdariffa*): rich in anthocyanins and organic acids, which exhibit pronounced antioxidant properties and promote vasodilation. Anise (*Pimpinella anisum*): contains essential oils (anethole), which have anti-inflammatory potential and the ability to modulate metabolic processes.

Histological examination is the "gold standard" for assessing the effectiveness of anti-atherosclerotic therapy, as it allows visualization of morphological changes in the vascular wall, the degree of lipid infiltration, and the preservation of the structural components of the intima and media.

The aim of this study is to comparatively evaluate the effects of alfalfa, hibiscus, and anise extracts on the morphofunctional state of the aorta and the dynamics of atherosclerotic changes in experimental hyperlipidemia.

Materials and Methods

Objects of study: Alfalfa (*Medicago sativa*), Hibiscus (*Hibiscus sabdariffa*), Anise (*Pimpinella anisum*), plants growing in the mountainous regions of Uzbekistan.

Rosovustatin Agio, produced by the Indian company Agio Pharmaceuticals Ltd., was used as a comparison drug.

Extraction technology. Samples of crushed Hibiscus Sabdariffa raw materials (dried petals) and Pimpinella anisum were placed in an enamel container, filled with water in a ratio of 1:10, covered with a lid, and extracted for 60–90 min at a temperature of 60 °C. Then, the extract was filtered and freeze-dried. 25 g of dried crushed dry mass of Medicago sativa L. herb was placed in a two-necked flask and poured with 500 ml of distilled water, extracted for 1.5 hours at a temperature of 70°C. Then, the extract was filtered. A second stage of extraction was carried out under the same conditions for 45 min, the extracts were combined, evaporated on a rotary evaporator, and freeze-dried.

Atherosclerosis model. The animals were administered crystalline cholesterol (STOCK-MED MCHJ, China) in a dose of 200 mg/kg in cottonseed oil in a volume of 4.0–4.5 ml using an automatic pipette for a long period (3 months). After 1 month of cholesterol administration, vitamin D2 (ergocalciferol), a 0.0625% solution in oil, was added to the animals' diet at a dose of 0.256 ml/kg for 30 days to enhance aortic lipidosis. In order to enhance sclerotic changes in the aorta and reduce the time of atherosclerosis induction, 1 month after the start of cholesterol administration and for the next 30 days, the animals were administered adrenaline at a dose of 0.04 mg/kg of body weight intravenously every 5 days (i.e., 6 injections). After three months, the surviving animals were divided into three groups: Group 1 (control) received water orally, Group 2 received a combination of extracts at a dose of 40 mg/kg, and Group 3 received the reference drug Rosuvastatin-Adgio at a dose of 0.4 mg/kg for 30 days. At the end of the experiment, the animals were transferred to the Republican Pathological Anatomy Center in Tashkent for histological examination of their organs and vessels.

Blood was collected dropwise from the rabbit's ear vein into microtubes over time. A study of the baseline parameters was conducted after 3 months of challenge and after 15 and 30 days of treatment of the following parameters: total protein, glucose, lipid spectrum: triglycerides (TG), total cholesterol (TC), high-density lipids (HDL), low-density lipids (LDL), on a biochemical analyzer using kits from CYPESS DIAGNOSTICS (Germany).

The content of very low density lipoprotein cholesterol (VLDL, mmol/l) and the atherogenic coefficient of cholesterol (AC_{xc}, O.U.) were calculated using the Friedwald formula:

$$AC_{xc} = \frac{TC - HDL}{HDL}, \quad VLDL = \frac{TG}{5}, \quad (1)$$

total protein, glucose, lipid profile: triglycerides (TG), total cholesterol (TC), high-density lipids (HDL), low-density lipids (LDL), very low-density lipoproteins (VLDL), and cholesterol atherogenic index (CAI)

Statistical analysis. All data were presented as mean ± standard error of the mean (SEM). Statistical analyses were performed

using R software (version 4.4.2; R Core Team, 2024) with the necessary statistical packages. Descriptive analysis was used to summarize the parameters. Inferential statistics, specifically Student's t-test, were conducted to compare the means of parameters between the control and CE groups, with a significance level of p-value < 0.05. Analyses across time periods were performed for water and food intake using appropriate statistical tests based on the assumptions of each dataset.

Results and discussions

Table 1 presents the results of studies of biochemical parameters of rabbit blood on the cholesterol model.

Table 1. *Blood biochemical parameters of rabbits administered atherogenic substances and after treatment with extracts and rosuvastatin in a rabbit model of atherosclerosis (M ± m, n = 4)*

Tests	Exodus	Treatment of baiting, month		Treatment duration, days			
				Composition of extracts		Rosuvastatin	
		2	3	15	30	15	30
Total protein, g/l	85,2±4,2	90,0±6,0	83,0±6,2	85,0±4,4	86,0±4,6	86,0±4,4	84,0±4,4
Glucose, mmol/l	4,0±0,2*	5,1±0,2*	5,8±0,3*	4,2±0,3#	4,2±0,3 #	4,6±0,4#	4,2±0,3#
TC, mmol/l	2,4±0,2	19,4±1,5*	25,4±1,8*	8,1±0,6*#	2,8±0,2#	9,2±0,6#*	7,8±0,6#*
TG, mmol/l	1,6±0,1	6,8±0,5*	8,1±0,6*	2,3±0,2*#	2,0±0,2*#	3,6±0,2*#	4,1±0,6*#
VLDL mmol/l	0,32±0,02	1,36±0,12*	1,6±0,14*	0,46±0,03*#	0,4±0,03*#	0,72±0,05*#	0,82±0,03*#
LDL, mmol/l	0,38±0,03	1,6±0,10*	2,66±0,12*	0,33±0,02#	0,40±0,02#	0,38±0,02#	0,32±0,02#
HDL, mmol/l	0,76±0,06	1,1±0,08*	1,1±0,08*	0,85±0,06#	0,75±0,06#	0,72±0,05#	0,81±0,06#
AC_{xc}	2,2 ±0,18	16,6±1,34*	23,0±1,7*	8,5±0,64#*	2,7±0,64#*	11,8±1,3*#	8,7±0,64#*

*Note: *p ≤ 0.001 relative to outcome. #p ≤ 0.001 relative to control*

Administration of atherogenic substances for 2 and 3 months resulted in significant changes in the lipid profile and carbohydrate metabolism: The atherogenic index (AOX) in the control group increased sharply from 2.2 to 23.0, confirming the successful creation of a model of severe atherosclerosis.

In the experimental group, after 15 days of using the extract composition, the TC indicator decreased by 3.1 times compared to the 3-month control (p ≤ 0.001), and after 30 days, it was not significantly different from the baseline. TG and VLDL decreased by 3.5 and 4.1 times, respectively, compared to the control after 15 and 30 days. AOX decreased by 2.7 times after 15 days of treatment, and after 30 days, TG, VLDL, and AOX approached baseline values. Treatment with Rosuvastatin for 15 and 30 days resulted in a 2.8- and 3.3-fold reduction in TC levels, and a 2.3- and 2.0-fold reduction in TG and VLDL levels, respectively, compared to control levels. AOX decreased by 2.0 times after 15 days of treatment and by 2.6 times after 30 days.

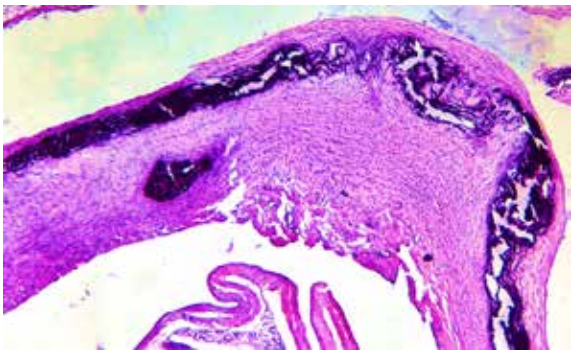
Thus, the therapeutic effect of the extract on the 30th day almost completely normalized the lipid profile of rabbits with cholesterol-induced atherosclerosis, and AOX approached baseline values. Rosuvastatin also had a positive effect on lipid profile, but TC and TG levels remained 2–3 times higher than baseline values. According to the table, the extract composition demonstrated a more pronounced and rapid normalization of total cholesterol levels and the atherogenic index

compared to rosuvastatin over this period (30 days). All changes after treatment were highly significant ($p \leq 0.001$) compared to the control group ("treatment").

Under the conditions of this experiment, the plant extract demonstrated a more pronounced ability to reduce total cholesterol and normalize the atherogenic index compared to rosuvastatin by day 30 of treatment.

Histological studies were conducted on control and experimental animals. Figure 1 shows the histopathological morphological picture of aortic arch atherosclerosis in a control animal.

Figure 1. *Aortic arch of a rabbit in which atherosclerosis was induced by administration of cholesterol powder and vitamin D2. General appearance of large foci of atherocalcinosis in the aortic arch at low magnification (Size: 10 × 4. Staining: Hematoxylin-Eosin)*



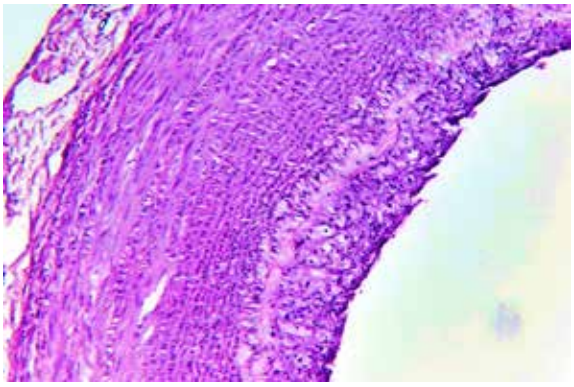
Large foci of atherocalcification are detected in the vessel wall. These calcifications are clearly located in the tunica media (middle layer) of the vessel. Massive plaques protruding into the lumen are visible, almost blocking blood flow. The microscopic specimen clearly shows large foci of calcification (atherocalcinosis), stained dark purple/black. This is the final stage of atherosclerotic plaque formation, characterized by the deposition of calcium salts in necrotic tissue. Around the calcification foci, metachromasia, defragmentation, and depolymerization of elastic fiber bundles are observed, as well as disorganization of the matrix between these bundles. The calcification foci are intensely stained with hematoxylin (blue-violet), and homogenized areas stained with eosin (pink) are visible around them. Significant thickening and destruction of the aortic layers is observed. The atherosclerotic plaque protrudes into the vessel lumen, which, in vivo, leads to hemodynamic compromise.

Pathological features of the classic stages of lipofibromatosis and atherosclerotic ulceration are fully expressed, indicating the development of atherocalcinosis. An example of this is the intense hematoxylin staining of elastic fiber bundles that are not yet fully fragmented, making them resemble calcification foci (normally, elastic fibers do not stain intensely with hematoxylin). Between these two zones, focal proliferation of cells of mesenchymal origin is observed: smooth muscle cells, fibroblasts, fibromyocytes, and macrophages. Connective tissue proliferation and destruction of the elastic framework of the vessel are visible, characteristic of severe stages of atherosclerosis induced by vitamin D2, which accelerates vascular calcification. Administration of vitamin D2 along with cholesterol explains the presence of massive calcifications visible at low magnification.

The microscopic picture (figure) fully confirms the results of laboratory tests from the table.

This image provides visual evidence of the profound organic vascular pathology that developed during the "priming" phase. The effectiveness of treatment (Extract or Rosuvastatin), as demonstrated by biochemical analyses, was aimed at preventing further progression of these lesions and normalizing lipid metabolism to halt the growth of these plaques.

Figure 2. *Aortic arch of rabbits in which atherosclerosis was induced by administering cholesterol powder and vitamin D2 for 3 months and which were then treated for 30 days with the extract composition (Size: 10 × 10. Staining: Hematoxylin-Eosin)*



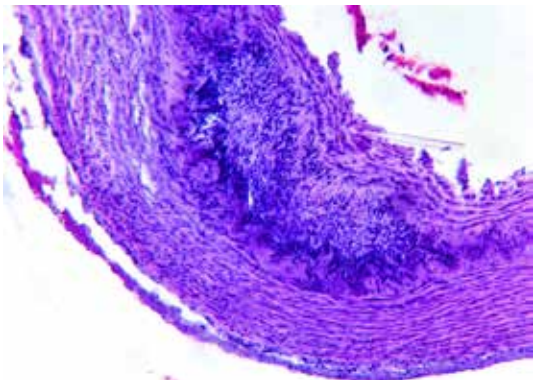
In Group 2, which received treatment with the extract composition for 30 days, Figure 2 demonstrates positive dynamics in aortic structure during therapy.

The ordering of the layers was restored, and the wall appeared more structured. Calcification foci were virtually absent or significantly reduced in volume. A decrease in vacuolization and lipid infiltration of the intima was observed. The improvement in wall structure is explained by a sharp reduction in atherogenic fractions. For example, in the extract group, TC decreased to 2.8±0.2 mmol/L, and LDL to 0.40±0.02 mmol/L, which halted the destructive process and initiated mechanisms for partial tissue restoration.

Group 3: A group of rabbits treated with Rosuvastatin for 30 days.

The surface of the endothelial layer of the aortic tunica intima is uneven, with focal deendothelialized (desquamated) areas. In the subendothelial layer, fibrolipomatous plaque formation is observed due to the proliferation, infiltration, and lysis of foam cells, myocytes, and fibroblasts. Partial focal changes are observed in the aortic media tunica, including disorganization and depolymerization of elastic fibers. Fibrinoid necrosis and plasma edema are found between the media and intima. The adventitia appears normal.

Figure 3. *Aortic arch of rabbits in which atherosclerosis was induced by administration of cholesterol powder and vitamin D2 for 3 months and then treated with rosuvastatin for 30 days (Group 2). General appearance of fibrolipomatous plaque in the subendothelial layer of the tunica intima (Size: 10 × 10. Staining: Hematoxylin-Eosin)*



Conclusion

The study results demonstrated that the experimental model of atherocalcinosis was successfully created and effectively treated. Morphological restoration of the aortic wall is fully consistent with a biochemical reduction in total cholesterol by ~89% in the extract group and normalization of the atherogenic index. Histopathological analysis clearly confirms that severe atherocalcinosis developed during the experiment is significantly corrected by treatment with the extract composition, resulting in stabilization of the vascular wall structure and a reduction in the rate of calcification.

References

Zyryanov, S. K. New possibilities for reducing low-density lipoprotein cholesterol levels: comparative characteristics of PCSK9-targeted therapy / S. K. Zyryanov, O. I. Butranova // Russian Journal of Cardiology. 2022. – Vol. 27. – No. 11. – P. 93–105.

Dyslipidemia and atherosclerosis / I. V. Sergienko, A. A. Ansheles, M. V. Yezhov [et al.]. – Moscow: PatiSS, 2020. – 57 p.

Klesareva E. A., Afanasieva O. I., Sherstyuk E. E., Tmoyan N. A., Razova O. A., Tyurina A. V., Afanasieva M. I., Ezhov M. V., Pokrovsky S. N. The relationship between the level of Lp(a) and the prevalence of atherosclerosis among young patients. Terapevticheskii Arkhiv (Ter. Arkh.). 2022. – 94(4). – P. 479–484.

Voevoda, M. I. New perspectives in the treatment of atherosclerosis / M. I. Voevoda, S. S. Bazhan // Novosibirsk Bulletin of Medical Science. 2018. – No. 1 (9). – P. 55–60.

Akhmedov V. A., Shevchenko A. S., Isaeva A. S. Modern views on the factors of occurrence and progression of atherosclerosis // RMJ. Medical Review. – 2019. – Vol. 3, No. 1–2. – P. 57–62.

ESC/EAS Guidelines for the Treatment of Dyslipidemia: Lipid Modification to Reduce Cardiovascular Risk / Working Group of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS) on the Treatment of Dyslipidemia // Russian Journal of Cardiology. 2020. – No. 25 (5). – P. 121–193.

Visseren F. L.J., Mach F., Smulders Y. M., et al. ESC National Cardiac Societies; ESC Scientific Document Group. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2021. – 42(34). – P. 3227–3337.

Emelkina V. V., Karasov I. A. Modern concepts of the pathophysiology of atherogenesis // International Student Scientific Bulletin. 2018. – No. 4–2. – P. 260–263.

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