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THE STUDY OF ANTI-ULCER EFFECT OF DEALCOHOLIZED EXTRACT OF ACORUS CALAMUS LEAVES (*Acorus calamus* L.) UNDER THE CONDITIONS OF ALCOHOL-PREDNISOLONE GASTRIC ULCER IN RATS

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Abstract

The use of herbal medicines is a promising way to improve treatment of the gastrointestinal tract diseases. At the same time, the leading role of oxidative stress is observed in the pathogenesis of several nosologies and diseases of the gastrointestinal tract in particular. A promising medicinal plant raw material with established antioxidant action is the leaf extract of Acorus calamus leaves (Acorus calamus L.)

Materials and methods. The object of research was dealcoholized extract of Acorus calamus leaves (DEAL). Model alcohol-prednisolone gastric ulcer was reproduced by a single intragastric administration of a combination of 80% ethanol (0.6 mL per 100.0g of animal weight) and prednisolone (20 mg/kg) in rats. 24 h after modeling of the control pathology, rats were euthanized, stomachs were removed, cut along a large curvature, washed with saline, macroscopic examination of the gastric mucosa and biochemical tissue examinations were performed. The DEAL and reference drug ranitidine ("Ranitidine-Zdorovya", tab. 0.15 № 20) were administered intragastrically prophylactically once daily at a dose of 1 mL/kg and 20 mg/kg for 3 days before the modeling of the pathology. The presence of antimicrobial activity of DEAL was also investigated by the double serial dilution method

Results and discussion. The use of DEAL in the prophylactic mode against the background of experimental alcohol-prednisolone gastric ulcer in rats contributed to reliable prevention of the development of all studied clinical indicators, sometimes 100% ($p < 0.05$). According to some indicators (swelling, hyperemia of the gastric mucosa), the studied extract was statistically significantly superior to the effectiveness of ranitidine ($p < 0.05$). The use of DEAL reduced the level of TBA-AP by 2.2 times (almost to the level of intact animals), the level of reduced glutathione (GSH) increased 1.9 times, and the Succinate dehydrogenase (SDH) content increased 1.33 times, which is probably due to the optimization of the process of energy supply to cells and reduce the intensity of free-radical oxidation processes. The presence of antimicrobial properties for DEAL has been established.

Conclusions. Dealcoholized extract of sweet flag leaves has antiulcer and antimicrobial activities.

Keywords: *extract of acorus calamus leaves, alcohol-prednisolone gastric ulcer, anti-ulcer activity*

Introduction

The improvement of the gastrointestinal tract (GIT) diseases treatment is an urgent problem today [1, 2]. Gastrointestinal pathologies are characterized by wide prevalence, chronic course with seasonal exacerbations, high comorbidity with pathologies of other organs and systems, the presence of many non-specific symptoms that complicate the differential diagnosis [3]. However, the treatment of gastrointestinal diseases is not always successful because they have complex pathogenesis and symptomatic treatment is not sufficient, and in most cases causes unwarranted polypragmasia [4].

Regardless of the type of medicines used in the treatment of peptic ulcer, to achieve the goal - rapid and complete, without recurrence, recovery of the gastric or duodenum mucosa - it is necessary to solve the following clinical tasks:

1) elimination of neurohumoral regulation disorders of the gastroduodenal system;

2) reduction of aggressive properties of gastric juice;

3) normalization of motor-evacuation activity of the upper gastrointestinal tract;

4) achievement of H. pylori eradication ;

5) increasing the resistance of the mucous membrane;

6) stimulation of reparative processes [1, 2, 5].

One of the possible ways to improve the therapy of the gastrointestinal tract, in particular peptic ulcer of the stomach and duodenum, is the use of medicinal products of natural origin [6, 7]. They act comprehensively, providing high efficiency, especially in chronic diseases [8]. Also, drugs of natural origin are characterized by high safety, polytropic mechanism of action, which avoids unreasonable polypragmasia. Also, drugs of natural origin have a general healing effect on the body [9, 10].

In connection with the above, the complex treatment of peptic ulcer of the stomach and duodenum should include plants that have antimicrobial, anti-inflammatory, analgesic, enveloping, film-forming, antitoxic, antacid, stimulating mucus secretion, reparative, and hemostatic effects, ie able to affect pharmacological "targets".

At the same time, the leading role of oxidative stress is observed in the pathogenesis of several nosologies and diseases of the gastrointestinal tract in particular [11,12]. The latter indicates the feasibility of using drugs with the antioxidant mechanism of action in diseases of the gastrointestinal tract.

A promising medicinal plant raw material with established antioxidant action is the leaf of sweet flag (Acorus calamus L.) [13]. At the Department of Botany of NUPh under the supervision of Professor T. M. Gontova a liquid alcohol-water extract of sweet flag leaves was obtained [14]. By evaporating the liquid extract on a rotary evaporator, a dealcoholized extract of Acorus calamus leaf (DEAL) was obtained. In our previous studies, the presence of anti-inflammatory, hepatoprotective, neurotropic, and antioxidant effects of this extract [15, 16, 17, 18] and favorable safety profile [19, 20] have been established.

This study aimed to establish the antiulcer activity of DEAL under the conditions of experimental alcohol-prednisolone gastric ulcer in rats and the study of antimicrobial properties of DEAL.

Methods

Model alcohol-prednisolone gastric ulcer was reproduced by a single intragastric administration of a combination of 80% ethanol (0.6 mL per 100.0g of animal weight) and prednisolone (20 mg/kg) in rats [21]. Ethanol is an alimentary gastrotoxic substance that causes dehydration and coagulation of the gastric mucosa and its necrosis. Prednisolone is a glucocorticoid hormone that potentiates the ulcerogenic action of ethanol. Prednisolone leads to oxidative stress, accumulation of free radicals in the gastric mucosa, disruption of plastic and energy metabolism, trophic of gastric mucosa, and its reparation. The mechanism of the damaging effect of prednisolone on the gastric mucosa is the inhibition of phospholipase activity and the synthesis of group E prostaglandins, which provide blood supply to the gastric mucosa. Besides, ethanol and prednisolone are metabolized by the same enzyme, 3-isoform of alcohol dehydrogenase. As a result of the synergism of the ulcerogenic effect, the strength of the damaging effect of strong alcohol on the gastric mucosa increases tenfold [22]. The drug combination is administered to rats after

24 h of fasting (free access to water). One day after the introduction of the combination of alcohol + prednisolone, the animals were euthanized with further examination of the gastric mucosa [21].

Experimental animals were divided into IV groups of 6 animals each: I - intact control (IC); II - control pathology (CP); III - CP + ranitidine; IV - CP + DEAL. DEAL was administered intragastrically prophylactically once daily at a dose of 1 mL/kg for 3 days before the modeling of the pathology. The reference drug ranitidine ("Ranitidine-Zdorovya", tab. 0.15 No. 20) was administered intragastrically at a dose of 20 mg/kg in a manner similar to DEAL [21].

At the time of ethanol/prednisolone mixture administration and 24 h after, the general condition of the animals was assessed: skin and coat condition, the color of mucous membranes, reaction to light and sound stimuli, orientation, food reflexes, etc.

24 h after modeling of the control pathology, rats were euthanized, stomachs were removed, cut along a large curvature, washed with saline, macroscopic examination of the gastric mucosa was performed [21]. The parameters of the stomach, intestines and gastric mucosa were evaluated: presence/absence of bloating, hemorrhage, hyperemia, swelling, folding disorders, and the degree of the mucosa damage. Also, a biochemical study of the gastric mucosa (determination of the content of TBK-AP, GSH, and SDH) was conducted by conventional methods [23].

The research was conducted based on the Educational and Scientific Training Center for Medical and Biological Research of the Educational and Scientific Institute of Applied Pharmacy of the NUPh. During the experiment, the animals were in the vivarium of the NUPh training center at an air temperature of 20-22 C, natural light regime "day and night", in standard cages, on a standard diet [24].

The study of antimicrobial properties of DEAL was conducted based on SI "II Mechnikov Institute of Microbiology and Immunology of the National Academy of Medical Sciences of Ukraine " by the method of double serial dilution [25]. As reference strains, museum strains of microorganisms Staphylococcus aureus ATCC 25923, Streptococcus pneumonia ATCC 49619, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Candida albicans ATCC 885 - 653 Bacillus cereus ATCC 10702 were used [26]. Meat - peptone agar, pepted meat broth, and Sabouraud medium were used as nutrient media.

The results were processed statistically using Student's t-test for normal distribution, Mann-Whitney Ť test in its absence, Fisher's angular transformation φ - taking into account the alternative form (presence/absence of a sign) [27].

Results and discussion

Normal reflex excitability, satisfactory condition of fur and skin, mucous membranes of normal color, and degree of moisture were observed among animals of the IC group. All animals responded normally to light and sound stimuli. No deaths of animals of the IC group were observed. Macroscopic examination of the gastric mucosa indicated the absence of hemorrhages, redness, swelling, ulcers. Bloating of the stomach and intestines was not observed (Table 1).

24 h after modeling of experimental gastric ulcer in 5 of 6 animals (83.33%) of control pathology inhibition and general weakness, piloerection of various degrees, dryness of mucous membranes, and lack of eating behavior were noted. In 1 animal of the CP group (16.67%) the condition was close to the characteristic physiological norm of animals. Hyperemia of the gastric mucosa was found in all animals of control pathology (100%), hemorrhage with leukocyte infiltrate - in 5 (83.33%), hemorrhage without leukocyte infiltrate - in 1 (16.67%), violation of the gastric mucosa folding - in 5 (83, 33%), bloating stomach- in 5 animals (83.33%), intestines - in 5 (83.33%).

Prophylactic administration of the comparison drug ranitidine was characterized by a tendency to reduce the number of animals with all the clinical signs of control pathology. However, such a decrease was achieved only in terms of hemorrhages with leukocyte infiltrate (0/6 vs. 5/6). Most animals of the ranitidine group (85.7%) had a normal appearance, satisfactory condition of the skin and mucous membranes, fur. The reflex activity was within normal limits. Only in 14.3% of animals, there was slight piloerection and reduced food reflexes.

The use of DEAL in the prophylactic mode against the background of experimental gastric ulcer contributed to reliable prevention of the development of all studied clinical indicators, sometimes 100% ($p < 0.05$). According to some indicators (swelling, hyperemia of the gastric mucosa), the studied extract was statistically significantly superior to the effectiveness of ranitidine ($p < 0.05$).

All rats receiving DEAL were characterized by a satisfactory condition, reflexes were normal.

Given that the processes of free-radical oxidation are one of the factors of ulceration, we have studied the effect of DEAL on the level of TBA-AP, SDH, and GSH in homogenates of the gastric mucosa of rats under ethanol-prednisolone gastric lesions (Table 2).

It has been found that in the animals of the control group the content of TBA-AP increased by 2.02 times, the content of GSH decreased by 1.88 times, the level of SDH decreased by 1.6 times as compared with intact animals ($p < 0.05$).

The use of DEAL reduced the level of TBA-AP by 2.2 times (almost to the level of intact animals), the level of GSH increased 1.9 times, and the SDH content increased 1.33 times, which, in our view, is due to the optimization of the process of energy supply to cells and reduction of the intensity of free-radical oxidation processes (Table 2).

The effect of ranitidine on the state of FRO/AOS processes and SDH content was tendentious ($p < 0.05$).

When determining the antimicrobial action of the DEAL extract by the method of double serial dilution, the following results were obtained, which are shown in table 3.

As a result of the conducted researches it has been established that the minimum inhibitory concentration of the studied preparation for reference - strains made: S. aureus ATCC 25923 - 6.25%; E. coli ATCC 25922 - 6.25%; P. aeruginosa ATCC 27853 -6.25%; S. pneumoniae ATCC 49619 - 6.25%; C. albicans ATCC 885 - 653 - 6.25% and B. cereus ATCC 10702 - 12.5 %.

The results obtained indicate the presence of antimicrobial properties in the extract of sweet flag leaf.

Thus, the studied dealcoholized liquid alcohol-water extract of sweet flag leaf at prophylactic intragastric administration at a dose of 1.0 ml/kg has a pronounced antiulcer effect on the model of alcohol-prednisolone ulcer in rats. The established presence of antimicrobial properties in DEAL advantageously complements the range of pharmacological properties of the studied extract.

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Table 1. The severity of general clinical symptoms of gastrointestinal lesions

Clinical sign	Experimental group, dose, n			
	IC, n=6	CP, n=6	Ranitidine 20 mg/kg, n = 6	DEAL, 1 ml/ kg, n = 6
Flatulence	0/6 [0%]	5/6* [83.33%]	3/3 [50%]	0/6** [0%]
Intestines flatulence	0/6 [0%]	5/6* [83.33%]	3/3 [50%]	1/6** [13.33%]
Hyperemia	0/6 [0%]	6/6* [100%]	4/6 [66.7%]	1/6**/*** [13.33%]
Swelling	0/6 [0%]	6/6* [100%]	4/6 [66.7%]	0/6**/*** [0%]
Violation of the folding of the gastric mucosa	0/6 [0%]	5/6* [83.33%]	2/6 [33.3%]	1/6** [13.33%]
Hemorrhages without leukocyte infiltrate	0/6 [0%]	1/6 [16.67%]	2/6 [33.3%]	1/6 [13.33%]
Hemorrhages with leukocyte infiltrate	0/6 [0%]	5/6* [83.33%]	0/6** [0%]	0/6** [0%]

Note. statistically significant differences ($p < 0.05$): * - with the IC group; ** - with CP group; *** - with the ranitidine group. In square brackets - the value of the indicator in %. IC - intact control, CP - control pathology, GM - gastric mucosa, n - number of animals in the group.

Table 2. The effect of DEAL on the content of TBA-AP, SDH, and GSH in the homogenates of the stomach of rats under conditions of experimental ethanol-prednisolone lesions of the stomach, n = 6, M ± m

Indicator	Experimental group, dose, n			
	IC, n=6	CP, n=6	CP + DEAL, 1 ml/kg, n = 6	CP + Ranitidine, 20 mg/kg, n = 6
TBA-AP, µmol/L	53.83±4.66	108.76±7.708*	49.18±4.522**/***	99.2±5.29
GSH, µmol/g	4.45+0.25	2.36±0.30*	4.58+0.224 **/***	2.58±0.38
SDH, µmol/g	1.78±0.144	1.105±0.06*	1.47±0.056	1.127±0.06

Notes: * - differences are statistically significant for the values of intact control, $p < 0,05$; ** - differences are statistically significant for the values of control pathology, p0.05; *** - differences are statistically significant for the values of animals from the group of ranitidine, p0.05; n is the number of animals in the group.

Table 3. Antimicrobial properties of DEAL

strains sample experiment	1/2 50%	1/4 25%	1/8 12.5%	1 /16 6.25%	1/32 3.125%	Culture control	Drug control	Control of the nutrient medium
S. aureus ATCC 25923	-	-	-	-	+	+	-	-
S.pneumoniae ATCC 49619	-	-	-	-	+	+	-	-
Escherichia coli ATCC 25922	-	-	-	-	+	+	-	-
Pseudomonas aeruginosa ATCC 27853	-	-	-	-	+	+	-	-
Bacillus cereus ATCC 10702.	-	-	-	+	+	+	-	-
Candida albicans ATCC 885-653	-	-	-	-	+	+	-	-

Notes: "+" the presence of growth (turbidity of the nutrient medium in the test-tube); "-" absence of growth (transparent nutrient medium).