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CHEMICAL AND PHARMACOLOGICAL STUDY OF THE PANCREOPROTECTIVE REMEDY

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ABSTRACT

The researches has shown that the complex plant remedy conventionally named "Pancreophyt" contains tannins from 4,76 to 5,20% (including 0,12% of ellagic acid), flavonoids (including 0,10% of quercetin, 0,67% of rutin, 0,18% of luteolin, 0,009% of luteolin-7-glycoside), polysaccharids from 4,0 to 4,2%, 0,04% of oleanolic acid, ascorbic acid from 3,6 to 3,9%. The extract "Pancreophyt" has the marked pancreoprotective effect in the triton-induced pancreatitis, decreasing the activity of α-amylase (by 24% on the 21st day of the experiment; $p<0,05$) and reducing the intensity of inflammatory reaction: the leucocyte content is decreased by 25% at the average ($p<0,05$), ESR index is 1,4 and 2,1 times decreased on the 14th and 21st days of the experiment respectively ($p<0,001$).

KEYWORDS: Pancreoprotective complex plant remedy, HPLC, Bidens tripartita L., Gnaphalium uliginosum L., Calendula officinalis L., Inula helenium L., Pentaphylloides fruticosa L., Hypericum perforatum L., Vaccinium myrtillus L.

1. INTRODUCTION

A conservative treatment of pancreatitis includes a set of arrangements aimed to pain elimination, suppression of secretory-enzymatic activity of the pancreas, correction of circulation pathology, protein-energy disturbances, as well as detoxication and antibacterial

therapy.[1, 2] The use of multicomponent herbal remedies, which have a wide range of therapeutic efficacy, comprehensively acting on organism as a mean of additional therapy during pancreatitis exacerbation period, as well as medical and preventive agents during incipience of the disease and at the stage of anti-relapse treatment, is pathogenetically substantiated.[3] Based on the above-mentioned, it was developed a complex plant remedy in the form of combination plant medicinal product and dry extract, conventionally named "Pancreophyt", which has antioxidant and pancreoprotective properties. [4, 5] It consists of the next medical plants – *Bidens tripartita* L. (herb), *Gnaphalium uliginosum* L. (herb), *Calendula officinalis* L. (flowers), *Inula helenium* L. (rhizome), *Pentaphylloides fruticosa* L. (burgeon), *Hypericum perforatum* L. (herb), *Vaccinium myrtillus* L. (burgeon) and others.[4, 5] Pharmacological activityof plant remedies is associated with the presence of biologically active substances: polyphenols, polysaccharides, minerals, amino acids, organic acids, vitamins, etc., which provide a wide spectrum of pharmacological activity of plant remedies. The herbal medicinal product "Pancreophyt" contains a complex of biologically active substances; the study has found tannins, flavonoids, polysaccharides, saponins; carbohydrates; amino acids, ascorbic acid. Using thin layer chromatography (TLC) it was identified at least five natural flavonoid compounds, including myricetin and quercetin, two phenolcarboxylic acids. [6] The research found the content of some phenolic compounds in the extract "Pancreophyt": cinnamic, gallic, caffeic, chicoric acids, and flavonoids - luteolin-7-glycoside, apigenin, hyperoside, rutin, vitexin, dihydroquercetin and quercetin. [5] The aim of this research– chemical and studying of pancreoprotective combination plant medicinal product.

2. MATERIALS AND METHODS

The quantitative content of tannins was determined by permanganometry [7] , polysaccharides – by gravimetry [7], ascorbic acid – by UV spectrophotometric method using Pharmacopoeial monograph 42-2668-95 "*Rosae extract siccum*". The research was carried out using eight repeated analysis of similar materials (Table 1).

Qualitative composition and quantitative content of some substances in the complex plant remedy was determined by HPLC methodusing "Agilent 1200" with tandem mass spectrometric detector "Ion trap" 6330 (electrospray ionization method). Zorbax Eclipse Column C18, 5 microns, 4,6x150mm. The powdered sample of complex plant remedy 1.5 g (accurately weighed) was placed in a conical flask and extracted with 70% ethanol on a steam

bath for 1 hour. The obtained extract was filtered into a volumetric flask of 50 ml, made up to volume with 70% ethanol, and shaken well. In parallel, a set of solutions with standard samples was prepared, 10 mg of standard samples of ellagic acid, oleanolic acid (Sigma-Aldrich), quercetin, rutin, luteolin, luteolin-7-glucoside (VILAR) were dissolved in 50 ml of 70% ethanol (for oleanolic acid - 95% ethanol). 2.5 ml of obtained solutions were placed in volumetric flasks of 50 ml, made up to volume with the same solventand shaken well. Elution was carried out in linear gradient, the composition of starting buffer (A) - a water solution of formic acid (0.1%), elution buffer (B) - 100% acetonitrile. From1st min (10% (A): 90% (B)),5th min (10% (A): 90% (B)), 15th min (90% (A): 10% (B)), 20th min (90% (a): 10% (B)),25th min (10% (a): 90% (B)), 30th min (10% (a): 90% (B)). The volumetric flow rate of eluent - 1.0 ml / min, injection volume-10mcL, elution time - 30 min. The analysis was conducted in a registration mode of negative ions, with Total Ion Current (TIC), with mass charges of characteristic (MS) and product (MS2) negative ions. UltraScan 50-1300 m / z, AutoMS mode. The analysis of obtained chromatograms was carried out by using standard samples [8]. Statistical processing of experimental results was conducted in accordance with the requirements of the State Pharmacopoeia XIII ed. using 5 repeated analyses of similar materials.[7]

Pancreoprotective effect was investigated on a model of acute pancreatitis which was induced in laboratory animals by injection of 0.3 ml of a 3% solution of Triton X-100 into pancreas under thiopental anesthesia (50 mg / kg) affected by a 24-hour food deprivation, as a result of which ahaemorrhagic form of acute pancreatitis occurred.[9] The research was conducted using 112 white Wistar rats of both genders, with initial weight of 200-220 g. The conditions for animals comply with "Good laboratory practice» (GLP) and the Order of the Ministry of Healthcare of Russian Federation N708N d.d. 23.08.2010 "On approval of Good laboratory practice". The experimental work was carried out in accordance with the Order of the Ministry of Health of Russia N 267 d.d. 19.06.2003 "On approval of Good laboratory practice" and the Rules accepted by the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes: EST N123» d.d. 18.03.1986 (Strasbourg, 1986). The research protocol was approved by the Ethics Committee ofInstitute of General and Experimental Biology of SB RAS (Report N5 d.d. 05.09.2010). Animal Euthanasia was performed by instant decapitation under brief ether anesthesia. Each group contained 8 animals.

3. RESULTS AND DISCUSSION

As a result the conducted study found out the quantitative content of tannins, polysaccharides, ascorbic acid in a combination plant medicinal product “Pancreophyt”. Within-test reproducibility was elucidated on the example of one batch of pancreoprotective complex plant remedy in 8 replicates (Table 1). The different batches of combination plant productcontained tannins from 4.76 to 5.20%; polysaccharides from 4.0 to 4.2%, ascorbic acid from 3.6 to 3.9%. Using HPLC method the research defined 0.12% ellagic acid, 0.04% oleanolic acid, 0.10% quercetin, rutin 0.67%, 0.18% luteolin, 0.009% luteolin-7-glucoside in “Pancreophyt". As a result the research of a complex plant remedy "Pancreophyt" detected tannins - from 4.76 to 5.20% (including 0.12% of ellagic acid), flavonoids (including quercetin 0.10%, 0.67% rutin, luteolin 0.18%, 0.009% luteolin-7-glucoside), polysaccharides from 4.0 to 4.2%, 0.04% oleanolic acid, ascorbic acid from 3.6 to 3.9% using various methods.

Previously it was found that the dry extract "Pancrephyt” in 300 mg/kg dose has an antioxidant and pancreoprotective effects on a model of pancreatitis caused by cooling pancreas of rats using chlorethyl. [4, 5] Therefore, in these batches of experiments, the extract "Pancreophyt" was administered in a dose 300 mg / kg intragastrically for operated animals from the first day of experiment during 21 days. The research was conducted onthe 1st, 3rd, 7th, 14th and 21st days of experiment. The efficiency of pancreoprotective phytoremedy was estimated according to the dynamics of changes in activity of pancreatic enzyme –α-amylase in blood serum by enzymatic kinetic method using automatic biochemical analyzer «Sapphire-400" (Japan) and reagents from «Chronolab» company (Switzerland). [10] The number of leucocytes was measured using an automated hematology analyzer "Abacus" with a standard set of reagents (Austria), blood sedimentation rate –by Pachenkov’s method. [10] The significance of the differences between these parameters among experimental groups wasestimated using the nonparametric Mann-Whitney’s criteria.[11] The differences were considered significant at p≤0, 05.

The results showed that the use of Triton X-100 into the pancreas led to the development of pancreatitis, as evidenced by the increasing of α-amylase’s activity in serum of animal blood on the 1st day of experiment, in control and experimental groups in average 3 times compared with the data of intact group of animal (Table 2), which is an important diagnostic feature. In following periods of observation the activity of this enzyme continued to decline rapidlyin a control group, maintaining a high activity relatively to the index of intact group of rats on 21st

day of experiment. A protracted use of dry extract "Pancreophyt" for animals with a dose of 300 mg / kg affected by acute triton-induced pancreatitis caused a decrease of α-amylase's activity in blood serum from the 3rd day of experiment in average on 14%, and on the 21st day the difference between variables of experimental and control groups of animals was 24% (Table. 2).

The increase of blood sedimentation rate and the number of leucocytes compared to the intact group of animals indicated about the severity of inflammation in control group of animals (Table. 3). Affected by the use of "Pancreophyt" the number of white blood cells of animals was average on 25% lower than the variables of control animalson the 7th, 14th and 21st days. The reduction of blood sedimentation rate in 1.4 and 2.1 times, also indicated about the less expressed inflammatory processes during the application of investigated remedy on the 14th and 21st days in comparison to control. Thus, the research revealed tannins from 4.76 to 5.20% (including 0.12% of ellagic acid), flavonoids (including quercetin 0.10%, 0.67% rutin 0 18% luteolin, 0.009% luteolin-7-glucoside), polysaccharides from 4.0 to 4.2%, 0.04% oleanolic acid, ascorbic acid from 3.6 to 3.9% in a complex plant remedy "Pancreophyt". A dry extract "Pancreophyit" has an expressed pancreoprotective activity, reducing the activity of pancreatic enzymes and the intensity of inflammatory reactions duringtriton-induced pancreatitis of rats.

Table 1: The quantitative content of some biologically active substances in a complex plant remedy "Pancreophyt"

Substances	Content, %	Metrological characteristic
Tannins	4,76	f=7; $X_{av,}$%=4,76; p,%=95; t (p, f)=2,36; S = 0,0301; Δx=0,2336; E,%=±4.89
Polysaccharides	4,05	f=7; $X_{av,}$%=4,05; p,%=95; t (p, f)=2,36; S = 0,0221; Δx=0,1657; E,%=±4.09
Ascorbic acid	3,85	f=7; $X_{av,}$%=3,85; p,%=95; t (p, f)=2,36; S = 0,0254; Δx=0,0921; E,%=±2,39
Ellagicacid	0,12	f=4; $X_{av,}$%=0,12; p,%=95; t (p, f)=2,77; S = 0,000837; Δx=0,002317; E,%=±1,93
Oleanolic acid	0,04	f=4; $X_{av,}$%=0,04; p,%=95; t (p, f)=2,77; S = 0,000115; Δx=0,00032; E,%=±0,79
Quercetin	0,10	f=4; $X_{av,}$%=0,10; p,%=95; t (p, f)=2,77; S = 0,000178; Δx=0,000479; E,%=±1,19
Rutin	0,67	f=4; $X_{av,}$%=0,67; p,%=95; t (p, f)=2,77; S = 0,001786; Δx=0,004947; E,%=±0,74

Luteolin	0,18	f=4; $X_{av,}$%=0,18 p,%=95; t (p, f)=2,77; S = 0,001440; Δx=0,003988; E,%=±2,20
Luteolin-7-glycoside	0,01	f=4; $X_{av,}$%=0,01; p,%=95; t (p, f)=2,77; S = 0,000027; Δx=0,000075; E,%=±0,83

Table 2: The influence of dry extract "Pancreophyt" on α-amylase's activity in blood serum of white rats with triton-induced pancreatitis.

Observation time, days	Group of animals		
	Intact n=8	Control n=8	«Pancreophyt» n=8
1	624,1±22,8	1990,0±29,8	1913,2±32,8
3	665,9±14,2	1663,8±70,0	1446,2±53,0
7	750,1±22,9	1410,4±61,3	1220,0±102,5
14	698,1±26,1	1304,0±70,9	1105,4±70,7*
21	660,6±12,8	1155,0±57,3	871,1±25,3*

Note: *- the difference was statistically significant compared with variables in control at P ≤ 0,05; n –the number of animals in a group.

Table 3: The influence of dry extract "Pancreophyt" on peripheral blood variables of white rats with triton-induced pancreatitis.

Variables	Observation time, days	Group of animals		
		Intact n=8	Control n=8	«Pancreophyt» n=8
White blood cells, $x10^9$/L	1	4,1±0,07	10,4±0,6	10,5±0,6
	3	3,9±0,06	8,7±0,3	8,7±0,3
	7	4,0±0,06	8,3±0,2	6,4±0,7*
	14	4,1±0,06	8,0±0,5	6,0±0,4*
	21	4,1±0,05	6,9±0,6	5,1±0,5*
Blood sedimentation ratemm/h	1	0,9±0,06	5,0±0,3	5,2±0,4
	3	1,2±0,08	6,75±0,2	6,0±0,4
	7	1,3±0,08	6,4±0,6	5,8±0,2
	14	1,0±0,04	6,6±0,6	4,2±0,6*
	21	1,4±0,13	6,0±0,7	2,8±0,4*

Note: * - the difference is statistically significant compared with variables in control at P ≤ 0,05; n –the number of animals in the group.

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