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PHARMACOLOGICAL EVALUATION POLYSACCHARIDE COMPLEX FLOWERS TANSY

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Abstract. From flowers tansy extracted polysaccharide complex. Installed its qualitative and quantitative composition, have developed a technique standardizing the content of reducing sugars. By thin layer chromatography and high pressure liquid chromatography after acid hydrolysis installed monosaccharide composition: glucose, xylose, arabinose, galactose and mannose. It is proved that the polysaccharide has a high content of uronic acid, which allows it to include the class of pectin. The investigation of gastroprotective activity of polysaccharide in the prophylactic administration at model destruction of the gastric mucosa to indomethacin. Introduction polysaccharide prevents various types of erosive and ulcerative destruction. According to anti-ulcer activity of the drug is superior to ranitidine and comparable to omeprazole. The study of anti-inflammatory activity of the polysaccharide on the model of exudative inflammation caused by the introduction of the formalin solution under aponeurosis posterior limbs rat. The polysaccharide after oral administration reduces the edema of inflamed tissues limbs, reducing leukocytosis, erythrocyte sedimentation rate normalizes. Anti-inflammatory activity comparable with diclofenac sodium. The study of hepatoprotective activity of the selected polysaccharide on the model of toxic carbon tetrachloride liver lesions. Polysaccharide complex reduces the level of aspartate aminotransferase and alanine aminotransferase; by gepatprotective activity inferior silymarin. The study of antioxidant activity was conducted on the model of acute toxic hepatitis and exudative inflammation model. Polysaccharide complexes exhibit pronounced antioxidant effect, it reduces the concentration of TBA-reactive products, increases the content of protein-free thiol groups and glutathione peroxidase activity. When coadministered with diclofenac sodium eliminates its prooxidant action.

Keywords: polysaccaharide, flowers of Tansy, anti-inflammatory activity, antioxidant effect, gastroprotective activity, gepatoprotective activity.

Introduction. A promising direction in the developing of pharmacology is the research of new drug sources, including medicinal plants. Medicinal herbal preparations have a number of advantages by the condition of efficient use: they have diverse pharmacological activity and sufficiently large breadth of therapeutic action, but at the same time, they have milder effect in comparison with synthetic drugs, especially it is important for long-term use; they are relatively safe and practically do not cause allergic reactions [1].

At the present time the most promising herbal remedies are considered drugs of individual substances, which are belonged to various classes of chemical substances such as alkaloids (atropine sulfate, codeine phosphate, pilocarpine hydrochloride), glycosides (digoxin, strofantin K), flavonoids (rutin, quercetin).

Polysaccharides are used in medical practice, mostly in the form of herbal medicines. However, at the present time it is carried out the active screening of non-starch polysaccharides of higher plants and algae in order to develop effective drugs on the basis of individual substances [2, 3].

Non-starch polysaccharides have diverse effect on the body normally and in various pathological conditions. They have adaptogenic effect [4], stimulate the physical performance [5], and the immune system [6, 7], enhance phagocytosis,

increase the production of antibodies, increase the number of lymphocytes in blood, demonstrate the anti-inflammatory activity [8, 9]. On the surface of the stomach mucosa and bowel polysaccharides form the gel which makes ambient and protective effect [10], moreover by means of swelling they modify the transit of chymus through the gastrointestinal tract, polysaccharides have a prebiotic activity [11], and they are promising enterosorbents [12].

Despite the relatively large amount of information about the biological activity of plant polysaccharide complexes, practically there are no publications reflecting the interaction between the characteristics of the specific polymer's structure, and the presence and the degree of their pharmacological action. In addition, one of the most important issue is the clarification and the elaboration of some possible mechanisms of polysaccharides action and their complexes in the treatment and prevention of various types of pathologies.

Tansy – is the officinal herb, which is used in medicine as a choleretic agent. In medical practice, they use the flower's infusion, as well as dry extract - drug "Tanatsehol". Tansy flowers have a complex chemical composition. They contain flavonoids, essential oils, hydroxycinnamic acids, tannins, polysaccharides [13].

The aim of the research was to separate polysaccharide complex from flowers of tansy and to study its composition and pharmacological activity.

Materials and methods.

Polysaccharide complex flowers tansy (PSP) isolated from air-dried flowers tansy ("Flowers of Tansy", "Zdorov'e" Ltd., Russia). Polyphenolic compounds isolated by preliminary extraction with 40% ethanol. From the resulting seed meal extraction three times with 1% ammonium oxalate solution for 1.5 hours recovered PSP, which was precipitated with excess ethanol. Extraction purification was done by successive washing with ethanol, acetone and ether. This production method makes better use of medicinal herbs as a polysaccharide is used as a source of meal after separation of flavonoids. A method for producing patented in the Russian Federation. Polysaccharide complex authenticity confirmed by qualitative reactions, identified pH, kinetic viscosity of a 3% solution and its solubilizing ability [14].

PSP monosaccharide composition was determined after acid hydrolysis by thin layer chromatography and high effective liquid chromatography. Uronic acids content was determined gravimetrically by precipitation reaction of polygalacturonic acid in the form of calcium pectate. The content of free carboxyl groups was determined alkalimetrically (phenolphthalein indicator). A technique has been proposed to standardize the PSP, based on the spectrophotometric determination of the content of reducing sugars by reaction with picric acid after acid hydrolysis of the polysaccharide [15].

The study of the pharmacological activity of the PSP was carried out on rats 126 outbred line SD (Sprague Dawley), weighing 150 - 200 g, obtained from the nursery "Stolbovaya", contained in the standard vivarium conditions. Animal studies performed in accordance with the "Rules of work with the use of experimental animals" (Order of the Ministry of Health 708n dated 23.08.2010).

Investigation of gastroprotective activity was conducted on 35 male rats, divided into 5 groups of 7 animals each. Ulcer simulated by intragastric administration of indomethacin (Indomethacin, "Sofarma" Ltd., Bulgaria) at a dose of 20 mg/kg twice at an interval of 4 hours [16]. The first group of animals administered daily PSPs for 3 days, twice a day for 4 – one hour before the 1st and 2nd indomethacin. Efficacy was evaluated 16 hours after the ulcerogenic action of the agent as a result of a macroscopic study on the presence of gastric ulcers. Destruction differentiated by size, counted Pauls index and anti-ulcer activity of the drug. The second and third group of animals was administered the reference drugs – omeprazole (Omeprazole, "Promed" Ltd., Russia) at a dose of 20 mg/kg [17] and ranitidine (Ranitidine, "Ozone" Ltd., Russia) at a dose of 25 mg/kg [18] mode purpose polysaccharide.

Study of anti-inflammatory activity was performed on 56 animals were divided into 4 groups of 14 animals each. 0,1 ml of 2,5% formalin solution was injected for the simulation of the inflammatory response by the fascia of the right hind limb rat [19]. The first group of animals was administered intragastrically PSP 2 hours before the injection of formalin solution, followed by 2 hours after injection, the next 7 days once. The second group of animals was administered reference drug diclofenac sodium (Diclofenac, "Hemofarm" Ltd., Russia) at a dose of 11 mg/kg [20] in the mode of appointment of the polysaccharide. A third group of animals in the same period was administered a combination of PSP at 0,3 g/kg body weight and diclofenac sodium in a dose of 11 mg/kg body weight. After 1, 2, 3, 4, 6 and 24 hours after the administration of formalin, then once a day for 7 days was conducted onkometric measurement values limb edema in rats on a digital plethysmometer StoeltingCo (USA). After 4 hours at 3 and 8 hours after the start of the experiment in all groups was determined leukocyte level and ESR value [21].

The study of hepatoprotective activity was conducted on 28 male rats were divided into 4 groups of 7 animals each. Simulation of acute toxic hepatitis was performed administration the oil 50% carbon tetrachloride solution intragastrically at a dose of 0,1 ml per 100 g body weight twice a day – control pathology series [19]. Beginning 1 day after the last administration of CCl_4 first group of animals on a daily basis for 7 days was administered PSP at 0,3 g/kg body weight. On the 8th day (24 hours after the final injection) evaluated the effectiveness of the drug on the following parameters: the activity of AST (U/l), ALT (U/l) and alkaline phosphatase (U/l), the level of total and direct bilirubin (mmol/l). Animals of the second group received a comparison drug – fruit extract of milk thistle ("Karsil", "Sopharma" Ltd., Bulgaria) - the equivalent of silymarin – 100 mg/kg in the polysaccharide assignment mode [22].

Effect of polysaccharide complex tansy flowers on the state of lipid peroxidation (LPO) and antioxidant defense cells was evaluated in two models of acute liver toxicity and acute exudative inflammation at the level of TBA-reactive products (TBA-RP), thiol groups (of GSH), glutathione-S-transferase (GST) and glutathione peroxidase (GPx) lysate of erythrocytes and blood plasma. The test parameters were determined as follows: RP-TBA concentrations (nmol/mg protein) by the reaction with thiobarbituric acid (method Stalnoy D.I., Gorishvili T.G. 1977), the level of GSH (mol/mg protein) by reduction reaction disulfide-5,5-dithiobis-2-nitrobenzoate (method Habeeb A.F.S.A., 1972) on spectrophotometer Shimadzu UV-150-02, GPx activity (nmol $NADFH_2$/min × mg protein) by enzymatic reduction reaction of tert-butyl hydroperoxide glutathione (method Paglia D.E., Valentine W.N., 1967, modification Lankin V.Z.), GST activity (HDBN nmol/min × mg protein) by the reaction of conjugation of glutathione with 1-chloro-2,4-dinitrobenzene (method Keen J.N., Iakoby W.B., 1978) using biochemical analyzer Humalyzer 2000.

Statistical processing of the results obtained in the course of chemical research carried out under the requirements of the State Pharmacopoeia of the Russian Federation XIII edition. Statistical processing of the results obtained in animal experiments were carried out using applications MS Exel 2010 and Statsoft Statistica 8.0. The data are shown as the arithmetic mean and standard error of the average value of (M±m) data in the normal distribution and a median upper and lower quartiles - in the distribution data other than the normal. The nature of the data distribution was assessed by the Shapiro-Wilk. The presence of a statistically significant between-group differences were determined using one-way ANOVA, differences between groups were determined by the criterion of Newman-Casely, reliable results are considered at a significance level of $p < 0,05$ [23].

Results.

Polysaccharide complex extracted from tansy flowers, amorphous material is light gray, in which the total content of the feed is 6.5%. The viscosity of the solution as measured under standard conditions was 2,2 Pa·sec, pH=6,70. PSP has a solubilizing ability for hydrophobic dyes. The polysaccharide is substantially free of free mono- and disaccharides in their determination by high performance liquid chromatography. According to the results of thin layer chromatography and high performance liquid chromatography, it was determined that the composition comprises PSP glucose, galactose, xylose, arabinose and mannose. Uronic acids content of 84,7±1,28%, free carboxyl groups – 15,66±4,79%; standardization of PSP held on the content of reducing sugars.

Introduction of indomethacin at a dose of 20 mg/kg twice at intervals of 4 h resulted in the formation of erosive-ulcerous lesions in 100% of the animals of all groups (table 1). Grossly destruction in rats differentiated into large (7,43±4,79), strip (5,00 ± 3,27) and chiseled (10,71 ± 2,93), the index of Pauls (for its calculation of the average number of ulcers was multiplied by the percentage of animals with ulcers, and divided by 100%) was 23,14.

Number of destructions in animals treated with a dose of PSP 0,3g/kg for 4 days course, reliable decreased compared to control data: the number of large ulcers decreased to 2,48 times, chiseled – in 1,6 times, strip-ulcer attended only one animal.

Antiulcer activity (ratio Pauls index in the control group to Pauls index in the experimental group), PSP was 2,31, indicating a marked gastroprotective effect at prophylactic use.

Introduction comparator drugs ranitidine and omeprazole reduced the number of large ulcerations in 3,72 times ($p<0,05$) and 6,52 times ($p<0,05$), chiseled destructions of 1,32 times and 1,41 times ($p<0,05$) respectively. In both groups significantly decreased the number of strip ulcers are observed upon administration of ranitidine in two animals, when administered omeprazole – one. Antiulcer activity of ranitidine was 2,25; omeprazole – 2,49.

Table 1.

Effect of polysaccharide complex flowers of tansy, omeprazole and ranitidine on the development of erosive-ulcerous lesions caused by indomethacin.

Series animals	Number of destructions 1 animal			Index Pauls	Antiulcer activity
	Large	Strip	Chiseled		
Control pathology, n=7	7,43±4,79	5,00±3,27	10,71±2,93	23,14	
PSP, n=7	3,00±1,63*	0,00 (0,00;0,00) **	6,71±1,49*	10,00	2,31
Ranitidine, n=7	2,00 (0,00;2,00)*	0,00 (0,00;3,00)*	8,14±2,91	10,29	2,25
Omeprazole, n=7	1,14±1,21*	0,00 (0,00;1,00) **	7,57±1,99*	9,28	2,49

Note: * - p<0,05; ** - p<0,005 - comparison of data from animal disease group control pathology

The introduction of formalin induced inflammatory response, accompanied by hyperemia and edema of the limbs in animals of all groups. The maximum value of edema in rats of the control group was observed after 4 hours after injection of formalin. In subsequent periods of observation, the gradual reduction of edema, limb volume normalization was observed on the 7th day of the experiment (figure 1).

The development of an inflammatory response accompanied by severe leukocytosis. After 4 hours after the administration of formalin, the number of leukocytes in rats of the control group increased by 3 times compared to the intact animals (p<0,05). ESR level in this group peaked on the third day of research and was 133,3% (p<0,05) relative to the level of intact animals.

The dynamics of edema in animals treated with PSP corresponded to that in the control series pathology, but its intensity decreased significantly. After 4 hours after the administration of formalin edema value significantly decreased by 8,97%, the level of leukocytes and ESR decreased by 38,67% (p<0,05) and 5,88% respectively, compared with the control group data. Normalization of these indices took place on the 7th day of research.

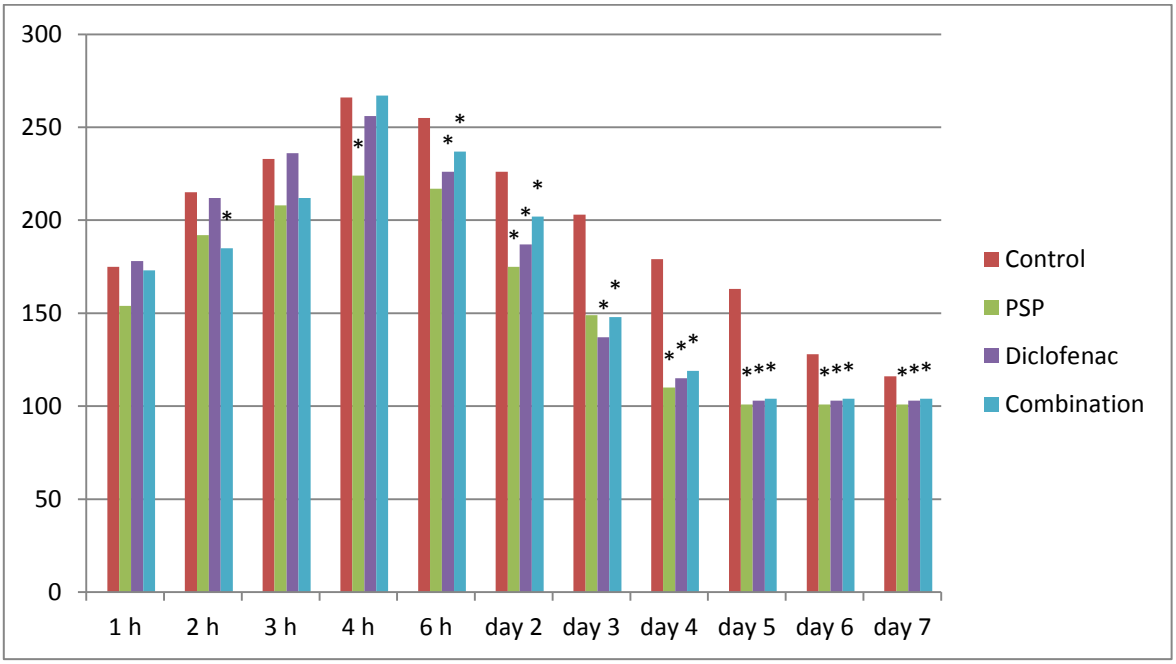

Figure 1. Dynamics of limb edema development (as a percentage of the norm), caused by the introduction of the formalin, without treatment and with therapy.

* - p<0,05 – comparison of data from animal disease control group.

Appointment of diclofenac sodium contributed significantly delay the development of the inflammatory process. As compared to the control group of rats, the intensity of edema disease through 4 hours after administration of formalin authentically decreased by 7,24%, the level of white blood cells to 52,79% (p <0,05) and ESR value is not changed.

Normalization parameters observed on the 5th day of the experiment.

The introduction of a combination of PSP and diclofenac sodium were not significantly changed the dynamics of limb edema as compared with the animals treated with diclofenac sodium and cap separately. The level of leukocyte and erythrocyte sedimentation rate was comparable with figures of animals treated with diclofenac sodium.

In intact animals the activity of AST was 81,03±6,02 U/l, ALT – 23,00±2,00 U/l, alkaline phosphatase – 306,33±14,74 U/l, the content of total bilirubin 0,1 (0,1; 0,11) mmol/l, direct bilirubin 0,033 (0;0,100) mmol/l.

The double introduction of carbon tetrachloride in rats led to development of acute toxic hepatitis. The animals of the control group in the plasma enzyme activity was statistically increased as compared to the intact animals: AST – 1,52 times, ALT – in 2,48 times, alkaline phosphatase (AP) – 2,73 times (figure 2) has also increased the content of total bilirubin 2-fold ($p<0,05$) and direct bilirubin in 2,51 times.

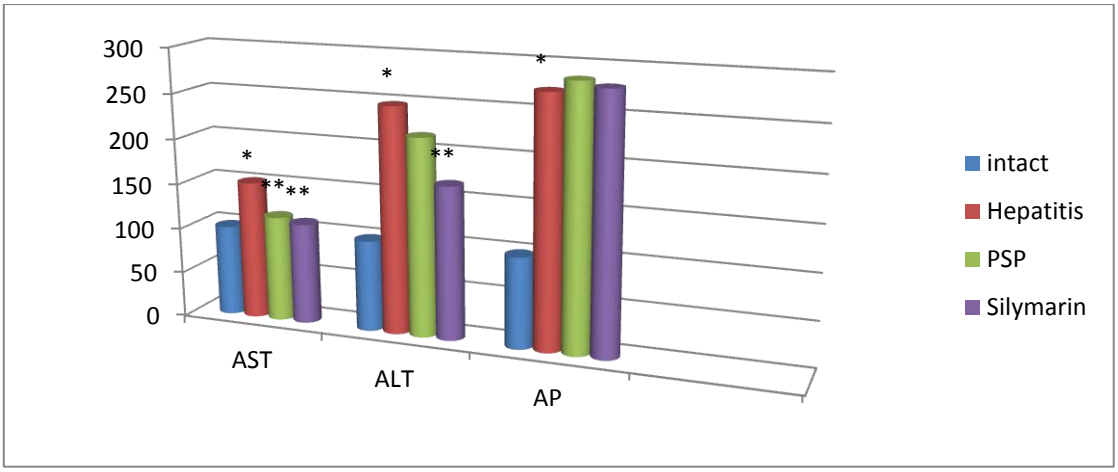

Figure 2. Activity (percentage of normal) of liver enzymes in the blood plasma at the 8th day in rats with hepatitis and during treatment with PSP and silymarin.

Hereinafter: * - $p<0,05$ – animals compared with data intact animals; ** - $p<0,05$ – comparisons with the data of disease control.

Introduction PSP at 0,3 g/kg for 7 days, reduced the toxic liver damage caused by carbon tetrachloride. ALT and AST activity was reduced compared to control in the pathology 1,31-fold ($p<0,05$) and 12,67% respectively.

Course administering silymarin 100 mg/kg also resulted in reduced CCl_4 toxicity in the liver. The animals of this group on the 8th day study statistically significantly decreased content of AST, ALT and total bilirubin (2,56; 1,48 and 2 times, respectively) in the blood plasma as compared to control these diseases.

Changes in the antioxidant defense system cells under the influence of PSP has been evaluated in two models: acute toxic hepatitis and acute exudative inflammation by Selye.

Indicators of the state of lipid peroxidation and antioxidant protection in intact animals are presented in table 2.

Toxic hepatitis, caused by the introduction of a 50% oil solution of carbon tetrachloride, was accompanied by the activation of the LPO. In the control group of rats TBA-RP blood plasma on the 8th day after the last injection of CCl_4 was statistically increased in 1,56 times, GSH levels in the lysate of erythrocytes and blood plasma decreased to 2,08 ($p<0,05$) and 1,56 ($p<0,05$) times, respectively compared with those of intact animals. Simultaneously there was a statistically significant decrease in the activity of GPx and GST in erythrocyte hemolysate 2,40 and 2,44 times, respectively (table 2).

Table 2.

Effect of polysaccharide complex flowers of tansy and silymarin on the state of lipid peroxidation and antioxidant protection in rats with experimental hepatitis.

Series animals / under investigation indicator		Intact animals, n=7	Control hepatitis, n=7	PSP course of 7 days, n=7	Silymarin course of 7 days, n=7
TBA-RP	hemolysate	6,90±0,55	13,89±5,50	9,01±1,06*	7,48±1,94
	plasma	29,12±1,97	45,41±8,57*	31,70 (28,27;39,83)**	33,84 (32,13;37,27)**
GSH	hemolysate	128,70 (128,22;129,18)	61,86±20,68*	135,55±24,76**	79,94±29,05
	plasma	102,49±13,37	65,50±9,79*	73,52±11,01*	85,78±10,68**
GST	hemolysate	12,85±4,53	5,36±0,74*	7,67±2,12**	5,11 (4,46;23,24)*
	plasma	257,85 (238,80;276,90)	210,95±41,30	176,83±31,10*	219,63±67,43
GPx	hemolysate	1,22±0,41	0,50±0,17*	0,91±0,34	0,93±0,29
	plasma	36,05 (31,50;40,60)	22,18±15,43	19,00±4,57*	27,07±2,11

The intensity of lipid peroxidation in animals treated with PSP at 0,3 g/kg of body weight 7 days course was less pronounced as compared to control disease. The level of TBA-RP hemolysate of red blood cells was reduced by 35,13% and 119,12% ($p<0,05$) increased the content of protein-free thiol groups, increased the activity of GST and GPh at 43,10% ($p<0,05$) and 82,00%, respectively. The blood plasma is a statistically significant decrease of 30,19% of the level of TBA-RP.

As compared to intact animals treated rats at a dose of PSP 0,3 g/kg of body weight 7 days of course, on the 8th day of TBA-RP erythrocyte hemolysate level remained statistically authentically increased to 30,58%, the content of GSH in blood plasma was lower by 28,27% ($p<0,05$) and GPh activity – by 47,29% ($p<0,05$). The rest of indicators studied were not statistically different from the intact animal data suggesting that the decrease in the processes of lipid peroxidation and antioxidant defense stimulation PSP.

Course introduction silymarin also led to a decrease in the severity of lipid peroxidation. The level of TBA-RP in hemolysate of red blood cells in rats in this series decreased by 46,14% ($p<0,05$), plasma – at 25,47% ($p<0,05$). Statistically significant increased content of protein-free thiol groups in blood plasma at 30,96%, GST activity was decreased by 4,66% in erythrocyte hemolysate and increased by 4,11% in the blood plasma as compared control diseases, GPx activity in erythrocyte hemolysate and in the blood plasma was increased by 86,00% and 22,05% respectively. Compared to intact animals when administered silymarin GST activity remained reduced to 60,23% ($p<0,05$), other indicators were not statistically different from the normal level.

Introduction formalin under foot aponeurosis of rats led to an increase in lipid peroxidation processes and a reduction in the antioxidant defense of cells. The animals in the control group 4 hours after formalin injection of TBA-RP levels in hemolysate of red blood cells increased by 35,29% ($p<0,05$), GST activity increased by 18,05%, the content of thiol groups is decreased by 19,41% ($p<0,05$) GPh activity – by 50,69% compared with those of intact animals (table 3). The blood plasma statistically significantly increased TBA-RP content to 71,28%, other indicators have changed in different directions and not statistically significant.

Itroduction PSP at a dose of 0,3 g/kg of course 7 days reduced the intensity of lipid peroxidation and increased antioxidant protection of cells: compared with the control of disease in 4 hours after the injection of formalin level of TBA-RP in hemolysate of red blood cells in rats decreased by 40,43% ($p<0,05$), the content of protein-free thiol groups authentically increased by 202,57%, the GST activity increased by 7,75% and GPh to 127,97% ($p<0,05$). The blood plasma is a statistically significant decrease in the concentration of TBA-RP at 32,14%, other indicators have changed not statistically significant. When comparing these results with the data of intact animals revealed normalization on the background of PSP levels of TBA-RP and protein-free thiol groups, the activity of GST and GPh in hemolysate of red blood cells increased by 10,73% and 40,08%, respectively.

Table 3.

Effect of polysaccharide complex flowers of tansy, diclofenac sodium, and combinations thereof on the state of lipid peroxidation and antioxidant protection in rats with experimental exudative inflammation.

Series animals / under investigation indicator		Intact animals, n=7	Control inflammation after 4 hours after the administra-tion of formalin, n=7	PSP, 4 hours after the administra-tion of formalin, n=7	Diclofenac-sodium, 4 hours after the administra-tion of formalin, n=7	The combination of PSP and diclofenac sodium in 4 hours after the administra tion of formalin, n=7
TBA-RP	hemo-lysate	8,49±1,06	11,51±1,69*	9,13±1,24**	10,4±0,54	11,2±0,59
	plasma	17,13±9,22	29,34±3,49*	19,91±1,57**	7,71±4,06**	7,07±3,88**
GSH	hemo-lysate	154,14±44,81	60,29±48,17*	182,45±22,44**	93,11±28,89	115,85±18,91 **
	plasma	106,04±27,92	129,93±30,22	91,64±13,04	74,41±30,96	78,42±13,10**
GST	hemo-lysate	12,46±1,87	14,71±2,26	15,85±2,77*	12,19±3,18	13,98±3,24
	plasma	188,75±29,98	181,40±33,00	209,00±27,65	254,67±23,73***	217,40±40,21
GPx	hemo-lysate	2,37±0,47	1,43±0,18	3,32±0,43**	2,23±0,42**	2,04±0,24**
	plasma	41,42±7,01	34,75±7,87	44,05±18,03	45,20±3,82**	45,23±5,41**

Course introduction of diclofenac sodium in a dose of 11 mg/kg for 7 days increased the intensity of lipid peroxidation. After 4 hours after the initiation of the inflammatory reaction against treatment diclofenac sodium TBA-RP levels remained elevated erythrocyte hemolysate (disease level control), the content of thiol groups increased by 39,97%, GST activity has decreased by 17,13% ($p<0,05$), GPh - increased by 56,64% ($p<0,05$) relative to control edema. Comparison of the results of this series with the data intact rats showed that TBA-RP levels remained elevated at 22,35%, a protein-free thiol groups was reduced by 45,23%, the GST activity and GPh remained lowered. Thus, diclofenac sodium has prooxidant effect early in the inflammatory response.

Co-administration of PSP and diclofenac sodium 4 hours after formalin injection resulted in a significant increase in the content of erythrocyte hemolysate thiol groups at 92,15%, TBA-RP level has not changed, GPh activity increased by 36,00% ($p<0,05$) , GST – has not changed relative to control edema. The plasma TBA-RP levels decreased by 75,90% ($p<0,05$), the content of thiol groups to 39,64% ($p<0,05$), glutathione peroxidase activity increased by 30,16% ($p<0,05$), the activity of glutathione-S-transferase is not significantly increased. As compared to the intact animals showed a significant increase of TBA-RP levels in hemolysate at 31,92%, reduction of protein-free thiol groups on 24,84%, GST activity increased and decreased GPh.

On day 7, the study of the control group animals TBA-RP levels in hemolysate of red blood cells remained significantly lower at 29,96% ($p<0,05$), in plasma, this figure does not change significantly. In all treatment groups studied parameters on the 7th day were close to the data of intact animals and statistically not different from them.

Discussion.

Dedicated extraction with a 1% solution of ammonium oxalate, polysaccharide complex flowers tansy is an amorphous light gray. When dissolved in water, it forms a viscous solution having a neutral pH. By thin layer chromatography and high performance liquid chromatography revealed that the composition comprises a polysaccharide: glucose, xylose, arabinose, galactose and mannose. The high content of uronic acids (84,7±1,28%) can be attributed to a class of PSP is pectin. Thus 15,66±4,79% of monosaccharide residues contain a free carboxyl group, which may account for some aspects of the pharmacological activity of the polysaccharide. Standardisation of the selected polysaccharide complex tansy flowers were carried out on the content of the amount of reducing sugars after acid hydrolysis.

On models indomethacin ulceration was found that the polysaccharide complex tansy flowers in prophylactic gastroprotective reception has a marked effect reducing the amount of degradation. For comparison, we choose the most effective and widely used antisecretory drugs with different mechanisms of action – omeprazole and ranitidine [24]. Comparative evaluation of the effectiveness of anti-ulcer activity coefficient showed that PSP is superior to ranitidine, but inferior to omeprazole.

3% solution of tansy flowers polysaccharide complex used in the experiments has a high kinematic viscosity and when administered in stomach envelops its walls, preventing the destructive action of hydrochloric acid on the mucosa. Pectic polysaccharides are surfactants [25] and can be oriented certain way of an interfacial of the hydrophilic and hydrophobic environment of the membrane of the stomach mucosa cells. The polysaccharide forms a protective film on the surface, which prevents corrosive environmental factors.

PSP, like other pectins, is a weak electrolyte, wherein it contains 15,66±4,79% of free carboxyl groups. Polysaccharide dissociation in the stomach is suppressed, molecular shape is formed, the concentration of hydrochloric acid is reduced. Thus, the possible effect of the antacid is another component providing gastroprotective action.

Plant polysaccharides exert wound-healing effect and stimulate tissue regeneration [26]. Calcium pectate in the experiment increases levels of proteolytic enzymes and concentration of nucleic acids [16], which according to the authors leads to activation of the synthesis of additional cells of the stomach on the principle of feedback. In view of the similarity of the structure can assume the existence of such action in the pectin polysaccharide flowers of tansy. In addition, PSP has an antioxidant effect, which reduces mucosal damage by free radicals formed as a result of increased lipid peroxidation under the action of indomethacin.

Study of anti-inflammatory activity of complex polysaccharide tansy flowers were carried out on the model of exudative inflammation caused by the introduction of a solution of formalin under the aponeurosis of the hind limbs of animals. The introduction of PSP in a dose of 0,3 g/kg rate to 7 days resulted in a marked inhibition of edema and exudation reduction, reduced leukocytosis and ESR normalized. As a comparison, the drug used most widely used NSAIDs – diclofenac sodium. Anti-inflammatory activity of the polysaccharide complex tansy flowers in the experiment was comparable to the effect of diclofenac sodium. However, he, like other NSAIDs, as a pro-oxidant has a damaging effect on the gastric mucosa. Therefore it was of interest to explore the possibility of co-administration of drugs, to identify a possible increase anti-inflammatory activity and to prevent adverse effects of diclofenac sodium.

Simultaneous administration of PSP and diclofenac sodium does not significantly alter the dynamics of the limb edema compared with separate administration. The level of leukocyte and ESR were not significantly different from that of animals treated with the polysaccharide and NSAID separately. Thus, we can conclude that there is no potentiation of the anti-inflammatory effect when administered to a combination of drugs. However, the use of such combinations is partially eliminates the prooxidant action of diclofenac sodium in the early period of development of the inflammatory response, as evidenced by the increase in free sulfhydryl groups and glutathione peroxidase activity in hemolysate of red blood cells.

The mechanism of antiinflammatory action PSP, as well as other pectin is probably related to activation of monocyte-macrophage system producing proteoglycans, glycoproteins and glucosamine, which leads to an acceleration of the maturation of T-and B-lymphocyte precursor cells. In turn, produces lymphocytes increases the activity of phagocytosis process. Under the influence of plant polysaccharide is an increase of plasmatic cells spleen and increased synthesis of γ-globulin, which also leads to the activation of phagocytosis. Polysaccharides are surfactants, they may interact with cell membranes, increasing the peroxide resistance [27]. In addition, PSP displays antioxidant activity, allowing you to reduce the damaging effects of free radicals, resulting in inflammation. This is consistent with the findings of our study: reduction of TBA-RP levels, increasing the number of free sulfhydryl groups and the activity of glutathione-S-transferase and glutathione peroxidase using PSP for the treatment of exudative inflammation. Integrated action reduces edema, normalization of leukocytes and ESR.

The study of hepatoprotective activity of complex polysaccharide tansy flowers were carried out on the model of acute toxic liver injury by carbon tetrachloride. Against the background of a course of PSP occurred statistically significant reduction in elevated levels of AST, however, ALT level decreased significantly not. Lowering transaminase activity indicates a decrease and stabilization of hepatocyte cytolysis membranes.

Silymarin was selected as comparison drug, because it is a widely used herbal hepatoprotectors. Underlying mechanism of action of silymarin is the stabilization of hepatocyte membranes, inhibition of cAMP, which leads to inhibition of calcium-dependent phospholipase and improving metabolic processes in the liver, increase in protein synthesis and cell regeneration acceleration. In addition, silymarin has antioxidant activity [28].

The effect on cytolysis polysaccharide complex flowers of tansy inferior silymarin. However, the

main role in protecting liver cells administered at course SAPs apparently plays its pronounced antioxidant activity.

As is known, carbon tetrachloride toxicity generally associated with the formation of free radicals during its metabolism in the cytochrome P-450 monooxygenase system. The indicators of free radical processes are intermediate oxidation products – diene conjugates and TBA-RP, and the concentration of the latter is directly proportional to the intensity of lipid peroxidation [28].

Introduction of the complex polysaccharide tansy flowers at 0.3 g / kg decreased the levels TBA-RP in hemolysate of erythrocytes and blood plasma, significantly increased the content of free thiol groups, and activity of glutathione-S-transferase in erythrocyte hemolysate. Free sulfhydryl groups play an important role in the redox homeostasis in the cell due to the ability to reversibly move from the reduced form (GSH) to oxidized (GSSG), changing the conformation, catalytic and regulatory functions of proteins [29].

Thus, the polysaccharide complex flowers of tansy has a strong antioxidant effect exceeding the activity of silymarin. PSP reduces the activity of lipid peroxidation and stimulates antioxidant cell system.

PSP, having a high potential for adsorption may bind free radicals and lipid peroxidation products formed when damaged cells, thereby providing a direct antioxidant effect and reducing the toxic load on the liver. By increasing the content of sulfhydryl groups and the activity of glutathione-S-transferase increases neutralizes function of hepatocytes, thereby realized indirect antioxidant effects.

Several studies have proved the presence of a stabilizing membrane activity of pectin polysaccharides [27]. The physiological effects on the cell membrane is mainly determined by the structure and physicochemical properties of the substance, as well as membrane receptors. The antioxidant effect can be realized by the presence of anti-inflammatory and immunomodulatory effect.

Conclusions.

1. The polysaccharide complex tansy flowers isolated on the patented technique, belongs to a class of pectin. According to the results of thin layer chromatography and high performance liquid chromatography it revealed that the polysaccharide composition comprises glucose, galactose, xylose, arabinose and mannose. Pectin fraction contains up to 85% polygalacturonic acid, 16% of which have a free carboxyl group. The method of standardization polysaccharide complex content of reducing sugars. Polysaccharide complex tansy flowers has a high solubilizing capacity. Its solution has high kinetic viscosity and neutral pH.

2. The polysaccharide complex tansy flowers at preventive oral administration for 4 days at a dose of 0,3 g/kg body weight has a significant gastroprotective effect of reducing the amount of destructive erosive and ulcerative lesions caused by indomethacin. In the experiment, antiulcer activity polysaccharide comparable to that of omeprazole and superior to that of ranitidine.

3. The polysaccharide complex tansy flowers when administered orally 7 days a course dose 0,3 g/kg has a pronounced inhibitory effect on the development of exudative component of the inflammatory response induced by administration of formalin aponeurosis limb rats, reduces leukocytosis and the level ESR. Polysaccharide complex inflammatory activity comparable with the activity of diclofenac sodium. Simultaneous administration of diclofenac sodium and polysaccharide complex flowers tansy does not significantly alter the dynamics of the limbs edema, leukocyte count and ESR value.

4. The polysaccharide complex flowers tansy has hepatoprotective effect, reduces cytolysis, reduces the activity of AST in the blood. The effect is inferior silymarin.

5. The polysaccharide complex flowers tansy exhibits anti-oxidant action, causing a reduction in the concentration of TBA-reactive products, elevated levels of protein-free thiol groups and the activity of glutathione-S-transferase and glutathione peroxidase in oxidative stress, caused by the introduction of carbon tetrachloride and inflammatory response. Polysaccharide complex by superior antioxidant activity of silymarin and reduces prooxidant effect of diclofenac sodium in a joint application early in the inflammatory response.

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