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CHOLERETIC EFFECT OF EXTRACTS OF *CARTHAMUS TINCTORIUS* L., *TAGETES ERECTA* L. AND *CALENDULA OFFICINALIS* L.

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The aim of the study was to determine the choleretic effect of extracts of safflower dye (*Carthamus tinctorius* L.), African marigold (*Tagetes erecta* L.) and pot marigold (*Calendula officinalis* L.) in experiments with intact white male rats of the Wistar line initially weighing 180–200 g. The obtained extracts in experimental therapeutic doses of 50–200 mg/kg were injected as aqueous solution into the duodenum of anesthetized animals (40 mg/kg of sodium thiopental, abdominally). For control group the equi-volume amount of distilled water was administered. It was established that extracts of safflower and pot marigold have a pronounced choleretic effect when administered once, due to the significant content of phenolic substances i.e. flavonoids.

Keywords: safflower dye (*Carthamus tinctorius* L.), African marigold (*Tagetes erecta* L.), pot marigold (*Calendula officinalis* L.), extracts, choleretic effect

One of the ways to supplement domestic herbal medicines is to study the pharmacological effects of well-known plants that are often used by the public, as well as pharmacopoeial species used in clinical practice for restricted indications.

The presence of a wide range of biologically active substances in them implies a multi-sided effect on the human body, affecting many functional systems. In this regard, additional studies of their chemical composition and pharmacological action are required, which will allow to identify the additional properties and to expand the indications for their use in clinical and preventive medicine. In this aspect, safflower dye (*Carthamus tinctorius* L.), African marigold (*Tagetes erecta* L.) and pot marigold (*Calendula officinalis* L.) are of interest.

The purpose of this study was to determine the choleretic effect of extracts of *Carthamus tinctorius* L., *Tagetes erecta* L. и *Calendula officinalis* L. in experimental conditions.

MATERIALS AND METHODS

Dry extracts made of these types of plant raw materials were obtained in the laboratory of chemical and pharmaceutical studies of the Institute of General and Experimental Biology of the SBRAS by Doctor of Pharmaceutical Sciences Olennikov D.N. and Candidate of Pharmaceutical Sciences Kashchenko N.I. in 2017 by extracting

the plant material (above-ground part) with 70% ethanol in the "raw material: extractant" ratio equal to 1: 15 using ultrasonic treatment for 60 minutes with sequential concentrating and drying in a vacuum drying cabinet.

The experiments were performed using intact white male Wistar rats with an initial weight of 180–200 g, obtained from the Scientific Center for Biomedical Technologies of the Russian Federation. The animals were kept in a certified vivarium of the Institute of General and Experimental Biology SB RAS with free access to water and food. When studying, we were guided by the requirements of the following regulatory documents: Guidelines for pre-clinical studies of medicinal products (2012); Order of the Ministry of Health of the Russian Federation No. 199n "On approval of the rules of good laboratory practice" dated 01.04.2016; Rules for performance of work using experimental animals; Rules adopted by the European Convention for protection of vertebrates used for experimental and other scientific purposes (Strasbourg, 1986). Bile was collected from anesthetized animals (sodium thiopental, 40 mg/kg, abdominally) every hour for 4 consecutive hours. Dry extracts made of these types of plant raw materials were administered to rats of the corresponding group directly into duodenum at doses of 50, 100 and 200 mg/kg in the form of aqueous solution. In the control group, the equi-volume amount of distilled water was injected to animals into the duodenum. The severity of the choleric effect of these extracts was estimated by the rate of secretion and the total amount of bile secreted, as well as by the content of its main ingredients such as bile acids, cholesterol and bilirubin [2,4,5]. Significance of differences in the data of experimental and control groups of animals was evaluated using the nonparametric Mann-Whitney U test. The study protocol was approved by the Ethics Committee of the Institute of General and Experimental Biology SB RAS (Protocol No. 3 of 02.02.2018).

RESULTS AND DISCUSSION

The data obtained during the study is presented in tables 1 and 2. Based on this data, it follows that the extract of calendula officinalis at a dose of 50 mg/kg increases the rate of bile secretion in rats by 16.0–44.0%; at a dose of 100 mg/kg – by 28.0–44.0% with increase in secreted bile by 35.0%. When administered to rats at a dose of 200 mg/kg, the rate of bile secretion increased by 33.0–36.0%, and the content of cholates exceeded the control data by 17.5–30.0% with increase in the excretion of cholesterol with bile (Tables 1, 2). The obtained data on the choleric effect of the obtained extract of calendula officinalis are consistent with the works [1, 3], testifying to the choleric properties of extracts from this raw material.

Administration of safflower dye extract to another group of rats was also accompanied by acceleration of bile secretion in animals. Thus, at a dose of 100 mg/kg, the rate of secretion increased by 24.0–29.3%, and at a dose of 200 mg/kg – up to 31.0%. Along with this, in this experiment, there was a tendency for active synthesis of cholates by hepatocytes, and at a dose of 50 mg/kg, increase in concentration of cholesterol in the secreted bile in rats was observed (Tables 1, 2).

The dry extract of African marigold in our experiments did not show a significant effect on the rate of bile secretion in intact rats, as well as on the total content of cholates in bile. It was noted only a moderate effect on the synthesis by hepatocytes and emission of cholates in bile. To some extent, there was acceleration of cholesterol excretion with bile in animals when African marigold extract was administered at the specified doses (Tables 1, 2).

Thus, the administration of pot marigold extract in experimental therapeutic doses of 50–200 mg/kg into duodenum of white rats anesthetized with sodium thiopental is characterized by a pronounced choleric effect with increase in the rate of bile secretion

Table 1

EFFECT OF PLANT EXTRACTS ON THE RATE OF BILE SECRETION IN WHITE RATS

Experimental conditions	The rate of bile secretion for 4 hours, mg/min per 100 g			
	1 h	2 h	3 h	4 h
1. Control (H ₂ O), n=8 2. African marigold: 50 mg/kg, n=8 100 mg/kg, n=8 200 mg/kg, n=8	5,2+0,3 5,6+0,4 5,5+0,3 6,0+0,3	5,3+0,4 5,6+0,4 5,5+0,2 6,0+0,3	5,0+0,3 5,1+0,3 5,2+0,3 6,2+0,4	5,2+0,3 4,5+0,2 4,7+0,2 5,7+0,3
1. Control (H ₂ O), n=8 2. Safflower dye: 50 mg/kg, n=8 100 mg/kg, n=8 200 mg/kg, n=8	6,2+0,3 6,2+0,3 6,4+0,2 6,6+0,4	5,8+0,4 6,1+0,3 7,5+0,1* 7,8+0,2*	5,2+0,4 5,3+0,2 6,4+0,2* 6,3+0,3*	5,2+0,3 5,3+0,3 6,2+0,2* 6,0+0,3
1. Control (H ₂ O), n=8 2. Pot marigold: 50 mg/kg, n=8 100 mg/kg, n=8 200 mg/kg, n=8	5,7+0,2 5,1+0,1 5,8+0,4 5,2+0,2	5,0+0,2 5,8+0,2 6,4+0,4* 5,9+0,4	4,8+0,2 6,6+0,5* 6,5+0,4* 6,4+0,4*	4,1+0,3 5,9+0,4* 5,9+0,4* 5,6+0,4*

Note: * – Hereinafter it means that the difference between the experimental and control data is significant at $P < 0.05$; n is the number of animals in the group.

and increase in the concentration of cholates in the secret. The identified choleric effect of the resulting extract is due to the content of a complex of biologically active substances, primarily flavonoids. Thus, the pot marigold extract contains up to 40.0% of flavonoids, carotenoids, terpenoids, as well as other natural compounds [1], which provide its choleric effect. The resulting dry extract of safflower dye shows the presence of luteolin, neocartamine and kaempferol derivatives, as well as contains halcones, which, along with other natural compounds [6], stipulate the observed choleric effect in white rats. The dry extract of African marigold showed a moderate choleric effect in our experiments, despite the significant content of flavonoids such as quercetetrin, patuletin, patulitrin, quercetetrin [7].

As you know, most flavonoids are characterized by a wide range of their effects on the body's functions. In particular, they stimulate the choleric reaction, have an anti-inflammatory effect, reduce the tone of smooth muscles, provide energy production in cells due to stabilization of membrane formations against the inhibition of free radical processes and mobilization of the endogenous antioxidant defense system [3]. Obviously, the content of significant amounts of flavonoids in extracts of pot marigold and safflower dye provides a pronounced choleric effect in white rats when the extract is directly administered into duodenum. The obtained study results can serve as a basis for expanding the indications for the use of extracts of pot marigold and safflower dye in clinical and

**EFFECT OF PLANT EXTRACTS ON THE TOTAL AMOUNT
AND BIOCHEMICAL COMPOSITION OF BILE IN WHITE RATS**

Experimental conditions	Total amount of bile for 2–4 hours of experiment	Bile acids	Bilirubin	Cholesterol
	mg/100 g	mg%		
1. Control (H ₂ O), n=8	930+36,1	507,3	14,0	54,5
2. African marigold:				
50 mg/kg, n=8	912+35,7	587,1	13,0	69,6
100 mg/kg, n=8	924+35,3	559,1	14,0	52,8
200 mg/kg, n=8	1074+56,4	564,3	10,0	56,3
1. Control (H ₂ O), n=8	972+37,0	832,2	16,0	85,1
2. Safflower dye:				
50 mg/kg, n=8	1002±38,6	934,8	17,0	115,9
100 mg/kg, n=8	1206±30,4*	877,8	15,0	97,2
200 mg/kg, n=8	1194+40,1*	866,4	17,0	74,3
1. Control (H ₂ O), n=8	834+36,0	552,9	24,0	22,8
2. Pot marigold:				
50 mg/kg, n=8	1098+34,0*	649,8	21,0	25,1
100 mg/kg, n=8	1128+39,0*	718,2	21,0	35,0
200 mg/kg, n=8	1056+41,0*	706,8	22,0	26,6

preventive medicine, as well as for their use at the stage of rehabilitation treatment in health resort organizations.

CONCLUSION

1. Pot marigold extract has a pronounced choleric effect at experimental therapeutic doses in intact white rats due to significant content of flavonoids and other natural compounds.

2. Administration of safflower dye extract to white rats at experimental therapeutic doses is accompanied by acceleration of the choleric reaction with increase of cholates in the secreted bile, which is due to the content of a complex of biologically active substances, including substances of phenolic nature.

3. The African marigold extract in our experiments did not show a statistically significant effect on the course of the choleric reaction in intact white rats; there was a certain tendency to increase of bile acids and cholesterol in the secreted bile, which requires additional studies.

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