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## Pharmacodynamic activity of Hepavitol: Mechanisms of Hepatopathy correction in animals.

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### ABSTRACT

Article presents the results of pharmacodynamic effects of the preparation Hepavitol in the treatment of chronic hepatitis in dogs. It is shown, that it has a pronounced hepatoprotective effect, improving the clinical state of animals and also correcting the basic metabolic processes in the body by optimizing the protein-synthetic, pigment-forming, enzyme-forming and detoxification functions of liver.

**Keywords:** pharmacology, medicine, hepatoprotectors, liver, hepatitis, selenium, phospholipids, dogs

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## INTRODUCTION

Diseases of the liver in animals occupy one of the leading places in the list of diseases from the total number of non-contagious pathologies. The liver is the main organ in maintaining homeostasis, maintaining the dynamic constancy of the internal environment of the animal's organism, and takes the most active part in eliminating the consequences of the disease. The physiological role of the liver is important and multifaceted. Any changes in its functional activity are reflected in the homeostasis system [4].

Analysis of recent data on the functioning of organs shows that in all pathological processes in the liver, membrane structures of cells are damaged. Cells and membranes of cells and cellular organelles (nuclei, mitochondria, microsomes) contain phospholipids, which form the structural basis of the membrane, the phospholipid bilayer. The regime of normal functioning of the membrane depends on many factors – the microviscosity of the phospholipid bilayer and the mobility of phospholipid molecules, the phase state of membrane lipids. Deviations of the biophysical characteristics of the phospholipid bilayer from the norm easily cause or, conversely, depend on various pathologies. All this determines a wide range of biological and pharmacological effect of phospholipids and an interest in phospholipid preparations of medical and pharmaceutical use [7].

The protective effect of many hepatoprotectors – drugs, that have the ability to display a wide range of pharmacological activities, including the normalization of metabolic processes and antioxidant effect, is based on inhibition of lipid peroxidation processes, which are considered as one of the leading pathogenetic mechanisms of liver lesion development. Therefore, the use of antioxidants in medicines, such as selenium and beta-carotene, is not only appropriate, but also pharmacologically justified [6].

Selenium plays an important role in normalizing the physiological and biochemical processes, mainly in the regulation of lipid peroxidation that provides normal antioxidant defense [2]. Carotenoids act as photoprotectors and antioxidants, at the cellular and molecular level preventing the transformations, induced by oxidizing agents and genotoxic agents, significantly increase immunocompetence and contact interaction of cells, contribute to normalized spending of vitamins and enzymes, show antistress properties [1,3].

**The purpose** of this work was to conduct clinical trials in the development of indications for the use of the Hepavitol preparation for hepatosis in dogs.

Hepavitol is an oil solution: beta-carotene (obtained from the biomass of the *Blakeslea trispora* fungus culture (C<sub>40</sub>H<sub>56</sub>), manufactured according to Pharmacopeia article 42-3867), selenium (in the form of the seleno-organic preparation DAFS-25, which is diacetophenonylselenide, containing 25% organically bound selenium) and essential phospholipids (soy lecithin) [5].

## METHODOLOGY OF RESEARCH

The experiment involved 14 dogs of large breeds aged 2 to 5 years, belonging to private individuals and having symptomatic manifestations of chronic liver pathology (fatty hepatosis type).

Clinical examination of animals was carried out consistently according to the generally adopted in the veterinary practice scheme, which includes the registration of the animal, obtaining the history from the owner, the recording of previous illnesses, and a general and special study. All animals were subjected to thermometry, the pulse number and respiratory movements per minute were counted. The intake of water and feed, the frequency of urination and defecation in animals were taken into account when obtaining the anamnesis.

In addition, ultrasound examination of the abdominal organs was carried out, in which echogenicity of liver and gall bladder tissues, echostructure, shape, size, presence or absence of volumetric formations and free fluid were assessed.

At the initial clinical examination of animals, the general condition was suppressed, the dynamic activity and reaction to external factors decreased, limb weakness and a shaky gait, fatigue, a cachectic

appearance, and lack of appetite (according to the owners). In 57 % of cases, diarrhea, periodic vomiting and bowel meteorism were noted.

When assessing the condition of visible mucous membranes, dryness was detected, in 4 cases – anemic, and 6 – insignificant icteritiousness of the mucous membranes of the oral cavity and the sclera of the eyes. The skin of all sick animals was dry, the coat was dull, disheveled, alopecia was found in the region of the thighs, lower back and base of the tail. Itching was registered in most dogs, which led to scratching and breaking the integrity of the skin in these areas. Evaluation of liver boundaries in 8 dogs revealed moderate hepatomegaly without sharp pain.

Ultrasound examination of the liver and bile excretory system revealed an increase in the size of the organ, rounding of the edges and indistinctness of the contour. The echostructure of the liver was non-uniform, of fine-grained type, increased echogenicity. Sound conductivity was lowered, depletion of the vascular pattern was revealed.

Laboratory analysis of blood in dogs registered erythropenia (reduction of erythrocyte up to  $4.25 \pm 0.26 \times 10^{12}/l$ ), lymphocytopenia and eosinophilia, as well as a low level of hemoglobin ( $94.8 \pm 6.5$  g/l).

When studying the biochemical parameters of blood it was found that in the blood of dogs with hepatitis the concentration of total protein, on average, was  $52.9 \pm 1.8$  g/l. Hypoproteinemia develops when the parenchymal elements of the liver are damaged, which leads to a decrease in its protein-synthetic function. The level of urea was registered at the lower limit of the norm ( $3.4 \pm 0.8$  mM/l).

As a result of the evaluation of the content of indicator enzymes in the blood of dogs (AST, ALT and alkaline phosphatase), data were obtained that indicate a change in the concentrations of these indicators towards an increase from the physiological norm. Damage to the structural components of the liver and the intensity of metabolic processes are exacerbated by increased permeability of plasma membranes of hepatocytes, which explains the increased activity of serum transaminases. The quantitative content of ALT in the blood of sick dogs was, on average,  $73.5 \pm 3.1$  U/l, and AST –  $48.6 \pm 2.2$  U/l. Registered in a number of animals, hyperphosphatazemia indicated moderate cholestasis (its content was detected at  $110.5 \pm 4.6$  U/l, slightly exceeding the norm).

The results of pigment metabolism in dogs with hepatitis showed deviations from the norm in the direction of increasing the total bilirubin content to  $12.1 \pm 1.6$   $\mu$ M/l. The level of vitamin A was  $78.6 \pm 3.9$   $\mu$ g%.

Thus, a background examination of dogs showed that a violation of the functional state of the liver during hepatitis is accompanied by a wide spectrum of morpho-biochemical abnormalities in the blood system.

To study the hepatoprotective properties of the Hepavitol preparation, the experimental dogs were divided into two equal groups (experimental and control).

Therapy for hepatitis was carried out in a complex way: in animals with signs of dehydration (alternated vomiting and diarrhea), Ringer's solution was intravenously injected for dehydration control of the organism; all dogs were given vitamins of group B, animals with cholestasis as a cholagogue – Allochol (according to the instructions for use).

The animals of the first group individually 2-3 times a day used Hepavitol (with a total daily dose of 0.5 ml per kg of body weight) for 30 days. To dogs of the second (control) group, as a comparative preparation, Essentiale was used 1 capsule 3 times a day. The effectiveness of the treatment of the animals of the experimental group was evaluated in comparison with the control dogs.

## RESULTS

When assessing the clinical state of dogs under the experiment, it was established that during the treatment positive changes were registered in animals of both groups. But in the control group, the

stabilization of the clinical status occurred at the end of the experiment in only 4 dogs (57%), and three other animals continued to use the medications.

In the group using Hepaviton, in 5 animals the appetite regained in two weeks, vomiting and diarrhea ceased, and the intensity of itching decreased to a complete cessation. On the affected parts of the body, the skin dried up and was covered with crust followed by cleansing. The unpigmented visible areas of skin and mucous membranes were pale pink in colour. By the 30th day of therapy clinical indicators in all animals normalized. Water and food intake in dogs was with normal frequency, the act of urination and defecation was carried out without deviations from the norm. In the areas of alopecia, active hair growth was observed, but in the dogs of the experimental group the hair grew almost completely, and in the control group dogs – in part. With palpation, the soreness in the liver area and an increase in its size were absent.

As a result of the carried out studies of the morphological parameters of the blood, it has been established, that by the end of the experiment, all the dogs showed normalization of the peripheral blood factor. The level of leukocytes in both groups was within the norm ( $10.3 \pm 0.86$  and  $11.5 \pm 0.73 \times 10^9/l$ ) without significant difference between the groups. The leucocytal formula, both in the experimental group of animals and in the control dogs, did not deviate from the norm.

The concentration of erythrocytes and hemoglobin in the group of dogs with the use of Hepaviton increased by 46.5 % and 31.8 % relative to background indicators. The significant difference between the groups in these indicators was 17.3 % and 11.7 %.

The content of total protein in the blood of dogs in both groups increased after treatment, registering at the level of ( $68.3 \pm 1.5$  and  $56.1 \pm 1.7$  g/l). However, this increase was unequal. Thus, the use of Hepaviton allowed to increase this indicator relative to the analogues of the control group by 21.8 %.

The dynamics of urea levels in the blood of dogs of both groups was positively correlated with the concentration of total protein. After Hepaviton therapy, the level of urea in the blood of the dogs on the 30th day of experiment increased from  $3.3 \pm 0.5$  mM/l (background) to  $4.5 \pm 0.3$  mM/l. In control animals, the concentration of urea in blood was varied within narrow limits. If, prior to the beginning of the experiment, its content was  $3.5 \pm 0.8$  mM/l, it reached the level of  $3.7 \pm 0.2$  mM/l on the 30th day.

The content of cholesterol in the blood of all animals was in the range of  $3.6-3.9 \pm 0.6$  mM/l, corresponding to the species norm.

Determination of the enzymatic activity of AST and ALT showed that all dogs had a reliable decrease in these parameters during the experiment. The concentration of AST in groups decreased to  $35.4 \pm 1.6$  and  $44.7 \pm 1.9$  U/l relative to background values ( $48.6 \pm 2.2$  U/l), ALT –  $37.3 \pm 1.2$  and  $45.1 \pm 1.4$  U/l, respectively, with background values of  $73.5 \pm 3.1$  U/l. In animals of the control group, the enzymatic activity of aminotransferases was at a higher level than in the dogs of the experimental group, with a significant difference of 20.9 % (ALT) and 26.2 % (AST).

The mean value of alkaline phosphatase before the beginning of the experiment in both groups was recorded at the upper limit of the norm. In the final period of the experiment, the level of this enzyme in the blood of dogs of all groups decreased, reaching the limits of  $58.9 \pm 3.7$  and  $65.4 \pm 2.8$  U/l, corresponding to the physiological norm.

Thus, the therapeutic regimen with Hepaviton had the most effective normalizing effect on the activity of ALT, AST and alkaline phosphatase due to a more pronounced recovery of the membrane structures of the liver cells and a decrease in the cytolytic syndrome.

Hepaviton therapy reduced the demonstration of cholestatic syndrome, which was manifested by a decrease in the level of bilirubin to  $5.4 \pm 0.2$   $\mu$ M/l in the experimental group and to  $6.1 \pm 0.5$   $\mu$ M/l in the control. However, the revealed changes in the content of bilirubin in the blood of the experimental dogs were statistically unreliable ( $P > 0.05$ ).

The vitamin content on the 30th day of the experiment in the animals of the experimental group reached  $110.8 \pm 2.6 \mu\text{g}\%$ . In dogs of the control group, the level of retinol in the blood was  $82.1 \pm 2.9 \mu\text{g}\%$ , with a statistically significant difference with animals treated with Hepavitol at 34.9 %.

### CONCLUSION

Thus, the results of the studies indicate that Hepavitol preparation has a pronounced hepatoprotective effect in hepatosis in dogs, providing a pronounced therapeutic effect, which is confirmed by the improvement of the clinical status of animals and the positive dynamics of laboratory indicators.

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