Composition and biological activity of polysaccharides of shoots and leaves of common raspberry (Rubus idaeus L.) D.K. Gulyaev, V.D. Belonogov, I.P. Rudakova (Perm State Pharmaceutical Academy of the Ministry of Health of the Russian Federation, Perm)

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SUMMARY

The article presents the results of a study of the monosaccharide composition of the water-soluble polysaccharide complex of shoots and leaves of common raspberry (Rubus idaeus L.). Using ascending chromatography on paper, it was found that the polysaccharide complex of raspberry shoots and leaves consists of arabinose, galactose and glucose. As a result of the study of acute toxicity according to the method of Prozorovsky V.V., it was found that the water-soluble polysaccharide complex of raspberry shoots and leaves belongs to the class of low-toxic substances, with LD50 - 5000 mg / kg. The anti-inflammatory activity of the polysaccharide complex of raspberry leaves was higher than that of raspberry shoots. Inhibition of the inflammatory response by the polysaccharide complex of raspberry leaves was 41.1%.

Keywords: common raspberry, shoots, leaves, polysaccharides, anti-inflammatory activity.

RESUME

The article presents the results of the monosaccharide composition of the water-soluble polysaccharide complex of shoots and leaves of raspberry (Rubus idaeus L.). With the help of an ascending chromatography on paper, it is established that the polysaccharide complex of shoots and leaves of raspberry consists of arabinose, galactose and glucose. As a result of the study of acute toxicity according to the method of Prozorovsky VV, it was established that the water-soluble polysaccharide complex of shoots and leaves of raspberry belongs to the class of low-toxic substances, with LD50 – 5000 mg/kg. The anti-inflammatory activity of the polysaccharide complex of raspberry shoots. The inhibition of inflammatory reaction by the polysaccharide complex of raspberry leaves amounted to 41.1%.

keywords:raspberry, shoots, leaves, polysaccharides, anti-inflammatory activity, Rubus idaeus L.

INTRODUCTION

Anti-inflammatory agents of plant origin are not widely used in medicine. However, to date, promising plant sources are known for obtaining anti-inflammatory drugs, which do not have a lot of side effects characteristic of synthetic drugs [1–6]. The search for new highly effective anti-inflammatory drugs of plant origin is topical.

The range of medicinal plant raw materials is represented by raw materials: raspberry fruits, black elderberry flowers, linden flowers.

Many types of medicinal plants accumulate biologically active substances in all organs, but only individual organs are used as medicinal plant materials, as a result of which natural resources are misused.

Therefore, research aimed at the integrated and waste-free use of natural resources is relevant.

It is of interest to evaluate the possibility of using common raspberry shoots to obtain biologically active substances.

The purpose of the study: to isolate the polysaccharide complex of raspberry shoots and leaves ordinary, to study its composition and anti-inflammatory activity.

MATERIALS AND METHODS

The objects of the study were samples of leaves and shoots of common raspberry, collected on the territory of the Ilyinsky district of the Perm Territory in the undergrowth of the green moss spruce forest in early July 2016. Raspberry shoots and leaves were dried in the air-shadow method.

The water-soluble polysaccharide complex (WPC) was extracted from the dried raw material. The method of isolation of polysaccharide fractions by N.K. Kochetkov [7]. A sample of air-dry raw material was crushed to a particle size of 2 mm in diameter. To remove low molecular weight sugars and phenolic compounds, about 100 g of raw materials were preliminarily extracted with 80% alcohol in a ratio of 1:10 for 1 hour in a Soxhlet apparatus.

After extracting low molecular weight sugars and phenolic compounds from the meal, it was extracted with purified water in a ratio of 1:10 at a temperature of 80 °C. The extraction was repeated twice under the same conditions. The combined extracts were evaporated in vacuo and precipitated by the addition of 3 times the amount of ethyl alcohol 96%.

The resulting fractions were purified by repeated washing with 96% ethyl alcohol.

To determine the monosaccharide composition of common raspberry polysaccharides, they were acid hydrolyzed with a 2M sulfuric acid solution in sealed ampoules at 105°C for 8 hours. The monosaccharide composition of the hydrolysates was determined by ascending paper chromatography in solvent systems: BUV 4:1:5, ethyl acetate-acetic acid-formic acid-water 18:3:1:4. Chromatograms were treated with an aniline phthalate reagent and developed in an oven at a temperature of 100–105°C until color appeared [8].

Determination of the acute toxicity of raspberry polysaccharides was carried out on white mice of both sexes, weighing 24–30 g, kept on a normal vivarium diet. Mice were kept in special cages in a stationary vivarium. The work complied with the rules for the maintenance, protection, use of laboratory animals, as well as recommendations from the manual for the experimental (preclinical) study of new pharmacological substances [9].

For the study, the express method for determining the average lethal dose of Prozorovsky V.V. was used [10]. Substances were administered once at doses of 1000 to 5000 mg/kg, orally using a gastric tube. After the introduction of the extract, the condition of the animals was assessed for 6 hours continuously. The absence or presence of cases of lethality of animals was noted. According to the results of the experiment, the substances were assigned to the hazard class according to GOST 12.1.007-7.

Determination of anti-inflammatory activity was carried out on rats weighing 180–250 g of both sexes (the group included 6 animals) on the model of acute inflammatory edema caused by subplantar injection of 0.1 ml of 1% aqueous solution of carrageenan into the hind paw of a rat.

An increase in the volume of the foot, indicating the development of edema, was assessed oncometrically before the injection and 4 hours after the injection of the carrageenan solution. The test substances were administered orally at a dose of 50 mg/kg 0.5 hours before the administration of the phlogogenic agent. Animals that did not receive the drug served as controls. Statistical processing was carried out according to the Student's method.

Based on the results obtained, the effect of inhibition was determined as a percentage of the control level. The presence of anti-inflammatory action was judged by the severity of inhibition of the inflammatory response. If this indicator was more than 30%, the result was taken into account as positive [3].

RESULTS AND DISCUSSION

Common raspberry is a branchy thorny shrub 1-2 meters high. In the first year of life, the stems are grassy, green, planted with thorns. In the second year, the shoots form inflorescences and bear fruit.

From the shoots and leaves of common raspberry, a water-soluble polysaccharide complex was obtained, which is an amorphous powder of yellowish-white color, bitter taste, with a characteristic odor.

In order to establish the monosaccharide composition of the obtained fractions, acid hydrolysis was performed followed by chromatographic determination of monosaccharides in hydrolysates.

According to the results of the chromatographic analysis of hydrolysates of polysaccharide fractions of shoots and leaves of common raspberry (Table 1), it was found that the PRP of shoots and leaves of raspberry consists of

arabinose, galactose and glucose residues.

Table 1

Factions	arabinose	Galactose	Rhamnoza	Glucose
VRPC shoots	+	+	-	+
FRP leaf	+	+	-	+

Monosaccharide composition of hydrolysates of polysaccharide fractions shoots and leaves of common raspberry

"-" - the absence of a component

The result of the study showed that the water-soluble polysaccharide complex of raspberry shoots and leaves belongs to the class of low-toxic substances, according to GOST 12.1.007-76, with LD50more than 5000 mg/kg (Table 2), which will be an advantage over synthetic drugs.

table 2

Determination of acute toxicity of raspberry polysaccharides

Fraction	LD50, orally (mg/kg)	Toxicity class according to GOST 12.1.007- 76
FRP leaf raspberries	> 5000	Low toxicity
VRPC shoots raspberries	> 5000	Low toxicity

After the introduction of the phlogogenic agent, the animals of the control group developed severe edema and hyperemia. The maximum development of an acute inflammatory reaction was observed 3 hours after the introduction of 0.1 ml of a 1% aqueous solution of carrageenan.

In the group of animals that 0.5 hours prior to the administration of the phlogogenic agent were injected with 50 mg/kg of raspberry leaf CRP, a significant decrease in the amount of edema was observed. Inhibition of the inflammatory response was 41.1% compared with the control (Table 3). In the group of animals that were injected with raspberry shoots CRP, there was no significant decrease in the amount of edema.

Table 3

Determination of anti-inflammatory activity of raspberry polysaccharides

Fraction	Increase in foot size after 3 hours, %	Reaction inhibition after 3 hours, %
Raspberry Leaf CRP	38.9±5.5 p < 0.02	41.1
Raspberry shoots FRPK	58.2 ± 2.2 p < 0.05	11.9
Nimesulide 33.9 ± 6.8 p < 0.05		48.7
Control	66.1 ± 6.7	

p - reliability of comparison with control

Based on the study, we can talk about the prospects for further research on the water-soluble polysaccharide complex of common raspberry leaves and the production of an anti-inflammatory agent based on it.

CONCLUSIONS

1. The composition of the water-soluble polysaccharide complex of raspberry leaves and shoots was determined, which is represented by arabinose, galactose and glucose.

2. It has been established that the water-soluble polysaccharide complex of raspberry leaves and shoots belongs to the class of low-toxic substances with LD50> 5000 mg/kg.

3. The water-soluble polysaccharide complex of raspberry leaves has a more pronounced anti-inflammatory effect (41.1% inhibition of the inflammatory response) than the water-soluble polysaccharide complex of raspberry shoots (11.9% inhibition of the inflammatory response).

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