Anti-opisthorchiasis activity

some species of the genus Cornflower (Centaurea) of the flora of Western Siberia

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Antiopisthorchiasis activity of some Siberian flora Centaurea species

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SUMMARY

In experiments on golden male hamsters, the anti-opisthorchiasis activity (against Opisthorchis felineus) of extracts obtained from Centaurea scabiosa L., Centaurea pseudomaculosa Dobrocz., Centaurea jaceae L. and Centaurea phrygia L. of the Asteraceae family was studied. The presence of anti-opisthorchiasis activity was confirmed in the sesquiterpene lactone of the guaian type, cinaropicrin, isolated from Centaurea scabiosa L. It was suggested that the sesquiterpene lactones grosshemin and repin have anti-opisthorchiasis activity.

Key words: Opisthorchiasis, Opisthorchis felineus, Centaurea scabiosa, Centaurea pseudomaculosa, Centaurea jaceae, Centaurea Phrygia, sesquiterpene lactones, cynaropicrin, anti-opisthorchiasis activity.

RESUME

In experiments on the golden hamstersmales antiopisthorchiasis activity (with respect to Opisthorchis felineus) of extracts obtained from Centaurea scabiosa L., Centaurea pseudomaculosa Dobrocz., Centaurea jaceae L. and Centaurea phrygia L. Asteraceae family was investigated. Antiopisthorchiasis activity of sesquiterpene lactone guainolidetype cynaropicrin isolated from Centaurea scabiosa L. was confirmed. It's suggested that sesquiterpene lactones grosshemin and repin has an antihelminthic activity.

Keywords: Opisthorchis felineus, Centaurea scabiosa, Centaurea pseudomaculosa, Centaurea jaceae, Centaurea Phrygia, sesquiterpene lactones, cynaropicrin, antiopisthorchiasis activity.

INTRODUCTION

In the last decade, hepatic helminth infections (enterobiasis, ascariasis, giardiasis, opisthorchiasis, etc.) are gradually turning from a local problem in individual regions into a widespread problem of a global nature. This trend may be associated with the increasing intensity of traffic flows and the migration of infected carriers [1].

Opisthorchiasis, the causative agents of which are Opisthorchis felineus and Opisthorchis viverrini -trematodes belonging to the Opisthorchiidae family are classified as zooanthroponotic diseases [2]. The degree of infection with this helminthiasis is determined by ethnic or traditional dietary habits and the consumption of non-disinfected fish. The world's largest focus of opisthorchiasis has formed in the Ob-Irtysh river basin. In Russia, the area of opisthorchiasis caused byO. felineus [3], stretches from the Yenisei basin to the western borders of Europe. A number of cases of infection with the indicated helminth were noted in the EU countries: Italy, Germany, Poland, Portugal and Spain [4]. Foci of opisthorchiasis caused byOpistorchis viverrini, available in countriesSoutheast Asia (Thailand, Laos, Vietnam, Cambodia) [5].

The huge invasion of the population of different regions and countries is a serious hygienic, environmental and social problem. It is known that opisthorchiasis invasion is accompanied by hepatomegaly, cholangitis, fibrosis of the periportal system, cholecystitis, and other diseases [6]. In addition, by the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO), the causative agent of opisthorchiasis is classified as the first group of carcinogens, because is an etiological factor in the development of bile duct cancer and cholangiocarcinoma [7].

In the treatment of opisthorchiasis of any origin, the synthetic antiparasitic drug praziquantel is used, which has a number of side effects and contraindications [8]. In this regard, the relevance of the search for highly effective and low-toxic drugs is increasing.

for the treatment of opisthorchiasis. Despite the existing tools for modeling the chemical structure of a substance depending on the properties of a molecular target, screening for molecules of natural origin is still relevant and is a source of many promising drug candidates. This is mainly due to the fact that the native chemical structure of such compounds, due to the proximity of the metabolism of plant and animal cells, is capable of providing effective pharmacological interaction, along with a low probability of occurrence of undesirable side reactions of the body.

The group of sesquiterpene lactones produced by plants of the Asteraceae family is one of the striking examples of biologically active substances that have found wide application in medical practice. Of greatest interest are antiparasitic (opisthorchiasis, giardiasis, malaria) and antitumor types of activity of sesquiterpene lactones [9].

This article is devoted to a review of the results of experimental anti-opisthorchiasis activity of sesquiterpenoid-containing plants of the genus Centaurea as wellthe activity of their individual compounds.

MATERIALS AND METHODS

Research objects: Centaurea scabiosa L. (CS), Centaurea pseudomaculosa Dobrocz. (CP), Centaurea jaceae L. (CJ) and Centaurea phrygia L. (CPH).

The aboveground part of these plants was collected in the phase of mass flowering in various regions of the Siberian Federal District of Russia during 2015: CS and CP - in the Tomsk region (outskirts of Tomsk); CJ and CPH - in the Kemerovo region (near the village of Novobalakhonovka). The plant material was dried using an air-shade method and crushed to a particle size of no more than 2 mm.

Extractive plant complexes were obtained by bismaceration using purified water, 40% and 70% ethyl alcohol. The solvents from the obtained extracts were removed under vacuum in a rotary evaporator at temperatures up to 50 -C.

Dry extracts of CS, CP, CJ, and CPH in 70% ethanol were a dark green viscous mass with a specific odor, and upon extraction with 40% ethanol and water, brown powders with a specific odor. The detection of sesquiterpene lactones in dry extracts was carried out by high performance liquid chromatography on a HEWLETT PACKARD Agilent 1100 Series (USA) in isocratic mode under the following conditions: analytical column filled with Zorbax SBC18 sorbent (4.6 - 150 mm, particle size - 5 μm); the composition of the mobile phase: methanol - water in a 50:50 ratio; detection at a wavelength of 204 nm; column temperature - 25 -C; the speed of the mobile phase is 0.5 ml / min; the volume of the injected sample is 20 μl.

To isolate cynaropicrin, which, according to the literature, can exhibit specific anti-opisthorchiasis activity [10], the crushed CS raw material was subjected to 3-fold extraction with a 4: 1 chloroform ethanol mixture at a feed-to-extract ratio of 1: 8, 1: 7, 1: 7 and heating to 60 - WITH. The duration of the first extraction was 2 h, the subsequent two - 1 h. The chloroform-ethanol extracts were combined and the solvent was removed on a rotary evaporator at a temperature of 40 -C. The obtained condensed extracts were dissolved in 2 parts of hot ethanol, 1 part of water heated to 70 -C was added and left in a dark place for a day. The separated precipitate was filtered off and treated twice under the indicated conditions.

Separation of the fraction of sesquiterpene lactones was carried out by column chromatography on KSK silica gel with a particle size of 120/220 μm. The eluent was a mixture of petroleum ether with an increasing gradient of ethyl acetate. The separation was monitored by chromatography in a thin layer on Sorbfil PTSKhPAUF plates (Russia) in the mobile phase of hexaneacetoneacetic acid (20: 10: 0.1). For comparison, we used working standard samples of sesquiterpenoids, isolated and identified by us earlier. When the column was eluted with a mixture of petroleum ether and ethyl acetate in a ratio of 80:20, fractions were obtained

containing the guaian-type sesquiterpene lactone cinaropicrin [11–13]. The purity of the isolated compound, determined by high performance thin layer chromatography, was 85.4%.

The model of experimental opisthorchiasis was reproduced on golden male hamsters weighing 40–45 g according to the generally accepted method [14]. To do this, from the infectedO. felineus fish, mainlydace and roach were isolated metacercariae by digestion in artificial gastric juice O. felineus. Infection of animals was carried out by intragastric administration of viablemetacercariae in the amount of 50 copies. in 0.5 ml of isotonic sodium chloride solution. Golden hamsters were kept in vivarium under natural light conditions on a standard diet with free access to food and water.

On the 30th day of invasion, a control necropsy was performed on several animals. As a result of the study of the organs of the hepatobiliary system (liver, gallbladder), a significant number of opisthorchiasis marits were revealed, which confirmed the fact of the development of opisthorchiasis.

The anti-opisthorchiasis effect of dry extracts of CS, CP, CJ, and CPH obtained in 70%, 40% ethanol and purified water, as well as cynaropicrin, was evaluated on 120 infected animals, the weight of which increased to 90–110 g on the 30th day of invasion.

The experimental animals were divided into 11 groups: group No. 1 (intact) - healthy animals; group No. 2 (control) - animals with opisthorchiasis. Group No. 3 - animals with opisthorchiasis who received the reference drug - praziquantel, in the form of a suspension of 1% starch mucus at a dose of 20 mg / kg of animal weight 2 times a day for 1 day. This dose and the scheme of administration are most effective in the treatment of opisthorchiasis caused byO. felineus [15].

Groups Nos. 4–6 - animals with opisthorchiasis treated with dry CS extracts in 70%, 40% ethanol and purified water, respectively.

Groups Nos. 7, 8 - animals with opisthorchiasis treated with dry CP extracts in 70% and 40% ethanol, respectively.

Groups No. 9, 10 - animals with opisthorchiasis treated with dry extracts of CJ in 70% and 40% ethanol, respectively.

Group No. 11 - animals with opisthorchiasis treated with CPH dry extract in 40% ethanol.

Since these extracts are non-toxic (LD_{50} more than 5000 mg / kg), then they were administered at the maximum possible single dose of 2.0 g / kg, limited by the physiological capabilities of experimental animals, in the form of a suspension of 1% starch mucus three times a day for five days.

Group No. 12 - animals with opisthorchiasis who received cinaropicrin at a dose of 40 mg / kg of animal weight in the form of a suspension in apricot oil, similar to the administration of praziquantel - twice a day for one day.

All studied samples were injected intragastrically through a metal probe; the latency period after the end of the course of treatment was 14 days. During the specified time, there is a natural excretion of marite opisthorchus, which have lost their viability as a result of treatment. After this period, the animals were euthanized with carbon dioxide, the liver was removed, and the opisthorchiasis marits were counted in the organs of the hepatobiliary system (the intensity coefficient was calculated, which is a criterion for anti-opisthorchiasis activity) [14]:

where K is the average number of maritas O. felineus in the control group;O - the average number of marits of opisthorchus in the experimental group.

RESULTS

As a result of the study, it was found that extracts have the greatest anti-opisthorchiasis activity. C. scabiosa, C. pseudomaculosa and C. jaceae obtained using inas an extractant 40% ethanol: the coefficient of IE was 71.6%, 69.7% and 68.11% (Table 1). At the same time, the CS extract showed a level of activity comparable to that of the reference drug praziquantel (IE praziquantel - 75.31%).

Extract C. scabiosa obtained on 70% ethanol, showed slightly less activity - its intensity rate was 64.11%. Water extractC. scabiosa showed moderateanti-opisthorchiasis activity (IE indicator - 53.13%).

The lowest indicators of anti-opisthorchiasis activity were demonstrated by extracts C. pseudomaculosa and C. jaceae obtained on 70% ethanol: their IE values were 32.86 and 30.33%, respectively. ExtractC. phrygia showed practically no activity (IE - 14.28%).

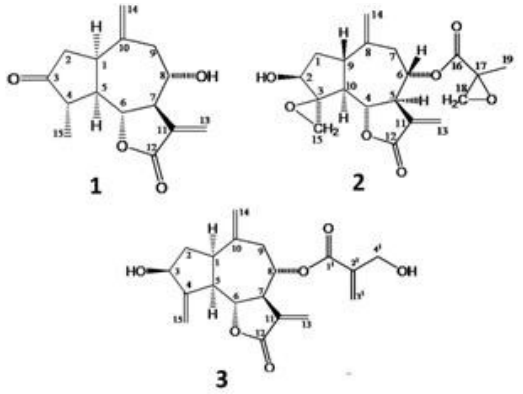
Table 1

Results of a study of the anti-opisthorchiasis activity of extractive complexesCentaurea scabiosa L., Centaurea pseudomaculosa Dobrocz., Centaurea jaceae L., Centaurea phrygia L.

Experimental group (test sample)	Qty animals, PCS.	Dose, g / kg (introduction scheme)	IE,%
Group # 1 (intact - healthy animals)	ten	-	-
Group no. 2 (control - animals with opisthorchiasis)	ten	-	-
Group No. 3 (praziquantel)	ten	0.02 (2 times a day - 1 day)	75.31 ± 1.24
Group No. 4 (70% ethanol extract CS)	ten	2.0 (3 times a day - 5 days)	64.11 ± 2.17
Group No. 5 (40% ethanol extract CS)	ten	2.0 (3 times a day - 5 days)	71.6 ± 2.99
Group No. 6 (aqueous extract CS)	ten	2.0 (3 times a day - 5 days)	53.13 ± 1.84
Group No. 7 (70% ethanol extract CP)	ten	2.0 (3 times a day - 5 days)	32.86 ± 2.55
Group No. 8 (40% ethanol extract CP)	ten	2.0 (3 times a day - 5 days)	69.70 ± 2.79
Group No. 9 (70% ethanol extract of CJ)	ten	2.0 (3 times a day - 5 days)	30.33 ± 1.87
Group No. 10 (40% CJ ethanol extract)	ten	2.0 (3 times a day - 5 days)	68.11 ± 3.45
Group No. 11 (40% ethanol extract of CPH)	ten	2.0 (3 times a day - 5 days)	14.28 ± 8.08
Group No. 12 (cynaropicrin)	ten	0.04 (2 times a day - 1 day)	34.11 ± 4.95

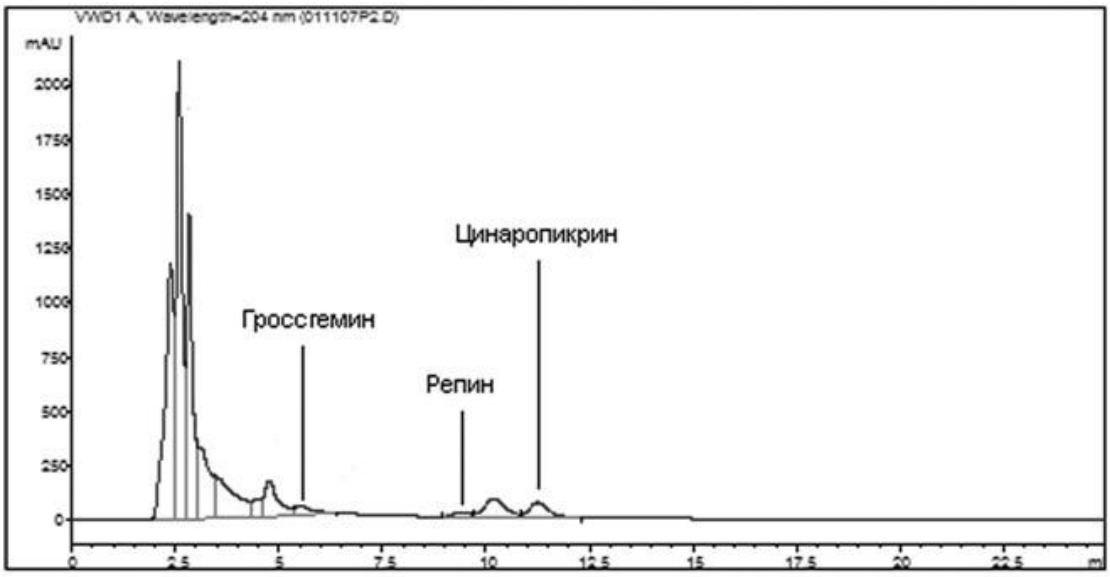
DISCUSSION

To substantiate the results obtained for determining the anti-opisthorchiasis activity, a comparative study of the chemical composition of the most effective extracts was carried out. C. scabiosa and C. pseudomaculosa by HPLC. As part of an extractive complexCS obtained using 40% ethanol, three guayan-type sesquiterpene lactones were found - grosshemin, repin, and cinaropicrin (Fig. 1) with a quantitative content of 0.83 in the sample; 0.45 and 1.23%, respectively (Fig. 2). The CP extract obtained with the same solvent contains only grosshemin and cinaropicrin with a quantitative content of 0.7% and 1.2% in the sample, respectively (Fig. 3).

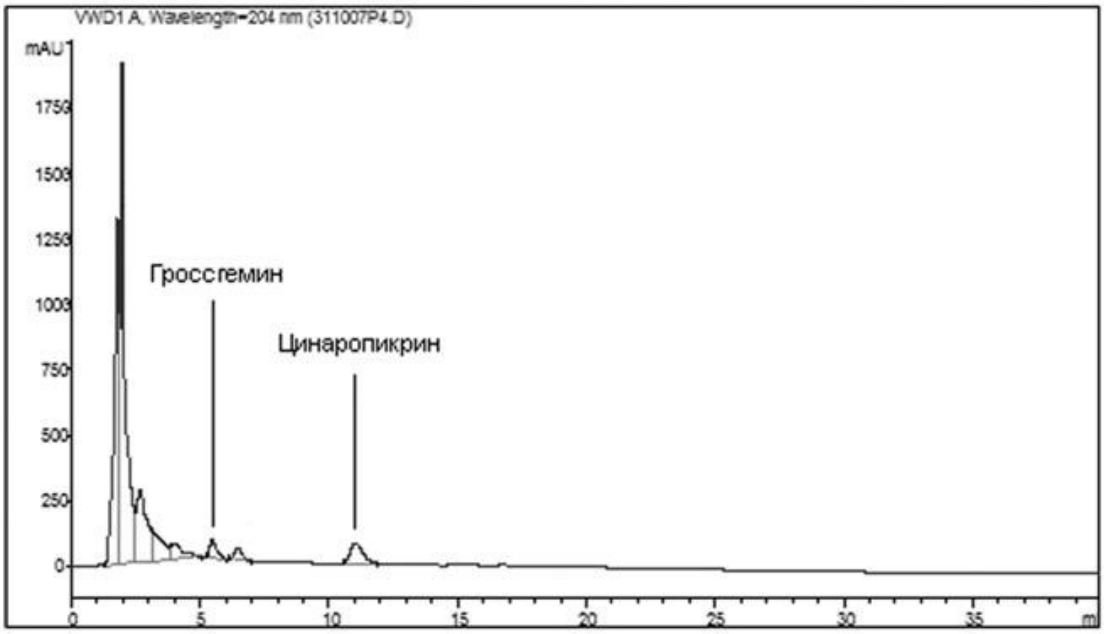


Rice. 1. Structural formulas of sesquiterpene lactones found in plants of the genusCentaurea (1 - grosshemin; 2 - repin; 3 - cynaropicrin).

These sesquiterpene lactones are known compounds and are found in many members of the genus Centaurea. Grossgemin [12], repin [13] and cinaropicrin [14] have a very similar chemical structure (Fig. 3) and, probably, are secondary metabolites of one biochemical pathway of the studied plants.



Rice. 2. Chromatogram of the extractCentaurea scabiosa on 40% ethanol (retention times of grossgemin - 5.52 minutes, repin - 9.4 minutes, cinaropicrin - 11.21 minutes).



Rice. 3. Chromatogram of the extractCentaurea pseudomaculosa on 40% ethanol (retention times of grosshemin - 6.08 minutes, cinaropicrin - 11.65 minutes).

To confirm the literature data on the anti-opisthorchiasis activity of cinaropicrin, it was isolated from C. scabiosa followed by a pharmacological study.In experiment in vivo, the anti-opisthorchiasis effect of isolatedcinaropicrin (the rate of intensity was 34.11%).

Thus, the results of a chemopharmacological study do not demonstrate a clear dependence of the anti-opisthorchiasis activity of the most effective extractive complexes CS and CP with the presence of cynaropicrin in their composition. This suggests that not only this substance contributes to the target activity.

Taking into account the similarity of the chemical structure of the sesquiterpene lactones contained in CS and CP extracts, it is possible that all these compounds have anti-opisthorchiasis activity. Thus, the presence of three sesquiterpene lactones (cinaropicrin, grosshemin, and repin) in a 40% ethanol extract of CS causes a higher activity of this sample compared to the CP sample, in which there is no repin, and, accordingly, the total quantitative content of sesquiterpenoids is lower.

On the other hand, the efficiency of isolated cynaropicrin in the experiment turned out to be significantly lower than the efficiency of the extractive complexes CS and CP. This is probably due to the complex effect of biologically active substances that make up these plants on various links in the pathogenesis of opisthorchiasis. Sesquiterpene lactones, in particular cinaropicrin, are capable of exerting a direct effect on the maritides of opisthorchids, as a result of which the latter die or their vital activity is significantly impaired, which leads to their detachment from the walls of the bile ducts of the liver. Along with sesquiterpene lactones, the extracts of these plants include phenolic compounds, polysaccharides, carotenoids, ascorbic acid, etc., exhibiting antioxidant, hepatoprotective, choleretic, detoxifying, anti-inflammatory and other types of activity.

In this regard, preliminary studies allow us to consider Centaurea scabiosa L.,Centaurea pseudomaculosa Dobrocz., Centaurea jaceae L. promising for further study in order to create new drugs for the treatment of opisthorchiasis.

CONCLUSION AND CONCLUSIONS

As a result of research in conditions in vivo high rates

anti-opisthorchiasis activity of extracts Centaurea scabiosa, Centaurea pseudomaculosa and Centaurea jaceae on 40% ethanol (IE - 71.6%, 69.7% and 68.11%, respectively). Similar extract Centaurea Phrygia showed no anti-opisthorchiasis activity. Confirmedpreviously described in the literature specific activity of cynaropicrin (IE - 34.11%) by HPLC in the most active extract Centaurea scabiosa sesquiterpene lactones discoveredguayana type: grosshemin (0.83%), repin (0.45%) and cinaropicrin (1.23%); in extractCentaurea pseudomaculosa (40% ethanol) - grosshemin (0.7%) and cinaropicrin (1.2%). Experimentally substantiated the assumption about the possible presence of anti-opisthorchiasis activity in other sesquiterpene lactones of plants of the genusCentaurea.

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