

ANTI-INFLAMMATORY PROPERTIES OF RASPBERRY SHOOT EXTRACT

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Abstract

Raspberry fruits contain some phenolic compounds, the predominant of which are anthocyanins and ellagitannins, with significantly lower concentrations of flavonoids, phenolic acids, and flavan-3-ols. It is known that the inflammatory process is a typical pathological process that accompanies most diseases and is associated with a complex of structural, functional, and metabolic disorders, both at the organ level and at the cell level and is accompanied by pain and edema. This work aims to study the anti-inflammatory properties of raspberry shoot extract (RSE).

Material and methods. The antiexudative activity of RSE was studied on 56 white outbred male rats weighing 180-220 g, in which a model of acute inflammation induced by subplantar injection of 0.1 ml of 1% carrageenan (Fluka, Switzerland) into the right hind paw of rats was reproduced. All animals were divided into 6 groups. I - control pathology (animals that were subplantarly administered solution of carrageenan and intragastrically administered with 0.5 mL/kg of distilled water); Groups II and III - animals that were administered carrageenan solution subplantarly and the studied RSE was administered intragastrically at a dose of 0.5 mL/kg and 1 mL/kg, respectively. Animals of groups IV and V were administered intragastrically drugs of comparison against the background of the introduction of carrageenan: diclofenac sodium at a dose of 8 mg/kg or indomethacin at a dose of 2 mg/kg; Group VI was administered intragastrically infusion of raspberry leaves at a dose of 1 mL/kg against the background of carrageenan; VII and group consisted of intact animals, which were administered 0.1 mL of saline subplantarly.

Results and Discussion. Analysis of the data in the table showed that RSE at a dose of 0.5 and 1.0 mL/kg has a decongestant effect throughout the entire period of the experiment. A particularly pronounced antiexudative effect of RSE at a dose of 1.0 mL/kg was exerted during the first three hours after the introduction of phlogogen. So, at a dose of 0.5 mL/kg its activity by the third hour of the experiment was 38.1%, and at a dose of 1.0 mL/kg 49.8%. 8 hours after the introduction of phlogogen, RSE inhibited the development of edema somewhat weaker than the traditional NSAIDs - indomethacin and diclofenac sodium (30.3% and 36.7%, respectively). After 24 hours, the antiexudative activity of RSE was 7.1% (for a dose of 0.5 mL/kg), 19.6% (for a dose of 1.0 mL/kg), 21.8% for indomethacin and 24.7% for diclofenac sodium. Thus, the studies carried out indicate the presence of anti-inflammatory, antioxidant, and anticytolytic properties of raspberry shoot extract.

Keywords: *anti-inflammatory activity, raspberry shoot extract.*

Introduction

Raspberry (European red raspberry) (*Rubus idaeus* L.) belongs to the Rosaceae family and is distributed almost throughout Europe, the Caucasus, Siberia, and Central Asia [1, 2]. Raspberry blooms in May and June; fruits ripen mainly in July and August. Raspberry grows in the undergrowth of mixed forests, in clearings, clearings and very often forms continuous thickets [2, 3]. In folk medicine, red raspberry (flowers, leaves) is very widely used. It is used as an anti-inflammatory, antipyretic agent (due to the presence of salicylic acid, and other BAS). Raspberry decoction is used for gargling in throat diseases. Raspberry fruits (aggregates of drupelets) are used as a high-vitamin remedy to improve appetite after illness [4]. Many publications are devoted to antidiabetic, cardioprotective, antitumor, and other properties of raspberry [5].

Raspberry fruits contain some phenolic compounds, the predominant of which are anthocyanins and ellagitannins, with significantly lower concentrations of flavonoids, phenolic acids, and flavan-3-ols [1,2, 5].

The pharmacological activity of natural compounds is due to their low toxicity, the ability to comprehensively affect the body, and rarely cause serious adverse reactions. This allows for the long-term treatment of chronic diseases. The unconditional advantage of herbal preparations is the availability of raw materials and a cost-efficient way of obtaining a medicinal product [4].

It is known that the inflammatory process is a typical pathological process that accompanies most diseases and is associated with a complex of structural, functional, and metabolic disorders, both at the organ level and at the cell level and is accompanied by pain and edema [6].

This work aims to study the anti-inflammatory properties of raspberry shoot extract (RSE).

Methods

The antiexudative activity of RSE was studied on 56 white outbred male rats weighing 180-220 g, in which a model of acute inflammation induced by subplantar injection of 0.1 ml of 1% carrageenan (Fluka, Switzerland) into the right hind paw of rats was reproduced [7]. Measurement of paw edema in rats was carried out after 1, 2, 3, 8, 24 hours, taking into account that after the administration of

carrageenan maximum edema is observed by the third hour, then the edema gradually decreases during the day [7]. For this, the volume of the paws in cm³ was measured using a digital plethysmometer Panlab (Spain) model LE 7500 version V29/10/2014. The amount of edema in each case was determined by the difference in volume between the edematous and healthy paws and was expressed in %, indicating how much the study drug inhibits the development of edema compared to the control, where the amount of edema was taken as 100%. The activity of RSE and reference drugs was calculated using the formula [7]:

$$(Ms-Mh) \times 100$$

$$A = 100\% - \frac{\text{-----}}{Msc-Mhc}, \text{ where}$$

A - antiexudative activity, %; Ms is the volume of the swollen paw in the experiment;
Mh is the volume of a healthy paw in the experiment; Msc-the volume of the swollen paw in the control;

Mhc is the volume of a healthy paw in the control.

The choice of this model of inflammation is because various mediators of inflammation are involved in the development of carrageenan edema: biogenic amines, kinin system, and prostaglandins: in the first 30-90 minutes - serotonin and histamine, in the interval between 1.5-2.5 hours - kinins, and between 2.5-5.5 hours - prostaglandins [7].

Since not only NSAIDs but also phytopreparations are widely used in the treatment of inflammatory diseases [4-6], the purpose of our research was a comparative study of the activity of anti-inflammatory drugs with a different from the traditional mechanism of antiphlogistic action. For this, we have chosen an infusion of raspberry leaves, as well as classical antiphlogistics - sodium diclofenac and indomethacin, administered at ED₅₀ (for anti-inflammatory effect) [7]. All animals were divided into 6 groups.

I - control pathology (animals that were subplantarily administered solution of carrageenan and intragastrically administered with 0.5 mL/kg of

distilled water); Groups II and III - animals that were administered carrageenan solution subplantarily and the studied RSE was administered intragastrically at a dose of 0.5 mL/kg and 1 mL/kg, respectively. Animals of groups IV and V were administered intragastrically drugs of comparison against the background of the introduction of carrageenan: diclofenac sodium at a dose of 8 mg/kg or indomethacin at a dose of 2 mg/kg; Group VI was administered intragastrically infusion of raspberry leaves at a dose of 1 mL/kg against the background of carrageenan; VII and group consisted of intact animals, which were administered 0.1 mL of saline subplantarily.

The use of several NSAIDs as reference drugs is due to some differences in the mechanisms of drug action [6].

The raspberry shoot extract obtained with 60% ethyl alcohol (RSE) was obtained at the Department of Pharmacognosy of the NUPh under the supervision of Professor A.N. Komisarenko.

RSE and reference drugs were administered 1 hour before the introduction of phlogogen (prophylactic regimen) [7, 8].

The activity of FRO processes was determined by the content of TBA-active products (TBA-AP) in the blood serum by the method of ID Stalnaya, T. Garishvili, and the intensity of cytological processes by the level of AsAT [7].

The reliability of the results obtained was assessed according to the Student's *t*-test using the software package (Statistica 6.0, MS Office 2010) [8]. During the experiment, the animals were kept in the vivarium of the NUPh training center at an air temperature of 20-22 °C, a natural day-night light regime, in standard ventilated cages, on a standard diet [10].

All experiments on animals were carried out following the regulations of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (1986, Strasbourg) [11].

Results and Discussion

The results of these experiments are presented in table 1 and Fig.1.

As a result of the experiments, it was found that by the first hour of the experiment, compared with

the initial background, the edema in the group of animals with model pathology increased by 1.5 times, by the second, third, and fourth hours - by 3.4 and 3.2 times, respectively, after one day, there was a tendency to a decrease in edema, but the size of the paws in the rats did not reach the norm and was higher than the initial background.

Analysis of the data in the table showed that RSE at a dose of 0.5 and 1.0 mL/kg has a decongestant effect throughout the entire period of the experiment. A particularly pronounced antiexudative effect of RSE at a dose of 1.0 ml/kg was exerted during the first three hours after the introduction of phlogogen (table. 1).

So, at a dose of 0.5 ml/kg its activity by the third hour of the experiment was 38.1%, and at a dose of 1.0 ml/kg 49.8%. 8 hours after the introduction of phlogogen, RSE inhibited the development of edema somewhat weaker than the traditional NSAIDs - indomethacin and diclofenac sodium (30.3% and 36.7%, respectively). After 24 hours, the antiexudative activity of RSE was 7.1% (for a dose of 0.5 mL/kg), 19.6% (for a dose of 1.0 mL/kg), 21.8% for indomethacin and 24.7% for diclofenac sodium.

Comparative analysis of the effectiveness of the application of the raspberry leaf infusion showed that against the background of its administration, the intensity of edema decreased by 13.6% at the 1st hour, by 26.2% at the 2nd hour, and by 22.2% at the 3rd hour. After a day, the effect of the drug was not observed. The results obtained suggest that the inhibition of biogenic amines (histamine, serotonin), kinins, and also prostaglandins plays an important role in the mechanism of RSE antiexudative action because RSE was the most active for 1-3 hours of the experiment. The anti-edema effect of RSE is due to the presence in its composition of salicylic acid, hydroxycinnamic acids, flavonoids, and other BAS.

Thus, the analysis of the results obtained indicates that in terms of the average daily antiexudative activity RSE is somewhat inferior to traditional antiphlogistics, but surpasses the infusion of raspberry leaves.

Since both RSE doses had an antiexudative effect, we studied their effect on the processes of free radical oxidation and cytolysis under conditions of carrageenan edema in rats. The results are shown in Fig. 1.

It was found that the administration of carrageenan increased the intensity of free radical oxidation (FRO) by 1.8 times, cytolysis - 2 times (in terms of AsAT activity). The data presented in Fig. indicate that RSE reduces the intensity of cytolytic processes, as evidenced by the decrease in AsAT activity under the influence of RSE at a dose of 1 mL/kg by 1.6 times and by 1.48 times at a dose of 0.5 mL/kg as compared with CP.

A decrease in the intensity of FRO processes in our studies was manifested by a 1.5-fold decrease in the level of TBA-AP in blood serum under the action of RSE at a dose of 1 mL/kg and by 1.4 times at a dose of 0.5 mL/kg.

Infusion of raspberry leaves lowered the activity of AsAT - 1.3 times and the level of TBA-AP - 1.21 times. Diclofenac sodium level decreased the activity of AsAT and the level of TBA-AP - by 1.2 times (Fig. 1). The influence of indomethacin on the processes of cytolysis and FRO was minimal.

In our opinion, due to the presence in the extract of raspberry shoots of polyphenols, salicylic acid, ellagitannins, anthocyanins, and other biologically active substances [2, 3, 5, 12, 13], which have anti-inflammatory and antioxidant properties, this extract not only reduces the formation of inflammatory mediators but also helps to reduce the intensity of the processes of FRO-destruction, cytolysis, etc. [12-15].

Thus, the studies carried out indicate the presence of anti-inflammatory, antioxidant, and anticytolytic properties of raspberry shoot extract.

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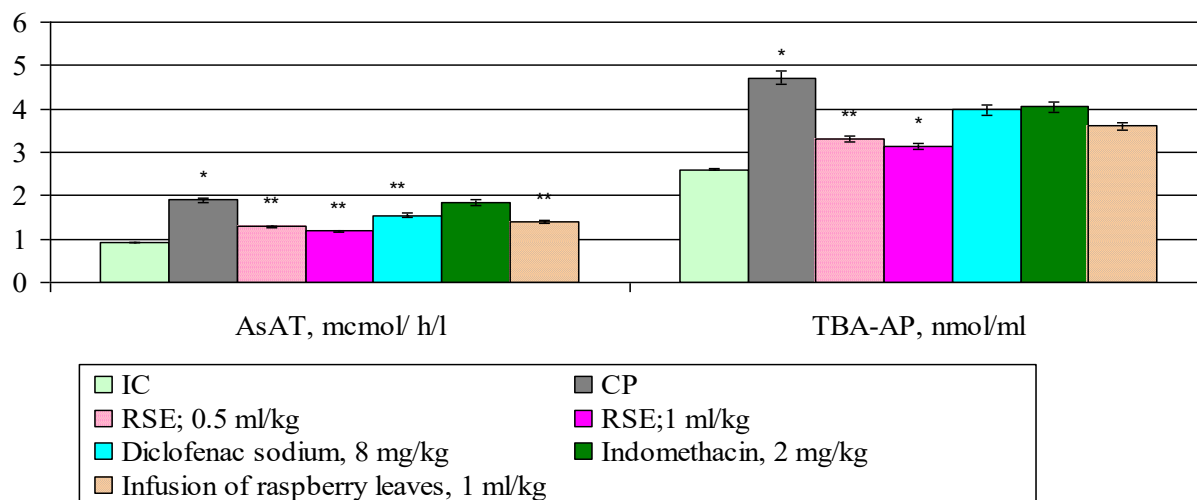
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Table 1. Effect of RSE on the exudation process in carrageenan inflammation in rats ($M \pm m$, $n = 8$)

Studied preparation	Dose	% of edema inhibition compared to control during				
		1 hour	2 hours	3 hours	8 hours	24 hours
RSE	0.5 mL/kg	28.6 \pm 6.1*	30.2 \pm 4.1	38.1 \pm 2.8	16.1 \pm 6.1	7.1 \pm 1.1 *
RSE	1 mL/kg	43.1 \pm 2.6 *	46.4 \pm 7.2 *	49.8 \pm 4.4 *	24.2 \pm 3.8 *	19.6 \pm 7.2 *
Indomethacin	2 mg/kg	39.1 \pm 4.1 *	42.8 \pm 4.4 *	53.1 \pm 5.2 *	30.3 \pm 7.1 *	21.8 \pm 2.4 *
Diclofenac sodium	8 mg/kg	32.4 \pm 3.7 *	40.5 \pm 5.1 *	54.6 \pm 4.8 *	36.7 \pm 5.9 *	24.7 \pm 3.3 *
Infusion of raspberry leaves	1 mL/kg	13.6 \pm 1.5	26.2 \pm 1.3	22.2 \pm 1.5	11.6 \pm 1.4	0

Note: $p \leq 0.05$ compared with the indicators of animals treated with infusion of raspberry leaves

Figure 1. Effect of RSE on AsAT activity and TBA-AP level in blood serum in carrageenan inflammation in rats

- * $p \leq 0.05$ significant compared to intact control (IC);
- ** $p \leq 0.05$ significant compared to the control pathology (CP).