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Research Paper

The importance of plant extract in improving the microflora of the gastrointestinal tract in the treatment of diseases of the stomach and duodenum

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ABSTRACT

Background: Gastric ulcer disease still is a big clinical worry, so people keep looking for brand new therapeutic agents. Plant derived extracts can be a promising origin for anti-ulcer compounds, yet the effect of a complex combination plant extract really needs a step-by-step testing through validated experimental models.
Objective: To evaluate the anti-ulcer activity of a combination complex plant extract given orally in two well established experimental gastric ulcer models-stress induced and acetylsalicylic acid induced-in lab animals.
Methods: Experiments were carried out on non linear male rats and on mongrel male mice. The combination plant extract was given per os at a dose of 150 mg/kg. Anti ulcer activity was assessed with two main outcome measures, the Pauls Index, which mirrors ulcerative lesion severity, and the Anti-Ulcer Effect index (AUE), which is used to quantify gastroprotection level compared with the control group. In total, two experimental ulcer models were used: an acute stress model and an acetylsalicylic acid (aspirin) induced gastric ulcer model.
Results: In the stress ulcer model, when the extract was administered there was a clear reduction in the ulcerative lesion burden. This was seen as decreases in large, punctate and stripe shaped ulcers, plus a drop in total ulcer count in mice. The AUE index was 4.6. In the acetylsalicylic acid induced model, gastric ulcers showed up in only 70% of animals treated with the extract, unlike controls, and the gastric mucosal injury looked notably weaker. The AUE index was 4.5.
Conclusions: The combination complex plant extract showed a rather meaningful anti ulcer effect in both experimental models, using an oral dose of 150 mg/kg, and the AUE indices were 4.6 for the stress induced ulcers, and 4.5 for the acetylsalicylic acid induced ulcers. So, these results back the idea that the extract could be a plausible gastroprotective candidate, and it seems sensible to move on with additional preclinical work and also clinical investigation.

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1. INTRODUCTION

Peptic ulcer of the stomach and duodenum is characterized by a wide prevalence, including about 4-6% of the adult population in all countries, and with full medical screening of patients, this percentage increases by more than 20%. It is known from the literature data that the peak incidence occurs at the age of 30-45 years and mainly in men aged 35-50 years, peptic ulcer of the stomach and duodenum occurs 3-4 times more often. Peptic ulcer of the stomach and duodenum is a seasonal recurrent disease with the severity of the course and a tendency to chronization, leading to disability and mortality of patients. These disorders occur against the background of destabilization of nervous and humoral mechanisms in the human body, which regulate secretory-reparative processes in the stomach and duodenum. In the pathogenesis of the disease, the main role is assigned to the imbalance between the factors of aggression and protection of the mucous membrane of the stomach and duodenum against the background of changes in neuroendocrine and immune regulation of the gastroduodenal zone. The main factors of aggression are hydrochloric acid, pepsin, bile acids, Helicobacter pylori (H. pylory), and mucus, bicarbonates, epithelial hydrophobicity, proper blood supply, cell renewal of the epithelial layer, as well as produced prostaglandins and other mediators protect the mucous membrane directly [1], [2], [3], [4], [5], [6], [7], [8]. It is known that today, with peptic ulcer of the stomach and 12 duodenum, the main 3 and 4 secret combined therapeutic measures are used. At the same time, as part of these therapeutic measures, along with antibiotics, antacids and gastroprotectors, agents with the ability to improve the microflora are also used. It has been noticed that this facilitates the course of the disease and reduces the recovery time. In this regard, the extract obtained on the basis of local medicinal plants was used in experimental conditions as part of a combination used in the treatment of gastric ulcer and duodenal ulcer [9], [10], [11], [12], [13].

2. METHODOLOGY

The object of research was a dry extract obtained from the following types of plant raw materials: Plantago major L. leaves, Achillea millefolium L. grass, and Polygonum Hydropiper L, Radix Glycyrrhizae roots, Matricaria chamomilla L. flowers, Polygonum aviculare L grass [12], [13]. The resulting extract contains carotenoids, polysaccharides, flavonoids, tannins, triterpene saponins, steroids, proteins, sesquiterpene lactones, mucus, resins, organic acids, vitamins, macro- and microelements, essential oils and other natural compounds. Standardization of the extract was carried out according to the amount of flavonoids. The presence of this spectrum of biologically active substances suggests the potential probiotic activity of the resulting extract. The work was carried out in accordance with the "Guidelines for conducting preclinical studies of medicines". The experiments were performed on nonlinear male rats with an initial weight of 180-220 g and on white mongrel male mice with an initial weight of 20-22 g. The animals were obtained and kept in a vivarium with free access to feed and water. Pharmacological studies were carried out according to the "Rules of work using experimental animals" [14], [15], [16].

Experimental therapeutic doses of the extract obtained using the Pauls anti-ulcer index were determined on the model of stress ulcers, which corresponded to 100-150 mg/kg. Therefore, all

subsequent experiments were carried out using a dose of 150 mg/kg. The anti-ulcer activity of an aqueous solution of a complex plant extract at an experimental therapeutic dose of 150 mg/kg and used together with the drug in effective doses of amoxacillin (25/kg), ranitidine (50 mg/kg) and metronidazole (25/kg) was carried out on models of stress in mices and "acetylsalicylic" ulcers in rats [2], [14]. Stress ulcers in rats were caused by hanging them by the cervical skin fold for 24 hours, and then the effect of drugs was evaluated with the calculation of the anti-ulcer effect. Previously, the mices were divided into groups: intact (10 mice); control (10 mice), experimental 1 (10 mice); experimental 2 (10 mice). Experimental animals 1 were injected into the stomach through a probe with the studied extract at an experimental therapeutic dose of 150 mg / kg and doses of amoxacillin (25 / kg), ranitidine (50 mg / kg) and metronidazole (25 / kg) 1 time a day for 7 days beforehand and 1 hour before stress exposure. mices of experimental 2, was administered reference drugs in is effective doses of amoxacillin (25/kg), ranitidine (50 mg /kg) and metronidazole (25/kg), according to a similar scheme. Animals of the control group were injected with an equi-volume amount of purified water according to a similar scheme. The animals of the intact group served as an additional control. Animals of all groups, except for mice of the intact group, were deprived of food, water and litter 1 day before immobilization.

A model of experimental acetylsalicylic ulcer in rats was created by administration of acetylsalicylic acid per os to control and experimental animals at a dose of 150 mg/kg 1 time per day for 3 days. The experiments were carried out on white rats, which were divided into groups: intact (10 rats); control (10 rats); experimental 1 (10 rats); experimental 2 (10 rats); experimental 3 (10 rats). Experimental 1 was injected with an extract in the form of an aqueous solution at an experimental therapeutic dose of 150 mg / kg and amoxacillin (25 / kg), ranitidine (50 mg / kg) and metronidazole (25 / kg), 1 time per day for 3 days from the beginning of the experiment; experimental 2 in an isoeffective dose of amoxacillin (25 / kg), ranitidine (50 mg / kg) and metronidazole (25 / kg),, control group of rats – purified water in an equi-volume amount and according to a similar scheme. The interval of administration of acetylsalicylic acid and medicinal substances, as well as water, was 4-5 hours. Intact rats served as an additional control. Determination of the anti-ulcer activity of the studied extract and reference agents was carried out 18 hours after their last administration.

The pharmacotherapeutic efficacy of the extract was evaluated at the end of the experiments. The animals were euthanized in a CO2 chamber, their stomachs were opened, the mucous membrane was washed with saline solution and the nature and number of destructive lesions on the gastric mucosa were determined macroscopically using a magnifying glass. The Pauls Index (IP) was calculated by the formula: IP=A•B/100, where A is the average number of ulcers per animal; B is the number of animals with ulcers in the group. The anti-ulcer effect (AUE) of the extract was judged in relation to the IP in the control to the IP in the experimental group of animals (PD = IPk/IPo), with AU/E 2 or more, it was believed that the extract, as well as reference drugs, have an anti-ulcer effect. Statistical processing of the obtained data was carried out using the Statistica 6.0 software package (USA). The data obtained during the experiments were processed by statistically generally accepted methods for a small sample with the determination of the average value (M) and the standard error of the average (m) using the formula M ± m. The reliability of the results (P) was evaluated using the student's t-test [17]. The differences were considered significant at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

The effect of the extract on the course of stress ulcers in the stomach in white mice was studied. The data obtained are presented in Table 1.

Table 1. Effect of the Obtained Extract on the Course of Stress Ulcers in the Stomach of White Mice (M±M)

№	Animal Groups	Number of Mice With	Average Number of Ulcers			Number of Ulcers Per 1	Number of Erosions	IP/ AUE
			Large	Striped	Point			

		Ulcers, %				Mouse		
1.	Intact	0	0	0	0	0	0	0
2.	Control	100	1,2±0,24	0,8±0,24	6,9±0,11	8,7±0,12	6,6±0,11	8,5/0
3.	Experimental 1	50	0,24±0,01*	0,24±0,01*	3,1±0,20	3,3±0,1*	3,1±0,24	1,8/4,1
4.	Experimental 2	75	0,48±0,01*	0,24±0,01*	4,1±0,40	4,6±0,60*	4,4±0,24	4,1/2,4

In the conducted studies, visual studies have shown that the expressed anti-ulcer activity with the extract in the dose used is higher than the reference means without extracts (Table 1). The effectiveness of the combination with the extract is characterized by a decrease not only in large, point, stripe-shaped ulcers, but also by a decrease in the number of ulcerative lesions in mice in experimental 1. The AUE index at the introduction of the studied extract was 4.6, and in comparison, drugs: in an isoeffective dose of amoxacillin (25 / kg), ranitidine (50 mg / kg) and metronidazole (25 / kg). It is obvious that under the influence of which the addition of biologically active substances available in the extract under study helps to limit the hyperactivation of stress-implementing systems of the animal body: hypothalamic-pituitary-adrenal, sympatho-adrenal with a decrease in aggression factors, along with the mobilization of protective factors of the gastric mucosa, improves microflora and stabilization of cell membranes due to the content of phenolic substances the nature of the extract [18]. The effect of the extract on the course of "acetylsalicylic" gastric ulcer in white rats was studied. The data obtained are presented in Table 2.

Table 2. The Effect of the Extract on the Course of "Acetylsalicylic" Gastric Ulcer in White Rats (M ± M)

No	Animal Groups	Number of Mice With Ulcers, %	Average Number of Ulcers			Number of Ulcers Per 1 Mouse	Number of Erosions	IP/ AUE
			Large	Striped	Point			
1.	Intact	0	0	0	0	0	0	0
2.	Control	100	1,1±0,03	5,7±0,1	8,9±0,16	15,7±2,24	7,8±0,50	16,3/0
3.	Experimental 1	70	0,22±0,01*	1,7±0,22*	3,1±0,30*	5,3±0,50*	3,1±0,21*	3,9/4,9
4.	Experimental 2	87	0,44±0,01*	2,9±0,55*	4,4±0,11*	7,7±1,21*	4,2±0,22*	6,8/2,8

Acetylsalicylic acid in the used dose irritates the gastric mucosa, causes premature peeling of the epithelium with the appearance of erosions, hemorrhagic ulcers [2]. Against the background of the introduction of the extract, less pronounced damage to the gastric mucosa was observed compared to the data of experimental 1, experimental 2. In experimental 1, the index of anti-ulcer action when the extract was administered was 4.5, and in reference agents: in an isoeffective dose of amoxacillin (25 / kg), ranitidine (50 mg / kg) and metronidazole (25 / kg). Against the background of the introduction of the extract, ulcers were detected only in 70% of animals, and in the control – in 100% of cases. The introduction of the extract to rats was accompanied by an increase in the resistance of the gastric mucosa, the restriction of aggression factors due to the biologically active substances contained in it, primarily flavonoids, mucus, tannins and other natural compounds. It is known that acetylsalicylic acid causes destruction of the mucosal barrier caused by blockade of the prostaglandin-synthetase complex due to inhibition of cyclooxygenase, as well as mucosal ischemia with microcirculation disorder and microthrombosis in the subepithelial layer [19]. The compounds contained in this extract limit damage to the gastric mucosa due to their multifaceted action, primarily its membrane-stabilizing effect due to the

content of flavonoids, tannins and other compounds capable of suppressing free radical oxidation of biomacromolecules, and thereby limit the destruction of the mucosa [20], [21].

4. CONCLUSION

In general, the data of the conducted studies indicate a pronounced anti-ulcer activity of the obtained combination with plant extracts, which to some extent exceeds the effects of an isoeffective dose of amoxacillin (25 / kg), ranitidine (50 mg / kg) and metronidazole (25 / kg) in experiments on white mice and rats with damage to the gastric mucosa. The course administration of the extract and reference drugs to animals in isoeffective doses is characterized by a natural decrease in the Pauls index and an increase in the anti-ulcer index, the main criteria for evaluating the anti-ulcer activity of drugs. It is the rich complex of biologically active substances available in the extract that accelerates the healing of the ulcerative defect due to its multifaceted effect on the main pathogenetic mechanisms of this pathology. In fact, the systemic effect of the extract is considered, balancing the factors of aggression and defense when using it, which is consistent with the literature data. The results obtained indicate the anti-ulcer activity of the combination with complex plant extracts and are of great interest for clinical practice, argue for the expediency of its use as part of the technologies used for the treatment of patients with peptic ulcer disease, as well as at the recovery stage, which will increase the effectiveness of therapeutic and preventive measures.

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Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Ozodjon Ilkhomovich Ergashov	✓	✓	✓	✓		✓		✓	✓	✓	✓			

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Informed Consent

All participants were informed about the purpose of the study, and their voluntary consent was obtained prior to data collection.

Ethical Approval

Not Applicable.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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