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Gastroprotective effect of *Ferulopsis hystrix* (Bunge) Pimenov in ethanol-induced gastropathy

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ABSTRACT

The aim of the study was to evaluate the gastroprotective effect of dry extracts from the roots and rhizomes of *Ferulopsis hystrix* in ethanol-induced gastropathy.

Materials and methods. The studies were carried out on 68 white *Wistar* rats. Ethanol-induced gastropathy was simulated by a single intragastric administration of ethanol in the dose of 10 ml/kg. Animals of the experimental groups received medicinal forms from the roots and rhizomes of *F. hystrix*: I – decoction in a volume of 10 ml/kg; II–V – dry extracts in the dose of 200 mg/kg, obtained by extraction with purified water, 30, 40 and 70% ethanol, respectively; VI – dry extract, prepared by double extraction with 40% and single extraction with 30% ethanol for 7 days before the modeling of gastropathy. Number of structural changes was determined in the gastric mucosa. They were differentiated into small, large, and strip-like erosions. The Pauls' index was calculated for structural changes. Pathomorphological studies of the stomach were carried out.

Results. The total number of structural changes in the stomach of animals in experimental groups I, II, IV and V is 44% lower on average, in experimental group III it is 67% lower and in experimental group VI it is 3.6 times lower than in the control. The Pauls' index for large erosions in experimental groups I–V is 38–75% lower, in experimental group VI it is 83% lower than the index in the control animals. No strip-like erosions are detected in animals of experimental groups III–VI. Pauls' index for these destructions in experimental groups I and II is 7.0 and 6.5 times lower than the index in the control animals. Microscopic morphological examination registered the increase of shallow-like erosions in the stomach of animals of experimental groups. Shallow-like erosions do not reach the *muscularis mucosae*. Microcirculation disorders and leukocyte infiltration are less pronounced than in the control group.

Conclusion. *F. hystrix* has the gastroprotective effect, increasing the resistance of the gastric mucosa to the effect of ethanol. The *F. hystrix* extract prepared with 30% and 40% ethanol shows the most pronounced pharmacotherapeutic effect in ethanol-induced gastropathy.

Key words: ethanol-induced gastropathy, *Ferulopsis hystrix* (Bunge) Pimenov, gastroprotective effect.

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Гастропротективное влияние *Ferulopsis hystrix* (Bunge) Pimenov при экспериментальной этаноловой гастропатии

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РЕЗЮМЕ

Цель. Оценка гастропротективного действия сухих экстрактов, полученных с использованием различных экстрагентов из корневищ с корнями *Ferulopsis hystrix* (Bunge) Pimenov при этаноловом повреждении желудка у белых крыс.

Материалы и методы. Эксперименты проведены на 68 самцах и самках крыс линии Вистар. Этаноловую гастропатию моделировали однократным внутрижелудочным введением этанола в дозе 10 мл/кг. Животные опытных групп (I– VI) в течение 7 сут до моделирования гастропатии получали лекарственные формы из корневищ с корнями *F. hystrix*: I – отвар в объеме 10 мл/кг; II–V – сухие экстракты в дозе 200 мг/кг, полученные путем экстракции водой очищенной, 30-, 40- и 70%-м этанолом соответственно; VI – сухой экстракт, приготовленный двукратной экстракцией 40%-м и однократной экстракцией 30%-м этанолом. В слизистой оболочке желудка определяли структурные изменения, которые дифференцировали на мелкие, крупные и полосовидные эрозии. Проводили патоморфологические исследования желудка.

Результаты. Установлено, что в I, II, IV и V опытных группах общее количество повреждений в желудке было в среднем на 44% меньше, чем в контроле, в III опытной группе – на 67% и в VI опытной группе – в 3,6 раза. Индекс Паулса для крупных эрозий в I–V опытных группах был ниже контрольного показателя на 38–75%, в VI – на 83%. Полосовидные эрозии не выявлялись у животных III–VI опытных групп; индекс Паулса для данных деструкций в I и II опытных группах был в 7,0 и 6,5 раза ниже показателя контрольных животных. В стенке желудка животных отмечались неглубокие эрозии, не достигающие мышечной пластинки слизистой оболочки; нарушения микроциркуляции и лейкоцитарная инфильтрация были менее выражены относительно контроля.

Заключение. *F. hystrix* оказывает гастропротективное влияние, повышая резистентность слизистой оболочки желудка к действию этанола. Наиболее выраженный фармакотерапевтический эффект проявляет экстракт, приготовленный двукратной экстракцией 40%-м и однократной экстракцией 30%-м этиловым спиртом.

Ключевые слова: этаноловая язва, *Ferulopsis hystrix* (Bunge) Pimenov, гастропротективное действие.

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INTRODUCTION

Ferulopsis hystrix (Bunge) Pimenov is a perennial plant of *Umbelliferae* family growing in Russian South Siberia and Far East [1]. The given plant was formerly classified as *Phlojodicarpus turczaninovii* Sipl. The roots and rhizomes of *F. hystrix* are used in folk and traditional medicine. For a long time, the roots of *F. hystrix* known under the name "*chuksugbai*" have been in the common use in Tuvinian folk medicine and now it holds a leading position as anti-inflammatory, wound-healing remedy, as well as in the treatment of tuberculosis and cancer [2]. In Mongolian and Buryat traditional medical systems, *F. hystrix* is a substitute of *Costus speciosus* (Tibet. *ru rta*) used in Tibetan medicine for the treatment of *rlung* of the blood, diseases of the lungs and throat, for amelioration of "compression" in the stomach and stopping of necrosis [3].

Rhizomes and roots of *F. hystrix* contain various groups of biologically active substances among which coumarins play a leading role in the pharmacological activity of the plant; their total content is 3.9–4.6% [4, 5]. Coumarins have antiviral, antibacterial and antifungal properties [6, 7]; also, they have anti-inflammatory [8] and antioxidant activity [9, 10]. The experiments on animals have shown a marked gastro-protective effect of natural and synthetic coumarins [11]. In this regard, the study of the gastro-protective effect of the *F. hystrix* roots and rhizomes is of great interest.

The aim of the study was to estimate the gastro-protective effect of the dry extracts derived from the *F. hystrix* rhizomes and roots with the use of various extraction solvents.

MATERIALS AND METHODS

The experiments were carried out on 68 white male and female Wistar rats weighing 180–200 g. The animal care was compliant with the rules of "Good Laboratory Practice" (GLP) and the Order of the Russian Health Ministry "On approval of Rules for Laboratory Practice" (No. 199N, 01.04.2016). Before the start of the experiments, the animals meeting the criteria were divided into groups taking into account the randomization principle. The experimental work followed the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasburg, 1986).

The animals were divided into 7 groups: a control group and 6 experimental ones. The animals of group I received the decoction of *F. hystrix* in the volume 10 ml/kg prepared according to the General Monograph 1.4.1.0018.15 Infusions and decoctions. The animals of groups II–V received the dry extracts (200 mg/kg) obtained by extraction with purified water, 30, 40 and 70% ethyl alcohol respectively; the raw material-to-extraction agent ratio was 1 : 10; they were prepared at room temperature by vigorous shaking and successive vacuum drying. The animals of experimental group VI received the extract prepared by double extraction with 40% ethanol and the third extraction with 30% ethanol. The given choice of extraction solvents allowed us to obtain the extract with maximum content of extractive substances and good physical qualities [12].

According to the data of high-performance liquid chromatography, coumarins are the main components of the *F. hystrix* dry extract including peucenidin (3′-O-acetoxy-4′-O-senecioloxy-2′,3′-dihydroroselol) and skimmin (umbelliferone-7-O-glucoside; 1.26%), their content was 16.65 ± 0.33 and 1.26 ± 0.03%, respectively. The quantitative analysis of coumarins in the *F. hystrix* dry extract was performed with the HPLC method on the microcolumn liquid chromatograph Milichrom A-02 (Econova, Novosibirsk, Russia) on the column ProntoSIL-120-5-C18 AQ (2 × 75 mm, Ø 5 um; Metrohm AG, Herisau, Switzerland); mobile phase: 0.2 M $LiClO_4$ in 0.006 M $HClO_4$ (A), acetonitrile (B); gradient mode (% B): 0–26 min 5–100, 26–29 min 100; flow rate 150 µl / min; the column temperature was 35 °C; UV-detector, 330 nm. The content of coumarins was calculated with the use of calibration curves constructed using commercial reference samples of skimmin and peucenidin (> 95%; Wuhan ChemFaces Biochemical Co., Ltd., Wuhan, Hubei, PRC). The results are presented as the mean of triplicate ($M \pm SD$).

The tested medicinal forms of *F. hystrix* were introduced intragastrically to animals for 7 days and the last dose was introduced 1 hour before the injection of ulcerogenic agent. The animals of the control group received purified water according to the same scheme. The lesion of the stomach mucosa was induced by a single introduction of absolute alcohol to animals in the dose of 10 ml/kg against the background of 24-hour food deprivation [13]. One hour after the alcohol introduction, the rats were decapitated under light ether narcosis. To estimate the state of the gastric mucosa, the stomach was cut along the greater gastric curvature and the number of destructions was determined. The destructions were differentiated as small erosions (≤ 2 mm), large erosions (2–5 mm) and stripe-like

erosions (>5 mm). The Pauls' index was calculated for each kind of lesions according to the formula [13].

For patho-morphological studies, the material was fixed in 10% neutral buffered formalin, dewatered in ascending alcohol and embedded in paraffin. The sections were stained with hematoxylin and eosin.

The results obtained were processed with the use of the program Statistica for Windows 6.0. To describe statistical differences, nonparametric Mann – Whitney *U*-test was used. The results were presented as interquartile range median *Me* (Q_1; Q_3). To compare the lesion frequency in comparison groups, the Fisher test was used. The differences were significant with $p < 0.05$.

RESULTS AND DISCUSSION

Small erosions have been revealed in all control animals; large erosion in 8 animals and stripe-like erosions in 6 animals. The Pauls' index was 4.0, 2.4 and 1.1 respectively (Table 1). In the gastric mucosa of the control animals, hemorrhages and erosions in the form of narrow dehiscences resulted from desquamation of necrotic cells in surface and glandular epithelium were noted. Five in ten animals had deep lesions reaching up to muscular layer of mucous tunic that were classified as ulcers. At the bottom and edges of the ulcers, necrotic masses with signs of desquamation were revealed. In the boundary zone, massive leucocytic infiltration was noted, as well as cell elements of the glands in the state of marked dystrophy up to necrosis. In the vessels of microvasculature, nuclei of endothelial cells were bloated and plasma-impregnated and had signs of plasmarrhage and diapedetic hemorrhages found partially along the vessels or at considerable range in the form of homogeneous pink masses with a touch of erythrocytes and leucocytes. In the vessel lumina sludge phenomenon, erythro- and leucostasis were noted.

The use of the *F. hystrix* decoction and extracts prepared with the use of various solvents had gastro-protective effect increasing the resistance of gastric mucosa to ethanol impact. In the animals that received the decoction and extracts prepared with the use of water and 70% ethanol, the total number of gastric mucosa lesions was 44% less on average than in animals of the control group. Small erosions were revealed in all animals of the experimental groups; their quantity and Pauls' index were in line with the indices of animals in the control group. Large erosions were noted in 7-8 animals out of 10 in the given experimental groups. The Pauls' index for large erosions in the experimental groups I, II, and V was less than in the control group by 38, 46 and 42%, respectively. Stripe-like erosions were noted in 2 out of 8 animals in the group, which received the water extract, and in 2 out of 10 animals in the group, which received the decoction. The Pauls' index for stripe-like ulcers in the given experimental groups was 7.0 and 6.5 times lower than that in the control group. In most animals of the experimental groups I, II, and V, microscopic studies revealed hemorrhages, erosions injuring no more than two-thirds of their muscular layer of mucous tunic. Ulcers with deep necrosis of the muscular layer of mucous tunic and circumscribed by the marked leucocytic infiltration were revealed in two animals of the experimental groups I and II. The areas with signs of plasmarrhage and diapedetic hemorrhages were less marked.

In animals of the experimental groups III and IV, which received the extracts prepared with the use of 30% and 40% ethanol, the total number of destructions in the muscular layer of mucous tunic was 67 and 45% less than that in the control animals (Table 1). Small erosions were noted in 8 animals of the experimental groups III and IV; large erosions – in 6 and 8 out of 10 animals which received the extracts prepared with the use of 30% and 40% ethanol, respectively. No stripe-like erosions were noted in the animals of the given experimental groups. The Pauls' index for small and large erosions was significantly lower in the experimental group III and was 1.4 and 0.6, respectively, as compared to 2.1 and 1.6 in the experimental group IV; it was 2.9 and 4.0 times lower than these indices in the control group. Histological studies of the muscular layer of mucous tunic in the animals of the experimental groups III and IV revealed not deep erosions injuring only gastric superficial-foveolar epithelium. Single erosions penetrated into half of the muscular layer of mucous tunic. Leucocytic infiltration around erosions was marked moderately. Vessel dilatation in microvasculature along with erythro- and leucostasis and small areas of plasmarrhage and diapedetic hemorrhages were noted.

The extract of *F. hystrix* prepared with the use of 30 and 40% ethanol combination demonstrated more marked gastro-protective effect. The number of all destructions per one animal in the given experimental group was 2.5 (Table 1) that was 3.6 times less than that of the control group. No stripe-like erosions were found. Small and large erosions were noted respectively in 7 and 5 animals out of 10; the Pauls' index was 3.6 and 6.0 times lower than that in animals of the control group. Patho-histological studies of the

stomach wall in animals of the group VI revealed destructive alterations only in the gastric superficial-foveolar epithelium; its desquamation resulted in small erosions with slight leucocytic infiltration. The vessels of microvasculature were moderately dilated and plethorical with signs of erythrostasis and erythrodiapedesis; no sludge phenomenon and plasmarrhages were noted.

Table 1

The effect of *Ferulopsis hystrix* on the severity of lesions in the gastric mucosa of white rats in ethanol-induced gastropathy

Indices	Groups of animals						
	Control, $n = 10$	Experimental I, $n = 10$	Experimental II, $n = 8$	Experimental III, $n = 10$	Experimental IV, $n = 10$	Experimental V, $n = 10$	Experimental VI, $n = 10$
Total number of gastric mucosa lesions, *Me* (Q_1; Q_3)	9 (7;11)	5 (2;8) $p \le 0.05$	6 (2;8) $p \le 0.05$	3 (2;3) $p \le 0.05$	4.5 (2;8) $p \le 0.05$	5 (2;7) $p \le 0.05$	2.5 (0;4) $p \le 0.05$
Small erosions							
Number of animals with erosions, %	100	60	100	80	80	100	70
Number of destructions, *Me* (Q_1; Q_3)	4 (3;5)	3.5 (2;4)	3.5 (2;4)	2 (1;3) $p \le 0.05$	2 (2;4) $p \le 0.05$	3 (3;4)	2* (0;3)
Pauls' index	4.0	1.9	3.3	1.4	2.1	3.4	1.6
Large erosions							
Number of animals with erosions, %	80	70	75	60	80	80	50
Number of destructions, *Me* (Q_1; Q_3)	3 (2;4)	2.5 (0;4)	2 (0;3)	1 (0;1) $p \le 0.05$	2 (1;3)	2 (1;2)	0.5 (0;1) $p \le 0.05$
Pauls' index	2.4	1.5	1.3	0.6	1.6	1.4	0.4
Stripe-like erosions							
Number of animals with erosions, %	60	30	25	0 $p \le 0.01$	0 $p \le 0.01$	0 $p \le 0.01$	0 $p \le 0.01$
Number of destructions, *Me* (Q_1; Q_3)	1 (0;3)	0 (0;1)	0 (0;2) $p \le 0.05$	0	0	0	0
Pauls' index	0.84	0.12	0.125	0	0	0	0

Note: *n* – number of animals included in the analysis.

The gastro-protective effect of the medicinal forms from the roots and rhizomes of *F. hystrix* is due to the content of phenolic compounds, particularly, coumarins and flavonoids, which have antioxidant, anti-inflammatory, anticoagulant and other effects. Coumarins inhibiting the enzyme COX-2 in the lesion suppress the synthesis of inflammation mediators (histamine, serotonin), proinflammatory cytokines – IL-1β, TNF-α and other biological substances; due to this, they have an anti-inflammatory effect promoting the decrease of vascular permeability and leucocyte migration [14, 15]. The vessel-dilating effect of coumarins is due to the decrease of intracellular calcium concentration in smooth myocytes in microvasculature vessels. The decrease of thromboxane B2 synthesis in thrombocytes due to cyclooxygenase inhibition plays an important role in the mechanism of coumarin anti-coagulating effect [16]. Hence, the inhibition of thrombocyte aggregation indirectly promotes microcirculation in the stomach mucosa, which, together with the vessel dilating and anti-inflammatory effects of coumarins, explains the presence of gastro-protective activity of *F. hystrix* in ethanol-induced stomach injury in white rats. This gastro-protective effect is probably due to antioxidant properties of coumarins and flavonoids contained in *F. hystrix* and their capability to chelate mixed-valent metal ions participating in the reactions of free radical formation [17], bind formed radicals [9] and increase the resistance of cell membranes [11].

CONCLUSION

Thus, the decoction and dry extracts from rhizomes and roots of *F. hystrix* prepared with the use of various solvents have gastro-protective effect increasing the resistance of the stomach mucosa to the impact of ethanol, limiting the development of dystrophic and necrotic processes in the gastric superficial-foveolar

and glandular epithelia and preventing the development of inflammatory processes in the stomach wall of white rats. The given medicinal forms may be arranged according to their pharmacological efficiency in ascending order: water extract of *F. hystrix* < extract of *F. hystrix* prepared with the use of 70% ethanol; < extract of *F. hystrix* prepared with the use of 40% ethanol < extract of *F. hystrix* prepared with the use of 30% ethanol < extract of *F. hystrix* prepared with the use of 40 and 30% ethanol.

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Authors contribution

Salchak S.M., Toropova A.A. – conception and design development, analysis and interpretation of data, critical revision for important intellectual content. Razuvaeva Ya.G., Arakchaa K.D. – substantiation of the manuscript, critical revision for important intellectual content, final approval of the manuscript for publication. Olennikov D.N., Nikolaeva I.G. – analysis and interpretation of data, critical revision for important intellectual content.

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