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Research Article

Correction of Toxic Liver Damage with a Multicomponent Herbal Extract in an Animal Experiment

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ABSTRACT

Background: Digestive diseases constitute a significant part in the overall structure of human diseases. Herbal cholagogues are indicated for a treatment of chronic liver, gall bladder, and bile ducts diseases. The aim of the work is to determine the choleretic effect of a new multicomponent plant extract. **Materials and Methods:** Multicomponent plant extract was obtained from the following types of plant materials: 300 g of immortelle flowers (*Helichrysum arenarium* L.), 100 g of tansy flowers (*Tanacetum vulgare* L.), 100 g of rose fruits (*Rosa* sp.), 100 g of leaves of common nettle (*Urtica dioica* L.), 50 g of mint leaves (*Mentha piperita* L.), 50 g of licorice roots (*Glycyrrhiza glabra* L.). The extract was standardized by the total flavonoid content. It was calculated and expressed in terms of luteolin and isosalipurposide standards (total flavonoids content: not less than 4% and 15% respectively). The animal experiments being done in 80 nonlinear male rats with initial body weight 180-200 g. In order to study a choleretic effect of multicomponent herbal extract, naive rats recieved the single experimental dose of 250 mg/kg. Pharmacotherapeutic activity was studied in white rats with CCl_4-induced hepatitis. **Results:** Studies indicate a pronounced choleretic effect of the studied plant extract, that is comparable with the effect of "Allochol" in intact rats experiments. The course administration of a per os (peroral) multicomponent plant extract in a dose of 250 mg/kg to white non-linear rats with tetrachloromethane liver damage has a choleretic effect: it increases the rate of bile secretion, stimulates the synthesis and secretion of cholates with bile, and also the excretion of cholesterol and bilirubin. **Conclusion:** The obtained research results argue the feasibility of using a multicomponent plant extract containing biologically active substances of phenolic nature in the prevention and comprehensive treatment of liver diseases.

Key words: Multicomponent extract, Choleretic effect, Experimental hepatitis.

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INTRODUCTION

Digestive diseases constitute a significant part in the overall structure of human diseases. This diseases category is characterized by a relapsing course, functional disorders in the case of organic nature of disease.[1,2]

Herbal cholagogues are indicated for a treatment of chronic liver, gall bladder, and bile ducts diseases.[3] A range of herbal drugs with choleretic activity is not wide and includes species, tablets containing purified extracts of "Flamin" (Helichrysi arenarii floridis flavonoids), "Caleflonum" (Calendulae officinalis floridis extract) and "Chophytol" (Cynarae scomuli foliae extract), Chophytol oral solution, "Allochol" tablets *et al.*[4]

In view of this, the expansion of the list of herbal drugs with choleretic activity is a promising way. Within this framework, it is reasonable to create new effective plant-based medicines.[5-7]

As is well known, phytopreparations have a mild, moderate and natural (physiological) effect on the body, have a gradually, but steadily developing therapeutic effect, unlike synthetic drugs. Herbal remedies have a small number of contraindications or practically do not have them. When taking herbal remedies, side effects, cases of intolerance are observed relatively rarely.

From this perspective, the purpose of our study was determining the choleretic activity of multicomponent plant extract.

MATERIALS AND METHODS

Based on the literary analysis and data collected from a preliminary phytochemical study of plant material, the components of extract were substantiated including the contribution of every ingredient.[8-11]

The object of study was dry extract from plant material: 300 g of immortelle flowers (*Helichrysum arenarium* L.); 100 g of tansy flowers (*Tanacetum vulgare* L.); 100 g of rose fruits (*Rosa* sp. – Rosa majalis Herrn., Rosa acicularis Undl., Rosa canina L. and others); 100 g of leaves of common nettle (*Urtica dioica* L.); 50 g of mint leaves (*Mentha piperita* L.); 50 g of licorice roots (*Glycyrrhiza glabra* L.).

The components mixture was extracted with hot water (75-85° C). The resulting extract contained polysaccharides, flavonoids, carotenoids, organic acids, vitamins, macro-and microelements, essential oils, and other natural compounds. The total flavonoid content was calculated and expressed in terms of luteolin and isosalipurposide standards (total flavonoids content: not less than 4% and 15% respectively). This biologically active substances are known for potential choleretic activity of the extract.

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The study was carried out in accordance with the Russian Federations's Federal Law "On Circulation of Medicines", "Guidelines for Preclinical Trials of Medicinal Products". The experiments being done in 80 nonlinear male rats with initial body weight 180-200 g. Animals were received from Federal State-Funded Institution of Science "Scientific Centre of Biomedical Technologies" of Federal Medical and Biological Agency of Russia and kept in animal facility with free access to food and water. Pharmacological research was carried out in compliance with the Order of the Ministry of Health of the Russian Federation No. 199n of April 01, 2016 "On Approval of Rules of Good Laboratory Practice" and in accordance with GLP.[12] The studies were approved by local Bioethics Committee (Protocol No. 7 of October 01, 2018).

In order to study a choleretic effect of multicomponent herbal extract, naive rats recieved the single experimental dose of 250 mg/kg. "Allochol" (a herbal cholagogue) registered in Russian State Register of Medicinal Remedies was chosen as a standard medication at dose of 250 mg/kg.[4]

Bile samples were taken from anesthetized animal (thiopental sodium, 45 mg/kg) through polyethylene cannula inserted into the common bile duct. The samples were collected every hour during 4 hours. The cholagogue activity of the extract was measured by the speed of secretion and the quantity of the total excreted bile, and also by the levels of bilirubin, bile acids, and cholesterol.

Pharmacotherapeutic activity was studied in white rats with CCl_4-induced hepatitis. The water extract was given intragastrically at a dose of 250 mg/kg per day during 10 days starting from day 2 after impairing agent exposure. Liver damage was inducted by intragastric administration of 50% oil solution of tetrachloromethane (0,4 ml/100 g body weight per day during 4 days.[13] Control group received an appropriate amount of purified water on a similar basis. A measure of activity was performed on days 7, 14, 21, and 28 of experiment.

Statistical data processing was done by using Statistica software program version 6.0 (USA).[14] Differences were considered statically significant at $P \leq 0,05$.

RESULTS AND DISCUSSION

Pharmacological study of choleretic activity of the resulting extract compared to "Allochol" was carried out in intact white rats. The results are shown in the Tables 1-5.

From the results given in Table 1, it can be seen that the herbal extract at a specified dose has a stronger choleretic activity than "Allochol". Receiving the extract, the increase of bile excretion in white rats appeared quickly and lasted for 4-5 hours, while receiving "Allochol" the effect lasted for 3-4 hours.

We researched the effect of the extract and "Allochol" on the amount of excreted bile for 2-5 hours in the experiment. It was established that receiving the extract the amount of bile excreted increased in the midst of intense bile excretion. "Allochol" had a lower choleretic effect.

This appearing effect in rats is accompanied by changes in the levels of major bile components – bile acids, cholesterol and bilirubin. The levels of bile acids, cholesterol and bilirubin in the bile excreted increased after extract administration. Given the herbal extract white rats with toxic hepatitis had an increased bile excretion rate.

The achieved results indicate a significant effect of the studied extract on the choleretic activity in white rats with induced liver damage. So, on Day 7 the rate of bile excretion increased, on average, by 46%, compared with the control group (Table 6). On Day 14 the rate of bile excretion increased by 41,5%, Day 21 – 44%, and Day 28 – 38% (from hour 2 to 4).

The analysis of the bile composition showed an increase of bile acids, bilirubin and cholesterol under the influence of the studied extract.

Cholates level increased 2.5 times, 1.5 times, 2 times on Day 7-21 of the experiment. While white rats were receiving the studied extract, bile bilirubin excretion increased 2.3 times on Day 7, 1.5 times on Day 14-21 in comparison with control group. Amount of bile cholesterol increased by 50, 18 and 12% on Day 7, 14 and 21 respectively in comparison with control group (Table 7). Multicomponent herbal extract represses bile formation and excretion at early stages of liver damage. These positive changes lead to reduce the severity of pathological process.

It was established that per os administration of the extract at dose of 250 mg/kg during the course produce choleretic effect in white rats with CCl_4-induced liver damage. Effect of the extract precedes an effect of "Allochol" in a number of indicators. The presence of biologically active substances (particularly phenolics) induces a choleretic effect and subsequent increase of liver capacity.[15-22]

Table 1: The effect of multicomponent herbal extract on the rate of bile secretion in intact rats.

№	Drug name	Dose, mg/kg	Rate of bile secretion (mg/min per 100,0 g)					
			1 h.	2 h.	3 h.	4 h.	5 h.	6 h.
1.	Distilled water (control)	-	2,6 ± 0,2	2,8 ± 0,1	2,5 ± 0,1	2,7 ± 0,2	2,2 ± 0,2	1,8 ± 0,2
2.	Herbal extract	250	2,6 ± 0,3*	5,0 ± 0,4*	5,0 ± 0,3*	4,2 ± 0,2*	3,2 ± 0,2*	2,5 ± 0,1*
3.	"Allochol"	250	2,7 ± 0,2*	4,1 ± 0,3*	4,3 ± 0,2*	3,8 ± 0,3*	2,9 ± 0,4*	2,2 ± 0,2

Note: Here and elsewhere below asterisk * denote significant differences at $P<0,05$.

Table 2: Effect of a complex extract on the amount of bile excreted during hours 2-5 of the experiment in intact rats.

№	Drug name	Dose, mg/kg	Amount of bile excreted, mg/100 g				Total amount of bile excreted during 4 hours, mg/100 g
			2 h.	3 h.	4 h.	5 h.	
1.	Distilled water (control)	-	168	150	162	132	612
2.	Herbal extract	250	300	300	252	192	1044
3.	"Allochol"	250	246	258	228	174	906

Table 3: Effect of a multicomponent herbal extract and "Allochol" on the bile acids levels in intact rats.

№	Drug name	Dose, mg/kg	Amount of bile acids within 1 hour, mg/100 g				Total amount of bile acids during 4 hours, mg/100 g
			2 h.	3 h.	4 h.	5 h.	
1.	Distilled water (control)	-	1,51	1,23	1,30	1,01	5,05
2.	Herbal extract	250	3,09	2,76	2,14	1,54	9,53
3.	"Allochol"	250	2,58	2,37	1,94	1,40	8,29

Table 4: Effect of a multicomponent herbal extract and "Allochol" on the bile cholesterol levels in intact rats.

№	Drug name	Dose, mg/kg	Amount of cholesterol within 1 hour, mg/100,0 g				Total amount of cholesterol during 4 hours, mg/100,0 g
			2 h.	3 h.	4 h.	5 h.	
1.	Distilled water (control)	-	0,020	0,016	0,019	0,011	0,066
2.	Herbal extract	250	0,036	0,042	0,045	0,023	0,146
3.	"Allochol"	250	0,044	0,036	0,027	0,015	0,122

Table 5: Effect of a multicomponent herbal extract and "Allochol" on the bile bilirubin levels in intact rats.

№	Drug name	Dose, mg/kg	Amount of bilirubin within 1 hour, mg/100,0 g				Total amount of bilirubin during 4 hours, mg/100,0 g
			2 h.	3 h.	4 h.	5 h.	
1.	Distilled water (control)	-	0,018	0,015	0,015	0,013	0,061
2.	Herbal extract	250	0,033	0,030	0,030	0,023	0,116
3.	"Allochol"	250	0,027	0,025	0,026	0,017	0,095

Table 6: Bile excretion rate dynamics under effect of a multicomponent extract in white rats with experimental hepatitis induced by CCl_4.

Experimental conditions	Bile excretion rate during 4 hours, mg/min per 100 g of body weight			
	1 hour	2 hours	3 hours	4 hours
Intact rats	5,4 ± 0,3	5,2 ± 0,2	5,2 ± 0,4	5,2 ± 0,4
		Day 7		
Control rats (CCl_4 +H_2O)	3,7, ± 0,3	3,2 ± 0,3	2,9 ± 0,2	2,6 ± 0,2
Experimental rats (CCl_4 + an extract)	5,3 ± 0,2**	5,0 ± 0,3**	4,1 ± 0,4*	3,8 ± 0,3**
Experimental rats (CCl_4 + "Allochol")	5,2 ± 0,3*	4,8 ± 0,2*	4,0 ± 0,3	4,3 ± 0,2*
		Day 14		
Control rats (CCl_4 +H_2O)	4,4 ± 0,4	4,4 ± 0,3	4,3 ± 0,3	4,0 ± 0,4
Experimental rats (CCl_4 + an extract)	6,0 ± 0,5*	5,8 ± 0,4*	6,2 ± 0,3**	6,2 ± 0,2**
Experimental rats (CCl_4 + "Allochol")	5,8 ± 0,4	5,6 ± 0,3*	5,8 ± 0,4	5,8 ± 0,3
		Day 21		
Control rats (CCl_4 +H_2O)	4,3 ± 0,1	4,0 ± 0,2	4,5 ± 0,2	3,9 ± 0,2
Experimental rats (CCl_4 + an extract)	6,6 ± 0,3**	6,0 ± 0,5**	5,8 ± 0,4*	5,6 ± 0,5**
Experimental rats (CCl_4 + "Allochol")	6,2 ± 0,2*	5,7 ± 0,4	5,5 ± 0,3*	5,3 ± 0,3*
		Day 28		
Control rats (CCl_4 +H_2O)	4,7 ± 0,4	4,4 ± 0,4	4,4 ± 0,4	3,3 ± 0,3
Experimental rats (CCl_4 + an extract)	4,8 ± 0,2	5,5 ± 0,1*	5,6 ± 0,3**	5,0 ± 0,1**
Experimental rats (CCl_4 + "Allochol")	4,5 ± 0,2	5,2 ± 0,2*	5,3 ± 0,3*	4,7 ± 0,2*

Note: Here and elsewhere below asterisk *denote that the differences between control and experimental groups are significant at $P < 0,05$: asterisks **denote that the differences between control and experimental groups are significant at $P < 0,01$.

Table 7: Bile composition dynamics under the influence of multicomponent herbal extract in white rats with experimental hepatitis induced by CCl_4.

Experimental conditions	Total amount of bile during hours 1-4	Total amount of bile acids	Bilirubin	Cholesterol
		mg/ 100 g body weight		
Intact rats	1202 ± 77	6,1 ± 0,5	0,10	0,11
	Day 7			
Control rats (CCl_4 +H_2O)	818 ± 34	3,13 ± 0,2	0,040	0,050
Experimental rats (CCl_4 + an extract)	1055 ± 82*	7,79 ± 0,4**	0,092	0,076
Experimental rats (CCl_4 + "Allochol")	1000 ± 70*	7,0 ± 0,3*	0,080	0,065
	Day 14			
Control rats (CCl_4 +H_2O)	980 ± 11	5,71 ± 0,3	0,074	0,078
Experimental rats (CCl_4 + an extract)	1310 ± 105**	8,30 ± 0,2**	0,113	0,092
Experimental rats (CCl_4 + "Allochol")	1200 ± 80*	7,8 ± 0,2*	0,92	0,087
	Day 21			
Control rats (CCl_4 +H_2O)	1011 ± 51	4,12 ± 0,3	0,076	0,080
Experimental rats (CCl_4 + an extract)	1415 ± 57**	8,11 ± 0,4**	0,111	0,090
Experimental rats (CCl_4 + "Allochol")	1315 ± 45*	7,21 ± 0,3**	0,095	0,085
	Day 28			
Control rats (CCl_4 +H_2O)	1086 ± 86	6,77 ± 0,4	0,110	0,076
Experimental rats (CCl_4 + an extract)	1181 ± 30	6,12 ± 0,2	0,113	0,076
Experimental rats (CCl_4 + "Allochol")	1100 ± 40	6,32 ± 0,3	0,10	0,076

CONCLUSION

Altogether, these data demonstrate a significant choleretic effect of a herbal extract, which is comparable to effect of "Allochol" in experiment in intact rats. Per os administration of the extract at dose of 250 mg/ kg during the course produce choleretic effect in white rats with CCl_4-induced liver damage. The extract increases the rate of bile excretion, stimulates synthesis and excretion of the cholates, and also induces excretion of cholesterol and bilirubin. The given results prove that it is reasonable to use the multicomponent herbal extract containing biologically active substances of phenolic nature for combined therapy and preventive care of hepatobiliary system diseases.

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CONFLICTS OF INTEREST

None.

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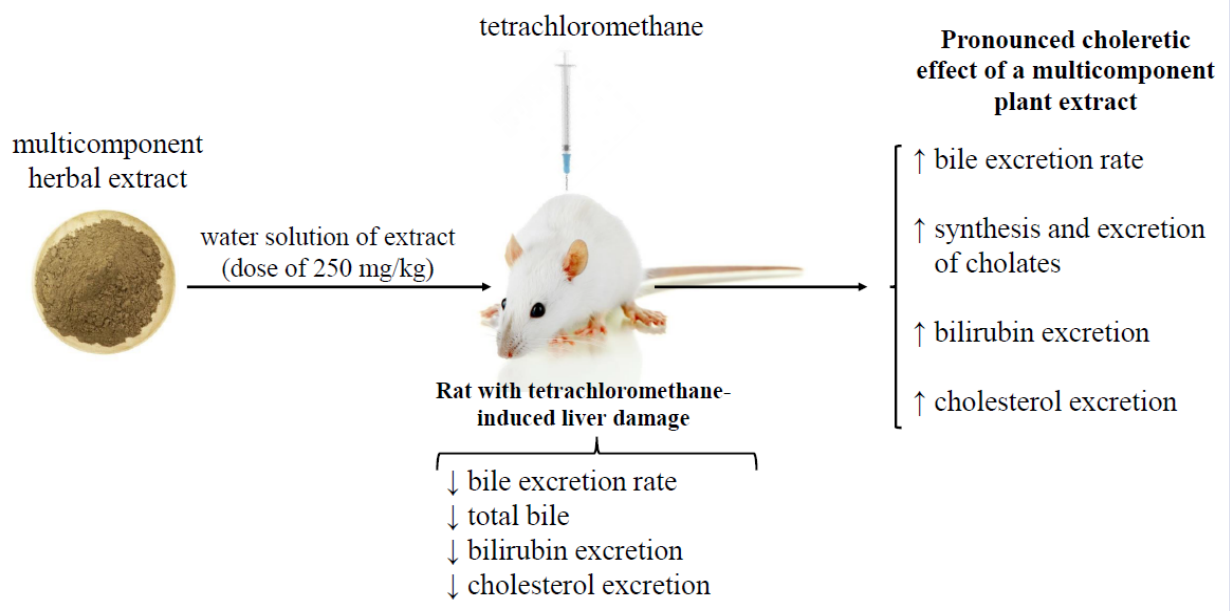
GRAPHICAL ABSTRACT



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