STUDY OF CHRONIC TOXICITY OF DRY EXTRACT ON THE ARTICHOKE PRICKLY BASIS

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In medical practice at treatment of diseases of liver and bilary tract preparations on the basis of an artichoke prickly (Cynara scolymus L.) take a special place. Preparations on the basis of an artichoke are successfully applied as effective bile-expelling and hypatoprotector remedies. Pharmacological activity of an artichoke prickly is defined by complex action oxycoric acids, flavonoids, vitamins, tannins, macro-and microcells, polysaccharides and aminoacids. Artichoke preparations possess good shipping and practically full absence of side-effects.

Keywords: diseases of liver, artichoke, biologic active substances

In support of the organism's homeostasis liver plays a great role because in pathological conditions it has the leading place not only in metabolism but in rendering harmless of endogen and exogenous toxic substances. Liver plays a role of universal metabolic barrier in the interaction process of a man with environment [1]. Liver diseases for today are one of actual problems of medicine as the liver, carrying out the functions, plays the important role in ability to live of an organism [2]. The purpose of this work is study of chronic toxicity of dry extract on the artichoke prickly basis grown up in Uzbekistan.

Materials and research methods

The study of dry extract chronic toxicity on the artichoke prickly basis was conducted in experiments on 40 white non-thorough-bred rats – males with mass of 150–185 gr. The preparation was daily brought into stomach in doses of 50, 250, 500 mg/kg during 2 months. Every dose was tested on 10 rats. In similar conditions to the control group of animals the solution (water) was brought in. All experimental and control animals were in the same conditions and usual ration.

During the experiment animals were under every day control; their total state, behavior, having food and water, hair cover, mucous membranes were recorded. Animals were weighed once a week. The morphological and biochemical analysis of peripheral blood was conducted. After the experiment animals were decapitated, their macroscopic examination was conducted, the coefficient of internal organs mass was determined. Parts of internal organs and the brain were fixed in 10% neutral formalin for the further histological research.

Results of research and their discussion

The conducted results have demonstrated that prolonged per oral use of dry extract in doses of 50, 250, 500 mg/kg is tolerated well by the experimental animals. All experimental animals didn't differ from control rats in state, behavior, increasing body mass (Table 1) and in hematological parameters (Table 2).

Table 1

Increasing body mass of rats taking the extract in chronic experiment (M ± m)

Introduction time	Control	50 mg/kg	250 mg/kg	500 mg/kg
Initial	161 ± 11,6	160 ± 14,0	165 ± 11,8	168 ± 12,3
After 1 month	234 ± 20,0	232 ± 15,0	235 ± 16,4	238 ± 14,5
After 2 months	282 ± 14,0	280 ± 16,4	277 ± 15,6	282 ± 16,8

N o t e . Statistically there are no reliable differences (P > 0,05).

According to biochemical parameters the decrease of urine, ß-lipoproteides, cholesterol and lipids have been recorded (Table 3).

In macroscopic examination of internal organs and the brain of experimental rats during the prolonged 60 days use of preparation in the above-mentioned doses there have not been the considerable changes in comparison with the control group of animals (Table 4).

Results of macroscopical and microscopical morphological researches of organs and tissues at introduction of dry extract have not revealed visible changes. Results of the general survey of the animals who have received a dry extract, have shown absence of visually distinguished deviations relatively the control group. All animals have a correct constitution, a tidy look, a brilliant woollen cover, the centres of growing bold or ulcers have not been revealed. Visible mucous membranes are damp, of light pink colour, shining and smooth by sight. Chest glands of females are without tumour formations and consolidations, in regular intervals soft to the touch. External genitals of males have no visible deformations or deviations from the control group.

Table 2

Picture of peripheral blood of rats taking the extract in chronic experiment

After 10 days of beginning the experiment				
Parameters	Control	50 mg/kg	250 mg/kg	500 mg/kg
Haemoglobin, g/l	130 ± 8,8	130 ± 10,8	123 ± 11,1	125 ± 9,8
Leukocytes, th/mkl	11,8 ± 1,3	11,4 ± 1,3	12,0 ± 1,15	12,2 ± 1,0
Erythrocytes, mln/mkl	6,8 ± 0,52	6,31 ± 0,71	6,34 ± 0,59	6,41 ± 0,66
Lymphocytes, %	64,0 ± 1,8	56 ± 2,1	60,0 ± 2,0	58,0 ± 2,15
Monocytes, %	4,0 ± 0,3	5,0 ± 0,3	4,5 ± 0,4	6,0 ± 0,2
Eosynophyls, %	2,5 ± 0,2	2,5 ± 0,2	3,0 ± 0,2	2,0 ± 0,3
Segment-nuclear neutrons, %	27,5 ± 1,8	30,5 ± 1,7	29,5 ± 1,8	31,0 ± 1,5
Bacillas-nuclear neutrophyls, %	2,5 ± 0,4	5,0 ± 0,5	3,0 ± 0,3	3,0 ± 0,3
After 30 days of beginning the experiment				
Haemoglobin, g/l	132 ± 8,5	130 ± 9,5	128 ± 10,2	131 ± 9,6
Leukocytes, th/mkl	10,9 ± 1,2	11,6 ± 1,07	11,1 ± 1,0	11,9 ± 1,1
Erythrocytes, mln/mkl	6,59 ± 0,61	7,45 ± 0,66	7,16 ± 0,49	6,84 ± 0,56
Lymphocytes, %	58,0 ± 1,8	56,0 ± 2,0	60,0 ± 1,6	61,0 ± 1,6
Monocytes, %	5,0 ± 0,3	5,0 ± 0,2	4,0 ± 0,3	6,0 ± 0,2
Eosynophyls, %	2,0 ± 0,2	2,0 ± 0,2	2,0 ± 0,2	1,0 ± 0,1
Segment-nuclear neutrons, %	29,0 ± 1,8	31,0 ± 1,6	30,0 ± 1,7	28,0 ± 1,9
Bacillas-nuclear neutrophyls, %	5,0 ± 0,3	5,0 ± 0,3	4,0 ± 0,4	6,0 ± 0,2
After 60 days of beginning the experiment				
Haemoglobin, g/l	130 ± 8,9	133 ± 8,8	135 ± 9,5	132 ± 7,7
Leukocytes, th/mkl	12,5 ± 0,9	12,7 ± 1,2	12,6 ± 0,9	12,7 ± 1,1
Erythrocytes, mln/mkl	6,8 ± 0,2	6,4 ± 0,4	6,7 ± 0,4	6,5 ± 0,4
Lymphocytes, %	60,8 ± 1,6	61,0 ± 1,6	62,0 ± 1,5	58,1 ± 1,8
Monocytes, %	4,2 ± 0,22	4,2 ± 0,22	4,8 ± 0,25	5,0 ± 0,2
Eosynophyls, %	2,8 ± 0,2	2,5 ± 0,3	2,7 ± 0,25	2,2 ± 0,24
Segment-nuclear neutrophyls, %	27,7 ± 1,8	27,6 ± 2,0	26,5 ± 2,2	29,1 ± 1,6

N o t e . Blood picture is in physiological norm. The difference in comparison with the control group is not reliable. (P > 0,05).

Table 3

Biochemical data of blood serum of rats taking the extract in chronic experiment (the data after the end of experiment)

Parameters	Control	50 mg/kg	250 mg/kg	500 mg/kg
AlAT, mmole/	1,46 ± 0,08	1,14 ± 0,14	1,3 ± 0,12	1,5 ± 0,19
AsAT, mmole/	3,16 ± 0,09	3,17 ± 0,14	3,6 ± 0,18	3,2 ± 0,16
Glucose, mmole/l	5,15 ± 0,57	5,11 ± 0,82	4,9 ± 0,63	4,9 ± 0,76
Total protein, g/l	78,0 ± 1,8	77,0 ± 2,4	76,0 ± 1,9	79,0 ± 1,7
Urine, mmole/l	5,9 ± 0,61	5,3 ± 0,63	4,7 ± 0,61	4,1 ± 0,54*
Lipoproteids, g/l	0,67 ± 0,03	0,52 ± 0,03*	0,56 ± 0,02*	0,55 ± 0,02*
Cholesterol, mg%	69,4 ± 4,6	62,5 ± 4,9	57,9 ± 4,1*	54,4 ± 4,6*
Lipids, g/l	2,08 ± 0,16	1,77 ± 0,23	1,42 ± 0,2*	1,11 ± 0,2*

N o t e . * P < 0,05 in comparison with the control.

In a thorax – visceral and parietal leaves of pleura and bodies of a thorax are without visible changes. Lungs are of light pink colour, airy, without consolidations or destructive changes. Heart is of usual size, without signs of ischemia or hypertrophy. The aorta and pulmonary arteries are smooth, anomalies of development or aneurysms have not been found

out. In heart cavities the small amount of liquid blood contained. Muscles of a myocardium are of brownish colouring, turgor is kept.

In a belly cavity – the liver is not increased in size, of usual form, has a soft consistence and a smooth surface. Glissonic capsule is thin, transparent, not strained. On a cut – histoarchitectonics of liver is not changed, parenchyma is moderately sanguineous. A stomach, a pancreas, loops of thin and thick intestines are without visible pathological changes. Kidneys are of usual size and shape, of brown colour and dense at palpation. On a cut of kidneys there are distinctly differentiated covering and brain substances, nephritic cups and tubs are without stones and pathological changes. Thimus, a thyroid gland and adrenal glands have no macroscopical differences from corresponding organs of control animals. At cranium opening – a brain is of greyish-white colour, damp, without signs of the expressed hypostasis. The soft brain cover densely lies to substance of a brain, in some places is observed moderate expansion and full blood of venules and small veins. Ventricles of brain are not increased in size, contain moderate quantity of transparent, colourless liquor.

Table 4

Mass coefficient of internal organs of rats taking the extract in chronic experiment

Organs	Control	50 mg/kg	250 mg/kg	500 mg/kg
Brain	6,14 ± 0,38	6,54 ± 0,39	6,22 ± 0,28	6,75 ± 0,25
Heart	3,40 ± 0,3	3,22 ± 0,2	3,81 ± 0,22	3,15 ± 0,25
Lungs	5,66 ± 0,26	5,52 ± 0,34	5,42 ± 0,28	5,9 ± 0,22
Liver	30,4 ± 2,4	30,9 ± 1,9	33,2 ± 2,2	31,6 ± 1,7
Kidneys	7,7 ± 0,23	6,8 ± 0,32	7,1 ± 0,29	7,42 ± 0,3
Spleen	3,9 ± 0,31	4,0 ± 0,45	3,65 ± 0,4	3,82 ± 0,4
Adrenal glands	0,14 ± 0,0026	0,17 ± 0,002	0,15 ± 0,002	0,16 ± 0,0018

N o t e . Statistically there are no reliable differences $P > 0,05$.

Photo optic microscopic research of internal organs and brain of all groups of animals, irrespective of a dose of the examined preparation, has shown the development of the same changes.

In brain tissues in some cases were observed moderated local microcircular frustration of vessels of a soft brain cover in the form of arterioles spasm, expansions of venous capillary with blood stasis in them. Intercellular substance of the big hemispheres bark and a cerebellum places are with signs of a small hypostasis. Cytoarchitectonics of barks of big hemispheres and a cerebellum is well kept, arrangement density of neurons and thickness of separate layers of a bark have no distinctive features in comparison with the control. Neurocytes of the big hemispheres barks as a whole are painted in regular intervals, some cells are a little increased in volume. Cytoplasm of neurocytes is basically fine-grained, with various distribution of chromatophilic Nissle substance. Kernels of neurocytes are of the roundish form, hyperchrome, with accurately expressed, intensively basophilic painted kernel. In some neurocytes the moderate swelling of kernels is noted. Often round the vessels, pyramid and basket cerebellum cells were found out narrow semi-moon-shaped unpainted sites. Neurons of brain kernels, pear-shaped Purkinje cells of a cerebellum, and also glyocytes of brain grey substance as a whole had characteristic structure for them. It is noted as well any pathological changes from structural components of hematoencephalitic barrier.

In tissues of experimental groups of animals was kept usual histoarchitectonics. Signs of pathological changes of inflammatory or destructive character is not revealed. The wall of intrapulmonary bronchial tubes consists of the corresponding tissue components inherent in the big, average and small bronchial tubes. Respiratory bronchioles and alveolar courses are without pathological changes. Alveolar epitheliocytes of I and II types have characteristic for them structure and tinctorial properties. An interalveolar connecting tissue is without pathological changes, in it and in a gleam of alveoluses come to light individual macrophages with characteristic dense inclusions in cytoplasm. As a whole, the microscopic structure of all departments of a lung has no essential differences from the control.

Heart – at experimental groups of animals the same as at control, accurately differ endocardial, miocardial and epicardial heart covers. Endothelium covering of endocardium is not broken, in some places come to light bulked up and increased in size endotheliocytes. The myocardium contains cardiomiocytes which form the focused muscular fibres. Fibres are

in regular intervals painted, their cross-section drawness is well kept. Kernels of cardiomiocytes are oval or extended, hyperchrome also have the central localisation. Inserted disks between cardiomiocytes are defined distinctly enough. Signs of hypoxia and a myocardium ischemia have not been revealed. As well as in the control, between muscular fibres the set of blood capillaries settles down. Morphological signs of pathological changes in epicardium and a pericardium have not been defined.

Liver – at experimental animals in a tissue of liver the expressed pathohistological changes have not been revealed. The liver capsule is not thicked, contains the longitudinal focused bunches of collagenic fibres. Parenchyma of liver is formed by the classical hepatic segments consisting of hepatic plates radially focused to the central vein or beams. Interlobed connecting tissue is developed poorly, signs of inflammatory infiltration and fibrosis of liver have not been found out. Hepatocytes are of polygonal form, with central located kernel, that is quite often defined. Two-nuclear hepatocytes are often met. Tinctorial properties of hepatocytes are not broken, hepatocytes with signs of fatty or albuminous dystrophy are not found out. Sinusoid capillaries are of usual size, in a gleam are defined individual erythrocytes and leukocytes. In sinusoid haemocapillaries walls and in spaces of Disse, at the big increases, the individual Cupfer cells, having intact structure, come to light. In some cases moderate expansion and blood filling of sinusoid haemocapillaries, central and sublobed veins is noted. Endothelium covering is without destructive changes, in some places are marked bulked up endotheliocytes with hyperchrome kernels. Structure of cholangioles and interlobed bilious channels is without pathological changes. All this specifies that the studied preparation does not render considerable negative influence on microscopic structures of liver.

Histoarchitectonics of kidneys at experimental animals are without changes. A capsule is thin, without signs of hypostasis and destruction. In covering substance numerous nephritic little bodies are defined. Vascular balls contain basically capillary loops of open type. The cavity of Shumljansky capsule is of usual size, does not contain uniform elements of blood or any other pathological precipitations. Individual nephritic little bodies with the expanded cavities of a capsule and moderated blood filling capillaries balls are marked. Epithelium of proximal, thin and distal departments of nephron has characteristic structure for these departments, without signs of destructive changes. Epithelium of collective tubules is presented by the main and inserted cells in a usual parity. In gleams of nephron channels and collective tubules are not found out precipitates or other pathological precipitation. A connecting tissue of covering and brain substance of a kidney is gentle, without signs of hypostasis and inflammatory infiltrates. Microscopic changes of kidneys in comparison with the control are not revealed.

Spleen – a capsule and trabeculas are well developed, contain powerful enough bunches of smooth – muscular cells. In parenchyma red and white pulps which have a usual parity, characteristic for adult animals are distinctly differentiated. The white pulp is presented by lymphatic follicles of the various sizes on which periphery the central artery is defined. Structural zones of a white pulp are differentiated enough, the part of lymphatic follicles contains herminative or the jet centre. In the jet centres are often found out the cells which are at various stages of mytotic fission. The red pulp is rich in erythrocytes, in the same place come to light macrophages the cytoplasm of which contain the pigment – hemocyderin. Pathological changes of a spleen as a whole are not revealed.

Pancreas – a capsule is thin, in parenchyma cuts of segments of the various sizes are accurately differentiated. The basic part of segments occupy asinuses, consisting of asinar cells. Homogeneous and zymogenous zones of acinocytes are accurately distinguishable. In each segment the Langergans islet is defined, the size and topography of which varies in enough wide limits. Islets basically are presented by basophilic cells and the blood vessels located between them. The interlobed connecting tissue contains exit channels and blood vessels. The pancreas of experimental animals as a whole has no essential distinctions in comparison with the control group.

The thyroid gland has a lobular structure, is surrounded by a capsule from a dense fibrous connecting tissue. Its parenchyma is formed by follicles, the sizes and colourability of those are within norm.

Structure of goitre glands at experimental animals is normal: the accurate border between covering and brain substance is distinguishable. The covering substance is presented with densely located lymphocytes, brain – with reticuloepithelium with an impurity lymphocytes and presence of thymic little bodies of Gassal.

On histologic cuts of adrenal glands the body capsule is not changed. Dystrophic changes in ferruterous cells of covering and brain substance of adrenal glands are absent. The typical parity of ball, fasicled and reticular zones is completely kept. In brain part of an adrenal gland chromaffin cells keep characteristic structure and sizes. Venous sines are not changed or slightly expanded.

Integumentary epithelium of stomach it is covered by a slime layer in which are defined extrusive cells. In own plate are found out separate lymphocytes, plasmatic cells, lymphoid follicles. Stomach glands have a usual tubular structure, without signs of destruction and disorganisation. Vessels are moderately sanguineous.

Fibers of a thin gut are covered with single-layered prismatic epithelium, among cells of which there are in a considerable quantity glass-shaped cells. In own plate of a mucous membrane meet lymphocytes and plasmatic cells, and also lymphoid follicles. It is marked moderately expressed sanguineous of vessels.

Thick gut is without any pathological features. Crypts are of the enough correct form, settle down densely. The parity of prismatic and glass-shaped cells on a surface of crypts corresponds to. In some places of submucuos layer meet lymphoid systems. Blood vessels are filled with blood, were marked perivascular hypostases. Thus, on a condition of mucous and submucuos covers architectonics the gastrointestinal tract of experimental animals essentially does not differ from control animals.

Endometrium is covered with single-layered prismatic epithelium. Well differs functional and basal layers of endothelium. Meet various length uterus glands, some of them are expanded, epithelium glands are low cylindrical, cytoplasm is basophilic. Kernels are of the extended form, occupy the most part of cells, they are painted intensively and homogeneous. Mitoses are absent. Strom is rich in cells and argyrophil fibres. The mucous membrane passes in submucous layer of a muscular cover after which follows vascular and above-vessel layers. Cover and brain substances of ovaries are well identified. The brain substance is presented by the fibrous connecting fabric containing the main vessels and nerves. In covering parts of ovary settle down primordial follicles, and also follicles at different stages of maturing up to mature graaphic bubbles. Degenerate changes are noted. Hemorrhages and an atrophy are not present. Growing follicles of different degree of a maturity are without pathological changes. In a brain part of ovary the connecting tissue with main vessels and nerves, sclerosis signs, collagenisation and fragmentations are not marked.

The tissue microscopy of ovaries of rats receiving a preparation has not revealed any pathological changes in channels and strome. In testicles the curved channels contain epithelium of all spermatogenesis stages. Spermatogony, spermacytes of 1 and 11 order, prespermatides and spermatides are well differentiated, and also in a considerable quantity formed spermatozoons at various stages of maturing. Basal membranes are thin. Follicular Sertoli cells and intersticial Leudigue cells are without degeneration signs, their quantity corresponds to the control. Internal diameter seed channels is not reduced, sclerosis and ischemia signs are not noted. Curved channels as at the control animals receiving in similar conditions solvent are covered with multinuclear epithelium, including spermatocytes of the first, second order and spermatides. Dystrophic changes in cytoplasm epithelium of cells are not noted. Sertoli cells are met in a small amount in thickness spermatogenic epithelium. Their number is approximately identical in all investigated cases. Intersticial Leudigue cells are visible in the form of continuous group near to capillaries. The last are expanded and filled with blood [3].

Conclusion

In the result of conducted researches it has been demonstrated that the prolonged per oral use of dry extract in doses of 50, 250, 500 mg/kg is well tolerated by the experimental animals and they didn't differ from the control rats. As to the biochemical parameters the decreasing of urine, ß-lipoproteids, cholesterol and lipids has been noted. It should be noted that there have not been revealed distrophic, necrobiotic and inflammatory changes in experimental animals and also reliable differences in structure of internal organs among experimental and control groups. The noted structural peculiarities of the investigated tissues show normal functional activity of internal organs. On the basis of comparative macro and microscopical research it can be concluded that the prolonged introduction of sufficiently large doses of dry extract on the artichoke prickly basis haven't revealed the considerable pathological changes in organs and tissues of experimental animals.

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