

**ANTICOAGULANT AND HYPOTENSIVE ACTION OF SALIVARY GLAND
SECRETION OF *ORNITHODOROS TARTAKOVSKYI* AND *HYALOMMA ASIATICUM*
MITES**

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ABSTRACT

The scientists studied the anticoagulant actions of secretion from the salivary glands of ticks *Ornithodoros tartakovskyi* Olenov, 1931. and recorded the retardation of blood plasma coagulation in rats and sheep. Coagulation parameters depended on the concentration of secretion being tested (from 25 to 150 µg/ml). At a maximum concentration (150 µg/ml) the blood plasma coagulation rate (comparing with the test) drops to 79.7±5.6%. To determine the anticoagulant mechanisms of blood plasma with the deficiency of factors X, XI, IX & VIII the activated partial thromboplastin time was used. The platelet aggregation process was prolonged considerably in the test. The scientists also determined the role the ions [Ca²⁺] play in the regulation of the contractive activity of the smooth muscle cells in blood vessels, the process being directly connected with Ca²⁺. The researchers also determined the duration of the anticoagulant characteristics of blood plasma and the effect of the secretion concentration on the activity of the hemostasis system, which is very important for the medical and pharmacological areas. Part of this research was devoted to investigation of salivary glands' extracts of ticks *Hyalomma asiaticum* on contractile activity of rat aorta. It was found that the extract has a strong relaxing effect on the aorta and modifies the properties of voltage-dependent calcium channels.

KEYWORDS: Tick, *Ornithodoros*, salivary glands, anticoagulant, blood-vascular system, biologically active substances. *Hyalomma*, relaxant.

1. INTRODUCTION

In the last few years the interest in blood sucking ticks Ixodidae and Argasidae has grown. The two main research areas are the ticks' ability to transmit diseases and the toxicity of the secretion of their salivary glands.

It is well-known that the saliva of blood sucking ticks is composed of prostaglandins, vasodilators, anti-platelets, immunomodulators and anticoagulants connected with their basic adaptive mechanisms. Research into the structural and functional characteristics of saliva revealed poisonous components in it. The anticoagulants prevent the host's blood from coagulating.^[5,6] Most of the biologically active substances forming ticks' saliva are characterised by a wide range of activities.

The biologically active substances in the saliva of a number of tick species with anticoagulant and anti-platelet properties have been studied properly. However, data on the physiological action mechanisms of the saliva of ticks Argasidae are fragmentary and absolutely inadequate.

The object of this study was to research into the coagulant and relaxant effect of the secretion in the salivary glands of ticks *O. tartakovskyi* on the functional activity of the blood vascular system.

Main purpose of this study is to investigate anticoagulant and relaxant effect of salivary glands secretion of *O. tartakovskyi* and *H. asiaticum* ticks on the functional activity of the blood circulatory system.

2. MATERIAL AND METHODS

Ticks *Ornithodoros tartakovskyi* were collected and studied using the methods described by Agrinsky^[1] and Balashov.^[2] The anticoagulant effect of these ticks' salivary secretion was studied with the use of other popular methods.^[9,14] The blood of an albino rat of an unknown species and a sheep was used as a test object.

The experiments were being accomplished using aorta specimens (2-3 mm in diameter and 3-4 mm in length) extracted from the thoraces of the albino rats of an unknown species (200-250 g) and placed into a special container (5 ml) perfused by Krebs physiological solution (composition, molecular weight: NaCl-120;

KCl-4,8; CaCl₂-2; MgSO₄-1,2; KH₂PO₄-1,2; NaHCO₃-20; glucose 10), pH=7.4, which was oxygenated by a carbogene (O₂-95%, CO₂-5%) at a temperature of 37±0.5°C. The isometric contraction of the muscle specimen was measured using mechanotron F03 (Grass Instrument Co., USA). The initial stress equal to 10 mega Newtons was applied to each specimen. After the stabilization time (45 min) was over, muscle contraction was evoked using KCl (50 mega Newton) and noradrenaline (1 micron). All the experiments were carried out in these conditions. The signal from the tension indicator was sent to an amplifier and recorded using recorder Endim 621.02 (Czech Republic). The obtained data were processed with the use of the computer program OriginPro 7.0 (OriginLab Corporation, USA). The range of contraction responses was given in percent of the maximal response and calculated as an average from 4 to 7 different experiments (n=4-7). The value P<0.05 indicates statistically significant differences.

To study of salivary glands extracts of *H. asiaticum* we have used a previously described method, which analyzes the mechanisms of smooth muscle calcium channels' functioning at searching for pharmaceuticals with hypotensive action.

3. RESULTS AND DISCUSSION

The research that was carried out to study the anticoagulant action of the secretion in the salivary glands of ticks *O. tartakovskyi* revealed the retardation of the coagulation of the blood plasma in rats and sheep. Coagulation parameters depended on the concentration of secretion being tested (from 25 to 150µg/ml). At a maximum concentration (150µg/ml) the blood plasma coagulation rate (comparing with the test) drops to 79.7±5.6%.

To determine the anticoagulant mechanisms of blood plasma with the deficiency of factors X, XI, IX & VIII the activated partial thromboplastin time was used. The platelet aggregation process was prolonged considerably in the test. Figure 1 shows the platelet aggregation process in the blood of rats.

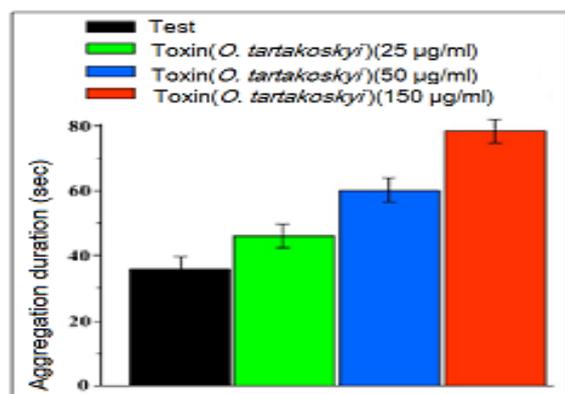


Fig. 1. The effect of various concentrations of the salivary gland secretion of ticks *O. tartakovskyi* on

the platelet aggregation process in the blood of the rat (original).

The recorded retardation of the anticoagulant process produced by biologically active substances in the secretion of ticks led to the decrease of the platelets' activity and inhibition of factors X. Some of the anticoagulant components caused the decrease of the formation of platelets^[16], while others – to the activation of inhibition factors Xa.^[15] The test also determined the action of the prothrombinase complex or Xa complex and the connection of thrombin with the coagulation effect present.^[7]

The components retarding blood coagulation processes found in the salivary glands of ticks *Ornithodoros moubata* *O. savignyi*, *Moubatin* and *Savignin* decrease the thrombin activation and have antiaggregation properties (prevent accumulation of platelets in the blood vascular system). The monobin discovered in the salivary glands of *Argas monolakensis* also has anticoagulant properties.^[15]

It was established that the TAP (tick anticoagulant peptide) of the protein molecule retards the effect of factors FXa on the blood plasma coagulation processes in the human being. Further the scientists discovered the formation of the active bonds of the TAP protein of factor FXa and proved their antiplatelet effect in the blood vascular system.^[10,11]

The results of our research and literary data demonstrate that the secretion of *O. tartakovskyi* (25-150 µg/ml) effects greatly the activity of the hemostasis bond and, to a lesser extent, the duration of the anticoagulant characteristics of blood plasma in conformity with the activated partial thromboplastin time test. The secretion of *O. tartakovskyi* retards factors X, XI, IX, VII in the blood vascular system.

At the same time, the KCl effect with the poison concentration equal to 150 µg/ml decreased the contraction of the muscle specimen comparing with the test.

Ions [Ca²⁺] play an important role in the regulation of the contractive activity of the smooth muscle cells in the blood vessels, this process being connected directly with [Ca²⁺]_i. The regulation of this process involves ducts in plasmolemma Ca²⁺_L^[8], activated receptor ducts effected by the sarcoplasmic reticulum inositol 1, 4, 5 – triphosphate (IP₃R), the calmodulin protein^[13], Ca²⁺-adenosine phosphatase in plasmolemma Ca²⁺-adenosine phosphatase and Na⁺/Ca²⁺-exchanger and protein kinase bonds.

To ensure the inflow of blood into ticks' organism their salivary glands include a number of vasodilators – agents dilating the walls of blood vessels.^[12,3] Prostaglandins with vasodilating properties were discovered in the

salivary glands of ticks belonging to the families Ixodidae and Argasidae. They have an additional effect, relaxing the smooth muscles of blood vessels and retarding platelet aggregation.^[3]

When the walls of blood vessels are damaged in the course of the platelet activation, ions Ca^{2+} increase, which is associated with the decrease of the activity of the smooth muscles in the blood vessels. In this connection, the protein molecules in ticks' salivary glands, calreticulin, bond ions Ca^{2+} and restrict the contraction of the smooth muscles of blood vessels.^[4]

The results of our research and literary data demonstrate that the secretion of the salivary glands of ticks *O. tartakovskyi* has a relaxant effect on the contractive activity of the specimen of the rat's aorta and the smooth muscles of the blood vessels. This is achieved through the vasodilating properties of the prostaglandins and the bonding of the calreticulin protein molecules with ions Ca^{2+} .

At investigation of *H. asiaticum* mite extract on rat aortic preparations in the calcium medium we have shown that this extract is a dose-dependent cause of muscle relaxation. In the experiments aorta preparations were reduced by 100% compared to the control with addition of 60 mM KCl to the medium. Salivary glands cause the relaxation of GMK in the range of 50-200 $\mu\text{g}/\text{ml}$ concentrations in a dose-dependent manner: a concentration of 50 $\mu\text{g}/\text{ml}$ of relaxation was $10 \pm 2,1\%$ as compared to controls at a concentration of 100 $\mu\text{g}/\text{ml}$ to $30 \pm 1,2\%$. At higher concentrations - 200 $\mu\text{g}/\text{ml}$ it was observed maximum relaxation of aorta drug to $55 \pm 3,6\%$ (Fig.1).

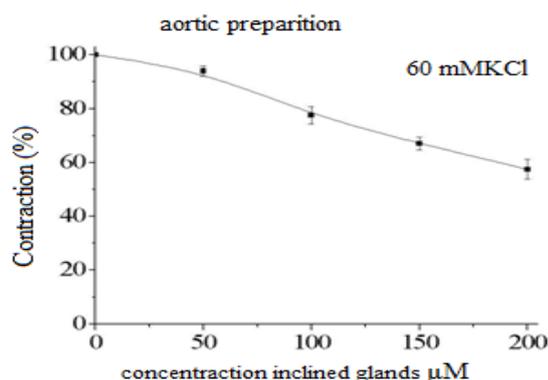


Fig.2. Dependence of relaxant action of salivary gland extracts from their concentration ($\mu\text{g}/\text{ml}$).

4. CONCLUSIONS

It was studied the degree of duration of anticoagulative features of plasma under the influence of different concentrations of a salivary glands secret of *O. tartakovskyi* tick on the homeostasis system activity. Salivary gland of *O. tartakovskyi* and *H. asiaticum* have a relaxant effect on the contractile activity of rat aorta preparation. Obtained results are pure preliminary

character. However, bioactive substances of of salivary glands secret of ticks deserve to use them as natural ingredients for the creation and improvement of drugs for vascular disease correction. We have studied the duration of anticoagulant characteristics of blood plasma, when different concentrations of the secretion in the salivary glands of ticks *O. tartakovskyi* effects the activity of the hemostasis system. The salivary glands of *O. tartakovskyi* (15-150 $\mu\text{g}/\text{ml}$) have a relaxant effect on the contractive activity of the specimen of the rat's aorta. The results of the research can be only regarded as preliminary. Nevertheless, the bioactive substances from the secretion of ticks' salivary glands can be used as natural components for the creation and development of remedies for the correction of vascular pathologies.

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