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Morphological examination of possible pancreotoxic and immunomodulatory action of Co-trimoxazole and corrective action on these effects of extracts of *Bupleurumaureum* and *Hill-growing Saltwort Herb* in comparison with Siliborum and Quercetin

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ABSTRACT

In the work are presented the results of the morphological examination of possible pancreotoxic and immunomodulatory actions of Bi-tol (co-trimoxazole) and corrective influence of alcohol extracts of Hill-growing Saltwort Herb and Bupleurumaureum at doses of 5 mg / kg and 10 mg / kg. Microscopic examination of the background to the introduction of Bi-tol in some acini of infant rats in some acinus among acini cells and in the lumen of some ducts were observed the appearance of cells that are similar to apoptotic, and in isolated pancreatic islets were observed small cell "loss" of beta-cell nucleus and pycnosis of nucleus and strong cytoplasmic eosino-philia. After intake of extract of Hill-growing Saltwort Herb at a dose of 5 mg / kg on a background of Bi-tol in infant rats condition of acinus and pancreatic islets did not change compared with intact control of the majority of infant rats, as in some animals from the group were found isolated pancreatic islets and a few internal-part ducts containing some similar to apoptotic cells. When using extract of Hill-growing Saltwort Herb at a dose of 10 mg / kg and Bupleurumaureum at doses of 5 mg / kg and 10 mg / kg of changes in the condition of exo- and endocrine part of the pancreas were observed. Thus, the examined extracts helped to reduce the presence of apoptotic cells in modified pancreatic islets and acynosis parenchyma of pancrea of infant rats and dominated by a similar effect of Quercetin were almost equal (especially extract of Bu- pleurumaureum), Siliborum. In immune organs (thymus and spleen) after intake of Bi-tol (antigenic stimulation) arose standard features of immune response of a definite degree of expressiveness. Examined extracts Hill-growing Saltwort Herb and Bupleurumaureum "soften" the severity of the standard features of the immune response to antigenic stimulation of Bi-tol and such "immune-modulating effect" was at the level of the comparative drugs Quercetin and Sili- borum.

Key words: drug-induced liver injury, sulfanilamide drugs, pancreotoxicity, immune- modulating effect, hepatoprotectors, extract of *Bupleurumaureum*, extract of Hill-growing Saltwort Herb, histology.

INTRODUCTION

Today one of the major problems of hepatology is the drug-induced liver injury since every year increases the spectrum of drugs that cause these injury [1-4]. If in the 1990's were known about 750 of such drugs, at the beginning of the 2000's - about 1000 of such drugs and the list is constantly updated [5-7]. A clear trend of increasing of the number of drug-induced liver injury was observed in recent years due to the constant expansion of the pharmaceutical market [8,9]. In particular, in Japan for the last 30 years was stated the 11-times increase of drug hepatotoxicity [10].

Drug-induced liver injury is described in the treatment with almost all pharmacological groups of drugs. Particularly noteworthy hepatotoxic effects of antimicrobial, antibacterial drugs and nonsteroidalantiinflammatory drugs [11]. Antibacterial sulfanilamide drugs take a strong position in the arsenal of modern drugs in the treatment of various

diseases [12]. Liver injury may occur after prolonged treatment with sulfanilamide drugs, both after a single use and even after withdrawal of it [13]. However, from the information from domestic and foreign literature it appears that the pathogenesis of liver injury with sulfonamides has not been specified completely yet. There is an assumption about a direct toxic injury to liver cells, about the reaction of increased sensitivity to drug, about damage to detoxifying function of the liver and the formation of insoluble acetylated compounds that can cause blockage of the bile tract and is not excluded the possibility of developing of autoimmune processes [4]. Along with liver antibacterial sulfonamides often cause damage to hematopoietic system also [10]. Despite the fact that these drugs cause hepatotoxic reactions with low frequency, their widespread medical use and significant amounts of use may cause the liver injury in a sufficiently large number of patients [14]. Clinical and morphological manifestations of drug-induced liver injury are different. Currently are distinguished 3 main groups of drug-induced liver injury: hepatocellular, cholestatic or mixed [15]. The target of toxic effect may be hepatocytes (necrosis), bile ducts and tubules (cholestasis) or sinusoidal cells (endothelium, Ito cells). Drug-induced liver injury may include parenchymal lesions in the form of functional impairment (induction of microsomal enzymes, hyperbilirubinemia) leading eventually to necrosis or apoptosis [16]. Other hepatotoxic effects of taking drugs include: formation of dyssebacia as acute fatty change and steatohepatitis; cholestasis; granulomatous changes; changes in the vascular system of the liver (enlargement of sinusoidal capillaries, purple hepatitis, non-cirrhotic portal hypertension, obstruction of venous outflow from the liver, nodular regenerative hyperplasi, etc. [17].

The liver takes bulk of impact at oral taking of drugs, especially those that have the phenomenon of "first pass" through the liver. Most xenobiotics are absorbed through nonspecific way by the diffusion of the sine through the membrane of hepatocytes. Back diffusion is usually difficult due to binding to specific intracellular proteins [18]. The liver is involved in many pathological processes, and its damage cause serious metabolic disorders, immune response, detoxification and antimicrobial protection. Liver belongs to the organs that are able to regenerate after damage due to cellular cooperation, the presence of the molecular mechanisms of the acute phase response and synthesis of a number of substances of protective nature. The most common liver injury is realized by chemical and immunological mechanisms. The chemical injury of the liver liver can be caused by natural substances and xenobiotics, including drugs. Causes of liver damage by means of molecular mechanisms, which are related to immune responses are: functioning of killer lymphocytes and cell cooperations, formation of autoantibodies and "house keeping" anti- bodys, action of mediators (cytokines, nitric oxide), activation of compliment system [19].

In connection with the mentioned above pharmacological science pays much attention to the search for new effective and harmless drugs with hepatoprotective, nephroprotectic, pancreopro- tectic and immunomodulatory effects [20, 21]. Today in Ukraine from the group sulfanilamides drugs is widely used domestic product Bi-tol produced by Zhytomyr Pharmaceutical Manufacturing Plant. Preliminary experimental examinations found that Bi-tol at a dose of 0.7 mg / kg at two-week course of introducing renders a hepatotoxic effect. In infant rats developed moderately distinct reactive drug subacute hepatitis with visually noticeable increase in the level of apoptosis of hepatocytes. Plant extracts of Bupleurumaureum and *Hill-growing Saltwort Herb*to some extent corrected hepatotoxic effect of Bi-tol, reduce the severity of hepatocyte apoptosis [22]. Through experiments on infant rats was also found that Bi-tol shows nephrotoxic effect. In the kidneys of experimental animals was observed an increased presence of apoptotic cells by modified "apoptosis protector" action in the kidney also extract of Bupleurumaureum had the advantage, which was up there with Quercetin and Siliborum. Previous studies have found that herbal extracts of Bupleurumaureum and Salsolacollina have marked antioxidant activity in vitro and in vivo [23].

The purpose of this examination was morphological examination of possible pancreotoxic and immunomodulatory actions of Bi-tol and corrective influence of alcohol extracts of *Bupleurumaureum*and *Hill-growing Saltwort Herb.*

EXPERIMENTAL SECTION

Examined the morphological structure of the liver, kidney, thymus, spleen, pancreas of infant rats with initial weight 60-100 g, which administered for 2 weeks Bi-tol in a dose mg / kg; similar organs of rats that together with bi- Tol took alcohol extract of Hill-growing Saltwort Herb and alcoholic extract of *Bupleurumaureum*at doses of 5 mg / kg and 10 mg / kg, or medications as a *Bupleurumaureum*at a dose of 100 mg / kg or Quercetin at a dose of 50 mg / kg, and also the same organs of intact infant rats of corresponding weight. The dropping from the experiment of rats was carried out by decapitation under light ether anesthesia according to the provisions of the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes [24]. The samples of organs fixed in 10% formalin solution, desiccated in alcohols of increasing concentration, embedded in paraffin, celloidin-wax. Sections were stained with hematoxylin and eosin [25]. View of micropreparations performed under a microscope Granum, microphotography microscopic images were made by the digital camera Granum DSM 310. Photographs were processed with the use of Computer Pentium 2,4GHz using Toup View.

RESULTS AND DISCUSSION

Pancreas of intact infant rats in the specimens was presented both exocrine and endocrine glandular tissue component. Exocrine part is represented by closely spaced acini. In the cytoplasm of acinal cells is clearly visible basophilic peripheral and central oxyphilic, which contains small zymogenic granule, areas. Value of differently painted areas in the cytoplasm of acinous cells varied in the range 1 : 2 - 1: 2,5. The nuclei are usually located in the basal part of the cells. System and interlobular internal excretory ducts are not changed. Duct epithelium is most common, sometimes in the lumen are visible clumps of condensed eosinophilic secretion. State of arteries and veins is normal. Depending on the caliber of the excretory ducts, arteries and veins of varying severity were observed periductal and peivasalstroma. Endocrine system presents different sizes of pancreatic rounded- oval islets, clearly distinguished from the surrounding of acinar parenchyma. Main mass of the cells in the islets were light polygonal beta cells, rather densely and evenly distributed in the central part. Between the strands of cells in a number of islands are visible common sinusoidal capillaries. On the periphery of the islands are chain alpha-cells (Fig. 1).

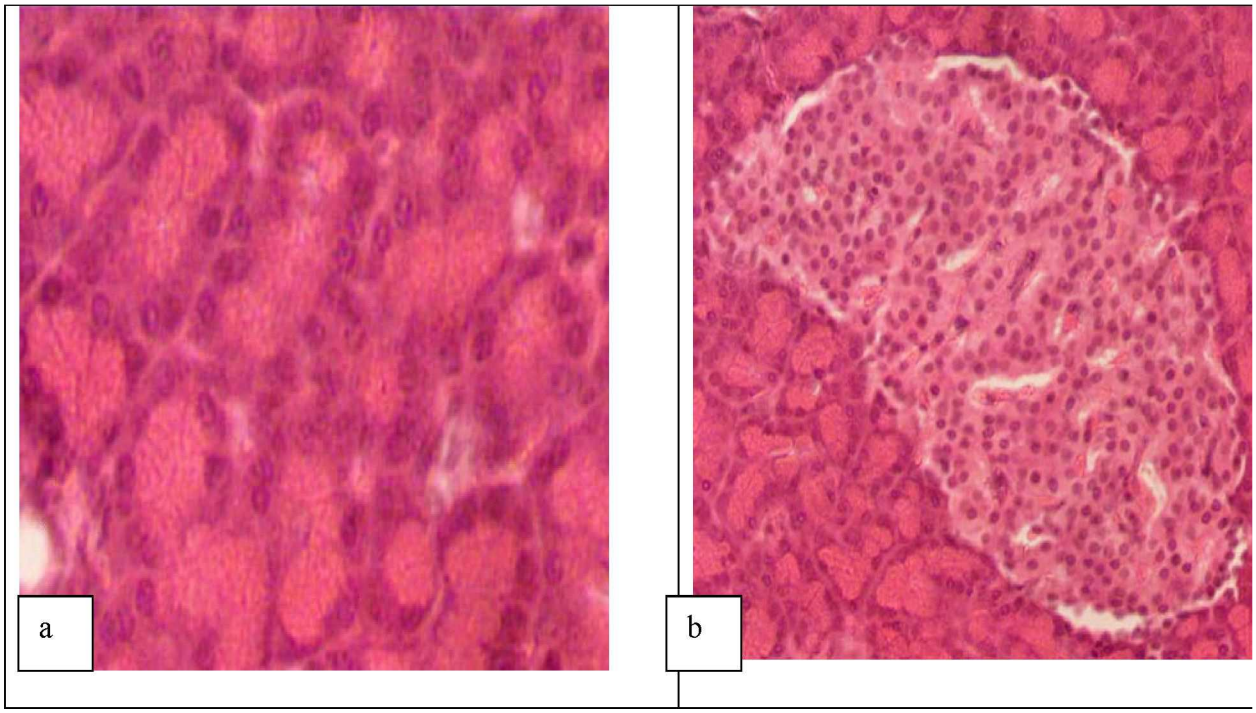


Fig. 1. The pancreas of intact infant rats: a - unchanged densely located acinus (x400); b - pancreatic islet with uniform distribution of normal form of beta-cells (x200). Hematoxy-lin-eosin

Administration of Bi-tol visually as a whole did not affect the status of acini and pancreatic islets of infant rats (Fig. 2).

However, in a number of acinus among acinous cells and in the lumen of some internal ducts was observed the appearance of cells that are similar to apoptotic (Fig. 3) and in isolated pancreatic islets was observed small cell "loss" of beta cells, pyknosis of nucleus and strong cytoplasm eosinophilia (Fig. 4).

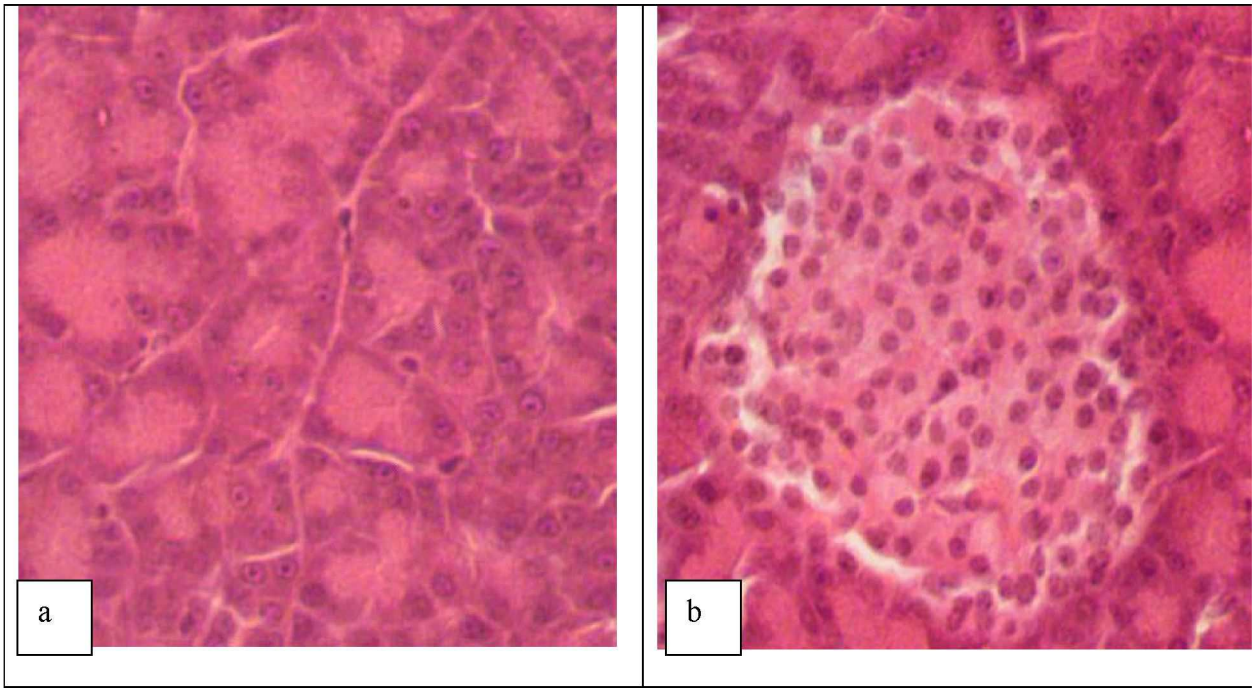


Fig.2. Pancreas of infant rats after administration of Bi-tol. State of acini (a) and pancreatic islets (b) is not changed. Hematoxylin-eosin.x400

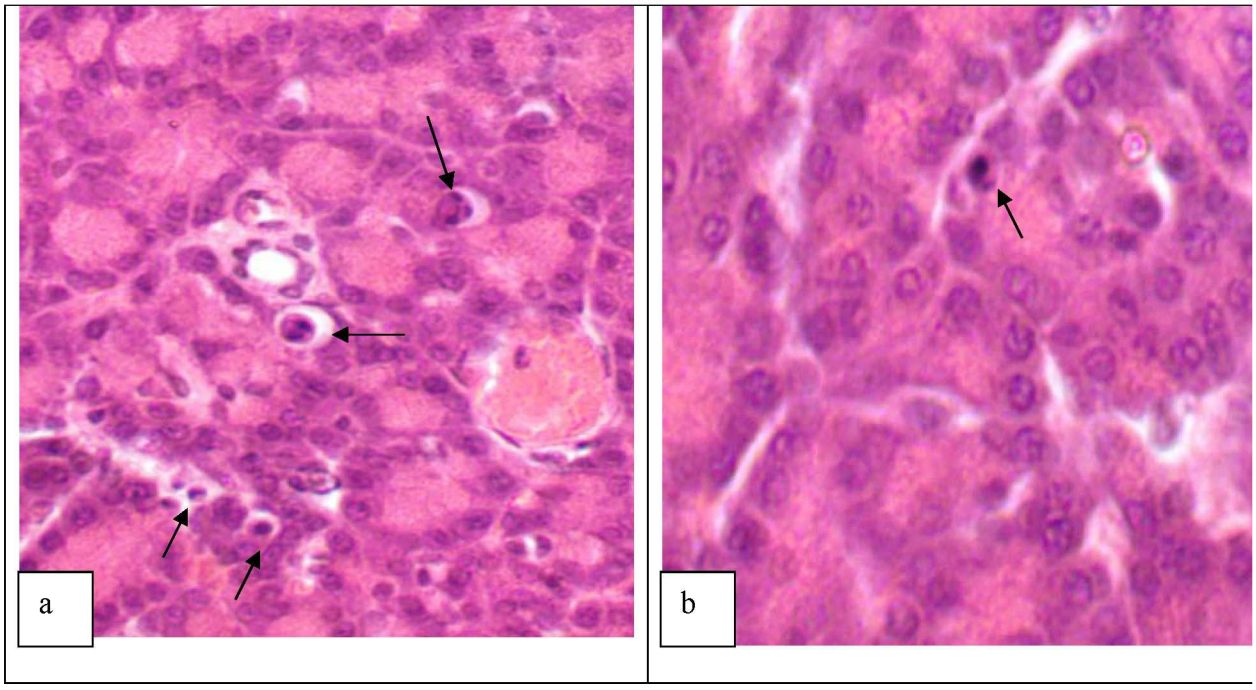


Fig. 3. Pancreas of infant rats after administration of Bi-tol. Cells resembling apoptotic in internal-part duct lumen (a), among acinose cells (b). Hematoxylin-eosin. x400

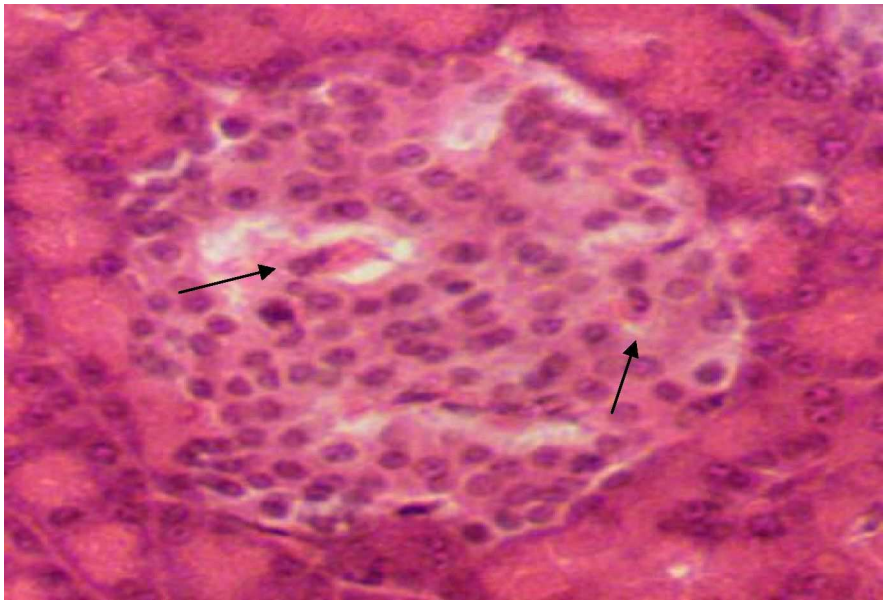


Fig. 4. Pancreas of infant rats after administration of Bi-tol. Pancreatic islet with the "fallout" and morphological changes in the state of individual beta cells

Condition of acini and pancreatic islets did not change compared with intact control in the majority of infant rats after administration of extract of *Hill-growing Saltwort Herb* in a dose of 5 mg / kg and Bi-tol. In two animals was found isolated pancreatic islets and a few internal-part ducts containing some similar to apoptotic cells (Fig. 5).

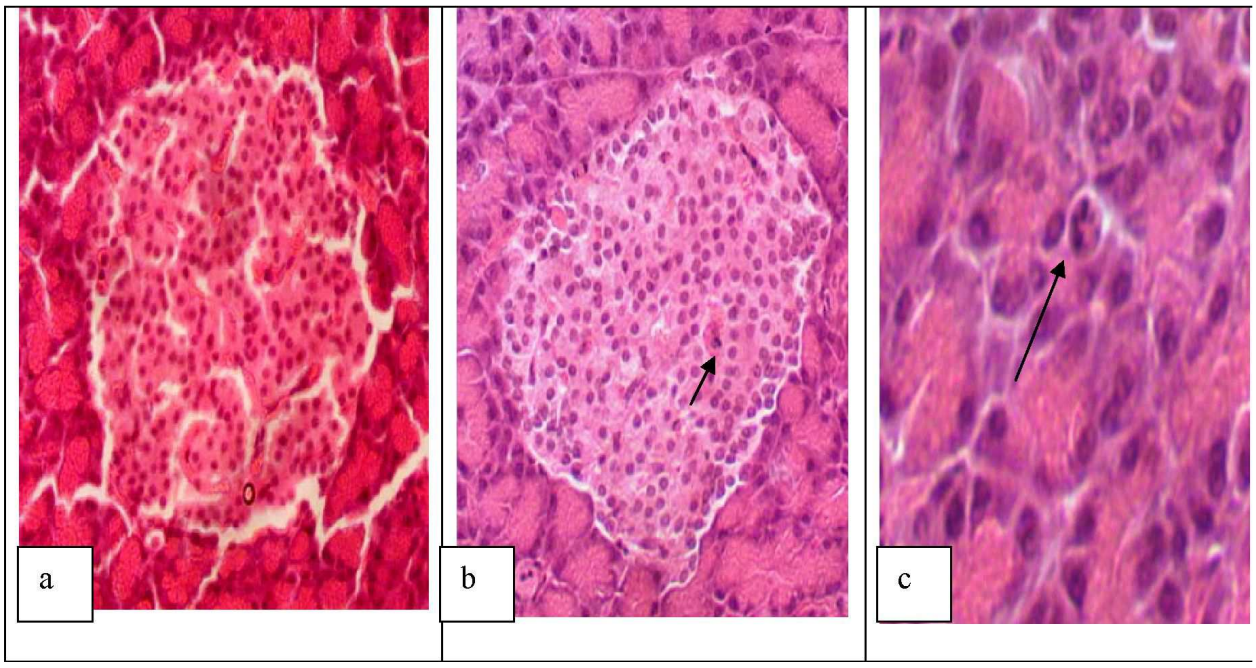


Fig. 5. Pancreas of infant rats after simultaneous administration of extract of *Hillgrowing Saltwort Herb* in the dose of 5 mg / kg and Bi-tol, unmodified pancreatic islet (a); single cell, similar to apoptosis, including visually not altered pancreatic beta cells (b) and in the lumen of the internal-part duct (c). Hematoxylin-eosin. a-b - x200, c - 400

No visible on light-optical level changes were detected after simultaneous administration of extract of *Bupleurumaureum* and Bi-tol (Fig. 6).

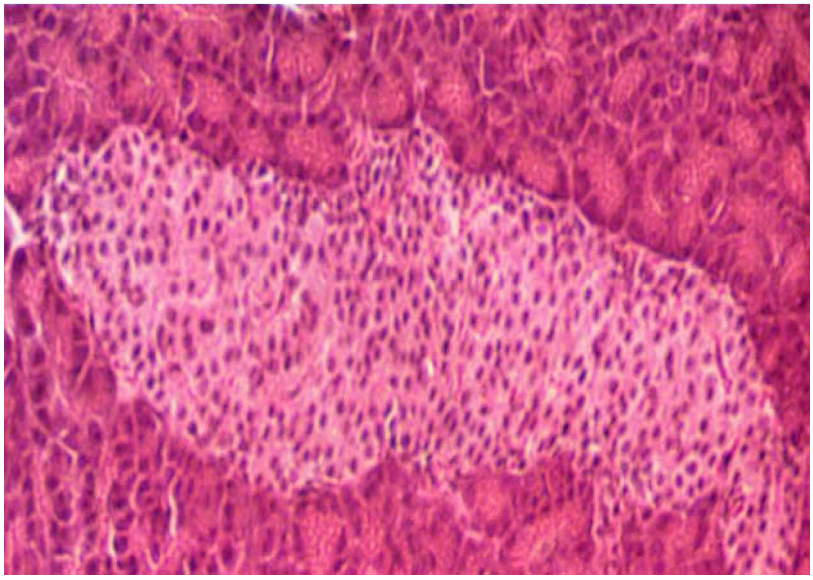


Fig. 6. Pancreas of infant rats after simultaneous administration of extract *Bu-pleurumaureum* in the dose of 5 mg / kg (a) and 10 mg / kg (b) and Bi-tol. Condition ofacini and pancreatic islets is normal. Hematoxylin-eosin. x200

No visible changes on light-optic level were detected after simultaneous administration of extract of *Bupleurumaureum* and Bi-tol (Fig. 7).

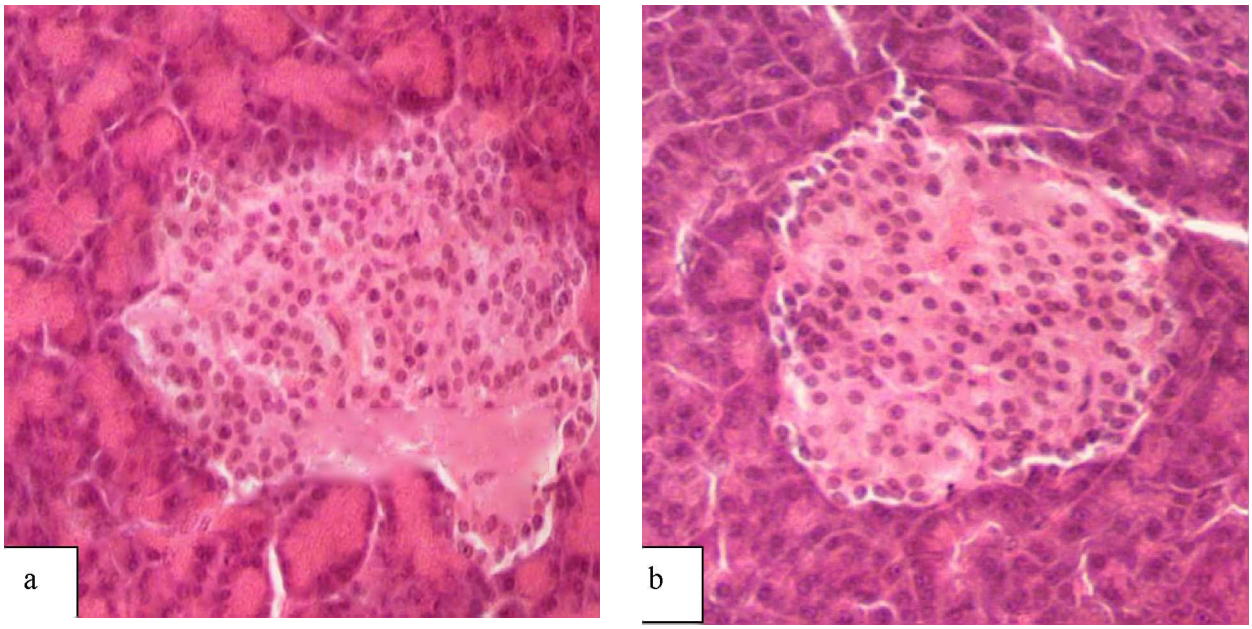


Fig. 7. Pancreas infant rats after simultaneous administration of extract o *Bupleu- rum aureum* at a dose 5 mg / kg (a) and 10 mg / kg (b) and Bi-tol. Condition of acinus and pancreatic islets as normal. Hematoxylin-eosin. x250

After the administration of Quercetin on the background of Bi-tol cells resembling apoptotic, was observed in non-isolated pancreatic islets, were visible mitosis even in the beta cell (Fig. 8), but in exocrine part of glandular tissue oacytosis cells are not visually changed.

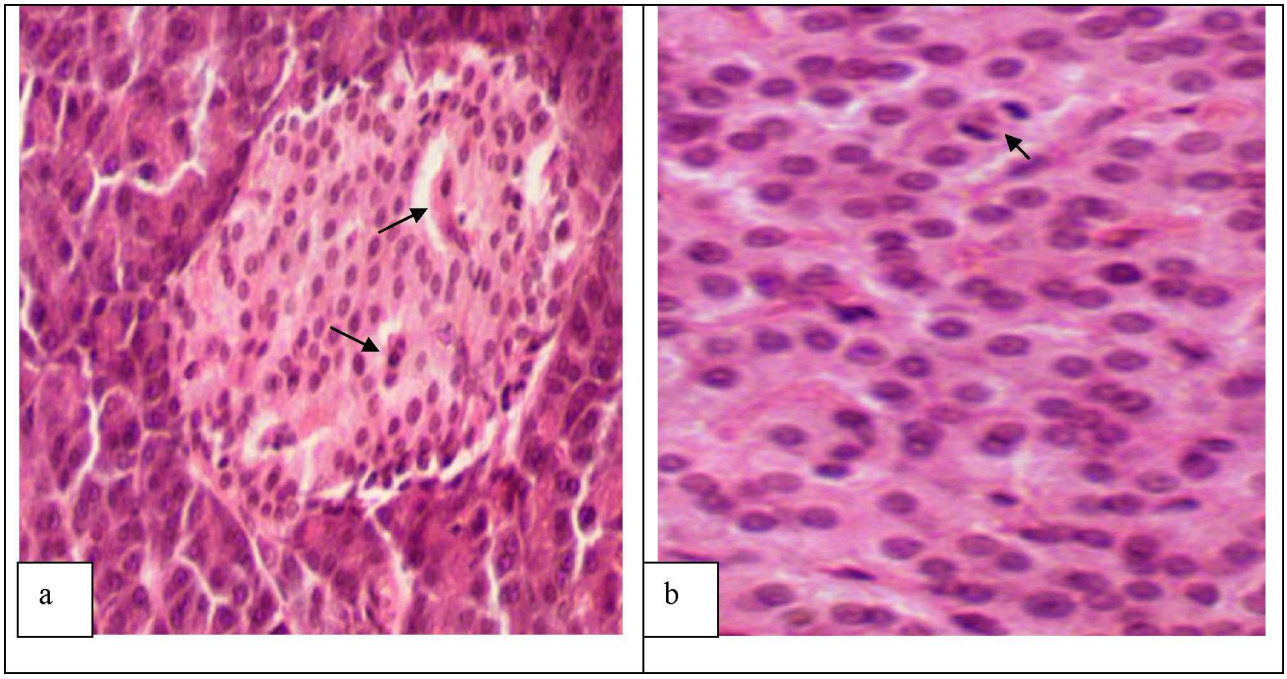


Fig. 8. Pancreas of infant rats after simultaneous administration of Quercetin and Bi-tol: a - cells resembling apoptotic in pancreatic islets (x250); b - beta cell mitosis (x400). Hema-toxylin-eosin

After administration of Siliborum against the backdrop of Bi-tol condition of all components of the glandular tissue of pancreas of infant rats was consistent to norm (Fig. 9).

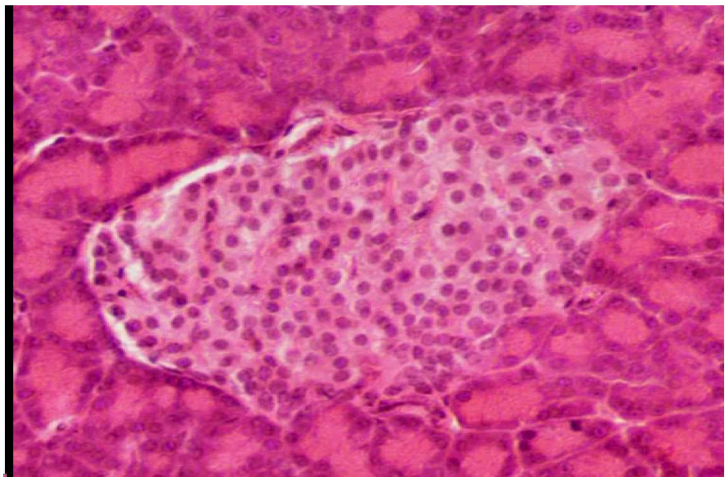


Fig. 9. Pancreas of infant rats after simultaneous administration of Siliborum and Bi-tol. Good condition of glandular tissue. Hematoxylin-eosin. x250

Thymus of all infant rats had well-defined lobed structure. Connective tissue capsule and septa were given sparingly. Particles are large enough. They clearly differentiated cortical and medulla. In intact infant rats the volume of cortex was about 1.5 - 2 times higher than the same in a brain. Density of thymic and medullary lymphocytes was within normal limits. Thymic cells are few, small, reticuloendothelial cells are usually masked by lymphocytes and not always observed (Fig. 10).

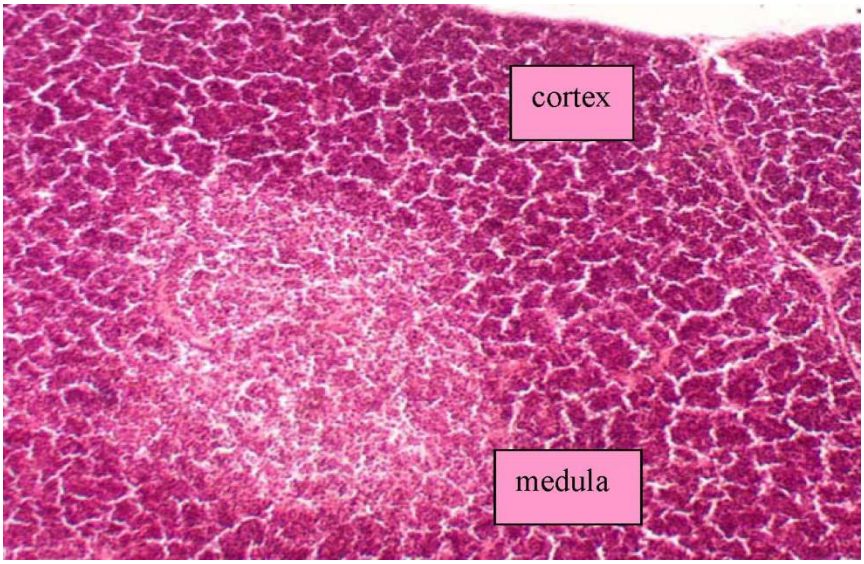


Fig. 10. Thymus of intact infant rats. Normal distribution of particles on the cortical and medulla, sufficient density of lymphocytes. Hematoxylin-eosin. x100

After the administration of Bi-tol noticeable changes in the clarity of the distribution of glandular tissue for cortex and medulla were not observed. Density of medullary and thymicthymocytes was not changed. Marked a moderate expansion in the medulla of the cloves, little expressive fine-focal proliferation of reticuloendothelium cells. In some infant rats was observed atypical structure of individual thymic cells that looked like "starry sky" - small dots of enlightenment due to adhesion of lymphocytes to macrophages (Fig. 11). This condition of thymus indicates the development of the in organ of standard reactive changes that occur in response to antigenic stimulation with Bi-tol (accidental transformation). Marked changes correspond to the 1st - 2nd phase of accidental transformation [15].

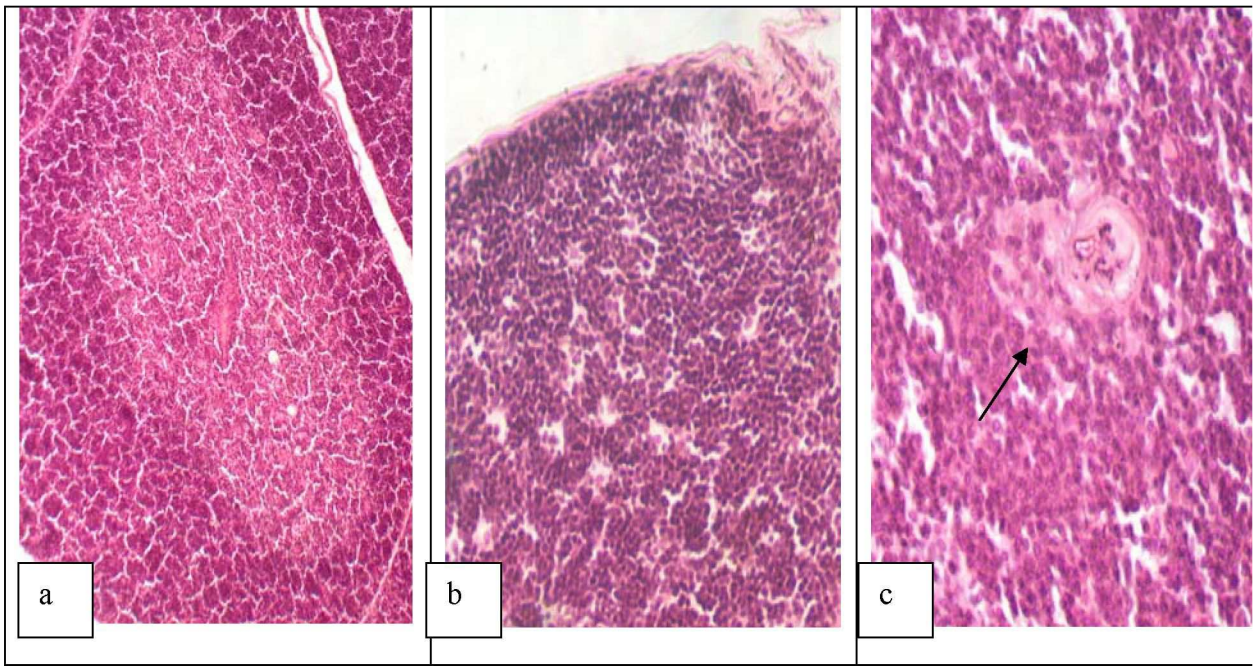


Fig. 11. Thymus of rats after administration of Bi-tol: a - moderate expansion of medulla (x100) b - picture of "starry sky" in the cortex (x150); c - atypical thymic body, small cell proliferation reticuloendothelium cells (x200). Hematoxylin-eosin

Simultaneous administration of Bi-tol and extracts of *licoroce (Glycyrrhiza)* or *Bupleurumaureum* in different doses and also Siliborum very moderate "softened" the severity of reactive changes in the body, almost invisible atypia of thymic cells, picture of "starry sky" in the cortex, marked only a slight increase in brain layer and small groups (2-3 cells) of reticuloendothelium (Fig. 12, Fig. 13). It features fully adequate immune response to antigenic stimulation.

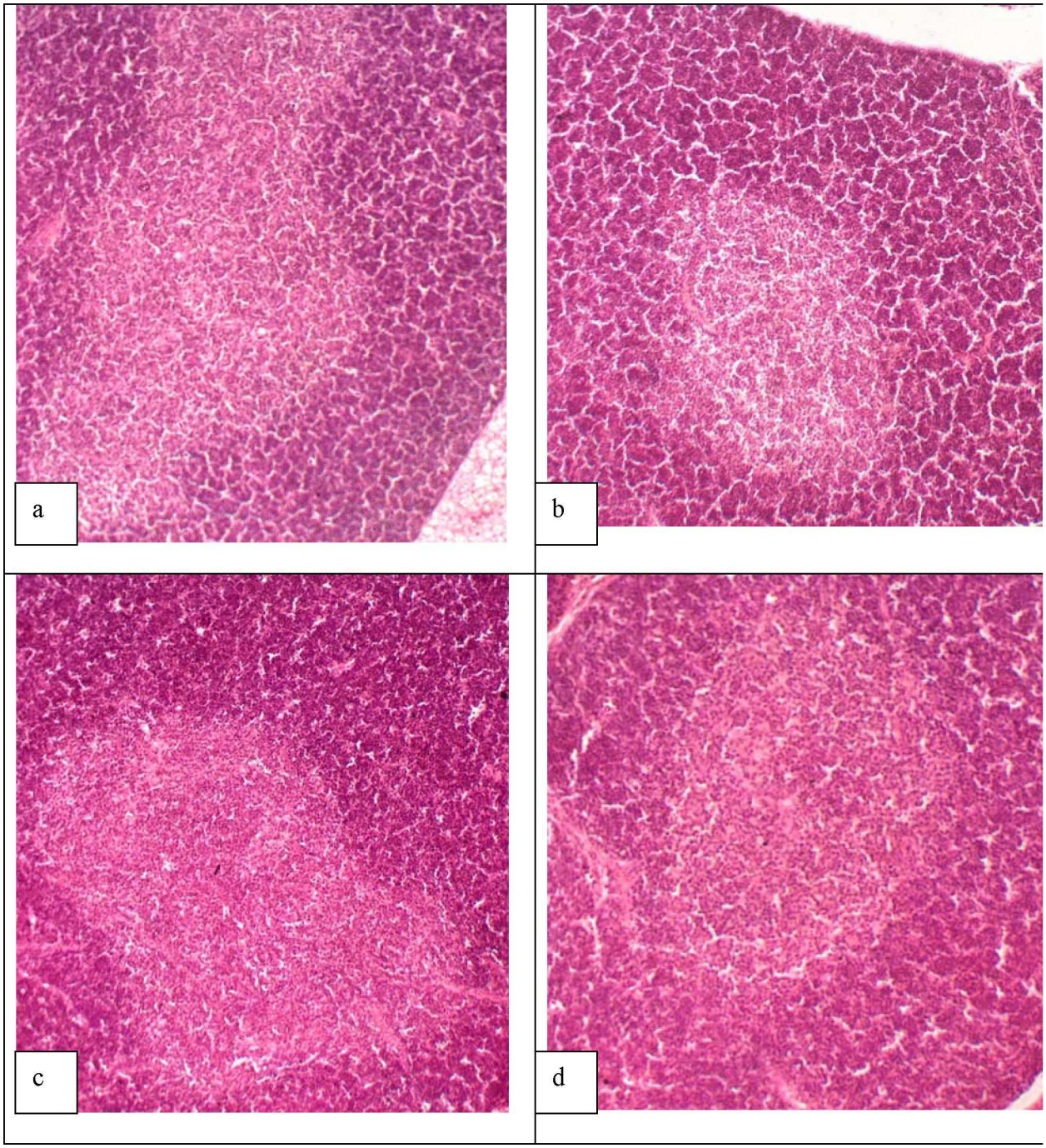


Fig. 12. Thymus of infant rats after simultaneous administration of extract of *Hill-growing Saltwort Herb* (a - 5 mg / kg, b - 10 mg / kg) and extract of *Bupleurumaureum* (c - 5 mg / kg, d - 10 mg / kg) and Bi-tol. A slight increase in the medulla. Hematoxylin-eosin. x100

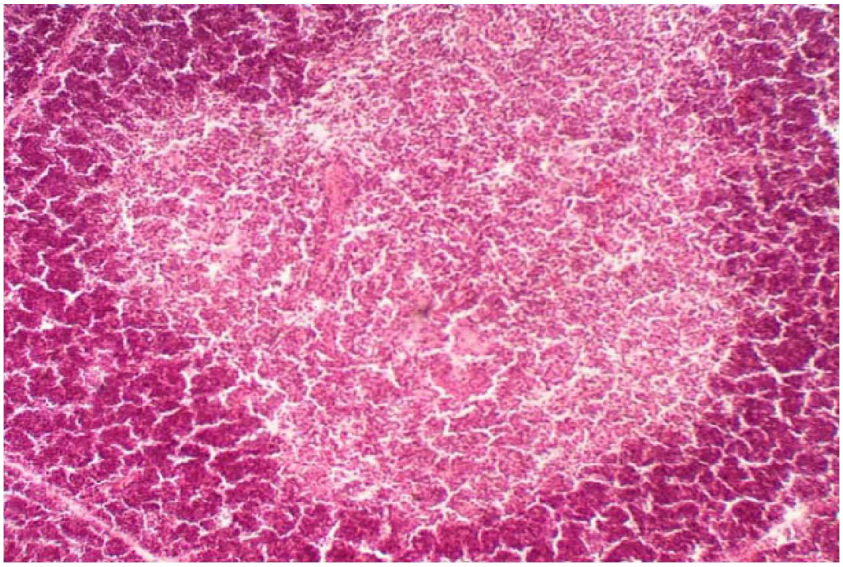


Fig. 13. Thymus of infant rat after simultaneous administration of Siliborum and Bi-tol. Moderate expansion of medulla. Hematoxylin-eosin. x100

After administration of Quercetin combined with Bi-tol microscopic picture of thymus was similar to that in mono administration of Bi-tol (Fig. 14).

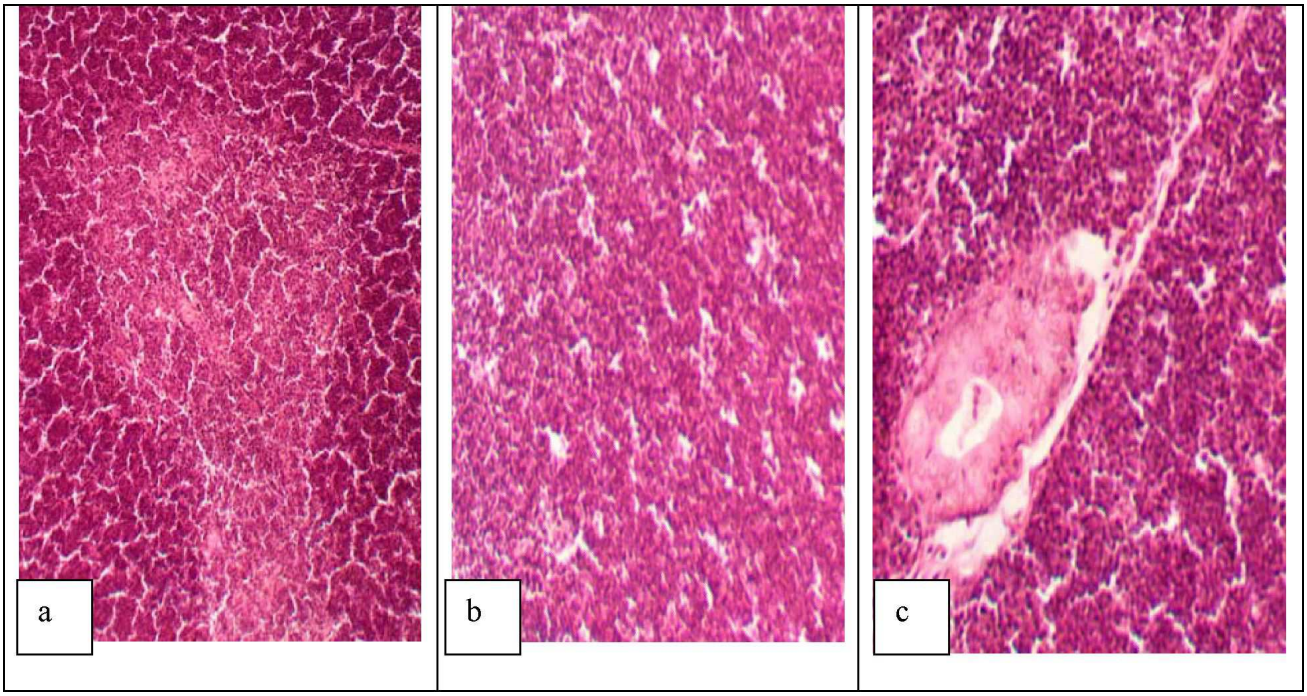


Fig. 14. Thymus of infant rats after simultaneous administration of Quercetin and Bi-tol: a - slight expansion of medulla; b - picture of "starry sky" in the cortex; c – atypical hymic body. Hematoxylin-eosin. a - x100, b-c - x150

Spleen. Expression of connective tissue capsule and trabeculae in all infant rats is moderate. White pulp with lymphatic nodules and perivascular lymph clutches of arteries and arterioles, reaching to the lymphatic nodule. Lymphatic nodules had often spherical, oblong- cut oval shape. Their number is sufficient in the cut. In the nodules is clearly visible eccentrically located central artery, sometimes several are seen prominent lumbar sections of it. In all nodules are clearly visible periarterial T -dependent zone, marginal zone, germinal center (cell reproduction)

- B-dependent zones. In intact infant rats within the same state lymph specimens have similar structures, although germinal centers in different animals varied according to the degree of activity. Density of lymphocytes in periarterial nodules and perivascular areas couplings is sufficient. Most cells in these areas are quite small. Germinal centers contain reticular cells of varying degrees of differentiation, lymphoblasts, large and medium lymphocytes,

macrophages. Marginal zones around the nodes and couplings are moderate in width. Lymphoid cells of these zones were larger and not so densely arranged as lymphocytes of periarterial zones of nodules and perivascular areas of couplings. Boundary between the white and red pulp is sufficiently expressive. Red pulp is able to bear plenty of nucleated cells, red blood cells (Fig.15a).

After the administration of Bi-tol germ centers of the modes were expanded, in them was clearly increased presence of macrophages, were visible lymphocytes contact with macrophages (outlets-picture of "starry sky"), more blast forms of cells. Marginal B-zone expanded, in it were increased lymphocytes, they are more tightly located (Fig. 15b). In the red pulp vaguely increased plasma reaction.

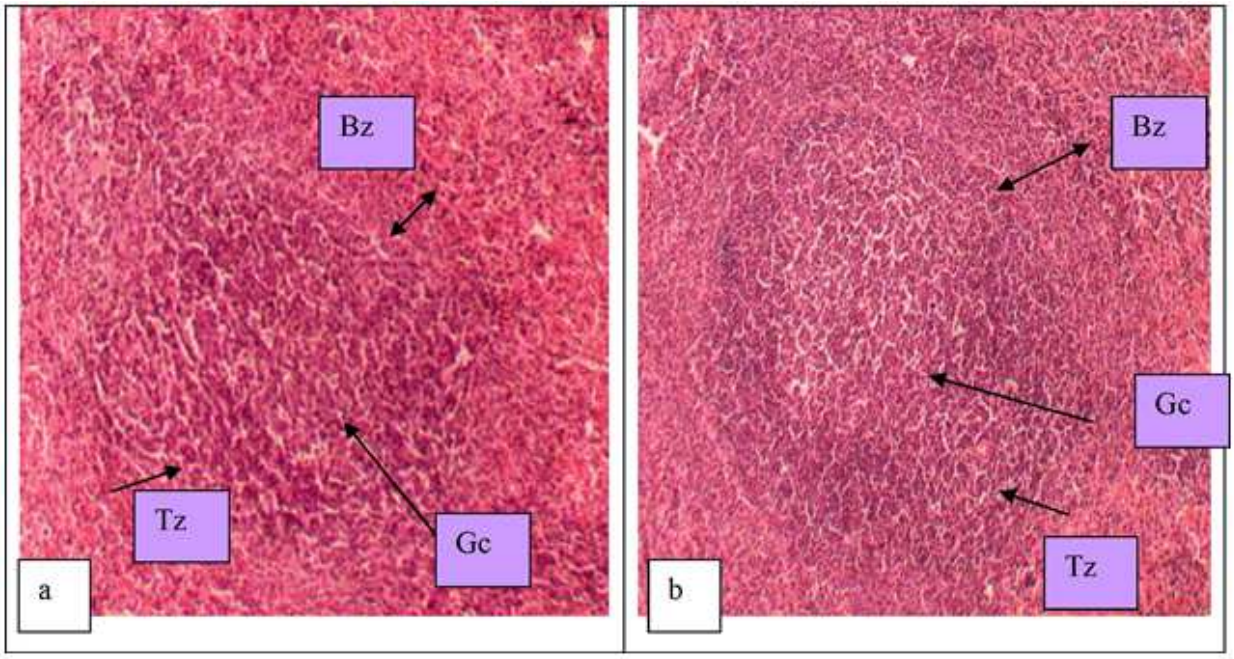
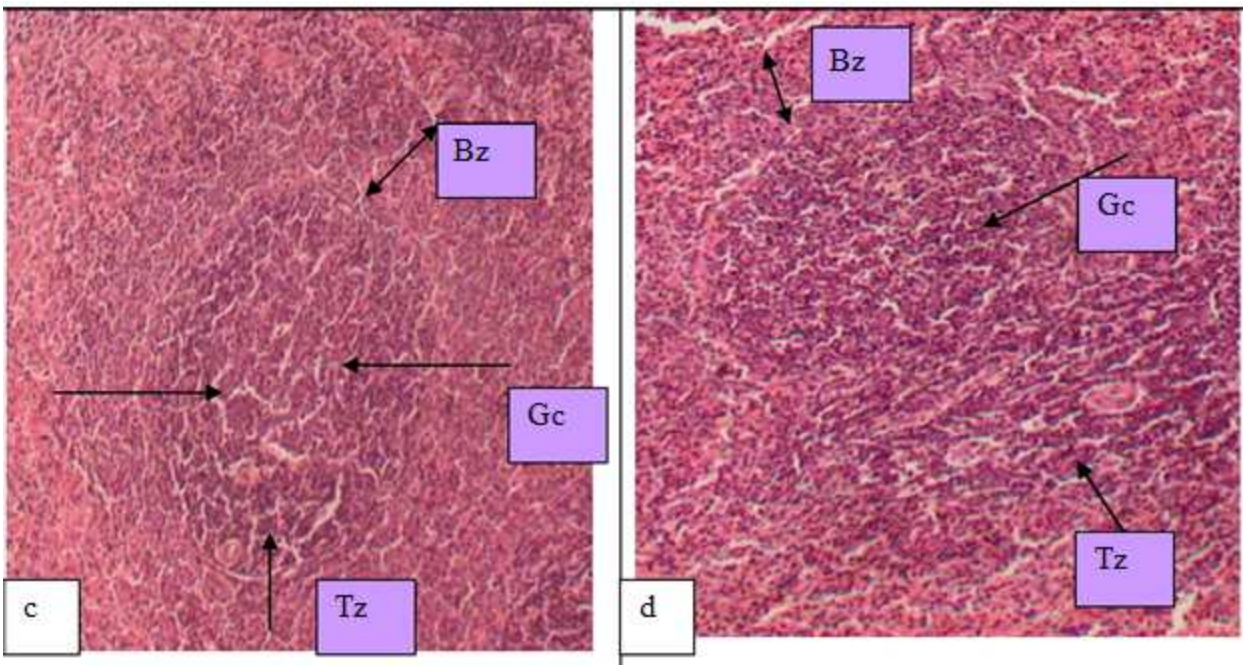


Fig. 15. Spleen of infant rats: a - intact - in lymph gland is visible perivascular T-zone, moderately active germinal center, marginal zone B; b - after the introduction of Bi- tol - more active germinal center of a lymph nodule, increasing of the width of the marginalzone. Hematoxylin-eosin.x200



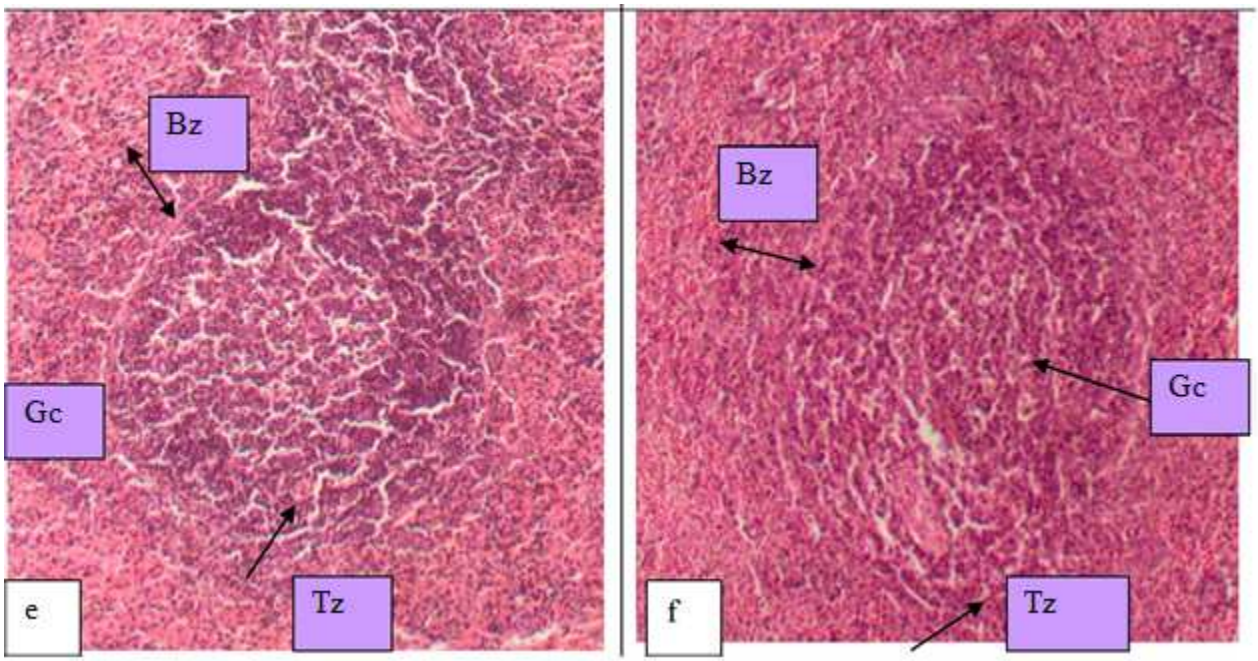


Fig. 16 Spleen of infant rats to which was administered extract of *licoroce (Glycyrrhiza)* (a -5 mg / kg, b - 10 mg / kg), *Bupleurumaureum* (c - 5 mg / kg, d - 10 mg / kg), Quercetin (e)and Siliborum (f). a-d - germinal center of lymph nodes in the less active, marginal zonewidth varies; e-f - distinct activation of germinal center. Hematoxylin-eosin.x200

It is known that marginal B-zone of the nodes is involved in the immune response, because it isthe place of "capture" of immune complexes, within this zone is made activation of B-lymphocyte in case of antigen stimulation and turn them into antibody-producing cells that lateraccumulate in red pulp [17]. As for germinal centers, the activation of them is in response to the activation of a large number of B-lymphocytes. The centers increase in size, they are a significant number of forms of blast cells and macrophages. The last remove the excess antigen, partially destroying them and giving them more immunogenic form [16,26].

Thus after the administration of Bi-tol (antigen) in the white pulp of the spleen of infant rats appear standard features of the immune response (antigenic stimulation), may be in increased from. After administration of the extracts of *Hill-growing Saltwort Herb*and *Bupleurumaureum*in a dose of 5 mg / kg and 10 mg / kg on a background of Bi-tol in the germinal centers of lymph nodes decreased blast cell form, disappeared the picture of "starry sky". As to the marginal zone - the width of them in different nodules ranged from normal (intact control) to increased (control with Bi-tol), lymphocyte density in the area was less than in the control with Bi-tol. Plasma response in the red pulp was inexpressive (Fig. 16 a-d).

Thus, the examined extracts of *Hill-growing Saltwort Herb*and *Bupleurumaureum*contribute to a "discreet" reaction of white pulp to the impact of antigen. After simultaneous administration of Quercetin and Siliborum with Bi-tol, the condition of germinal centers of nodules corresponded to that in case of mono administration of Bi-tol (in case of administration of Siliborum to a lesser extent). The width of the marginal zone, density of location of the lymphocytes in it ranged (Fig. 16 e-f).

CONCLUSION

1. Bi-tol shows no appreciable pancreatoxic action. At the same time, in the pancreas is also seen an increased presence of apoptotic modified cells (morphological features of apop- totic cells are common for all eukaryotic cells).
2. In the immunogenesis organs (thymus and spleen) after administration of Bi-tol (antigenic stimulation) arise standard features of immune response of a definite degree of expressiveness.
3. Examined extracts helped to reduce the presence of apoptotic cells in modified pancreatic islets and acinose parenchyma of pancreas of infant rats and outbalanced a similar effect of Quercetin and were almost on a level (especially, extract of *Bupleurumaureum)* of Siliborum.
4. Examined extracts of Hill-growing Saltwort Herb and Bupleurumaureum "soften" the expressivity of the standard features of the immune response to antigenic stimulation with Bi-tol and with such "immune-modulating effect" were at the level of the comparator agents Quercetin and Siliborum.

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