# ANTI-INFLAMMATORY ACTIVITY OF PORCINE AND BOVINE CARDIAC AND AORTIC TISSUES

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**Abstract – The investigation of porcine and bovine cardiac and aortic tissues on Wistar rats with a model of experimental atherosclerosis revealed the anti-inflammatory effect assessed by the markers of vascular endothelial inflammation using quantitative ELISA. Analysis of the obtained data showed the reliable reduction of the atherogenic index in the blood of the animals fed with raw material under investigation due to acceleration of lipid metabolism and elimination of residual cholesterol from animal blood, which was indirectly confirmed by the changes in apo-protein content in the rat blood. Serum concentrations of VCAM-1 and E-selectin in the animals from the experimental groups reduced on average by 52.9% and 54.1%, respectively; however, the ICAM-1 and vWf concentrations, on the contrary, increased by 60.7% and 62.8%, respectively. On the basis of the obtained data, we put forward a hypothesis on the curative anti-inflammatory activity of cardiac and aortic tissues.**

**Key Words –anti-inflammatory effect, cardiac and aortic tissues, experimental atherosclerosis**

## I. INTRODUCTION

Investigation of the curative-therapeutic properties of meat products is topical. The presence of amino acid sequences in muscle proteins, which have hypotensive, antioxidative, opioid, immunomodulatory, prebiotic, mineral binding, hypocholesterolemic and antimicrobial activities, is well-known [1-4]. It was interesting to study cardiac and aortic tissues to detect substances with anti-atherosclerotic activities. A systematic approach to investigation of the anti-atherosclerotic properties of the bovine and porcine cardiac and aortic tissues with prospects for developing functional foods on their basis consists in the analysis of the trends in changes of the main biological markers both during the course of disease and in the process of recovery. Hence, it is recognized with increasing frequency that along with the main indices of blood lipid spectrum such as concentration of total cholesterol (TC), triglycerides (TG), lipoproteins of high, low, intermediate and very low density (HDL, LDL, IDL, VLDL), atherogenic index (AI), the most informative is the ratio of apolipoprotein B100 concentration to apolipoprotein A concentration (ApoB/Apo A), reduction of which in serum indicates an anti-atherosclerotic effect of a test substance [5-6]. Apo A-I (apolipoprotein A-I) is the main protein component of HDL and ensures binding the HDL particle with the corresponding receptors. ApoB-100 (apolipoprotein B-100) is a constituent of VLDL, IDL and LDL and acts as a ligand for LDL receptors [7,8]. The anti-inflammatory effect is measured by assessing changes in concentration of the main inflammatory markers in animal blood. VCAM-1 (vascular cellular adhesion molecule-1) is expressed on the endothelium, macrophages, bone marrow stromal cells and some other cell types and promotes the adhesion of lymphocytes, monocytes and eosinophiles (but not neutrophils) to the activated endothelium with subsequent penetration into the inflammatory focus.

ICAM-1 (intercellular adhesion molecule-1) is expressed on the vascular endothelium, monocytes, B- and T- lymphocytes and mediates the adhesion of neutrophils, monocytes and lymphocytes to the activated vascular endothelium with their subsequent migration towards the inflammatory focus. Von Willebrand factor (VWF) is a plasma glycoprotein, which plays a significant role in attachment of platelets to the injured sites of blood vessel walls. E-selectin is expressed on the endothelium and mediates adhesion of leucocytes (neutrophils, monocytes and T-cell subpopulations) to the activated vascular endothelium at the initial phases of inflammation [9-12]. Previously, we developed a model of experimental hyperlipidemia and

atherosclerosis on laboratory rats of Wistar stock [13], by which the hypolipidemic and anti-inflammatory activities of the porcine and bovine cardiac and aortic tissues were assessed.

## II. MATERIALS AND METHODS

The subjects of the research were: bovine aorta, bovine heart, porcine aorta and porcine heart. The research was performed on male rats of Wistar line (n=80) at the age of more than 1 year and weighing 380±20 g. The animals were kept in the standard conditions of a vivarium with free access to water and feed. Eight groups were formed: intact (n=10), control (n=10), groups 1-6 – experimental ($n_{i=1-6}$=10). The animals of the control and experimental groups were subjected to disease simulation according to Chernukha et al, 2013 [14]. The intact rats were kept on a standard vivarium diet. On completion of the simulation, the animals of the experimental groups received the raw minced test samples with their diet on the basis of 5 g/head: group 1 – bovine cardiac tissues, group 2 – bovine aortic tissues, group 3 – a mixture of bovine cardiac and aortic tissues (1:1), group 4 – porcine cardiac tissues, group 5 – porcine aortic tissues, group 6 – a mixture of porcine cardiac and aortic tissues (1:1), the animals of the control group were kept on a standard vivarium diet.

On completion of the simulation, on the 14$^{th}$ day and 28$^{th}$ day of treatment, the rats were euthanized in the chamber for euthanasia VETtech according to the rules of humane handling of animals; the autopsy and blood collection for biochemical and hematological analyses were carried out. Biochemical analyses were performed using the semi-automatic biochemical analyzer BioChem SA (HTI, USA), hematological analyses were carried out using the semi-automatic analyzer Abacus Junior Vet (Diatron, Austria). ELISA was performed using analyzer ImmunoChem 2100 (HTI, USA) according to ELISA methods with reagents (Cusabio, China).

## III. RESULTS AND DISCUSSION

Already on the 14$^{th}$ day of treatment, the recovery of blood indices was recorded: cases of leucopenia were not observed in the animals of all groups; however, slightly pronounced cases of lymphopenia were recorded. On the 28$^{th}$ day, the concentration of lymphocytes in the blood of the experimental animals of all groups was normalized. On the 14$^{th}$ day of treatment, the most active reduction in total cholesterol and triglyceride concentrations were recorded in the animals consumed porcine tissues: blood cholesterol concentrations in the animals from groups 4, 5 and 6 were lower by 22.4%, 38.6% and 10.5%, respectively, compared to the control group, and triglyceride concentrations were 55.6%, 37.0% and 40.7% lower, respectively, in the blood of the animals from groups 4, 5 and 6 compared to the control group. The similar tendency was also observed on the 28$^{th}$ day of treatment. It is important to notice that on the 14$^{th}$ day of treatment, the residual cholesterol (VLDL and IDL) was not detected in the animals of all experimental groups, which suggests the acceleration of lipid metabolism. A sharp decrease in the atherogenic index (AI) was also observed as early as by the 14$^{th}$ day of treatment. AI was 47.6% on average lower in the animals of the experimental groups compared to control. The ApoB/ApoA ratio significantly decreased already on the 14$^{th}$ day of treatment, and was 4.48; 2.65; 2.13; 7.16; 4.18 and 10.61 times lower, respectively, in the blood of the animals from all experimental groups compared to control (Fig.1).

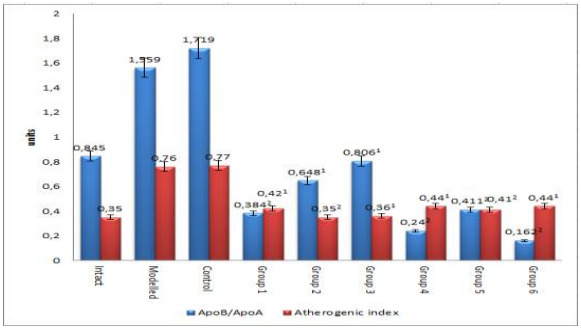


Figure 1. AI and Apo B/ApoA ratio on the 14$^{th}$ day of treatment

On the 28$^{th}$ day of treatment, the significant reduction in ApoB/ApoA ratio was also noted in the control group. This index was 1.37±0.13 times on average lower in the animals of the experimental groups excluding the animals received porcine cardiac tissues, in which this index decreased by 3.36 times (Fig.2).

Already on the 14th day of treatment, the blood concentration of VCAM-1 in the experimental groups decreased and was 39.4%, 37.6%, 41.4%, 48.2%, 25.8% and 52.9% lower, respectively, compared to control. On the 28th day, the tendency towards decrease in VCAM-1 concentration persisted with VCAM-1 concentration equal to 37.3% on the average compared to control (Fig.3).

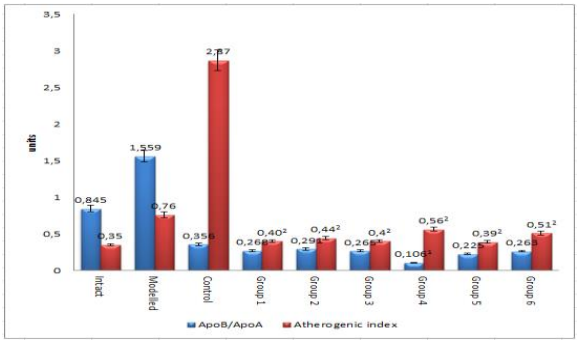


Figure 2. AI and Apo B/ApoA ratio on the 28th day of treatment

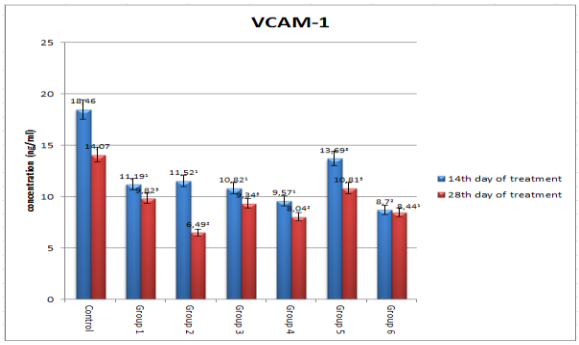


Figure 3. VCAM-1 dynamics on the 14th and 28th day of treatment

The reverse situation was observed regarding ICAM-1. On completion of disease simulation, ICAM-1 concentration sharply increased both in the animals of the control group and in the rats of the experimental groups in response to triggering the defensive functions of the body. On the 14th day of treatment, it was 14.0% and 33.5% lower, respectively, than in control, but significantly higher compared to the intact values only in the animals consumed bovine aortas and the mixture of bovine cardiac and aortic tissues. By the 28th day, ICAM-1 concentration continued to increase significantly exceeding the intact values. The greatest dynamics was observed in the animals consumed bovine mixture and was 60.7% versus control (Fig.4).

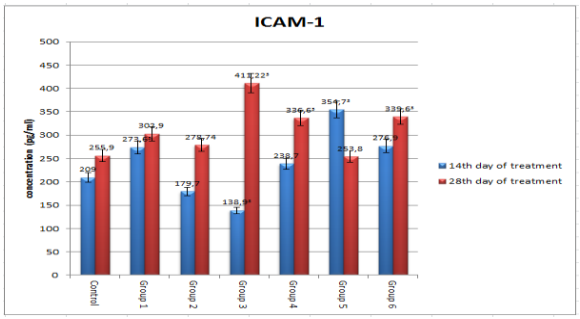


Figure 4. ICAM-1 dynamics on the 14th and 28th day of treatment

The similar picture was observed regarding vWf. On the 14th day of treatment, vWf concentration was 34.0% and 16.5%, lower, respectively, than in control, but was also significantly higher than the intact values only in the animals consumed bovine hearts and the mixture of bovine cardiac and aortic tissues. By the 28th day, vWf concentration continued to rise significantly exceeding the intact values. The greatest dynamics was observed in the animals consumed porcine mixture and was 62.8% versus control (Fig.5).

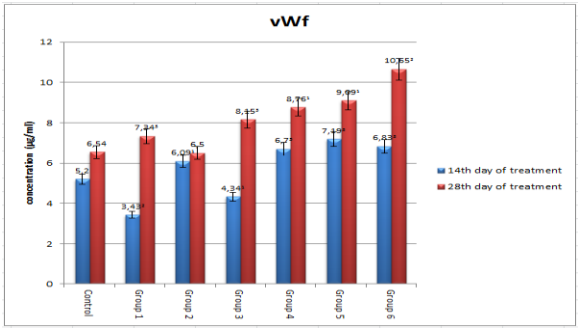


Figure 5. vWf dynamics on the 14th and 28th day of treatment

Concentration of E-selectin was normalized as early as on the 14th day of treatment both in the animals of the control and experimental groups; however, in the animals consumed bovine aorta, porcine heart and porcine mixture it was 30.4%, 29.6% and 48.1%, lower, respectively, than in control. Concentration of E-selectin continued to decrease also on the 28th day in all experimental groups; the best ultimate dynamics was observed

in the animals consumed bovine cardiac and aortic tissues, porcine cardiac tissues and porcine mixture and was 54.1%, 29.3%, 36.8% and 30.8%, respectively (Fig.6).

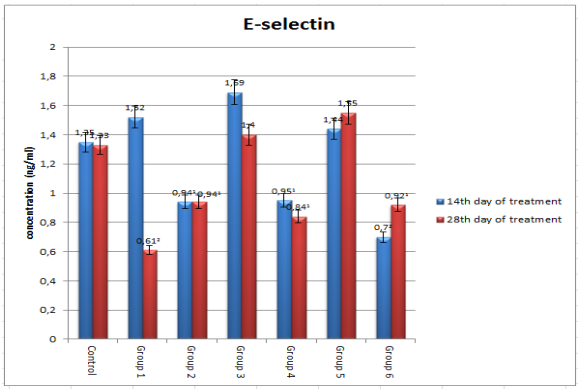


Figure 6. E-selectin dynamics on the 14th and 28th day of treatment

## IV. CONCLUSION

Thus, the hypolipidemic effect of the tested raw material resides in acceleration of lipid metabolism, which significantly reduces not only the atherogenic classes of lipoproteins but also atherogenic index (AI). The complex analysis of the obtained results shows that the porcine tissues have the highest hypolipidemic effect. The data on the ELISA analysis of the inflammatory markers makes it possible to put forward a hypothesis about the anti-inflammatory and curative action of the components of the investigated raw material regarding the injured site of the vessel. VCAM-1 and E-selectin are expressed on the endothelium and mediate adhesion and penetration of monocytes, B- and T- lymphocytes into the injured site, thus inducing inflammation and lysis of the atheromatic plaque with release of its content into the lumen of the vessel. These indicators in the animals consumed porcine and bovine cardiac and aortic tissues were reduced or in the normal range, which gives evidence about normal functioning of the vascular endothelium and enhancement of its defensive reaction. vWF is a key factor of blood coagulation and increase in its concentration blocks up thrombus growth in case of atheromatic plaque disruption. ICAM-1 is expressed not only by the endothelium, but also on the surface of monocytes, B- and T- lymphocytes, and its increase increases the adhesive capacity of the above mentioned hemocytes in the injured site. Thus, plaque resorption presumably occurs “in the rear”, which protects the lumen of the vessel, ensuring at the same time its normal functioning and elimination of injury from the extraluminal part.

In the future, we plan to study the action of high and low molecular weight ultrafiltrates of porcine aorta on a model of experimental atherosclerosis and assess the effect on C-reactive protein and VEGF (Vascular endothelial growth factor).

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