141. RU0002763761 - HEPATOPROTECTIVE HUMIC AGENT

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[RU]
Writing

[1] The invention relates to the field of medicine and pharmaceutics, and more particularly to humic preparations, and is intended to increase the resistance of the liver to pathological effects and various injuries, as well as to enhance its neutralizing function.

[2] The liver is an organ in which metabolism is most actively among other human organs. The liver is located between the digestive system and the circulatory system and protects all of the body from external influences. In addition, the liver is a very important organ responsible for metabolism, detoxification, decomposition, synthesis and secretion.

[3] In particular, the liver performs a function of energy exchange control, so all nutrients absorbed from food are metabolized into substances that can produce energy in the liver and supplied or accumulate throughout the body, as well as about 2000 species of enzymes, albumin and fats for blood clotting, such as serum proteins, bile acids, phospholipids and cholesterol factors are synthesized, stored and distributed in the liver.

[4] In addition, the liver performs the function of removing various metabolites into the duodenum through the bile duct and the immune function that plays an important role in maintaining the life expectancy. Also, because the ex vivo substance that enters the living organism once passes through the liver, the liver has a higher risk of exposure to many toxic substances in addition to nutrients than other organs, so the probability of damage is very high.

[5] The liver is an organ with excellent regenerative capacity, and if there is any injury, it is restored to a normal state sufficiently, but if the injury is maintained, a portion of the liver tissue is completely destroyed, and the liver function is also impaired, making it difficult to recover.

[6] When such liver damage becomes chronic, it progresses to fibrosis, cirrhosis and liver cancer, regardless of the cause. Fibrosis, cirrhosis and liver cancer are those diseases which are currently not of clear treatment and mortality from such diseases is also high. Therefore, it is very important to prevent and treat liver damage before liver damage is chronic to suppress progression to fibrosis, cirrhosis or liver cancer.

[7] The liver disease does not have subjective symptoms at an early stage, such symptoms appear only in severe hepatic injury, therefore the liver disease is difficult to detect at an early stage. For this reason, the liver disease takes place among the causes of death not only in Russia, but also in the world, and effective methods of treatment and diagnosis do not exist.

[8] Liver disease is one of the major diseases in modern life, which is a problem for modern people who are subject to excessive stress, smoking, alcohol and drug consumption. Therefore, there is a need to develop a method for liver protection, in particular a method for liver protection using natural products with less side effects or resistance problems.

[9] A multi-numerical group of substances having a hepatoprotective effect is known. Their number, in particular, include various substances improving metabolic processes in the body, for example vitamins, lipid peroxidation inhibitors, as well as antihypoxants [Essentiale preparations, etc.]. In medical practice, some preparations of the flavonoid structure [silibinin, et al] are used as selective hepatoprotectors in the structure to the vitamins of the P group and plant agents [Lib -52, etc.].

[10] The disadvantages of most of these substances are the narrow spectrum of action caused by a significant number of contraindications, insufficient efficiency, complex production technology.

[11] One of the most important problems of modern experimental and clinical pharmacology is the development and search of new agents for liver protection without such problems as side effects or tolerance, or therapeutic agent or dry functional food product for prevention, improvement or treatment of liver diseases.

[12] Humic acids, fulvic acids are known to have a powerful impact on any living organism due to the rich composition. They contain a complete set of amino acids, macro-and microelements, minerals, as well as polysaccharides of natural origin, vitamins, peptides, fatty acids, polyphenols, ketones, catechins, etc. All more than 70 useful components. Such a saturated formulation explains the positive biological effects of humic acid [see https:// fb. ru/Arabidopsis/288472/humanovyie-kislotvi-cho-tkoe-i-kak-oni-vliyavut-na-organizm].

[13] Humic acids are capable of stimulating certain functions of human neutrophils. For preparations based on humic and humic-like substances, antiviral activity is identified, for example, for simplex herpes simplex virus-HSV. Humic compounds can be used as microbiocides, preventive agents against HIV/AIDS distribution. Cytotoxic and antiviral properties of humic acids and fulvic acids isolated from coal and peat have been investigated. Studies have shown that

all studied compounds were low toxic and have a sufficiently high inhibitory effect on HIV infection [Al POPS, etc. Biological Activity and Biochemistry of Humic Substances. Part 2. Medico-Biological Aspect [Review of Literature]. In Russian Academy of Sciences, 2016/5, from 9-15]. Oxyhumate has been shown to increase Th -1 cell activity and reduces cytokine production Th -2 cells [Mariette et al, 2002]. The observed stimulation of human lymphocyte proliferation was associated with an increase in IL -2 production and IL -2 receptor expression together with a decrease in IL -10 amount by Oxyhumate [Joone et al, 2003]. In vivo it is shown that oral administration of humic substances improves the parameters of innate immunity in experimental animals: enhancement of antibacterial activity of blood serum, phagocytic activity, activity of lysozym and bacterial agglutination [Sangiguel et al , 2016]. This makes the humic substances with potential products for the development of multi-target immunoactive microbicides.

[14] Research in the field of development of new biologically active compounds has shown that humic substances of different genesis have immunomodulatory and anti-inflammatory effects, which makes it possible to use them for the prevention and treatment of chronic dermatoses, atopic dermatitis, allergic rhinitis and other diseases accompanied by inflammatory and allergic reactions. Promising is the use of humic substances as antifungal, antiviral agents. Anti-inflammatory activity of humic acids is studied in models of acute and chronic inflammation. The possible anti-inflammatory mechanism is explained by the ability of humic acids to reduce the generation of oxygen radicals and reduce oxygen consumption by activated phagocytes [Al SAUNKO et al Biological activity of humic substances: promising and problems of their use in medicine [review]. Medial, No. 1 [23] April, 2019, 54-60, DOI: http://dx.doi.org/10.21145/2225-0026-2019-l-54-60].

[15] The anti-inflammatory effect of humic compounds is linked to their ability to reversibly inhibit the excess synthesis of interleukin-ip hyperactivated macrophages, to level the enhanced yield of neutrophilic granulocytes from medullary depot in blood, to reduce oxygen consumption by activated phagocytes, followed by a decrease in the generation of oxygen radicals, which ultimately leads to a decrease in the intensity of the inflammatory reaction [Senova] The biological activity of humic substances derived from peat and the possibility of their use in therapeutic practice. International reviews: clinical practice and health, No. 4, 2017, from 114-122].

[16] The closest analogue of the claimed invention is patent RU 2372926 c1, from which a hepatoprotective humic agent is known, obtained by treating peat with 1.0% aqueous ammonia solution and 1.75% hydrogen peroxide solution in a ratio of 1: 5: 5 while heating to 140°C while continuously stirring to obtain a liquid phase. The liquid phase, which is a concentrated mother liquor, contains not less than 45000 mg/l of humic acids. However, the present invention provides a product having insufficiently high hepatoprotective activity due to incomplete extraction of humic substances from the feedstock.

[17] It is an object of the present invention to provide a humic agent comprising a broad spectrum of humic substances and having hepatoprotective activity, without the use of chemical reagents.

[18] The technical result of the claimed invention is the production of a hepatoprotective humic agent containing a broad spectrum of humic substances and having no chemical impurities, which can be used in the field of medicine and pharmaceutics.

[19] The claimed technical result is achieved in that the claimed humic agent having hepatoprotective activity is a colloidal solution containing water, humic substances, which are humic and fulvic acids and salts thereof, hydroquinone and chrysin, wherein the size of the colloidal particles is in the range of 30 nm to 10 microns, the weight of humic substances is from 1 to 20 wt %. %, Hydroquinone is present in an amount of not more than 3 wt. % by weight of humic substances and chrysin in an amount not exceeding 2% of the weight of humic substances.

[20] Also disclosed is the use of the resulting humic agent to increase the resistance of the liver to pathological effects and various lesions.

[21] Examples

[22] Example 1 Preparation of the Claimed Hepatoprotective Humic Agent [GM].

[23] Pre-ground raw material [Leonardite, Lignin, Coal, Peat, Sapropel] was used in a mixture with water, which was placed in an ultrasonic unit. The mixture of humin-containing raw material with water was heated to 30-80°C and treated with ultrasound at a pressure of 0.05-0.8 MPa when the required temperature was reached. After ultrasonic treatment, the solution was cooled to room temperature and filtered to separate particles with a size greater than 10 μm. The resulting product was diluted with water to a humic substance content of from 1 to 20% by weight. %, Wherein the amount of hydroquinone in the agent does not exceed 3 wt. %. % Of the weight of humic substances and chrysotile is not greater than 2 wt %. % by weight of humic substances. The size of the colloidal particles is in the range of 30 nm to 10 μm.

[24] The study of the composition of the obtained agent was carried out by GC-MS on a Chromes Analyzer consisting of a gas chromatograph [Chromes-Kristol 5000] and a liquid dispenser of DCF -2 M For identification of derivatives, an automatic database of search and identification of data of chromatography-mass spectrometry NIST17 MS Life was used.

[25] Study Conditions:

[26]

Капилярная колонка	Phenomenex ZB-DRUG-1 30 м*0.25 мм*0.25 мкм, (или аналогичная);
Условия МС-детектора:	Деление потока 5,0. Температура источника ионов = 200 °С. Температура переходной линии = 290 °С. Диапазон сканирования = 50-550 а.е.м., длительность скана = 0.3.
Объем пробы	1 мкл;
Условия ПИД-детектора	Температура, 270 °С. Расход водорода, мл/мин 20,0. Расход воздуха, мл/мин 200,0. Температура инжектора, °С 280,0 Колонка Agilent 5ms 30м×0,25мм×0,25мкм
Детектор	МС или ПИД;

[27] The results of the study are shown in Table 1

[28]

Перечень надёжно идентифицированных соединений в составе препарата гуминового напитка методом ГХ-МС

Название	Время	Высота	Площадь	Площадь, %	Вероятность	Match	R.Match
Oxalic acid, 2TMS derivative	10.62	41756018.01	75477275.30	58.40	81.54	825	938
Propanedioic acid, 2TMS derivative	11.90	11728138.47	21050841.43	16.29	83.53	890	917
Octanoic acid, TMS derivative	12.82	229615.86	486664.98	0.38	72.55	672	786
Benzoic Acid, TMS derivative	13.43	608462.64	1014714.07	0.79	82.5	832	854
Butanedioic acid, 2TMS derivative	14.12	3406595.08	6833765.55	5.29	69.77	851	880
2-Butenedioic acid, (E)-, 2TMS derivative	14.50	126856.29	129195.54	0.10	52.71	589	747
Nonanoic acid, TMS derivative	14.73	213604.31	280904.71	0.22	85.17	732	799

[29]

Methylmaleic acid, 2TMS derivative	15.51	309845.22	715036.39	0.55	65.56	606	910
Pentanedioic acid, 2TMS derivative	15.87	447922.11	887009.07	0.69	81.75	730	807
Decanoic acid, TMS derivative	16.54	117241.98	169249.45	0.13	51.7	600	766
Hexanedioic acid, 2TMS derivative	17.77	150364.36	230696.13	0.18	78.78	665	768
Undecanoic acid, TMS derivative	18.26	37835.19	24699.79	0.02	17.02	492	618
Dodecanoic acid, 1-methylethyl ester	19.96	65527.21	132130.63	0.10	42.75	541	685
4-Hydroxybenzoic acid, 2TMS derivative	20.11	120423.53	216000.55	0.17	74.89	714	774
Suberic acid, 2TMS derivative	21.32	82365.63	206289.69	0.16	56.5	542	769
Phthalic acid, 2TMS derivative	22.06	669813.68	1639401.99	1.27	96.52	877	916
Isophthalic acid, 2TMS derivative	23.05	208118.73	508161.57	0.39	89.98	795	872
Phthalic acid, 2TMS derivative	23.47	226871.03	553823.23	0.43	61.17	642	808
Palmitic Acid, TMS derivative	28.50	458588.18	1253716.69	0.97	93.57	822	844
Stearic acid, TMS derivative	32.42	340967.87	837880.68	0.65	88.01	697	759
Nonadecanoic acid, TMS derivative	33.96	61947.42	105976.86	0.08	64.98	572	731
Arachidic acid, TMS derivative	35.37	155566.96	309909.97	0.24	34.83	478	600
Lignoceric acid, TMS derivative	40.06	75749.44	134757.72	0.10	23.12	480	695
3-Methylsalicylic acid, 2TMS derivative	19.49	205157.78	339556.30	0.26	14.58	557	659

[30]

3-Methylsalicylic acid, 2TMS derivative	19.67	205018.47	545772.26	0.42	34.89	809	857
Aspirin, TMS derivative	15.43	156868.92	273560.52	0.21	65.54	662	722
Salicylic acid, 2TMS derivative	18.05	1479568.33	2487460.30	1.92	68.27	837	886
Chrysin, 2TMS derivative	27.78	898312.04	2813053.44	2.18	40.97	655	659
(Sulfanediylbis(4,6-dichlorobenzene-2,1-diyl)oxy)bis(trimethylsilane)	33.29	189131.42	417511.42	0.32	25.5	479	495
(Sulfanediylbis(4,6-dichlorobenzene-2,1-diyl)oxy)bis(trimethylsilane)	33.51	217405.45	546563.44	0.42	12.11	474	482
Hydroquinone, 2TMS derivative	11.72	894863.99	3817475.25	2.95	61.48	807	813
Benzaldehyde, 2,5-dimethyl-	14.43	113845.60	114932.74	0.09	41.7	712	833
2,6-Dihydroxyacetophenone, 2TMS derivative	19.40	54077.75	91889.63	0.07	10.64	444	584
2-Hydroxyphenethyl alcohol, 2TBDMS derivative	24.30	178719.73	364271.15	0.28	15.2	623	667
1-(2-Thienyl(2-((trimethylsilyl)oxy)-1-naphthyl)methyl)piperidine	25.99	70043.12	267004.05	0.21	11.72	525	576
1-Phenyl-1,2-ethanediol, 2TBDMS derivative	26.87	54016.05	139548.81	0.11	20.12	498	713
Trimethylsilyl O,O'-bis(trimethylsilyl)vanilpyruvate	31.31	1126005.84	2801566.68	2.17	33.03	554	724
4-Hydroxy-3-ethoxyphenylpyruvic	32.27	114537.34	361005.20	0.28	16.11	481	748

[31]

acid, tri-TMS							
9,10-Anthracenedione, 2-methyl-1,6-bis(trimethylsilyl)oxy-	27.10	203564.35	664701.54	0.51	24.61	669	884

[32] According to the present invention, in addition to humic and fulvic acids, phenolic derivatives, in particular hydroquinone, flavanoids [chrysin] and other active substances, are also included. Therefore, the claimed agent is characterized by a broad spectrum of active substances.

[33] Example 2 Testing for Toxicity

[34] Determination of indices of acute toxicity included experiments in mice. The animals were randomly distributed in groups by randomization. The absence of external signs of diseases and homogeneity of groups by weight of the body [± 10%] was considered as criteria of acceptability acceptability. Administration of the preparation was carried out intragastrically in increasing doses by Sprue-Wilcoxon. The highest dose was limited to the maximum possible volume of administration of the preparation. Groups of 10 animals of different sex were used to study each dose of the preparation. The observation period was 14 days. When administering the preparation at doses of 4000-8000 mg/kg (in humic acid) in animals, a change in the response to the hand is detected, the change in respiration, motor activity, and muscle tone, in some animals, a change in the consistency of feces was observed. When the preparation is administered at a dose of up to 4000 mg/kg (in humic acid), all animals were removed, when the preparation is administered at a concentration of 6000 mg/kg (in humic acid), 5 animals were died from 10, and when the preparation is administered at a dose of 8000 mg/kg (in humic acid), 8 animals of 10% surviving animals had satisfactory intoxication and upon completion of the preparation for 14 days of observation, the delayed effects are not observed. No signs of prolonged clinical intoxication were noted. The dynamics of the body weight of experimental animals did not differ from the control. At the end of the observation period-on day 14, surviving animals were taken to determine possible pathological changes after a single administration of the preparation. The inspection of the experimental and control groups showed that all animals in them were normally fed, had correct teletings, smooth and shiny hair, shiny, conventional painting, clean and spicy natural holes. The macroscopic examination of the internal organs of any features was not detected. Analysis of the mass coefficient values did not reveal any significant differences between the groups of animals receiving different doses of the preparation. Thus, the results obtained suggest that the resulting preparation can be attributed to a V class, ie, practically non-toxic drugs.

[35] Example 3 Study of Hepatoprotective Activity of the Claimed Agent (GS) in Experimental Toxic Liver Injury

[36] Studies were performed on 47 white outbred male rats with an average body weight of 279.2 ± 4.06 g, 5 groups-intact (7 heads), control (10 heads) and 3 experimental (10 animals in each).

[37] Carbon tetrachloride (hereinafter techloromethane, TMS) was administered to the animals of the control and experimental groups in the form of a 50% oil solution after daily food deprivation orally once with a metal gastric probe at a dose of 320 mg/kg in a volume of 0.064 ml of TXM per 100 g of body weight. The control group of animals after TMS no preparations were administered. The animals of the experimental groups were injected with GS of 1.0 mg/kg (1 group), 5 mg/kg (2 group) and 10 mg/kg (3 group) intramuscularly after 24 hours after administration of TCHM followed by daily administration in the same dosages. Intact group was not subjected to any experimental effects and blood and organs were taken simultaneously with control and experimental groups. On the 7th day after administration of TCHM, euthanasia was performed by overdosing with chloroform narcosis, blood sampling and pathoanatomical assessment of the state of the internal organs were performed. Evaluation criteria were biochemical blood analysis indicators: hepatic cytolysis and cholestasis-alanine aminotransferase [ALT] markers, aspartate aminotransferase [ACT], alkaline phosphatase [AP], gamma-glutamyl transpeptidase [GGTP];

total lipids, cholesterol total; creatinine, urea, total protein, albumins fractions of globulins [alpha, beta, gamma-globulins].

[38] In addition, on the 6th day after administration of TMS in animals of intact, control and experimental groups, an open field test was performed to assess the general clinical condition and behavioral responses. It has been found that in animals of the control group an increase in alkaline phosphatase activity is observed by 7.5% with a pronounced increase in GGTP by 3.21 times, which is the classical signs of cholestasis. Lipid content was significantly increased by 54.1%, cholesterol by 79.4%, which may be evidence of biliary obstruction. The ACT and ALT content was significantly increased by 36.7% and 78.0%, with the coefficient of De titration was 0.86, which generally indicates the development of hepatocyte cytolysis syndrome [Table 2]. The reduction in urea concentration was 38.9% and was reliable with respect to healthy animals, which characterizes the decrease in hepatic tissue capacity for deamination. The tendency to increase the creatinine content by 2.1%, however, may not be indicative of significant renal function disorder. A slight decrease in total protein content by 4.3% was observed, indicating a tendency for protein-synthetic liver function disorder. The percentage of albumins was reduced by 9.4%, while increasing the relative content of all fractions of globulins: alpha-globulins by 17.1%, beta-globulins by 29.9%, gamma-globulins by 12.0%, indicating a violation of the ratio of protein fractions.

[39] In general, a complex of classical markers of acute dysfunction of metabolic processes specific for toxic hepatitis is identified in animals of the control group.

[40]

Таблица 2. Биохимические показатели цитолиза и холестаза (7 сутки после введения ТХМ).

Показатель	Интакт	Контроль	1 группа	2 группа	3 группа
АСТ, ед	43,0±6,9	58,8±3,9	51,1±2	47,4±3,1	43,2±2,3
Разница с интактом,%	-	36,7	18,83%	10,20%	0,46%
Разница с контролем,%	-	-	-13,10%	-19,4	-26,5
АЛТ, ед	38,1±4,24	67,8±8,44	53,7±2,7	46,4±1,1	41,9±2,3
Разница с интактом,%	-	78	40,9	21,7	9,9
Разница с контролем,%	-	-	-20,7	-31,5	-38,2
ГГТП, ед	1,93±0,53	6,2±0,52	5,3±1,3	3,9±1,1	2,1±0,9
Разница с интактом,%	-	221	174,6	102	8,8
Разница с контролем,%	-	-	-14,5	-37	-66,1

[41] BACKGROUND OF THE INVENTION A significant reduction in ACT, ALT, and GGTP showed a significant decrease in the manifestation of cholestasis and cytolysis manifestations in all experimental groups after administration of the TCHM.

[42] The total lipid content compared to the control was reduced by 33.7% [1 group], 51.6% [2 group], 78.9% [3]. Cholesterol concentration was lower relative to the control, respectively, by 21.3%, 32.4% and 46.9%. Thus, the use of the studied agent promotes a significant reduction in cholesterol and total lipid content.

[43] In carrying out the test, the open field on the 6th day of the toxic hepatitis course revealed the presence of significant changes in all types of behavioral activity in the control and experimental groups when compared to healthy animals of the intact group. It should be noted that the defecation in the test period was absent in both the animals of the intact group and in the control and all 3 experimental groups. In the control group, a decrease in horizontal activity [SEG] was observed by 23.5%, while the number of vertical posts [VDA] was increased 2.9 times, the normal reflex by 84.2%, weight 3.5 times. This complex of changes may indicate a reduction in locomotor activity [reduction of horizontal activity] and an increase in anxiety level as increasing the vertical activity indicates an increase in the orientation reaction, increasing the number of ggling in the mink characterizes an increase in spontaneous research activity.

[44] In the first group, the horizontal activity was reduced as compared to the intaxis [53.8%] and with respect to the control animals by 39.6%, the vertical activity was completely absent. In this experimental group, a decrease in norm reflex by 42.9% was observed when compared to the control, however, the index slightly exceeded the values of intact animals by 5.3%. The number of weights was reduced by 71.4% when compared to the control. These changes characterize a decrease in locomotive measure along with a decrease in the level of anxiety in animals of this experimental group.

[45] Against the background of the use of the claimed GGS in the second group, the horizontal activity was also reduced by 38.3% when compared to the intaxis and 24.1% with respect to the control animals. A reduction in norm reflex by 2.9% was observed when compared to the control, however the index exceeded the values of intact animals by 1.5 times. The number of weights was reduced by 2 times as compared to the control. These changes tend to prevent the reduction of locomotor activity and reduce the severity of anxiety.

[46] The use of the claimed GGS in the third group was accompanied by a slight decrease in the horizontal motor activity by 29.2% when compared to the intaxis and by 7.4% with respect to the control. Vertical activity was significantly reduced compared to the control and intaxis by 72.4% and 20.0%, respectively. A significant increase in the number of reference reflex acts was observed in 2.51 times with respect to the control and 4.63 times as compared to the intaxis. During the observation period in the open field, the weight of the animals of this test group was absent. This complex of changes characterizes negligible locomotion of locomotion while reducing the level of anxiety and increasing the research activity of animals.

[47] In the course of pathoanatomical studies, it has been found that the toxic effect of TCHM on the 7th day in the animals of the control group exhibited a number of specific changes. The liver of loose consistency, yellowish-red color, liver weight is significantly reduced by 11.3% with respect to healthy animals of the intact group, which confirms the presence of destructive processes in the liver parenchyma and is consistent with these biochemical studies. In 50.0% of the animals in the mucous membrane and the gastric mucosa in the region of small curvature, massive hemorrhage from 25 to 160 mm 2 is detected, in 50% of the animals in the gastric mucosa, spot ulcers in an amount of 3-9 pcs with a total area of not more than 14.5 mm. in the mesentery of the small intestine, point hemorrhages. Spleen of dark-side color, thymus with point hemorrhages. The heart without visually determined pathology was increased by 10.9%. The lungs, lung, and lung tissue are reduced, and the weight is increased by 31.4% with respect to the subject. Kidney red, weight is increased by 8.3%, 50% of the animals in the bladder contained a yellow-reddish urine, turbid, with protein flakes. In the experimental groups, a tendency to prevent the

reduction of liver weight [within 1.0-2.8%] was detected. There was a general trend to prevent an increase in the weight of kidneys, lungs and heart, other specific changes compared to intact animals.

[48] These data indicate that in toxic hepatitis caused by carbon tetrachloride, most of the criteria of the claimed GGS is an effective hepatoprotective agent, simultaneously providing a reduction in the expression of cytolysis and cholestasis markers, normalising lipid and protein metabolism, and increasing the synthetic function of the liver. Therefore, the claimed agent can be used as a hepatoprotective agent, which confirms the achievement of the claimed technical result.