Microstructural assessment of organs and tissues in experimental immunosuppression and correction in animals

M. N. Drozd¹, V. M. Usevich²
¹ Ural State Agrarian University, Ekaterinburg, Russia
* E-mail: vus5@yandex.ru

Abstract. Modern technological conditions of keeping and exploitation of animals and poultry do not exclude the negative impact of stress on their body. This leads to a decrease in the body’s resistance, an increase in morbidity and mortality. In addition, the quantity and quality of the products obtained are reduced. Stress prevention helps to increase the natural resistance of the body. The search for effective means of preventing stress reactions of the body in animal husbandry and poultry farming is an urgent problem of practical veterinary medicine. Polymineral feed additives of natural and artificial origin may exhibit adaptogenic properties. The purpose of this study was to give a microhistochemical assessment of the effectiveness of a mineral feed additive on some internal organs during experimental immunosuppression in laboratory animals. In the conditions of experimental immunosuppression, to identify the effect of a feed additive on the structure of organ cells and their metabolism. Materials and methods. To confirm the effectiveness of the mineral adaptogen, histological, morphometric and histochemical studies of stress-determining organs (adrenal glands), organs of excretion of metabolic products (kidneys) and the central organ of homeostasis (liver) were performed. As a result of the conducted studies, the organoprotective role of the mineral adaptogen was revealed. The most sensitive organs to the action of adverse factors (liver, kidneys, adrenal glands) have been identified. Morphometrically determined the reliability of morphological changes in them. During prophylactic feeding of mineral adaptogen in the studied organs, the revealed changes did not have total destruction, approaching the structure and metabolic processes in cells to the structure of these organs in intact animals. Scientific novelty. For the first time, a comparative microstructural, histochemical and morphometric assessment of some internal organs in the body of laboratory animals under conditions of artificially induced immunosuppression was carried out, confirming the organoprotective effect of a mineral adaptogen – a feed mineral additive of domestic production.

Keywords: stress, immunosuppression, prevention, adaptogen, histology, histochemistry, morphometry, feed additives.


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manipulations and actions. By studying histochemical changes in tissues and cells, you can see a color chart of biochemical processes, which will allow you to compare immunobiochemical processes with the results of biochemical studies of blood serum.

Currently, to level the negative consequences of these factors, various drugs, feed additives, multivitamin complexes, probiotics, medicinal plants and biologically active substances are used in veterinary practice [4–6]. All of these funds can be attributed to the group of adaptogens, which is divided into mineral, organic – animals, microbiological, vegetable – natural and synthetic – pharmaceutical preparations. All these adaptogens increase the body’s natural resistance. The most interesting are those adaptogens that have the widest possible effect and are effective in relation to the natural resistance and safety of the livestock, as well as having a positive effect on the quantity and quality of the products obtained [1–3].

For a long time there has been a search for cheap and effective means of animal protection. Adaptogens of domestic production are obtained from by-products of the pharmaceutical, processing livestock, natural resources and metallurgical industries [1; 3]. The quality and safety of adaptogens for the life and health of humans, animals and birds is determined by the laboratories of medical and veterinary institutions. A large number of studied natural mineral adaptogens were used as enterosorbents that absorb enterotoxins of various origins. The mineral adaptogens used today belong to the group of zeolites of natural and artificial origin [2; 3].

Their main common advantage lies in the structure of the crystal lattice, capable of absorbing toxic and harmful substances, as well as the mechanism for enriching the body with essential minerals according to the principle of ion exchange. [3]. The fundamental difference between them lies not only in the quantitative and qualitative chemical composition, but also in the possibilities of their enrichment with the necessary minerals, as well as the stability of the mineral composition. All natural zeolites have a variable chemical composition, which depends on the depth of the formation being mined. Its chemical composition is influenced by the climatic conditions of the period of its formation. As a result of technological processing, artificial zeolites have a constant chemical composition, which can be changed in accordance with the requirements of technical regulations or animal husbandry requests. For artificial zeolites, the raw material is a by-product of aluminum production, the natural resource for which is natural bauxite. The level of occurrence of these minerals is much deeper; these are more ancient structures with constant formation conditions. In this regard, the most interesting mineral adaptogen with a stable mineral composition [3]. Previous studies on the chemical composition showed that the amount of minerals in it is more than thirty, four of which are neutral in relation to metabolic processes in the body. In addition, the mineral adaptogen is enriched with stabilized iodine. But until recently, there is no definitive information about the immuno- and organoprotective action of mineral adaptogens at the level of metabolic processes in general, at the cellular and intraorgan level.

In connection with all of the above, the search for effective means of preventing the negative effects of stress is an urgent task of practical veterinary medicine. As such a means, a feed mineral additive is used under the trade name “BSh-VIT”, produced by LLC “Sorbent-K” Russia. Previously used as an enterosorbent and a source of macro- and microelements. The positive results obtained from its use have led to its deeper study at the level of organs, tissues and cells.

The aim of the study was to give a microstructural and histochemical evaluation of the effectiveness of a mineral feed additive on some internal organs in experimental immunosuppression in laboratory animals.

The objectives of the study were:
- to determine the morphological structure of organs against the background of artificial immunosuppression and prophylactic use of mineral feed additives;
- to find out at the microscopic and histochemical level changes in the cells of the liver, the central organ of homeostasis, against the background of artificial immunosuppression and the prophylactic use of mineral feed additives.

Methods

Experimental studies were carried out in the laboratory of the Department of Infectious and Non-Contagious Pathology of the Ural State Agrarian University, in the laboratory of immunobiology of the FSBNU Urfa Research Center of the Ural Branch of the Russian Academy of Sciences, the histological laboratory of the Central Scientific Research Laboratory of the Ural State Medical University. For the study, white outbred rats of 3 months of age were selected with a live weight of 250 grams of both sexes equally, 15 heads in each group. Animals were selected into groups according to the principle of analogues, they were divided into 2 groups, control and experimental. In the experimental study, the control group included healthy intact animals that were not exposed to any effects for 3 weeks, the experimental group included animals that received a feed mineral supplement throughout the entire study period. The scheme of experience is presented in table 1.

At the beginning of the study, 6 animals, 3 heads of females and males, were taken out of the experiment to determine the morphological picture of the internal organs in intact animals. Throughout the study period, all animals received the same diet and drink ad libitum from automatic drinkers. Specialized feed “Littleone-rats” was used as feed. Animals of the experimental group were fed with the mineral feed additive “BSh-VIT”, at the rate of 0.3 g per 1 kg of live weight of the rat (0.075 g per head) once a day throughout the entire study period. Feeding animals in groups twice a day.
On 21st day from the start of the experiment, artificial immunosuppression was induced in animals of both groups by simultaneous intramuscular administration of 0.5 mg of dexamethasone and 2.5 % suspension of hydrocortisone acetate at the rate of 20 mg per 100 grams of rat live weight. The drugs used cause the involution of the organs of the immune system and the development of the process of glycolysis in the liver, while increasing the level of glucose in the blood [9].

After the creation of immunosuppression on the 14th day, 6 animals (3 females and 3 males) were removed from each group from the experiment. From these animals, material was collected for histological and histochemical studies. For microscopic studies, the liver, kidneys and adrenal glands were taken. Pieces of the selected organs were fixed with a formalin solution, dehydrated with alcohols of increasing concentration, and embedded in paraffin. All histological studies were performed according to generally accepted methods. To obtain histosections, a rotary microtome HM-450 Microm was used. The prepared sections were stained with hematoxylin and eosin according to standard methods. For the detection of mucopolysaccharides – PAS-reaction with Schiff’s reagent.

To study histosections, an Olympus CX41 light microscope was used at a magnification of x 100, 200, 400. Microphotography was carried out with a Levenhuk C130 NG digital camera to document the most common changes in the comparison groups.

The resulting digital material was subjected to statistical processing in the Excel program. The arithmetic mean error (M) and the arithmetic mean error (m) were read. The significance of the difference was assessed by Student’s t-test. The results were considered reliable at \( P \leq 0.05 \).

**Results**

To assess the immunosuppressive effect, we assessed the microstructure of the stress-modulating organ, the adrenal glands. The adrenal glands are classified as organs of the endocrine system, tk. they produce hormones. The morphostructure of the adrenal gland has a two-layer structure, while the cortical and medulla have a different origin, respectively, different functions and structure.

The cortical zone occupies a large area, corticosteroid hormones are synthesized in it, and catecholamines are synthesized in the medulla. The cortical substance is represented by three zones: the outer – glomerular, represented by adrenocorticoctyes, the middle – bundle, and on the border with the medulla there is a mesh zone, represented by endocrinocytes. Adrenalin produced by the adrenal cortex stimulates glycogenesis in the liver, and glucocorticoids stimulate gluconeogenesis. Therefore, the ratio of hormones produced by the adrenal glands ensures the development of suppressive states in target organs and tissues, and, in general, the development of a state of immunosuppression in the body. Chromaffin cells located in the medulla produce epinephrine and norepinephrine.

Mineralocorticoids (aldosterone) are produced by cells of the zona glomeruli, and cells of the zona fasciculata produce glucocorticoids (cortisol). Cortisol is a hormone that has the ability to suppress the function of the immune system and affects carbohydrate metabolism. The cells of the zona reticularis produce sex hormones. When studying the microstructure of the reticular zone, no noticeable changes were noted in all animals of the studied groups.

The cells of the medulla produce epinephrine and norepinephrine. Adrenaline is capable of aggregation in individual granules and bind to the phospholipids of the cell membrane. They are immediate type hormones, so they do not work for a long time. Also, under microscopy at the level of conventional light microscopy, they did not suffer noticeable changes.

In this regard, our attention was drawn to the morphostructure of the adrenal cortex and its glomerular and fascicular zones.

Since the structure was identical in the microstructure of the reticular zone in all groups of animals, it was not described separately. Artificially reproduced immunosuppression primarily affects the structure of the upper layer of the adrenal gland - the cortical layer and, to a lesser extent, the lower zones of the cortical and medulla layers. Probably, long-term chronic immunosuppression can lead to a change in the microstructure of all layers of the adrenal gland and have a greater effect on the microstructure of organs and systems of the animal body.

At the same time, it was found that the micropicture of the organ did not undergo changes in comparison with the control group of animals (intact healthy animals). The structure of the adrenal glands had a characteristic structure on the outside covered with a capsule, represented by connective tissue and divided into cortical (Fig. 1, 4) and cerebral zones.

With induced immunosuppression, there is a slight thickening of the connective tissue capsule of the organ and a slight increase in the number of lymphoid cells in the cortical zone (Fig. 2, 5). The brain area had a typical structure.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>“Little-one-rats” food and artesian drinking water</th>
<th>Immunosuppression for 21st day from the start of the study</th>
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<tbody>
<tr>
<td>Control – intact animals</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Experimental group mineral feed additive constantly at a dose of 0.075 g per head</td>
<td>+</td>
<td>+</td>
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With artificially induced immunosuppression against the background of feeding a feed mineral supplement, thickening of the connective tissue capsule of the organ was also noted, a slight increase in the volume of the cortical zone due to the migration of lymphoid cells into it (Fig. 3, 6). At the same time, the structure of the cerebral zone had a typical structure.

In the study of the microstructure of the kidneys in the intact control group, a typical structure of the glomerular epithelium and convoluted tubules was noted, while the brush borders, the epithelium of the convoluted tubules in a functionally active state were clearly visible (Fig. 7).

With immunosuppression in the convoluted tubules, there are areas with violations of the brush border, degenerative changes in the epithelium and desquamation of epithelial cells (Fig. 8).

When studying the microstructure of the kidney of the cortical zone in the group of animals with artificially induced immunosuppression against the background of feeding a mineral feed additive, focal disorders of the brush border of the convoluted tubules were noted (Fig. 9). The loss of the brush border leads to a violation of the reabsorption of primary urine and the loss of vital substances (protein, amino acids, glucose, vitamins, salt, water (up to 85% of the total reabsorption).
When conducting morphometric studies on the loss of the brush border per unit area occupied by the convoluted tubules, in the group of intact animals it was 19 %, in the group of intact animals after immunosuppression 51 %, and in the group that received a mineral adaptogen throughout the entire period of the study, these changes were 29 % (table 2).

Under immunosuppression, intact rats show dystrophic changes in the epithelium of the convoluted tubules; in the epithelium of the convoluted tubules, the percentage of loss of the brush border increases significantly compared with the intact control (differences are statistically significant).

When using a mineral supplement in the feed – in the convoluted tubules of the kidney, the loss of the brush border in the epithelium occurs more often than in the control (in 29 % versus 19 % in the control), but the changes are less pronounced than with immunosuppression in naive rats.

When studying the microstructure of the liver in naive rats, the structure of the organ has a typical structure. The lobules of the liver are well expressed, the vessels of the triad and the bile duct are visible and have a typical structure, the organ and hepatocytes are in a functionally active state. The cytoplasm of hepatocytes is fine-grained, the nuclei are round, small in size, there are single binuclear hepatocytes. The portal tracts are not dilated; single lymphocytes are visible in the portal stroma (Fig. 10). With induced immunosuppression in the liver of rats, pronounced dystrophic changes in hepatocytes are noted with the development of proteinaceous and small-drop fatty degeneration, the bile duct is significantly enlarged, and signs of vasodilation are noted (Fig. 11). In the group of immunosuppression against the background of feeding the mineral feed additive, dystrophic changes are also noticeable, but they are more smoothed. Hepatocytes undergo protein degeneration, the bile duct is moderately dilated. The vessels of the triad artery and vein are slightly dilated (Fig. 12).

<table>
<thead>
<tr>
<th>Group</th>
<th>Intact control</th>
<th>Immunosuppression</th>
<th>MA and Immunosuppression</th>
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<tbody>
<tr>
<td>% loss of the brush border</td>
<td>19</td>
<td>51</td>
<td>29</td>
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<td>of the tubule epithelium</td>
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Table 2
When conducting a histochemical study on the content of glycogen and mucopolysaccharides (MPS) in hepatocytes and intercellular substance – the connective tissue stroma of the organ in intact animals, the PAS-reaction revealed a significant content of glycogen and MPS in hepatocytes and connective tissue stroma, the bile duct was slightly enlarged, which corresponds to the state of the liver healthy animals (Fig. 13).

With immunosuppression, there was noted a significant decrease in the amount of glycogen in hepatocytes, due to glycolysis caused by immunosuppression. A sharp depletion of hepatocytes in glycogen. Glycogen and MPS are partially determined only in the stroma of the organ. The cytoplasm of hepatocytes contains a greater amount of fatty inclusions. The bile duct is significantly dilated, there is perivascular edema (Fig. 14). Significant losses of glucose lead to energy problems of these organs in intact animals in terms of structure and metabolic processes in cells.

When immunosuppressed against the background of feeding the mineral adaptogen, hepatocytes also lose glycogen, as a result of glycolysis caused by immunosuppression, but in some hepatocytes and liver stroma there is a small amount of glycogen and MPS, bile duct dilation and signs of edema in the triad region (Fig. 15).

Discussion and Conclusion

The analysis of the conducted studies showed that artificially induced immunosuppression has a negative effect on the structure of the studied internal organs of the animal organism. Pronounced changes were noted in the structure of kidney and liver cells. Less pronounced changes were recorded in the adrenal glands. The revealed changes in the organs of the animals of the experimental group corresponded to the organo-protective effect of the mineral feed additive used for prophylactic purposes, which can characterize it as a mineral adaptogen. Histochemical studies have established a change in metabolic processes at the level of cells in the liver.

There can be drawn the following conclusions:
1. During prophylactic feeding of a feed mineral supplement, changes in the liver and kidneys did not have total destruction, approaching the structure of these organs in intact animals in terms of structure and metabolic processes in cells.
2. With prophylactic feeding of a mineral feed additive with subsequent immunosuppression and a morphometric analysis of the state of the brush border of the convoluted tubules of the kidneys, it statistically significantly shows the preservation of the structure of these cells.
3. The prophylactic use of a mineral feed additive in the feed showed its organoprotective effect.
4. The mineral feed additive used has pronounced organoprotective and stress-neutralizing properties, which makes it possible to attribute it to the group of mineral adaptogens.

References


Authors’ information:
Marya N. Drozd', assistant of the department of infectious and non-infectious pathology, ORCID 0000-0001-2345-6789, AuthorID 843196; +7 904 542-52-25, umn100@yandex.ru

Vera M. Usevich', candidate of veterinary sciences, associate professor of the department of infectious and non-infectious pathology, ORCID 0000-0002-5389-9277, AuthorID 654193; +7 904 542-52-25, vus5@yandex.ru

1 Ural State Agrarian University, Ekaterinburg, Russia