Studying the Mechanism of Action of the Infusion of Serviceberries

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Abstract. The article presents the results of experimental studies to establish the effect of the preparation prepared from the infusion of berries on the hematological parameters of rabbits. The influence of different volumes of the preparation on some hematological parameters and nonspecific immunity of rabbits was determined. The experiment was carried out in the vivarium of the Faculty of Veterinary Medicine and Biotechnology of the Ryazan State Agrotechnological University named after P.A. Kostychev. The experiment involved male rabbits of the Californian breed in the amount of 50 heads, divided into 5 groups: control and 4 experimental. The animals of the control group were intact, the experimental ones received an orally infusion of raspberries of the same concentration, but as a result of the research it was found that the volume of the infusion of berries of berries is of decisive importance for the processes of hematopoiesis in the body of rabbits. Application of dosages of 15 and 20 ml per head per day. It has been proven that this is the best drug that promotes the activation of erythropoiesis and an increase in the formation of hemoglobin in the body of rabbits in a shorter time compared with the use of infusion of smaller volumes. The same volumes are administered to rabbits for 30 years. Days had a positive effect on the increase in leukocytes in the blood, which indicated an increase in the immune status of rabbits, since these cells performed a protective function.

1. Introduction
The study of the mechanisms of action of infusions prepared from plant materials containing biologically active substances is of interest from the point of view of the prevention and treatment of a number of diseases. Such infusions are available and cheap, which allows them to be used in veterinary medicine and animal husbandry. Infusions from fruits and vegetative parts of plants are widely used in farms and the private sector for the prevention and treatment of a number of animal diseases, increasing hematopoiesis and immunity [1, 2, 3, 4, 5, 6].

Serviceberry is an ordinary plant in the form of a shrub, widespread in central Russia, but little known to the majority of the population. Serviceberry ordinary grows in forest belts and in personal plots. The fruits of the plant are berries (figure 1), which are edible, tasty, used for food in natural and dried form. Juices, preserves, jams, marshmallows, jelly, kissel, compote and other products are made of berries [7].
The biological composition of the fruits is wide. These are vitamins, minerals, antioxidants, organic acids, anthocyanins and other components with a wide spectrum of action and unique properties. According to V.G. Leonchenko, E. Zhbanova and others [8, 9, 10, 11] sulfur berries contain a large amount of polyphenolic compounds, coumarins and beta-sitosterol.

Polyphenols, being plant compounds, contain antioxidants and anthocyanins. Their main ability is manifested in reducing the risk of developing cardiovascular diseases, lowering cholesterol levels, preventing blood clots and improving digestion, lowering blood sugar levels and stimulating brain function [2, 6, 7, 8].

The fruit contains coumarins, similar in function to polyphenols. They have a hypotensive effect, reduce blood clotting, help in the treatment of thrombophlebitis and cardiovascular diseases. In addition, they have diuretic and choleretic properties. Beta-sitosterol, which is part of the fetus, cleanses the blood vessels, preventing the deposition of cholesterol on the walls, promotes its excretion from the body, participates in the formation of testosterone or its own male sex hormone, and activates the synthesis of its own female sex hormones.

To make sure that the infusion of wormwood can be used as a prophylactic herbal remedy to improve hematopoiesis and increase the immunity of animals, an experiment was conducted on rabbits. Studying the effect of any drug on a living organism, it is necessary to determine the optimal frequency rate, volume and method of their application. Studies were carried out on clinically healthy animals using the oral route of administration of wormwood infusion. The aim of the study was to experimentally study the effect of the volume of infusion of wormwood berries on the morphological and biochemical parameters of blood and nonspecific immunity of rabbits.

2. Material and methods
The experiment was carried out on 50 male rabbits of the Californian breed, aged 4-5 months, with a live weight of 2,059.4±21.2 g in a vivarium of the Faculty of Veterinary Medicine and Biotechnology of Federal State Budgetary Educational Institution of Higher Education “Ryazan State Agrotechnological University Named after P.A. Kostychev”. The animals were formed into 5 groups: 4 experimental and the control one, 10 animals each. The rabbits were housed in the same room in individual single-tier cages. The studies were carried out in winter and lasted 30 days. The animals were housed in individual single-tier cages. The duration of the experiment was 30 days in winter. The basic diet (BD) contained 205 feed units, 2.25 MJ of exchangeable energy. BD was nutritionally balanced and fully met the needs of the animals [12].

The rabbits of the experimental groups were daily drunk with infusion of serviceberries, but of different volumes. Animals of experimental group 1 received 5 ml/head of infusion daily, experimental group 2 had 10 ml/head, experimental group 3 got 15 ml/head and experimental group 4 had 20 ml/head.

Figure 1. Serviceberries.
Control animals received distilled water as placebo instead of infusion. Blood sampling for hematological studies was carried out according to the generally accepted method from the saphenous vein of the thigh at the beginning of the experiment, on the 10th, 20th and 30th days of the study [1, 2, 13]. Blood analysis was performed on an Abacus Junior Vet automatic hematology analyzer. The infusion of serviceberries was prepared from dried and crushed fruits collected in forest belts of Ryazan region (figure 2).

3. Results
Before the experiment, hematological parameters of all animals were practically at the same level and corresponded to the norm. In further studies there was a tendency to an increase in the level of erythrocytes and hemoglobin in the blood of experimental rabbits that received an infusion of serviceberries as a biologically active additive in comparison with the control. Data on morphological and biochemical parameters of red blood are presented in table 1.

Table 1. Morphological and biochemical parameters of red blood.

| Indicator                      | Day of experiment | Group                                      |
|-------------------------------|-------------------|--------------------------------------------|
|                               | Experimental 1    | Experimental 2    | Experimental 3     | Experimental 4     | Control       |
| Erythrocytes, *10^{12}/l      |                   | 10              |                   |                   |               |
| 1                             | 5.957±0.13        | 5.978±0.11      | 5.939±0.17        | 5.944±0.15        | 5.999±0.12    |
| 10                            | 6.008±0.09        | 6.088±0.06      | 6.088±0.05        | 6.45±0.12         | 5.986±0.09    |
| 20                            | 6.023±0.05        | 6.501±0.12      | 6.505±0.11        | 6.037±0.05        | 6.014±0.06    |
| 30                            | 6.030±0.09        | 6.260±0.09      | 6.344±0.06        | 6.143±0.07        | 5.976±0.10    |
| Hemoglobin, g/l               | 10                | 10.72±4.29      | 108.3±5.03        | 108.2±4.89        | 107.2±5.25    |
| 20                            | 109.5±4.49        | 113.6±4.22      | 113.8±3.33        | 121.4±5.60        | 108.9±1.91    |
| 30                            | 110.4±4.88        | 127.8±4.16      | 110.7±3.06        | 109.5±2.84        | 109.3±4.01    |
| Erythrocyte hemoglobin content, pg | 1              | 21.06±1.18      | 21.15±0.85        | 21.14±0.92        | 21.18±1.21    |
| 10                            | 21.58±1.21        | 20.51±0.85      | 20.18±0.58        | 20.45±0.49        | 21.13±1.13    |
| 20                            | 21.44±1.16        | 21.95±0.59      | 21.67±0.77        | 22.03±0.99        | 21.19±1.16    |
| 30                            | 21.76±0.97        | 22.89±0.68      | 22.84±1.32        | 22.09±0.84        | 21.42±1.22    |
| Average erythrocyte volume, fl | 10               | 63.1±1.97       | 63±2.26           | 62.9±2.13         | 63±2.31       |
| 20                            | 62.9±2.23         | 62.3±1.89       | 59.6±1.51         | 59±1.49           | 63.0±2.49     |
| 30                            | 63±2.01           | 60.4±1.35       | 61.2±1.81         | 61±1.33           | 62.8±1.69     |
|                               |                   | 61.8±5.55       | 62.2±2.25         | 62.3±1.95         | 62.9±1.73     |

Note: hereinafter *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 in relation to the control group.
Fluctuations in the level of erythrocytes and hemoglobin in experimental group 1 were insignificant throughout the experiment and did not exceed the control values much. The number of erythrocytes in the blood of rabbits of experimental group 2, who received daily 10 ml/head, increased already on the 10th day of the experiment, that was 1.7 % higher compared with the indicators of control animals. On the 20th day it was 8.1 % higher and by the 30th day of the experiment, these animals had a decrease in this indicator, however, these values were still 4.7 % higher than the control ones. The number of erythrocytes in the blood of rabbits of experimental groups 3 and 4, who received increased volumes of the serviceberry infusion equal to 15 and 20 ml/head per day, increased on the 10th day of the experiment and exceeded the control values by 1.2 % and 0.4 %, respectively. Consequently, the amount of the infusion that entered the body was of decisive importance for the production of erythrocytes by the red bone marrow.

Thus, the number of erythrocytes in the blood of rabbits in the experimental groups increased in accordance with an increase in the dose of the preparation that entered the body.

When analyzing the level of hemoglobin in the blood of animals, a similar trend was observed. Throughout the entire study period, the hemoglobin value increased over the sampling periods and depended on the amount of infusion that entered the body. In experimental group 1 on day 10, this indicator was significantly higher than in the control by 0.60 %. On days 20 and 30, the difference between these groups and the control was 0.6 % and 0.7 %, respectively, in favor of the experimental groups. Thus, there was a tendency to a gradual increase in the hemoglobin content in the blood of the rabbits of the experimental groups, which got the infusion of serviceberries, in comparison with the control. High levels of hemoglobin content were in the second and third groups on the 20th day of the experiment, being significantly higher than that in the control. This indicator continued to increase in experimental group 2, reaching a maximum by 30 days. It reached the highest rates by day 10 in the fourth experimental group, whose animals received infusion in an amount of 20 ml/head daily, which was 11.4 % higher than that in the control. Such changes in the number of erythrocytes and the level of hemoglobin in the blood of animals of all experimental groups, apparently, are associated with the influence of the polysaccharide complex of serviceberries, which stimulated the formation of erythrocytes in the red bone marrow. The rapid increase in the indicator in the 4th experimental group by the 10th day of the experiment is explained by the quantitative volume of the infusion that entered the body. The larger the volume, the shorter the time period was the activation of the process of erythropoiesis and an increase in the hemoglobin content in the blood. The decrease in the above indicators in group 4 on the 20th and 30th days of the experiment is apparently associated with the oversaturation of the erythrocyte membrane with the polysaccharide complex, which influenced the stabilization of erythropoiesis in the blood of animals in a shorter time. All plant cells have a cell membrane of a polysaccharide nature, which is a product of the activity of the cytoplasm. The period of saturation of the erythrocyte membrane was in direct relationship with the volume of the preparation administration. The larger its volume was, the more active the process of hematopoiesis took place. The content of hemoglobin in the erythrocyte and the value of the average volume of the erythrocyte varied both in the control and experimental groups in periods of blood sampling, but were within the physiological norm (table 1).

The use of the infusion of serviceberry in dosages of 15 ml/head and 20 ml/head per day to the animals of experimental groups 3 and 4 stated higher indicators for the content of erythrocytes and hemoglobin in the blood, which were achieved in a shorter period of time and that is on the 10th day of the study, but subsequently these indicators decreased.

According to a number of researchers a significant increase in the level of erythrocytes and hemoglobin proves some improvement in gas and water-salt metabolism in the body of animals, stabilization of blood pH and acceleration of the flow of substances to cells [1, 2, 3, 4].

The leukocyte indices in animals of all groups before the study were identical. According to the periods of blood sampling, the leukocyte indices in the rabbits of the experimental groups increased in accordance with the amount of the infusion that entered the body. On the 10th day of study, there was a process of increasing the number of leukocytes in the blood of rabbits in the experimental groups. It was
3.0 % more in experimental group 1 compared with the control. It was 6.2 % more in experimental groups 2 and 4, and 9.6 % more in experimental group 3 in comparison with the control. A similar trend was noted on the 20th and 30th days of the study. The difference in the experimental groups compared to the control was in favor of the experimental groups. On the 30th day experimental group 4 had the highest leukocyte number. It was 20.2 % higher than in the control, 10.5 % higher than in experimental group 1, 3.7 % higher than in experimental group 2, and 3.5 % higher than in experimental group 3.

The increase in the number of leukocytes in the experimental groups in comparison with the control indicates an increase in phagocytosis and activation of the destruction of microbial cells under the influence of the serviceberry infusion.

It was found that the use of infusion of serviceberries in the amount of 20 ml/head for 30 days had a positive effect on the increase in the number of leukocytes, which indicated an increase in the immune status of rabbits. Leukocytes in the body perform a protective function against infections, participate in metabolism normalization, as well as in the stimulation of the process of formation of new cells.

Figure 3. Indicators of the total number of leukocytes for the study period (n=10).

4. Conclusion
As a result of research, it turned out that the volume of raspberry infusion is of decisive importance for the processes of hematopoiesis in the body of rabbits. Best of all, the use of doses of 15 and 20 ml / head per day has proven itself, contributing to the activation of erythropoiesis and an increase in the formation of hemoglobin in the body of rabbits in a shorter time, compared with the use of infusion of smaller volumes. The same volumes administered to rabbits for 30 days had a positive effect on the increase in leukocytes in the blood, which indicated an increase in the immune status of rabbits, since these cells perform a protective function.

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