The results of experimental studies of plant extract on the model of toxic liver damage in an animal experiment

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Results of experimental studies of plant extract on model of toxic damage of the liver in the experiment on animals

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SUMMARY

V article presented study results hepatoprotective actions a multicomponent plant extract obtained from the following types of plant materials: flowers of the immortelle sandy, tansy flowers, rose hips, stinging nettle leaves, mint leaves, licorice roots, in conditions of tetrachloromethane hepatitis. It was found that the course administration of the extract per os at a dose of 250 mg / kg to white nonlinear rats with carbon tetrachloride damage to the liver has a hepatoprotective effect that exceeds the effect of the reference drug allochol. With the pharmacotherapy of toxic hepatitis with a plant extract in the blood serum, the levels of activity of enzymes - markers of cytolysis syndrome are clearly reduced, and the manifestations of cholestasis syndrome are significantly reduced. The pharmacotherapeutic effect of the extract in liver damage is due to the presence in it of a complex of biologically active substances, primarily compounds of phenolic nature. The results obtained prove the advisability of using a plant extract containing biologically active substances of a phenolic nature in the prevention and complex treatment of liver diseases.

Key words: plant extract, toxic hepatitis, hepatoprotectiveaction.

RESUME

Results of studying of hepatoprotective effect of the multicomponent plant extract received from the following types of plant raw materials: sandwort flowers, tansy flowers, rose hips, dioecious nettle leaves, mint leaves, licorice roots in the conditions of model of toxic hepatitis are provided.

It is established that course introduction of per os of extract in a dose of 250 mg / kg to white nonlinear rats with toxic injury of a liver has hepatoprotective effect, somewhat exceeding effect of drug of comparison of Allocholum.

Results of the conducted researches demonstrated that pharmacotherapy of toxic hepatitis with plant extract significantly decreases levels of activity of enzymes in blood serum - cytolysis syndrome markers, lowers manifestations of cholestasia syndrome.

Pharmacoterapeutic influence of extract on damaged liver is caused by existence of a complex in it of biologically active agents and, first of all, components of the phenolic nature. The results of research suggest use of the plant extract containing biologically active agents of the phenolic nature in complex treatment and prevention of diseases of a liver.

Keywords: plant extract, toxic hepatitis, hepatoprotective action.

INTRODUCTION

The centuries-old experience of treating diseases with medicinal plants is described in many treatises, for example, in Tibetan medicine in "Zhudshi" and "Vaiduryaonbo". When analyzing them, it can be concluded that the composition of multicomponent mixtures includes from 3 to 53 components of plant origin [1]. In traditional medicine, the disease is considered as a complex process with a violation of the functions of vital organs. To correct these conditions, apply

multicomponent funds in the form of fees, and along with this, diet therapy, physiotherapy exercises, compliance with the work and rest regimen and other measures are recommended [2].

The advantage of multicomponent drugs is the mutual enhancement of the beneficial pharmacological properties of each incoming ingredient, the compliance with the polyvalence of the pathogenesis of the disease, the effect on the patient's body as a whole as a corrective system [3]. In this regard, the development of modern and effective drugs based on charges with a given pharmacological activity is relevant and expedient. Given this circumstance, the task of finding drugs with hepatoprotective action is urgent.

The purpose of this study was to study hepatoprotective action a multicomponent herbal extract intended for the treatment of liver diseases.

MATERIALS AND METHODS

Based on the information-analytical analysis of the literature and the data of a preliminary phytochemical study, we substantiated the components for obtaining the extract, taking into account the contribution to the total effect of each ingredient.

The object of research is a dry extract obtained from the following types of plant materials: sandy immortelle flowers (Helichrysum arenarium L.) - 300 g, flowers of common tansy (Tanacetum vulgare L.) - 100 g, rose hips (Rosa sp.) - 100 g, stinging nettle leaves(Urtica dioica L.) - 100 g, mint leaves (Mentha piperita L.) - 50 g, licorice roots (Glycyrrhiza glabra L.) - 50 g

The extract was obtained by joint extraction of the collection components with hot water 75–85 oC. The resulting extract contains polysaccharides, flavonoids, carotenoids, organic acids, vitamins, macro and microelements, essential oils and other natural compounds. Standardization of the extract was carried out according to the sum of flavonoids in terms of luteolinstandard and isosalipurposidestandard, while the content of the sum of flavonoids is regulated not less than 4% in the first case, and not less than 15% in the second case. The presence of the specified spectrum of biologically active substances (BAS) suggests the potential hepatoprotective activity of the obtained extract.

The work was performed in accordance with the Federal Law “On Medicines”, “Guidelines for Conducting Preclinical Research of Medicines”. The experiments were carried out on 40 non-linear male rats with an initial weight of 180-200 g. The animals were obtained from the Federal State Budgetary Institution Scientific Center for Biomedical Technologies of the FMBA of Russia and were kept in a vivarium with free access to food and water. Pharmacological studies were carried out in accordance with the "Rules for Conducting Work with the Use of Experimental Animals", "Rules adopted by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), Order of the Ministry of Health of the Russian Federation No. 199n dated 01.04.2016 " On the approval of the rules of good laboratory practice ”. The research was approved by the VILAR Bioethical Commission.

The determination of the pharmacotherapeutic efficacy of the extract was carried out with intra-gastric (1 time per day) course administration of the extract in the form of an aqueous solution at a dose of 250 mg / kg for 10 days with carbon tetrachloride hepatitis in white rats, starting from 2 days after the first injection of the damaging agent.

Liver damage was caused by intragastric administration to white rats of a 50% oily solution of carbon tetrachloride in a volume of 0.4 ml / 100 g of animal weight once a day for 4 days [4]. The herbal preparation allochol at a dose of 250 mg / kg was used as a reference drug. Animals of the control group received purified water in an appropriate volume according to a similar scheme.

The studies were carried out after 7, 14, 21 and 28 days from the beginning of the experiment. The functional state of the liver in animals was assessed by the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), the content of cholesterol, β lipoproteins, total bilirubin, direct bilirubin, indirect bilirubin and total protein in the blood serum using an analyzer for clinical chemistry. Clima MC15. Definition

thymol test was carried out by the method of color reaction with diacetyl monooxime.

A number of histological, histochemical, and histoenzymological methods were used to assess the morphofunctional state of the liver in experimental animals [4].

Statistical processing of the obtained data was carried out using the software packageStatistica 10.0 (USA). Differences were considered significant at P≤ 0.05 [5].

RESULTS

The effect of the multicomponent extract on the course of experimental hepatitis in white rats, caused by the introduction of carbon tetrachloride (CCl_4). Tetrachloromethane (CCl_4) is the most well-known hepatotoxin used to model liver diseases. Toxic liver damage caused by prolonged administration of CCl_4, is an adequate model of cirrhotic liver damage in humans [6].

The data characterizing the dynamics of changes in biochemical parameters in the blood serum under the influence of the studied extract in toxic hepatitis in rats are given in table. 1.

Table 1

The dynamics of changes in biochemical parameters in blood serum under the influence of the extract in the experimental (CCl_4) hepatitis in white rats

No.	The main biochemical indicators	Intact rats	Control rats (CCl4 hepatitis)	Experienced rats (CCl4 hepatitis + extract)	Experienced rats (CCl4 hepatitis + allochol)
1	2	3	4	5	6
7 days					
1.	ALT, μM	0.68 ± 0.11	4.96 ± 0.08	3.40 ± 0.17 **	3.80 ± 0.10
2.	AST, μM	0.41 ± 0.01	3.18 ± 0.22	2.48 ± 0.14 **	2.56 ± 0.21 *
3.	Total protein, g / l	7.45 ± 0.11	6.12 ± 0.22	7.04 ± 0.13 **	7.12 ± 0.15 *
4.	Thymol test, units	1.36 ± 0.19	7.68 ± 0.13	5.80 ± 0.70	6.00 ± 0.10
5.	Lipoproteins, units	0.90 ± 0.02	1.47 ± 0.04	0.99 ± 0.04 *	1.20 ± 0.04 *
6.	Cholesterol, mg%	56.0 ± 2.0	35.0 ± 2.0	64.0 ± 3.0 **	60.0 ± 3.0 *
7.	Alkaline phosphatase, units	144.0 ± 10.0	290.0 ± 7.5	248.0 ± 13.0 *	2400 ± 12.0 *
eight.	Total bilirubin, mg%	0.53 ± 0.03	11.2 ± 0.67	8.68 ± 0.26 **	8.9 ± 0.4 *
nine.	Direct bilirubin, mg%	0.22 ± 0.02	6.2 ± 0.46	5.04 ± 0.35 **	5.9 ± 0.4 *
ten.	Indirect bilirubin, mg%	0.31 ± 0.04	5.00 ± 0.31	3.64 ± 0.12 **	4.0 ± 0.3
14 days					
1	2	3	4	5	6
1.	ALT, μM	0.68 ± 0.11	4.04 ± 0.18	2.85 ± 0.18 **	3.0 ± 0.2
2.	AST, μM	0.41 ± 0.01	2.40 ± 0.13	1.73 ± 0.11 *	1.93 ± 0.14
3.	Total protein, g / l	7.45 ± 0.11	6.33 ± 0.08	6.80 ± 0.17 *	7.00 ± 0.15
4.	Thymol test, units	1.36 ± 0.19	6.76 ± 1.07	3.50 ± 0.25 *	4.10 ± 0.45 *
5.	Lipoproteins, units	0.90 ± 0.02	1.82 ± 0.13	1.19 ± 0.04 **	1.40 ± 0.09 *
6.	Cholesterol, mg%	56.0 ± 2.0	123.0 ± 10.0	64.0 ± 3.0 **	82.0 ± 5.0 *
7.	Alkaline phosphatase, units	144.0 ± 10.0	270.0 ± 16.0	162.0 ± 10.0 **	185.0 ± 13.0 *
eight.	Total bilirubin, mg%	0.53 ± 0.03	4.98 ± 0.16	4.08 ± 0.16 **	4.20 ± 0.14
nine.	Direct bilirubin, mg%	0.22 ± 0.02	2.48 ± 0.10	2.04 ± 0.01 **	2.15 ± 0.03 *
ten.	Indirect bilirubin, mg%	0.31 ± 0.04	2.50 ± 0.01	2.04 ± 0.03 **	2.25 ± 0.05
21 days					
1	2	3	4	5	6
1.	ALT, μM	0.68 ± 0.11	3.12 ± 0.14	2.02 ± 0.11 **	2.32 ± 0.14 *
2.	AST, μM	0.41 ± 0.01	1.69 ± 0.05	1.04 ± 0.13 **	1.39 ± 0.12 *

3.	Total protein, g / l	7.45 ± 0.11	6.52 ± 0.15	7.51 ± 0.13 **	7.60 ± 0.14 *
4.	Thymol test, units	1.36 ± 0.19	6.80 ± 0.10	2.10 ± 0.15 **	2.45 ± 0.17 *
5.	Lipoproteins, units	0.90 ± 0.02	1.55 ± 0.55	0.99 ± 0.08 **	1.12 ± 0.12 *
6.	Cholesterol, mg%	56.0 ± 2.0	101.0 ± 6.0	51.0 ± 5.0 **	65.0 ± 5.0 *
7.	Alkaline phosphatase, units	144.0 ± 10.0	226.0 ± 7.4	138.0 ± 6.3 **	150.0 ± 7.0 *
eight.	Total bilirubin, mg%	0.53 ± 0.03	1.96 ± 0.09	1.46 ± 0.09 *	1.62 ± 0.10
nine.	Direct bilirubin, mg%	0.22 ± 0.02	0.72 ± 0.04	0.54 ± 0.03 **	0.64 ± 0.05
ten.	Indirect bilirubin, mg%	0.31 ± 0.04	1.24 ± 0.15	0.92 ± 0.10	0.98 ± 0.10
28 days					
1	2	3	4	5	6
1.	ALT, μM	0.68 ± 0.11	2.82 ± 0.24	1.36 ± 0.24 **	1.52 ± 0.28 *
2.	AST, μM	0.41 ± 0.01	1.18 ± 0.09	0.88 ± 0.08 *	0.94 ± 0.04 *
3.	Total protein, g / l	7.45 ± 0.11	7.15 ± 0.01	7.46 ± 0.10 **	7.40 ± 0.08 *
4.	Thymol test, units	1.36 ± 0.19	3.22 ± 0.33	2.48 ± 0.22 *	2.64 ± 0.29 *
5.	Lipoproteins, units	0.90 ± 0.02	0.95 ± 0.02	0.91 ± 0.03	0.92 ± 0.03
6.	Cholesterol, mg%	56.0 ± 2.0	66.0 ± 3.0	51.0 ± 2.0	55.0 ± 2.0
7.	Alkaline phosphatase, units	144.0 ± 10.0	195.0 ± 8.7	129.0 ± 4.8 **	140.0 ± 5.4 *
eight.	Total bilirubin, mg%	0.53 ± 0.03	1.86 ± 0.04	0.79 ± 0.07 **	0.86 ± 0.06 *
nine.	Direct bilirubin, mg%	0.22 ± 0.02	0.62 ± 0.02	0.21 ± 0.05 **	0.23 ± 0.048
ten.	Indirect bilirubin, mg%	0.31 ± 0.04	1.24 ± 0.10	0.58 ± 0.07 **	0.63 ± 0.07 *
Note: * - means here and below that the differences compared to the control are significant at P - 0.05, ** - means here and below that the differences compared to the control are significant at P - 0.01.					

Based on the data in table. 1 it can be concluded that the introduction of the extract clearly affected the course of toxic hepatitis. Already in the early stages of the development of liver damage (day 7), the signs of cytolysis and cholestasis syndromes decreased with a simultaneous improvement in the functional state of the liver. The severity of cholestasis syndromes was judged on the basis of a comprehensive assessment of the indicators of cholesterol content and the activity of alkaline phosphatase and cytolysis - according to the activity of the enzyme markers AST and ALT. So, on the 7th day of the experiment in animals receiving the studied extract (Table 1), there was a significant decrease in the activity of ALT, AST (by 32% and 22%, respectively) compared with the data in animals of the control group. It was also found a decrease in the thymol test by 33%;

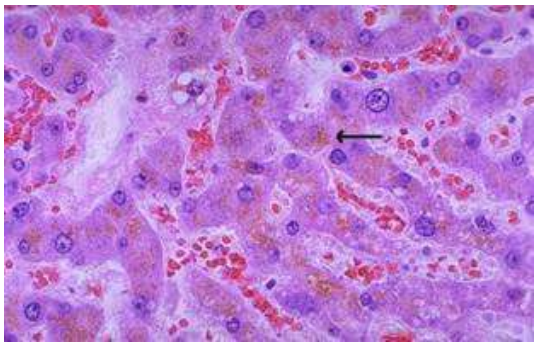
After 14 days of the experiment in the rats of the experimental group, the activities of ALT, AST, ALP; indices of total bilirubin, β-lipoproteins, cholesterol, thymol test were significantly lower than those in animals of the control group.

With the introduction of the extract on the 21st day of the experiment, the total protein value increased to 7.51 ± 0.13, i.e. approached the indicator of the intact group. The indicators of total bilirubin, direct bilirubin, indirect bilirubin significantly decreased by 26%, 25% and 26%, respectively, compared with the control.

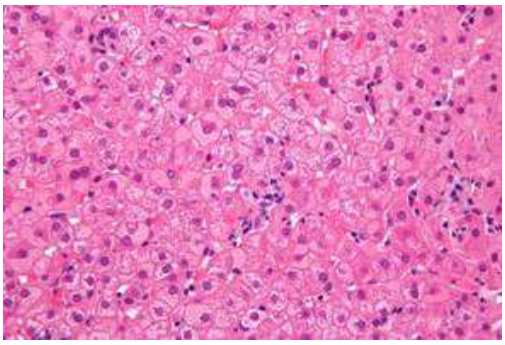
The results of the studies showed that on the 28th day of the study, during the pharmacotherapy of toxic hepatitis with the studied extract, the levels of activity of enzymes - markers of the cytolysis syndrome in the blood serum of white rats clearly decreased, and the manifestations of cholestasis syndrome significantly decreased under these conditions. It should be especially noted that the administration of the studied extract to rats with liver damage was characterized by a decrease in the thymol test parameters. While in the control group, a number of indicators (ALT, AST, thymol test) indicated an ongoing pathological process. At the same time, the effectiveness of the studied extract in some parameters was superior to that of allochol.

In a pathomorphological study of the liver on the 7th day of the development of toxic hepatitis in white rats of the control and experimental groups, pronounced hemodynamic

disorders. Moreover, in the control, the signs of destruction of liver tissue were sharply expressed and were reduced to the presence of extensive areas of necrosis, often filled with round cell elements, and in the experiment with pharmacotherapy with the studied extract, the severity of alterative processes was noticeably less. In addition, if in the liver of rats of the control group, lipofuscin was found in large quantities throughout the parenchyma of the organ (Fig. 1), then in the experiment the content of this pigment was found in a much smaller amount, and its granules were small in size. Glycogen in liver cells in the control could not be detected in any case, but in the group of experimental animals it was found in 3 cases out of 5. The DNA content in the liver of rats receiving the extract was significantly higher than in the control. In the central zones of the liver lobules in the control, pronounced large and medium-drop fatty degeneration was observed, and in the experiment, fat was detected in 2 cases out of 5. In dystrophic cells, both in the control and in the experiment, the activity of acid phosphatase was sharply reduced. The activity of alkaline phosphatase in the liver in the experimental group of rats was higher in the areas of proliferation and cell infiltration. A comparatively lower activity of this enzyme was observed in the zones of the vessel lining.



Rice. 1. The content of lipofuscin in the parenchymaliver control in the absence of treatment. Staining with hemotoxylin and eosinm, increase x400 (7th day).



Rice. 2. Dual-core and hypertrophiedhepatocytes in the liver of white rats during pharmacotherapy with an extract of experimental carbon tetrachloride hepatitis. Staining with hemotoxylin and eosin, x200 magnification (14th day).

On the 14th day of the development of experimental hepatitis in the control, there were still pronounced disorders in the structure of the organ - discomplexation of the beam structure of the lobules, hemodynamic changes, round-cell infiltration. Binuclear and polyploid cells were found in tissue in small numbers. The lipofuscin content continued to remain at a high level, in the center of the lobules, the phenomena of large-drop fatty degeneration were observed. Glycogen and DNA were less than in the group of animals that received the multicomponent extract.

As before, the levels of acid phosphatase activity remained high in the control compared with the data in the experiment. In the experimental group of animals, alterative changes in the liver were less pronounced. In particular, in 4 cases out of 5, normalization of the beam structure of the lobules was noted, the location of hepatocytes looked more ordered; only in 2 cases were the phenomena of cell degeneration by the type of "turbid swelling" found. At the same time, the characteristic extracts under the conditions of pharmacotherapy were the appearance of many hypertrophied (polyploid) and binuclear cells (Fig. 2). We also encountered cells with a low content of lipofuscin; glycogen, as a rule, was found in almost all cells, and the DNA content was higher than in the control. Moreover, against the background of the introduction of the extract in 3 cases out of 5, the activity of succinate dehydrogenase was close to normal.

On the 21st day of the experiment in the liver of the animals of the control group, the phenomena of plethora of blood vessels, granular degeneration, and also cellular infiltration along the course of the vessels were found. Kupffer's cells looked edematous; they found hypertrophied and binucleated cells in liver sections in a small amount. Lipofuscin was found in all cells, glycogen in 3

cases out of 5. In the experiment with the introduction of the investigated extract observed intensive processes of restoration of the structure of the organ. The hepatocytes were arranged in an orderly fashion, forming radically located hepatic tracts. There was a slight cellular infiltration along the vessels; only in some cases were dystrophically altered cells containing lipofuscin found. The acid phosphatase activity was lower than in the liver of control rats, and the succinate dehydrogenase activity was close to normal.

On the 28th day of the development of the pathological process, signs of granular dystrophy were preserved, discomplexation of the beam structure of the liver was observed, many nodular infiltrates in the parenchyma and along the vessels were observed, at the same time hypertrophied hepatocytes with dark-colored large nuclei were noted. In the foci of dystrophically altered cells, many adhered lipofuscin granules were detected, the glycogen content in the liver cells was low, and there were no signs of fatty infiltration. In the experiment against the background of the introduction of the extract, the structure of the liver practically did not differ from the structure of the liver in intact rats.

Consequently, experimental pharmacotherapy with multicomponent plant extract of toxic hepatitis in white rats is accompanied by a significant improvement in the functional state of the liver, a high level of recovery processes and prevention of disorganization of the organ structure.

DISCUSSION AND CONCLUSIONS

Based on the data obtained, it can be concluded that the resulting extract, when administered in courses, had a pronounced hepatoprotective (hepatoprojection) effect. Under the influence of a multicomponent plant extract, from the early stages of liver damage, the activity of enzymes decreased, and the severity of the inflammatory reaction decreased. These positive changes in the aggregate led to a decrease in the severity of the pathological process. Course introductionper os of the specified plant extract in a dose 250 mg / kg white nonlinear rats with carbon tetrachloride damage to the liver had a hepatoprotective effect, superior in a number of indicators to the effect of the reference drug allochol.

It can be assumed that the pharmacotherapeutic effect of the studied extract in liver damage is due to the presence of a complex of biologically active substances in it, first of all, phenolic compounds. Due to their dominant content, it seems that stabilization of biological membranes is ensured, followed by an increase in the functional activity of the liver, which was previously described using the example of other plant extracts [7].

The results obtained allow arguing the advisability of using the studied plant extract containing phenolic biologically active substances in the prevention and complex treatment of liver diseases.

LITERATURE

1. Nikolaev, S.M. Phytopharmacotherapy and phytopharmacoprophylaxis of diseases / S.M. Nikolaev. - UlanUde: BSU Publishing House, 2012 .-- 286 p.

2. Sokolov, S.Ya. Phytotherapy and phytopharmacology. A guide for doctors / S.Ya. Sokolov. - Moscow: MIA, 2000 .-- 976 p.

3. Lubsandorzhieva, P.N.B. Development and standardization of herbal remedies for the treatment and prevention of diseases of the digestive system / P.N.B. Lubsandorzhieva. - UlanUde: Publishing house of the BNTs SB RAS, 2016 .-- 280 p.

4. Guidelines for conducting preclinical studies of drugs. Part first. - M .: Grif i K, 2012 .-- 944 p.

5. Borovikov, V.P. A popular introduction to modern data analysis with STATISTICA / V.P. Borovikov. - M .: Hot line. Telecom, 2014 .-- 288 p.

6. Bueverov, A.O. Medicinal lesions of the liver / A.O. Bueverov // Russian medical magazine. - 2012; 3. - P.107-110.

7. Korsun, V.F. Medicinal plants and liver disease: a guide to clinical phytotherapy / V.F. Korsun [and others]. - M., 2014 .-- 327 p.

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