VILNIUS UNIVERSITY

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EXPERIMENTAL AND CLINICAL EVALUATION OF OXIDATIVE PROCESSES AND EFFECTIVENESS OF HERBAL TINCTURES IN THE GASTRODUODENAL MUCOSA

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VILNIAUS UNIVERSITETAS

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GASTRODUODENINĖS SRITIES GLEIVINĖS OKSIDACINIŲ PROCESŲ IR AUGALŲ TINKTŪRŲ POVEIKIO EKSPERIMENTINIS IR KLINIKINIS ĮVERTINIMAS

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List of abbreviations used in the text

AA – adjuvant arthritis
AOA – antioxidant activity
H_2O – water
H_2O_2 – hydrogen peroxide
CAT – catalase
MDA – malondialdehyde
ROS - reactive oxygen species
SOD – superoxide dismutase
AH – Horse chestnut
FU – Meadowsweet

1. INTRODUCTION

Despite of the well-developed pharmaceutical industry and high technologies which are being used for the production of various drugs, more and more researchers and clinicians tend their attention to research the biologically active substances of herbs and their effectiveness in the treatment of different diseases.
Use of herbs in the clinical practice is often based on the observations of folk medicine, but till now it is lack of consistent clinical studies, demonstrating their usefulness for the treatment of various diseases. Herbs are able to facilitate the side effects induced by chemical drugs. They can enhance the effects of drug action and may also be used for the prevention of the disease relapse.
For this reason, it is essential to determine the pharmaco-therapeutic impact of biologically active substances of plants on the all chains of pathological process for the successful application of herbs in the clinical practice.
Treatment of functional disorders and organic changes in the gastrointestinal tract with herbs can be one of the areas of herbs application beside the usual therapy. For this purpose, the use of herbs will be scientifically proved and the new opportunities will be unclosed to coordinate both therapies (i.e., phytotherapy and drug therapy).
It is known that the capacity of antioxidant system of organism and lipid peroxidation processes play an important role in the occurring of various trophic and proliferative pathological changes (including ulceration) in the gastric and duodenal mucosa. Products of lipid peroxidation markedly increase in the damaged tissues of gastric ulcer through the circulatory upset.
The rise of ulcers is associated with the presence of *Helicobacter pylori* (*H. pylori*) infection in the gastroduodenal mucosa that leads to the induction of free oxygen radicals during the pathological process.
The search of the ways to act on the pro/antioxidant system in the whole organism or in the ulcers with the purpose to accelerate their healing and reduce the likehood of ulcer relapse is very important. Some substances of herbs show antimicrobial activity and immune response against *H. pylori*. It is believed that one of the factors contributing the healing of ulcer is the antioxidant properties of the herbal substances.
The use of herbs in the treatment of gastrointestinal diseases is not enough based by experimental and clinical investigations. The horse chestnut (*Aesculus hippocastanum* (L.)) [AH] and meadowsweet (*Filipendula ulmaria* (L.) Maxim) [FU] flowers have the high amounts of flavonoids and other biologically active compounds, which possess an antioxidant effect, and therefore can positively influence the oxidative system of gastroduodenal mucosa. However, it was failed to find the data about the influence of tinctures from AH and FU flowers on the evolution of reactive oxygen species (ROS) in the gastroduodenal mucosa. Therefore, it can be stated that the investigation of those tinctures on the oxidative system of the gastroduodenal mucosa is an important area of scientific research because of their antioxidant properties which can improve the healing of the gastroduodenal ulcers.

Aim of the study

The aim of the study is to determine the changes of the oxidative system in the blood and mucosa of patients suffering from gastric and duodenal ulcers, *in vivo* evaluate the

impact of the herbal tinctures prepared from the *Aesculus hippocastanum* (L.) and *Filipendula ulmaria* (L.) Maxim flowers on the mucosal oxidative system of the rats with experimentally-induced ulcers, and *in vitro* – in the mucosal biopsies obtained from the experimental and clinical material.

Objectives of the study

1. To investigate the changes of pro/antioxidant system indices in the gastric mucosa and blood serum of rats with experimentally-induced ulcers during physiological conditions or in the case of systemic disease (adjuvant arthritis).
2. To assess the effects of *Aesculus hippocastanum* (L.) and *Filipendula ulmaria* (L.) Maxim tinctures on the indices of oxidative system of the gastric mucosa in the rats with experimentally-induced ulcers.
3. To investigate the changes of pro/antioxidant system indices in patients with gastroduodenal diseases, and to explore the dependence of these changes on *H. pylori* infection.
4. To assess the status of oxidant system in the blood serum and gastroduodenal mucosa (investigations *in vitro*) and evaluate the effects of herbal tinctures on these indices in patients with acute and healing ulcers.

Scientific novelty

The growing importance of natural non-toxic herbal preparations causing more sparing than crippling effects is seen during the recent years. Although the significance of oxidative system and the effects of chemical drugs in the treatment of gastroduodenal diseases are widely described in scientific literature, the number of patients suffering from them has not decreased. Therefore, the search of the new ways that could successfully affect the oxidative system is still remained.
For the first time, on the basis of *in vivo* and *in vitro* tests, the effects of tinctures from the AH and FU on the indices of oxidative system of the gastric and duodenal mucosa was investigated in patients with gastroduodenal pathology and in animals with experimentally-induced gastric ulcers.

Practical significance of the study

In addition to usual therapy the plants are a valuable material for the manufacture of herbal products and their application as adjunctive therapy for the treatment of some pathological conditions. Studies *in vivo* and *in vitro* of FU and AH tinctures will enable to broaden the potential use of these herbs in the field of gastroduodenal pathology.

Defensive theses

1. The development of experimentally-induced gastric ulcers changes the status of oxidative system of gastroduodenal mucosa during the physiological conditions as well as in the case of systemic disease.
2. A different degree of the changes in the oxidative system of organism reflects an acute or healing stage of ulceration process in the gastroduodenal mucosa.

3. During the course of ulcerative process the tinctures from blossoms of *Aesculus hippocastanum* (L.) and *Filipendula ulmaria* (L.) Maxim positively acted the oxidative system of the gastroduodenal mucosa and distinctly reduced the intensity of prooxidative indices.

2. MATERIALS AND METHODS OF THE SUDY

2.1. Experimental investigations

2.1.1. Herbal tinctures

Ethanolic tinctures prepared from the flowers of FU and AH were studied. Tincture obtained from FU flowers has been registered in Lithuania (Keturkienė et al., Patent EN 4880B, 2002).
The content of salicylic aldehydes in this tincture was 0.15 mg/ml (25°), flavonoids - 1.39 g per 100 ml. In AH tincture the content of flavonoids was 1.88 g/100 ml. No toxic effects on the rats by using the maximal (FU - 1.57 g/kg and AH - 1.55 g/kg) and repeated doses (5 mg/kg, 10 mg/kg, 20 mg/kg) on the 21^{st} day of the study were revealed [Keturkienė et al., 2000; Keturkienė et al., 2002] in the toxicity studies.

2.1.2. Animals

In this study 95 female Wistar rats weighing 200-240 g were used. The animals were purchased from Institute of Immunology (Vilnius, Lithuania). They were housed in standard cages under standard light conditions (12 h light/dark cycle) at 50-70% humidity and at room temperature of 18^0 C $\pm$ 2^0 C. Rats received standard chow ("Kėdainių grūdai") and water *ad libitum*. The animals had been allowed to acclimatize for 3 days before the experiments were started. All animals were used with the approval of Lithuanian Laboratory Animal Use Ethics Committee under the State Food and Veterinary Service.

2.1.3. *In vivo* experiments

Gastric ulcers in healthy rats and rats with adjuvant arthritis (AA) were induced with indomethacin (Balkanpharma, ser. No 594801) prepared in 2% of Tween 80® solution and injected *per os*. Two injections of indomethacin in doses 30 mg/kg/day and subsequent injection of drug in dose 60 mg/kg/day for 12 hours fasting rats were used. The treated groups of rats received herbal tinctures *per os* in the dose of 0.75 ml/100 g of body weight 1 hour before the administration of indomethacin. The rats were decapitated after 24 hours under the light anaesthesia. The stomachs were removed and rinsed by cold physiological solution, placed on the ice and opened along the greater curvature. Gastric samples were photographed and the development of indomethacin-induced gastric ulcers was evaluated. The percentage of ulcer areas observed macroscopically in the antrum zone of gastric was tabulated by using the computerized planimetry and examined histologically. Then the samples from the antral portion of stomach were frozen at -20° and kept for 3-7 days for the estimation of biochemical parameters. The gastric mucosa was scrapped, weighed and homogenized in the appropriate ice-cold

phosphate buffer. The end product of lipid peroxidation malodialdehide (MDA), activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and total antioxidant activity (AOA) were determined in the blood samples of the all groups.

2.1.4. Experiments *in vitro*.

For the determination of the indices of mucosal oxidative system the gastric mucosal samples were homogenized in the buffered solution (10%, pH = 7.4) by using a glass homogenizer and centrifuged at 13000 rpm for 15 min at 4 °C. The dose of the herbal tincture was chosen experimentally. After 1 hour of incubation at 37 °C the homogenate (0.2 ml) was supplemented with the tincture (0.1 ml).

2.1.5. Biochemical methods of investigation

2.1.5.1. Assay of lipid peroxidation

The end product of lipid peroxidation (MDA) was measured with 0.8% thiobarbituric acid in 2% solution of orthophosphoric acid according to the method described by Gavrilov and co-authors (1987). The data was expressed as nmol/mg of protein.

2.1.5.2. Detection of catalase (CAT) activity

CAT activity was measured by the following decomposition of 0.03% of hydrogen peroxide (H_2O_2) in the presence of 4% solution of ammonium molybdate as described by Koroliuk et al. (1988). The data was expressed as nmol/L/min.

2.1.5.3. Detection of superoxide dismutase (SOD) activity

SOD activity was measured by using nitrotetrasolium blue chloride as described by Csovari et al. (1991). The data was expressed as the units of the activity in mg of protein.

2.1.5.4. Assay of total antioxidant activity (AOA)

AOA was measured by applying 1% Tween 80® and 0.25% thiobarbituric acid as described by Galaktionova et al. (1998). The data was expressed as the percentage of reduction rate and compared with blank values.

2.1.5.5. Detection of protein content in the samples

The protein content of the samples was measured by commercial Kit B040 (Aqua Medica, Poland).

All chemicals used in the study were obtained from Sigma or Fluka.

2.2. Clinical investigations

2.2.1. Methods of sampling and assessment of biopsy material from the gastric and duodenal mucosa

The patients with complaints through illness of the upper gastrointestinal tract and who underwent the esophagogastroduodenoscopy were drawn in the study. During this procedure biopsies for the histological investigation were taken according the Sydney guidelines for the assessment of gastritis by using the Houston visual analogue scale.
The stages of ulceration in the study were evaluated in accordance with the histological investigation. High infiltration with polymorphonuclear cells was the main feature of the acute stage of ulceration. The healing ulcer's stage was determined as a slight infiltration with monomorphonuclear cells and plasma cells.

2.2.2. Patients' selection criteria

1. Patients with gastro-intestinal disorders suffering from the ulcers of gastroduodenal mucosa who were diagnosed by endoscopy.
2. Patients not treated with gastroprotective drugs for three months.
3. Patients who did not receive hormones, cytotoxic drugs, non-steroidal anti-inflammatory drugs for three months and non-smokers.
4. Patients free from the other diseases.

Patients without the visual endoscopic pathology and only with the complaints of dyspepsia served as a control.
All investigations were carried out after the permission of Lithuanian Bioethics Committee.

2.2.3. Incubation of mucosal samples with herbal tinctures and the measurement of the indices of oxidative system

Biopsy samples for the determination of hydrogen peroxide (H_2O_2) were stored at -70° C. Mucosal tissues were homogenized and prepared as 5% homogenates in the buffer of Amplex Red kit. During *in vitro* studies the dose of FU and AH tinctures for the incubation with gastroduodenal mucosa homogenates was chosen according to a standard adult (70 kg) dose of the tincture approved by Pharmacopoeia (2 μl).
For the assessment of levels of reactive oxygen species (ROS) in the gastroduodenal mucosa there was applied a detection of H_2O_2 using an Amplex Red Hydrogen peroxide/peroxidase Assay Kit **(A-22188** MOLECULAR PROBES).
The indices of oxidative system (MDA, CAT, and AOA) in the serum of patients were investigated in the same methods as described above.

2.3. Statistical analysis of data

All the data was expressed as mean values ± SEM. Statistical analysis was done using SPSS/PC software version 8.0. P values less than 0.05 were considered to be significant. The nonparametric Mann-Whitney test was used for the evaluation of histological changes in the test organs.

3. RESULTS
3.1. Experimental investigations

3.1.1. Experimentally induced gastric ulcers in rats and their treatment with herbal tinctures

A decrease of the ulcer index by 3% was observed after the treatment with FU tincture (Table 1). The histological investigation revealed the significant decrease ($P < 0.05$) of inflammatory infiltration with polimorphonuclear leukocytes, less count of erosions, less plethora after the administration of FU tincture.

Table 1. Area of gastric mucosal lesions in ulcer-induced rats treated with tincture of FU

Index	Groups		
	I **Gastric ulcer** **(n = 9)**	**II** **Gastric ulcer + FU tincture** **(n = 9)**	**III** **Control** **(n = 10)**
Total gastric area (mm^2)	649.97±25.58*	$^{+}$802.18±15.78*	1122.37±86.08
Area of antrum (mm^2)	172.89±10.82*	$^{+}$209.21±14.19	321.21±26.54
Damaged area in antrum (mm^2)	33.84±14.49	42.21±8.86	-
Ulcer index	**5.11**	**4.96**	-

Note: Indomethacin-induced experimental ulcers were performed as described in Material and Methods. The percentage of areas of antral ulcers was tabulated by computerized planimetry. Ulcer index is the ratio between the ulcer area and the total area of a glandular part of stomach. (*) – The differences are significant in comparison with the control group. (+) – The differences are significant between I and II groups.

It was found that in rats with experimental gastric ulcer the level of MDA in the blood serum decreased by 12.7% in comparison with the healthy rats. The activity of antioxidant enzymes (CAT and SOD) and AOA was also significantly diminished by 69.3%, 32.25% and 71.6% respectively.

Table 2. Indices of pro/antioxidant system in the serum of ulcer-induced rats treated with FU tincture

Groups		n	MDA (nmol/ml)	CAT (mmol/L/min)	SOD (ua/mL of serum)	AOA (% of reduction)
I	**Gastric ulcer**	9	5.49±0.26	26.95±0.76*	85.59±3.07*	9.09±3.32*
II	**Gastric ulcer + FU tincture**	9	5.41±0.21	24.78±0.77*	$^{+}$67.49±3.76*	12.38±1.41*
III	**Control**	10	6.29±0.37	$^{+}$87.73±0.89	$^{+}$126.33±4.13	$^{+}$32.00±1.14

Note: FU tincture (in dose 0.75 ml/100 g of body weight) was given daily *per os*. The first injection was performed 60 min before the induction of ulcers. MDA - malondialdehide, CAT - catalase, SOD – superoxide dismutase, AOA - total antioxidant activity. (*) - The differences are significant in comparison with the control group. (+) - The differences are significant in comparison with the I^{st} group.

The treatment with FU tincture reduced the level of MDA by 1.5% and SOD activity by 21.15% ($P < 0.002$) in the blood serum as compared with the intact animals. No

significant differences in the activity of CAT and AOA between the treated and untreated groups of animals were detected.

Because the tinctures were prepared in 40° ethanol solution, the impact of ethanol on the indices of pro/antioxidant system in the homogenates of gastric mucosa have been studied. Ethanol significantly increased the level of MDA in the homogenates of gastric mucosa of the ulcer-induced rats and significantly decreased CAT activity in the all tested groups of animals. Vehicle had no impact on AOA.

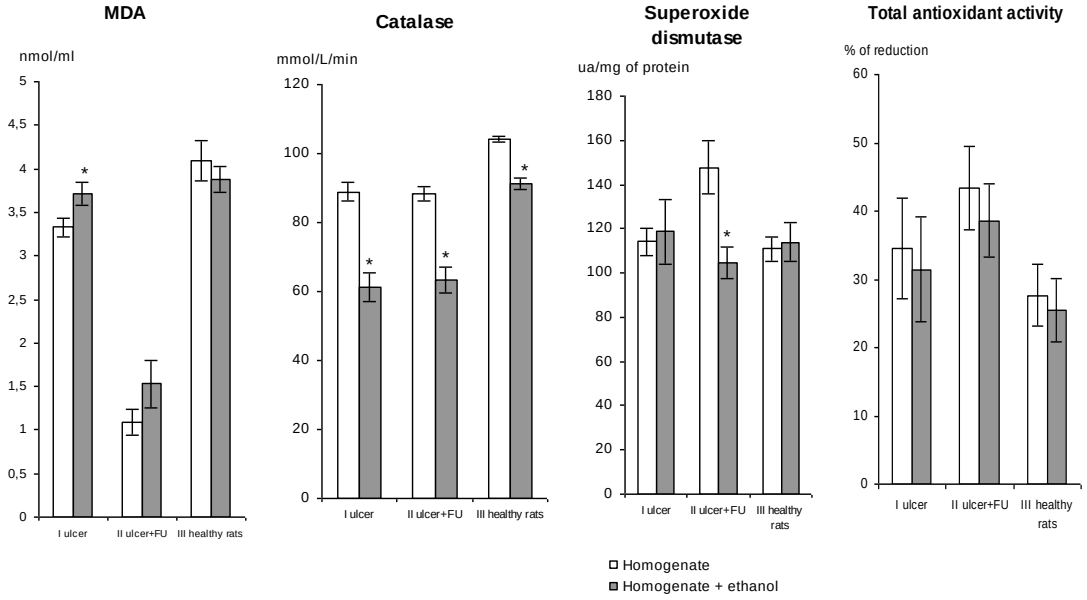

Fig. 1. Effect of 40º ethanol on the parameters of pro/antioxidant system in the homogenates of gastric mucosa of the ulcer-induced rats treated with FU tincture

10% gastric tissue homogenate produced with ratio 1:9 (one part of tissue and nine parts of buffer). (*) – The differences are significant between the pure homogenate and the homogenate with ethanol.

Incubation of homogenates from the healthy rats with FU tincture (Table 3) diminished the activity of antioxidant enzymes CAT and SOD by 81.8% and 10.5% respectively and enhanced AOA values by 2.4 times ($P < 0.001$).

Table 3. Indices of pro/antioxidant system in the homogenates of gastric mucosa of the ulcer-induced rats after their incubation with FU tincture

Index	**Groups**					
	I **Ulcer** (n = 9)		**II** **Ulcer + FU tincture** (n = 9)		**III** **Control** (n = 10)	
	Homogenate	**Homog.+FU**	**Homogenate**	**Homog.+FU**	**Homogenate**	**Homog.+FU**
MDA (nmol/ml)	333±0.11*	3.73±0.09^{+}	•1.09±0.15*	•1.39±0.20*	4.01±0.23	3.87±0.06
CAT (mmol/L/min)	89.88±2.77*	18.29±0.71^{+}	88.27±2.05*	20.16±0.60^{+}	104.24±0.74	18.99±0.73^{+}
SOD (ua/mL of serum)	114.07±6.33	83.82±7.78^{+}	•147.61±12.00*	77.02±8.51*$^{+}$	110.80±5.33	99.16±0.95^{+}
AOA (% of reduction)	34.54±7.29	64.41±2.41^{+}	43.33±6.13	68.93±1.58^{+}	27.65±4.57	61.23±4.49^{+}

Note: 10% gastric tissue homogenates were produced with ratio 1:9 (one part of tissue and nine parts of buffer). (*) – The differences are significant in comparison with respective groups of the control rats. (+) – The differences in the groups are significant between the pure homogenates and the homogenates incubated with FU tincture. (•) – The differences are significant between the subgroups of I and II groups.

A slight decrease of MDA by 3.62% was found. In the ulcer-induced groups a significant increase of MDA and AOA (by 12% and 86.5%, respectively) and a reduction of SOD ($P < 0.0001$) and CAT ($P < 0.01$) activity (by 79.65% and 26.5 %) were observed. After the incubation of homogenates with FU tincture, AOA significantly increased by 59.08% ($P < 0.001$) in the ulcer-induced rats orally treated with FU tincture. As a result, the treatment with FU tincture markedly decreased the level of MDA and significantly increased AOA in the gastric tissue.

Table 4. Indices of pro/antioxidant system in the blood serum of the ulcer-induced rats treated with AH tincture.

Groups		n	MDA (nmol/ml)	CAT (mmol/L/min)	SOD (ua/mL of serum)	AOA (% of reduction)
I	**Gastric ulcer**	9	5.51±0.27	46.59±2.82*	87.47±10.20*	14.76±5.12*
II	**Gastric ulcer + AH tincture**	9	5.01±0.21	$^{+}$67.52±3.18*	112.31±8.70	21.14±4.87*
III	**Control**	10	4.89±0.31	$^{+}$90.43±3.12	$^{+}$130.13±11.02	$^{+}$33.17±2.96

Note: (*) - the differences are significant in comparison with the control group. (+) - The differences are significant in comparison with the Ist group.

The ulcer-induced rats treated with AH tincture (Table 4) showed a significant increase of CAT activity by 44.9% ($P < 0.001$) and SOD activity by 28.4%. AOA in the blood serum was also elevated by 43.2%.

In homogenates from the healthy animals incubated with AH tincture a statistically significant decrease of CAT activity by 22.5% ($P < 0.001$) and the increase of SOD activity by 21.6% (the differences were near to significant, $t = 2.04$) were revealed (Table 5). Nevertheless, AOA after the incubation with AH tincture increased in the gastric mucosal homogenates about 1.8 times ($P < 0.002$). The incubation of the gastric mucosa homogenates obtained from the ulcer-induced rats with AH tincture showed an elevated level of MDA by 16.2% ($P < 0.02$), the increased SOD activity (by 19.5%) and AOA (by 23.85%). Accordingly, 2 times a lower activity of CAT ($P < 0.001$) was observed. Similar changes of pro/antioxidant indices in the gastric mucosa homogenates were observed in the animals of the experimental group treated *per os* with AH tincture. Only the activity of SOD was decreased by 27.53%. In each of the investigated group the incubation of homogenates with AH tincture slightly increased the levels of MDA, AOA and decreased the activity of CAT in comparison with their levels in the pure homogenates. SOD activity after the incubation with AH was elevated except the group of rats that received AH tincture *per os* where its activity decreased by 29.3%. The highest SOD activity in the gastric homogenates was revealed in the group of animals that received AH tincture *per os*.

To sum up, FU tincture significantly stimulated AOA and AH tincture AOA and SOD activity in the gastric mucosal homogenates. The oral pre-treatment with herbal tinctures markedly diminished the levels of MDA.

Table 5. Indices of pro/antioxidant system in the homogenates of gastric mucosa of the ulcer-induced rats after their incubation with AH tincture

Index	Groups					
	I Ulcer (n = 9)		II Ulcer + AH tincture (n = 9)		III Control (n = 10)	
	Homogenate	Homog.+AH	Homogenate	Homog.+AH	Homogenate	Homog.+AH
MDA (nmol/ml)	3.33±0.11*	$^{+}$3.87±0.18	$^{\bullet}$1.09±0.15*	$^{\bullet +}$1.62±0.17*	4.01±0.23	4.18±0.23
CAT (mmol/L/min)	89.88±2.77*	$^{+}$42.34±4.02*	88.27±2.05*	$^{+}$47.89±4.96 *	104.24±0.74	$^{+}$80.82±2.85
SOD (ua/mL of serum)	114.07±6.33	136.29±19.16	$^{\bullet}$147.61±12.00*	$^{+}$106.98±11.36*	110.80±5.33	134.75±10.46
AOA (% of reduction)	34.54±7.29	42.78±3.30	43.33±6.13	46.26±3.76	27.65±4.57	$^{+}$48.67±3.41

Note: 10% gastric tissue homogenates were produced with ratio 1:9 (one part of tissue and nine parts of buffer). (*) – The differences are significant in comparison with respective groups of the control rats. (+) – The differences in the groups are significant between the pure homogenates and the homogenates incubated with AH tincture. (•) – The differences are significant between the subgroups of the I and II groups

3.1.2. Experimentally induced ulcers in the rats with adjuvant arthritis and their treatment with herbal tinctures

3.1.2.1. The influence of FU tincture on the parameters of pro/antioxidant system in the blood serum and gastric mucosa of the ulcer-induced rats with adjuvant arthritis

It is known that the digestive tract is damaged during the development of autoimmune process. Thus the status of oxidative system in the gastric mucosa of the ulcer-induced rats with adjuvant arthritis (AA) was examined. The ulcer index decreased by 11.5% after the treatment with FU tincture (Table 6) and less mucosal oedema, inflammatory infiltration with macrophages and neutrophylic leukocytes were observed in the samples of gastric mucosa.

Table 6. Areas of the lesions in the gastric mucosa of the ulcer-induced rats with adjuvant arthritis treated with FU tincture

Index	Groups			
	I AA	II AA+ulcer	III AA+ulcer+FU	IV Control
Total gastric area (mm^2)	866.73±30.82*	839.41±26.60*	774.35±46.75	1122.37±86.08
Area of antrum (mm^2)	232.32±11.50*	265.22±15.53	211.87±20.84	321.21±26.54
Damaged area in antrum (mm^2)	-	51.85±10.80	46.74±11.39	-
Ulcer index	-	**5.12**	**4.53**	-

Note: Gastric samples were photographed and converted to a digital picture. The percentage of the areas of antral ulcers was tabulated by a computerized planimetry. Ulcer index is the ratio between the ulcer area and the total area of glandular part of the stomach. (*) – The differences are significant in comparison with the control group.

A statistically significant reduction of MDA by 21% and CAT activity by 62.4% in the blood serum of the ulcer-induced rats with AA was observed in comparison with the

control AA group (Table 7). The most obvious decrease of AOA by 94.8% was also revealed in this group ($P < 0.0001$). The treatment with FU tincture significantly increased the values of SOD activity by 45.46 % and AOA by 6.6 times ($P < 0.01$).

Table 7. Indices of pro/antioxidant system in the blood serum of the ulcer-induced rats with adjuvant arthritis treated with FU tincture

Groups		n	MDA (nmol/ml)	CAT (mmol/L/min)	SOD (ua/mL of serum)	AOA (% of reduction)
I	**Rats with AA**	**8**	$^{+}$6,67±0,09	$^{+}$95,53±1,11	138,04±15,23	$^{+}$37,41±1,13
II	**Ulcer-induced rats with AA**	**6**	5,27±0,09*	35,93±1,49*	126,39±2,94	1,95±1,36*
III	**Ulcer-induced rats with AA +FU ticture**	**9**	5,23±0,18*	34,35±1,57*	$^{+}$183,85±5,47*	$^{+}$12,89±4,43*

Note: AA – adjuvant arthritis was induced by a single injection of 0.1 ml of complete Froind's adjuvant into the left hind paw. The experimental ulcer was induced by the three injections of indomethacin. The treatment with FU tincture (dose 0.75 ml/100 g of body weight) was started 60 min. before the induction of ulcers and applied daily *per os*.
(*) - The differences are significant in comparison with the control AA group. (+) - The differences are significant in comparison with the II group.

The incubation of homogenates with ethanol solution significantly diminished CAT activity in the all tested groups, but it did not influence the other indices (Fig. 2).

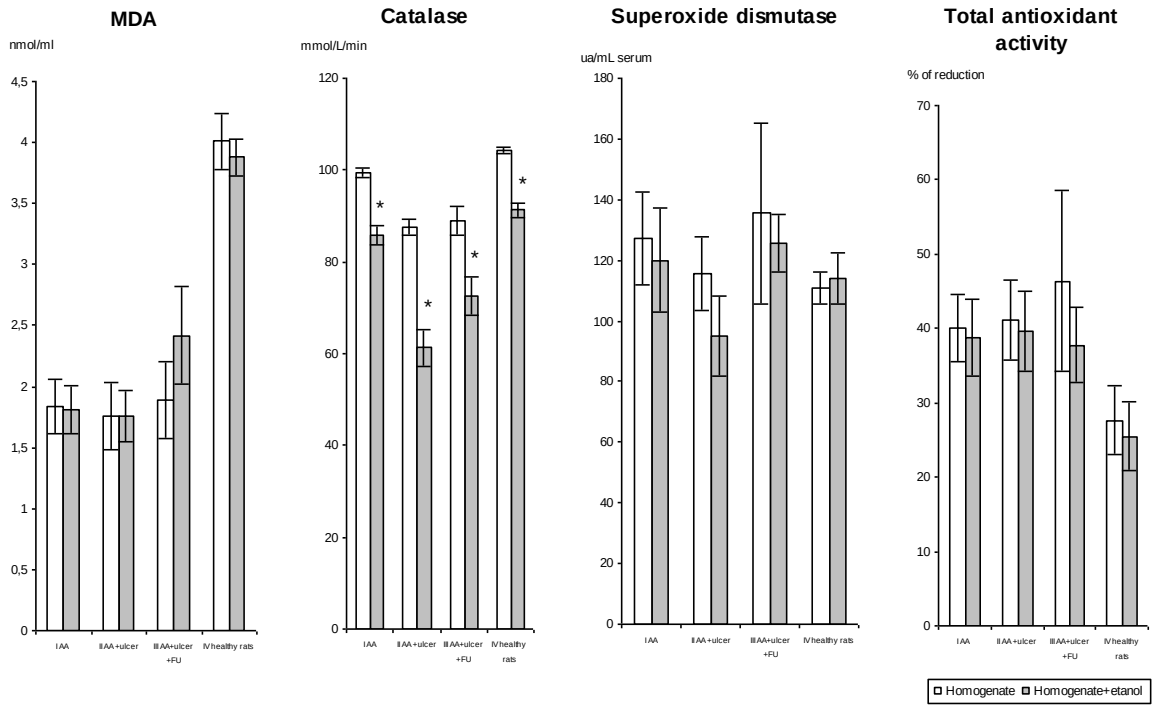

Fig. 2. Effect of 40º ethanol on the parameters of pro/antioxidant system in the homogenates of the gastric mucosa of the ulcer-induced rats with AA treated with FU tincture.
10% gastric tissue homogenates were produced with the ratio 1:9 (one part of the tissue and nine parts of the buffer). (*) – The differences are significant between the pure homogenate and the homogenate incubated with 40º ethanol.

The decreased level of MDA (in the all tested groups) which significantly differed from the healthy animals ($P < 0.001$) was observed in the pure homogenates and the homogenates incubated with FU (Table 8). CAT activity after the treatment with FU was about 4-5.5 times lower in comparison with the healthy animals ($P < 0.002$-0.001).

Table 8. The influence of FU tincture on the indices of pro/antioxidant system in the homogenates of gastric mucosa of the ulcer-induced rats with AA

Index	Groups							
	I AA (n = 9)		II AA + ulcer (n = 8)		III AA + ulcer + FU (n = 6)		IV Control (n = 10)	
	Homoge-nate	Homog. + FU	Homoge-nate	Homog. + FU	Homoge-nate	Homog. + FU	Homoge-nate	Homog. + FU
MDA (nmol/ml)	1.84±0.22*	2.29±0.36*	1.76±0.28*	2.29±0.16*	1.89±0.31*	2.28±0.24*	4.01±0.23	3.87±0.06
CAT (mmol/L/min)	99.47±0.94*	$^{+}$24.45±0.95*	$^{\bullet}$ 87.53±1.60*	$^{+}$19.61±1.14 $^{\bullet}$	$^{\bullet}$ 88.96±3.20*	$^{+}$18.35±1.23 $^{\bullet}$	104.24±0.74	$^{+}$18.99±0.73
SOD (ua/mL of serum)	127.21±15.55	92.28±14.18	115.79±12.18	$^{+}$78.88±9.48*	135.45±29.76	101.00±13.17	110.80±5.33	99.16±0.95
AOA (% of reduc-tion)	40.02±4.46	$^{+}$57.39±5.25	41.13±5.40	$^{+}$62.07±5.30	46.35±12.17	51.15±10.90	27.65±4.57	$^{+}$61.23±4.49

Note: (*) – The differences are significant in comparison with the respective groups of the control rats. (+) – The differences in the separate groups are significant between the pure homogenates and the homogenates incubated with FU tincture. (•) – The differences are significant between the subgroups of the II and III groups.

Although the incubation of homogenates with FU tincture in the all tested groups reduced SOD activity, the significant differences between the pure and the incubated homogenates were observed only in the ulcer-induced animals with AA ($P < 0.05$).
AOA activity in the pure gastric mucosal homogenates of the all tested groups was higher by 44.7%, 48.75% and 67.6% than in the healthy control animals. The incubation of mucosal homogenates with FU tincture increased AOA in all investigated groups. A significant increase was observed in I, II and the control groups ($P < 0.05$-0.001) (Table 8).

3.1.2.2. The influence of AH tincture on the parameters of pro/antioxidant system in the gastric mucosal homogenates of the ulcer-induced rats with adjuvant arthritis

The level of MDA significantly increased ($P < 0.02$) only in the homogenates of gastric mucosa of the rats with AA after their incubation with AH tincture (Table 9). In the ulcer-induced rats with AA decreased CAT activity in the all groups was observed ($P < 0.02$-0.001). The incubation of gastric homogenates with tincture suppressed CAT activity in the all investigated groups ($P < 0.001$). Probably this effect was related to ethanol presence in the tincture. No significant differences were detected in SOD activity neither in the target groups nor in the healthy animals. The most obvious increase of

SOD activity (by 17.8%) after the incubation of homogenates with AH tincture was observed in the group of healthy rats (differences were near to significant; t = 2.04).
AOA was higher in the gastric mucosal homogenates of the all experimental groups of the animals compared with the normal rats, but a significant increase by 1.8 fold (P < 0.002) was observed only in the healthy animals after the incubation of homogenates with AH tincture.

Table 9. The influence of AH tincture on the indices of pro/antioxidant system in the homogenates of gastric mucosa of the ulcer-induced rats with adjuvant arthritis

Index	Groups							
	I AA (n = 9)		II AA + ulcer (n = 8)		III AA + ulcer + AH (n = 6)		IV Control (n = 10)	
	Homoge-nate	Homog.+ AH	Homoge-nate	Homog.+ AH	Homoge-nate	Homog.+ AH	Homoge-nate	Homog.+ AH
MDA (nmol/ml)	1.84±0.22*	$^{+}$2.58±0.18*	1.76±0.28*	2.06±0.21*	1.89±0.31*	2.63±0.82	4.01±0.23	4.18±0.23
CAT (mmol/L /min)	99.47±0.94*	$^{+}$77.18±3.25	$^{\bullet}$ 87.53±1.60*	$^{+}$48.46±1.88* $^{\bullet}$	$^{\bullet}$ 88.96±3.20*	$^{+}$55.96±5.00* $^{\bullet}$	104.24±0.74	$^{+}$80.82±2.85
SOD (ua/mL of serum)	127.21±15.55	122.53±13.27	115.79±12.18	133.57±20.63	135.45±29.76	126.70±24.10	110.80±5.33	134.75±10.46
AOA (% of reduction)	40.02±4.46	44.00±5. 54	41.13±5.40	46.30±5.16	46.35±12.17	37.10±5.67	27.65±4.57	$^{+}$48.67±3.41

Note: (*) – The differences are significant in comparison with the respective groups of the control rats. (+) – The differences in the separate groups are significant between the pure homogenates and the homogenates incubated with AH tincture. (•) – The differences are significant between the subgroups of II and III groups.

3.2. Clinical investigations

129 patients with specific complaints of the upper gastroduodenal pathology and with gastric and duodenal ulcers and chronic gastritis were included in this study. Chronic gastritis, acute or healing stages of the ulcers were detected by histological examination. The indices of antioxidant system in the blood serum of the all patients and the level of hydrogen peroxide (H_2O_2) in the gastroduodenal mucosa of 92 patients were established.

3.2.1. Changes of pro/antioxidant system in the patients' blood serum with gastroduodenal diseases

The most significant increase of MDA (by 64.9%) in comparison with the healthy persons was found in the patients with duodenal ulcer (P < 0.01). The patients suffering from the gastric ulceration had 48.4% higher levels of MDA than the control subjects, but significant differences between these groups were not detected. The increased amount of MDA was also found in the patients with chronic gastritis (P < 0.03) although it was higher only by 16.5% than in the control group (Fig. 3).
CAT activity in the blood serum increased in the all patients suffering from gastroduodenal diseases. Although the highest increase by 36.9% was observed in the patients with duodenal ulcer, but significant changes were found only in the patients' group with chronic gastritis (P < 0.02) where CAT activity increased by 29.4%. AOA

was the highest in the control group and significantly decreased in the all tested patients' groups (P < 0.003-0.0001) suffering from the gastroduodenal diseases.

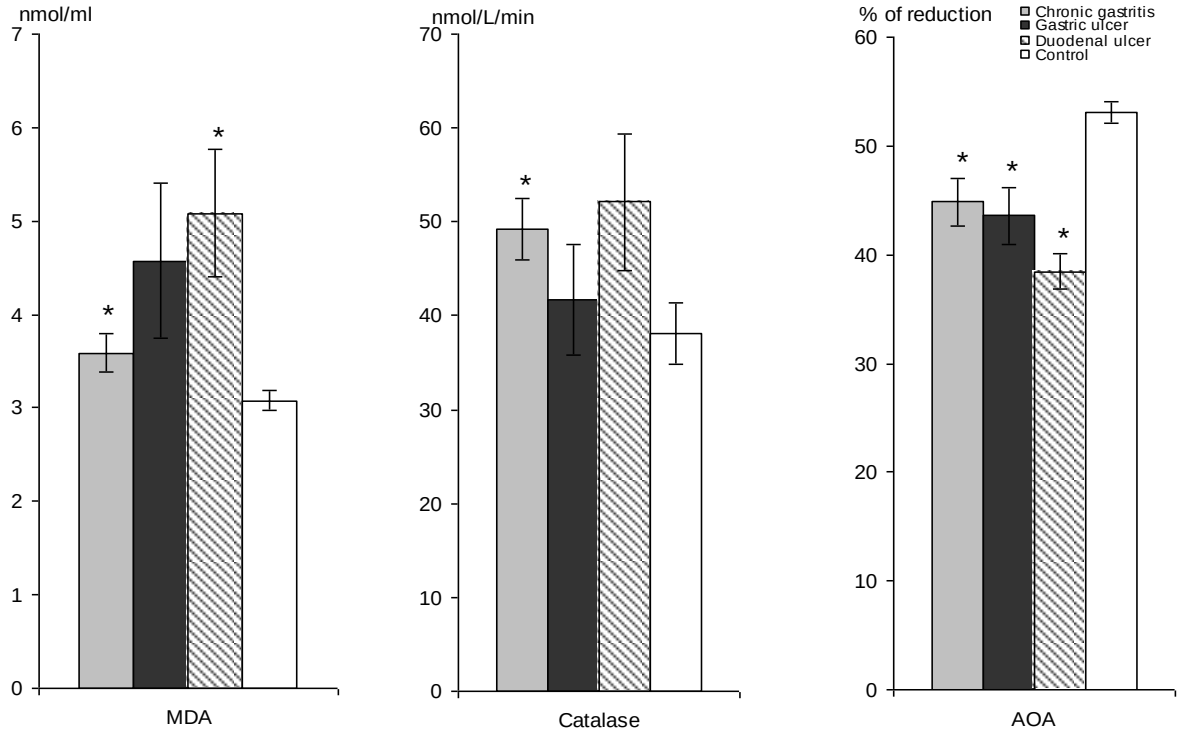

Fig. 3. Indices of pro/antioxidant system in the patients' blood serum with gastroduodenal diseases

(*) - The differences are significant in comparison with the control group.

No significant differences between the indices of oxidant system were revealed for the patients with acute and healing gastric ulcers, but the level of MDA was lower by 31.3% and AOA was higher by 9.4% in the patients' groups with healing gastric ulcers.

CAT activity which was increased by 9.6% in the patients with gastric ulcers decreased by 5.1% in the healing stage of disease and was just as control (Table 10). It should be noted that the all investigated indices came up to the control level during the ulcer healing process.

Table 10. Indices of pro/antioxidant system in the patients' blood serum with a gastric ulcer

Groups	n	Index		
		MDA (nmol/mg)	Catalase (nmol/l/min)	AOA (% of reduction)
Gastric ulcer	14	4.57±0.83	41.71±5.82	44.83±2.16* P < 0.0001
Healing gastric ulcer	8	3.14±0.34	39.59±11.04	47.65±3.49
Control	35	3.08±0.11	38.07±3.33	53.06±1.01

Note: (*) - The differences are significant in comparison with the control group

The same changes of the indices of pro/antioxidant system were observed in the patients' group with a healing duodenal ulcer (Table 11). A comparison of the investigated indices between the patients' groups with duodenal ulcer and healing duodenal ulcer showed that in last-mentioned patients a significant decrease of MDA ($P < 0.03$) and CAT activity ($P < 0.006$), and the increase of AOA ($P < 0.005$) were observed.

Table 11. Indices of pro/antioxidant system in the patients' blood serum with a duodenal ulcer

Groups	n	Index		
		MDA (nmol/mg)	Catalase (nmol/l/min)	AOA (% of reduction)
Duodenal ulcer	18	5.08±0.68* P < 0.01	52.13±7.26	38.47±1.57* P < 0.001
Healing duodenal ulcer	18	3.34±0.35^{+} P < 0.03	28.81±2.33^{+} P < 0.006	47.40±2.45^{+} P < 0.005
Control	35	3.08±0.11	38.07±3.33	53.06±1.01

Note: (*) – The differences are significant in comparison with the control group. (+) - The differences are significant in comparison with the duodenal ulcer group.

If MDA and AOA indices came up to the control level, the CAT activity was lower by 24.3% than in the control group and lower by 44.7% than in the patients with the acute duodenal ulcers. Such phenomenon was not observed in the patients with the healing gastric ulcers.

3.2.2. Reactive oxygen compounds in the gastroduodenal mucosal tissue

It should be noted that the level of H_2O_2 in the normal gastric mucosa was 5 times lower than in the duodenal mucosa (Fig. 4). In the cases of a chronic gastritis and a healing gastric ulcer the content of H_2O_2 in biopsy material was more than 2-fold higher and significantly differed ($P < 0.0001$) from the controls. The significant increase of H_2O_2 by 40.4% ($P < 0.0001$) was observed in the patients with the gastric ulcers.

In the cases of duodenal pathology the level of H_2O_2 in the gastric mucosa was lower compared to the normal control mucosa. A significant reduction of H_2O_2 (by 33%; $P < 0.015$) was observed in the acute stage of a duodenal ulcer. In the ulcer healing cases a decrease of H_2O_2 was only 14.8%. A pathological process proceeding in the gastric mucosa changes the intensity of oxidative reactions in the duodenal mucosa. In both processes of the chronic gastritis and the gastric ulcer a significant reduction of H_2O_2 was observed in the duodenal mucosa (by 47.8%, $P < 0.0001$ and 29.4% $P < 0.045$, respectively) in failing of any visible histological changes in them.

On the whole, the pathological process in the duodenal mucosa significantly decreased the intensity of oxidative reactions. In the case of the acute duodenal ulcer the H_2O_2 was lower by 31.6% ($P < 0.001$) and in the healing stage of the pathological process the decrease of H_2O_2 was 20.9% ($P < 0.04$).

The comparison of H_2O_2 in the gastric mucosa depending on the H. pylori infection (Table 12) showed that in the *H. pylori* negative samples the amount of H_2O_2 in the patients with a healing gastric ulcer significantly increased ($P < 0.003$) and exceeded the control level more than 2.4 times. In the patients' group with chronic gastritis this augmentation reached 72.7%.

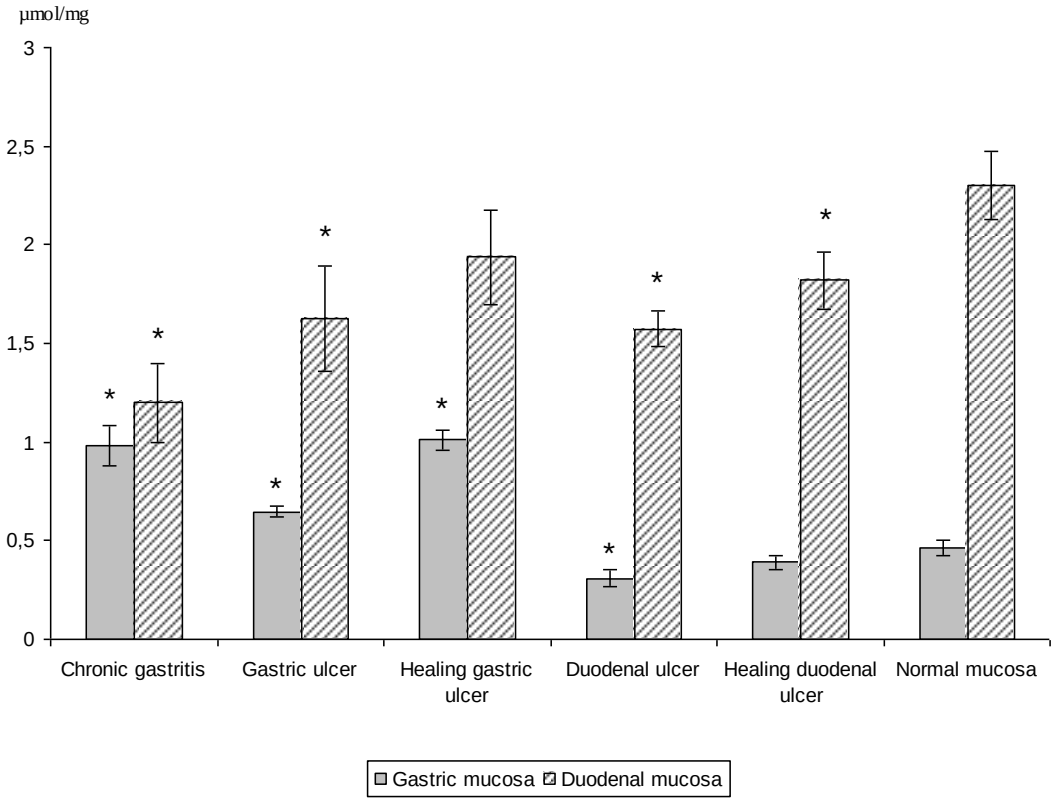

Fig. 4. Amount of hydrogen peroxide (µmol/mg) in the patients' gastric and duodenal mucosal tissue with different gastroduodenal pathology
(*) – The differences are significant in comparison with the normal mucosa

In the cases of chronic gastritis and healing gastric ulcer the amount of H_2O_2 in *H.pylori* positive samples of the gastric mucosa increased about 2 times compared with the control group ($P < 0.005$; $P < 0.003$).

Table 12. The amount of hydrogen peroxide in the patients' gastroduodenal mucosa with the gastroduodenal pathology, depending on the presence of *H. pylori* infection

Pathology	**Amount of hydrogen peroxide (µmol/mg)**			
	n/n %	***H. pylori* negative**	**n/n %**	***H. pylori* positive**
Chronic gastritis	19/7 36.8	0.76±0.08* P < 0.006	19/12 63.2	1.04±0.15* P < 0.005
Gastric ulcer	12/0 0	-	12/12 100	0.59±0.04
Healing gastric ulcer	8/3 37.5	1.05±0.07* P < 0.003	8/5 62.5	0.97±0.06* P < 0.003
Duodenum ulcer	16/2 12.5	0.56±0.13	16/14 87.5	0.27±0.04* P < 0.008
Healing duodenum ulcer	17/3 17.6	0.30±0.05	17/14 82.35	0.41±0.04
Control	20/11 55	0.44±0.04	20/9 45	0.51±0.06

Note: n/n – number on the left – the total number of the tested samples, number on the right – the number of cases without *H. pylori*. % - the patients' percentage with *H. pylori* infection. (*) - The differences are significant in comparison with the control mucosa.

Meanwhile, during the duodenal pathology the amount of H_2O_2 in the gastric mucosa was found lower by 47% in the patients suffering from the duodenal ulcer and lower by 19.6% in patients with the healing duodenal ulcer. Although the significant differences between the groups of patients with various pathology of the gastroduodenal zone in *H. pylori* negative or *H. pylori* positive groups were not detected, the higher levels of H_2O_2 were found in *H. pylori* infected patients suffering from chronic gastritis (an increase by 36.8%), gastric ulcer (100%), and healing duodenal ulcer (36.7%). The same was observed in the control group, where the increase of H_2O_2 was 15.9%.

3.2.3. Influence of FU and AH tinctures on the oxidative system of the gastric and duodenal mucosa in patients with gastroduodenal diseases

As for the production of tinctures 40° ethanol has been used, the impact of this solvent on the amount of H_2O_2 in the gastric and duodenal mucosal homogenates was studied (Fig. 5). The ethanol solution had no significant effect on the level of H_2O_2 in the patients' gastric mucosal homogenates that were suffering from the all investigated diseases, except the patients with gastric ulcer, where 40° ethanol significantly reduced H_2O_2 ($P < 0.01$).

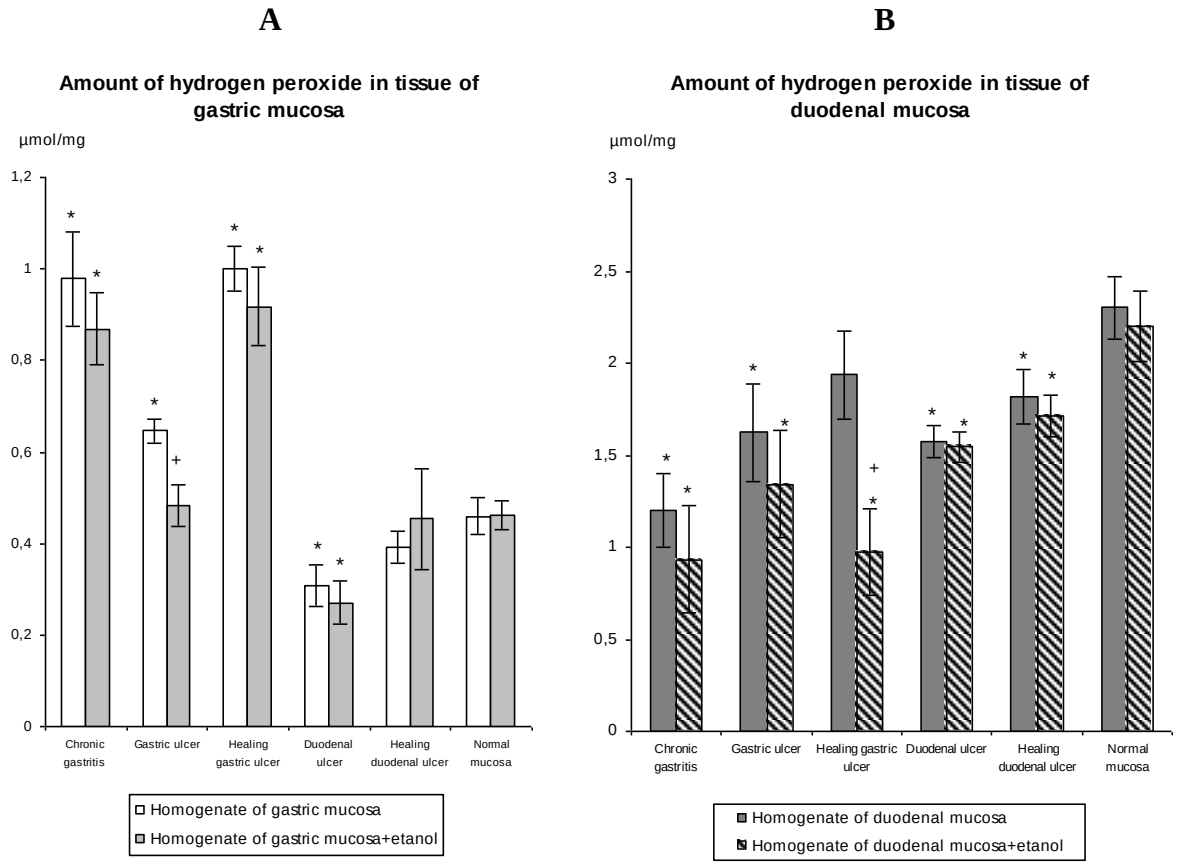

Fig. 5. Effect of 40° ethanol on the level of hydrogen peroxide in the gastric and duodenal mucosal tissues of patients with gastroduodenal diseases

5% gastric and duodenal mucosal tissue homogenates were produced with ratio 1:19 (one part of tissue and nineteen parts of the buffer solution). (*) - The differences are significant in comparison with the indices of normal mucosa. (+) - The differences are significant in comparison with the groups without ethanol.

In the homogenates of duodenal mucosa a significant difference between the amount of H_2O_2 in the pure homogenate and the homogenate with ethanol was found only in the patients' group with healing gastric ulcer ($P < 0.02$) where ethanol reduced the H_2O_2 by 49.7%.

The obtained data also revealed that in the patients' gastric tissue homogenates with chronic gastritis, gastric ulcer, healing gastric ulcer the amount of H_2O_2 was higher ($P < 0.001$-0.0001), but in the patients with the duodenal ulcer it was lower ($P < 0.01$) in comparison with the normal mucosa (Fig. 5A). Almost the all investigated pathological processes resulted in a significant decrease of H_2O_2 level ($P < 0.05$-0.001) in the homogenates of duodenal mucosa.

3.2.3.1. Effect of FU and AH tinctures on the level of hydrogen peroxide in the patients' gastric mucosa tissues suffering from gastroduodenal diseases

The amount of H_2O_2 decreased in the patients' gastric mucosal homogenates suffering from gastroduodenal diseases after their incubation with FU tincture (Table 13), except the patients with healing duodenal ulcer where H_2O_2 was higher by 19.9%.

Table 13. The amount of hydrogen peroxide in the gastric mucosal tissues incubated with FU and AH tinctures

Pathology	n	Amount of hydrogen peroxide (μmol/mg)		
		I Gastric tissue homogenates	II Gastric tissue homogenates + FU	III Gastric tissue homogenates + AH
Chronic gastritis	19	0.978±0.102*	0.789±0.089*	0.835±0120*
Gastric ulcer	12	0.646±0.026*	$^{+}$0.420±0.040	0.717±0.079
Healing gastric ulcer	8	1.000±0.050*	$^{+}$0.755±0.090*	$^{+}$0.599±0.034
Duodenum ulcer	16	0.308±0.044*	0.258±0.026*	$^{+}$0.566±0.073
Healing duodenum ulcer	17	0.392±0.036	0.470±0.050	$^{+}$0.725±0.080
Control	20	0.460±0.039	0.359±0.040	0.504±0.078

Note: 5% gastric mucosal tissue homogenates were produced with ratio 1:19 (one part of the tissue and nineteen parts of the buffer solution). (*) - The differences are significant in comparison with the control mucosa. (+) - The differences are significant in comparison with the I group.

A statistically significant reduction of H_2O_2 by 34.8% ($P < 0.001$) and by 24.5% ($P < 0.03$) was found in the patients' homogenates with gastric ulcer and healing gastric ulcer after their incubation with FU tincture.

In the gastric mucosal homogenates of patients with chronic gastritis and healing gastric ulcer, AH tincture reduced the amount of H_2O_2 by 14.62% and 40.1% ($P < 0.001$) respectively. But in the patients with gastric ulcer the level of H_2O_2 after the incubation with AH tincture increased by 11%, while in the homogenates of patients, suffering from the duodenal ulcer – by 83.76% ($P < 0.01$).

In the cases of healing duodenal ulcer, the level of H_2O_2 in the gastric mucosal homogenates incubated with AH was about 1.9 times (84.94%) higher and significantly differed from the levels of H_2O_2 in the gastric mucosal homogenates without incubation ($P < 0.0001$). Both herbal tinctures only slightly reduced (by 14.62% and 19.32%) the amount of H_2O_2 in the homogenates from the patients with chronic gastritis. It should be

noted that the effect of both tinctures on the level of H_2O_2 in the homogenates prepared from the normal gastric mucosa was different: FU tincture decreased, and AH – increased the amount of H_2O_2, but the obtained changes were not significant.

3.2.3.2. Effects of FU and AH tinctures on the oxidative system of the duodenal mucosa in patients with gastroduodenal diseases

After the incubation of the duodenal mucosal homogenates with FU tincture the level of H_2O_2 was slightly decreased in them (Table 14). A significant decrease of H_2O_2 by 21.9% ($P < 0.01$) in the normal duodenal mucosa was also observed.
AH tincture showed the same effect and reduced the amount of H_2O_2 by 27.3% ($P < 0.01$) in the normal duodenal mucosa.
In the cases of gastroduodenal pathology, FU tincture decreased the amount of H_2O_2 in homogenates, except in the patients with duodenal ulcer pathology, where H_2O_2 insignificantly increased.
Both herbal preparations significantly diminished the amount of H_2O_2 in the patients' duodenal mucosa with chronic gastritis in comparison with the control group ($P < 0.001$).

Table 14. The amount of H_2O_2 in the patients' duodenal mucosal tissues with gastroduodenal diseases after their incubation with FU and AH tinctures

Pathology	n	Amount of hydrogen peroxide (µmol/mg)		
		I Duodenum mucosa homogenates	II Duodenum mucosa homogenates + FU	III Duodenum mucosa homogenates + AH
Chronic gastritis	19	1.200±0.200*	1.132±0.170*	1.188±0.139*
Gastric ulcer	12	1.623±0.267*	1.394±0.230	1.459±0.188
Healing gastric ulcer	7	1.937±0.239	1.762±0.340	1.404±0.220
Duodenum ulcer	16	1.574±0.090*	1.604±0.100	1.837±0.088
Healing duodenum ulcer	18	1.819±0.147*	1.625±0.149	2.042±0.170
Control	19	2.303±0.170	$^{+}$1.799±0.179	$^{+}$1.674±0.140

Note: 5% duodenal mucosal tissue homogenates were produced with ratio 1:19 (one part of the tissue and nineteen parts of the buffer solution. (*) - The differences are significant in comparison with the control. (+) - The differences are significant in comparison with the I group.

A more marked decrease of H_2O_2 (Fig. 6 A) in the gastric mucosal homogenates obtained from the patients with gastric ulcer ($P < 0.01$), duodenal ulcer ($P < 0.001$), and healing duodenal ulcer ($P < 0.02$) after their incubation with FU tincture was observed in comparison with the effect of AH tincture.
The lower level of H_2O_2 was found in the duodenal mucosal homogenates after their incubation with FU tincture, although the significant differences between the effect of tinctures was not found. Only in the patients with healing gastric ulcer AH tincture more intensively than FU reduced the level of H_2O_2 in the gastric and duodenal homogenates (Fig. 6 A,B).

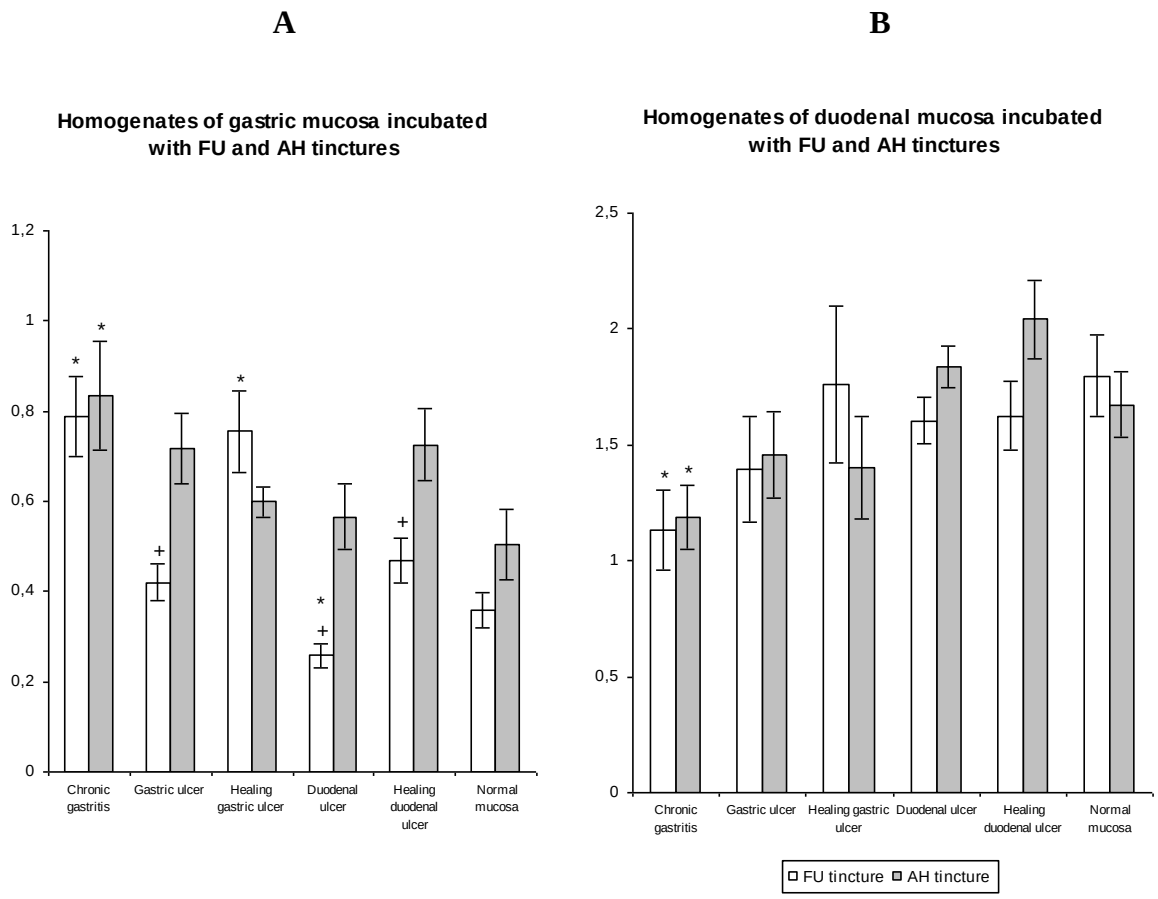

Fig. 6. Amount of H_2O_2 in the patients' homogenates of the gastric (A) and duodenal (B) mucosal tissue with gastroduodenal diseases after their incubation with FU and AH tinctures
Note: (*) - The differences are significant with the control groups (normal gastro or duodenal mucosa). (+) - The differences are significant between the homogenates after their incubation with different tinctures.

The obtained results from the clinical investigations which involve the status of pro/antioxidative system in the patients with gastroduodenal pathology and the influence of herbal tinctures on the amount of H_2O_2 in the mucosal homogenates are shown in the Tables 15 and 16.

Table 15. Changes of the indices of pro/antioxidant system in patients suffering from gastroduodenal diseases

Pathology	**Indices**			
	MDA (in serum)	**H_2O_2** (in mucosa)	**CAT** (in serum)	**AOA** (in serum)
Chronic gastritis	↑*	↑↑*	↑*	↓*
Gastric ulcer	↑↑	↑*	↑	↓*
Healing gastric ulcer	↑	↑↑*	↑	↓
Duodenum ulcer	↑↑*	↓*	↑↑	↓↓*
Healing duodenum ulcer	↑	↓	↓*	↓

Note: ↑- an increase of indices, ↓ - the decrease of indices, ↑↑- markedly increased values, ↓↓- markedly decreased values. (*) – The differences are significant in comparison with the control groups.

Table 16. Influence of *Filipendula ulmaria* (L.) Maxim [FU] and *Aesculus hippocastanum* (L.) [AH] tinctures on the amount of hydrogen peroxide in the gastroduodenal mucosa

Pathology	Variation of hydrogen peroxide (H_2O_2)	
	FU tincture	AH tincture
Chronic gastritis	↓	↓
Gastric ulcer	↓*	↑
Healing gastric ulcer	↓*	↓↓*
Duodenum ulcer	↑	↑↑
Healing duodenum ulcer	↓	↑
Control	↓	↑

Note: ↑- an increase of indices, ↓ - the decrease of indices, ↑↑- markedly increased values, ↓↓- markedly decreased values. (*) – The differences are significant in comparison with the control groups.

Data presented in the Table 15 showed, that the most significant increase of MDA level in the blood serum and H_2O_2 level in the gastric mucosa was observed respectively in the patients with chronic gastritis and duodenum ulcer, and with chronic gastritis and healing gastric ulcer. The highest decrease of AOA in the blood serum was found in patients suffering from duodenum ulcer.

Fu tincture (Table 16) markedly decreased H_2O_2 level in the gastroduodenal mucosa of patients with gastric ulcer and healing gastric ulcer, and AH tincture – in patients with healing gastric ulcer.

4. CONCLUSIONS

1. Indomethacin-induced ulceration in the animals markedly reduces catalase activity and total antioxidant activity in the blood serum, and superoxide dismutase activity in the gastric mucosal homogenates.
2. The tincture of *Filipendula ulmaria* reduces the pathological changes in the gastric mucosa, enhances the indices of antioxidant activity in the blood serum, and markedly reduces the concentration of malondialdehide and increases the total antioxidant activity in the homogenates of gastric mucosa.
3. The tincture of *Aesculus hippocastanum* positively acts on the indices of antioxidant system in the blood serum, increases catalase activity, and markedly reduces the amount of malondialdehide in the homogenates of gastric mucosa.
4. The amount of malondialdehide in the blood serum and hydrogen peroxide in the gastric mucosa increase in the patients suffering from the gastroduodenal diseases. This increase is related to *H. pylori* infection in the patients with chronic gastritis and gastric ulcer.
5. The tincture of *Filipendula ulmaria* distinctly reduces the amount of hydrogen peroxide in the patients' homogenates of gastric mucosa with gastric and duodenal ulcers, and in the patients' homogenates of duodenal mucosa with chronic gastritis, gastric ulcers and duodenal healing ulcers, but the increase of H_2O_2 is observed in the patients' homogenates with acute duodenal ulcers.
6. The tincture prepared from *Aesculus hippocastanum* more effectively than the tincture from *Filipendula ulmaria* decreases the amount of hydrogen peroxide in

the patients' gastric and duodenal mucosal homogenates with healing gastric ulcer and in the patients' duodenal mucosal homogenates with chronic gastritis, gastric ulcers, and increases it in the patients with duodenal ulcers.

5. PUBLICATIONS

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SUMMARY IN LITHUANIAN

GASTRODUODENINĖS SRITIES GLEIVINĖS OKSIDACINIŲ PROCESŲ IR AUGALŲ TINKTŪRŲ POVEIKIO EKSPERIMENTINIS IR KLINIKINIS ĮVERTINIMAS

1. ĮVADAS

Skrandžio ir dvylikapirštės žarnos gleivinės įvairių patologinių procesų atsiradimui (tarp jų ir opaligei) svarbią reikšmę turi organizmo pro/antioksidacinės sistemos būklė, antioksidacinės sistemos pajėgumas, lipidų peroksidacijos procesai. Šių procesų dinamikos pažinimas leistų išsamiau pažinti patologinio proceso grandis, bei suteiktų galimybę panaudoti antioksidantinius preparatus šių ligų gydymui. Pastaruoju metu mokslininkų dėmesys krypsta į natūralių antioksidantų panaudojimo galimybes klinikinėje praktikoje.
Dauguma augaluose randamų biologiškai aktyvių medžiagų pasižymi antioksidacinėmis savybėmis, tačiau trūksta nuoseklių mokslinių studijų, atskleidžiančių augalų vartojimo indikacijas, kadangi jie dažniausiai vartojami remiantis liaudies išmintimi. Paprastojo kaštono ir pelkinės vingiorykštės žiedų tinktūrų poveikio gastroduodeninės gleivinės oksidacinei sistemai tyrimai leistų įvertinti jų poveikį ir praplėstų šių augalų taikymo indikacijas klinikinėje praktikoje.

Tyrimo tikslas:
Nustatyti pakyčius gleivinės oksidacinėje sistemoje, sergant skrandžio ir dvylikapirštės žarnos opalige, ir įvertinti paprastojo kaštono (*Aesculus hippocastanum* (L.) žiedų bei pelkinės vingiorykštės (*Filipendula ulmaria* (L.) Maxim) žiedų tinktūrų poveikį gleivinės oksidacinei sistemai *in vivo*, sukėlus žiurkėms eksperimentinę opaligę, ir *in vitro*, panaudojant tyrimams eksperimentinių gyvūnų ir ligonių gleivinės biopsinę medžiagą.

Tyrimo uždaviniai:

1. Ištirti skrandžio gleivinės ir kraujo serumo pro/antioksidacinės sistemos rodiklių kitimą fiziologinėmis sąlygomis ar sisteminio susirgimo (adjuvantinio artrito) metu, sukėlus žiurkėms eksperimentinę opaligę.
2. Ištirti paprastojo kaštono (*Aesculus hipocastanum* (L.) ir pelkinės vingiorykštės (*Filipendula ulmaria (L.) Maxim)* žiedų tinktūrų poveikį skrandžio gleivinės oksidacinės sistemos rodikliams.
3. Ištirti pro/antioksidacinės sistemos pokyčius ligoniams, sergantiems gastroduodeninės srities ligomis, ir jų priklausomybę nuo infekuotumo *H. pylori*.
4. Ištirti paprastojo kaštono (*Aesculus hipocastanum* (L.) ir pelkinės vingiorykštės (*Filipendula ulmaria (L.) Maxim)* žiedų tinktūrų poveikį opalige sergančiųjų ligonių gleivinės oksidaciniams procesams.

Mokslinis naujumas:
Pastaraisiais metais vis didesnę reikšmę įgyja natūralūs netoksiški, daugiau saugančiu nei žalojančiu poveikiu pasižymintys augaliniai preparatai. Nors mokslinėje literatūroje pakankamai plačiai aprašyta oksidacinės sistemos reikšmė sergant įvairiomis virškinimo trakto sistemos ligomis, nustatytos proceso patologinės grandys, ištirtas cheminių vaistų poveikis, tačiau šių susirgimų nemažėja. Todėl išlieka svarbi naujų būdų, veikiančių oksidacinę sistemą, paieška. Atliktame darbe, remiantis *in vitro* ir *in vivo* tyrimais nustatytas oksidacinės sistemos rodiklių aktyvumas eksperimente ir klinikoje sergant gastroduodeninės srities ligomis bei įvertintas, paprastojo kaštono (*Aesculus hippocastanum (L.)*) ir pelkinės vingiorykštės (*Filipendula ulmaria* (L.) Maxim) poveikis šiems rodikliams. Pirmą kartą klinikinėje praktikoje nustatytas integralaus rodiklio - vandenilio peroksido kiekis pažeistoje gastroduodeninės srities gleivinėje bei jo korekcijos galimybės taikant paprastojo kaštono (*Aesculus hippocastanum (L.)*) bei pelkinės vingiorykštės (*Filipendula ulmaria* (L.) Maxim) tinktūras, kurios dėl savo potencialių antioksidacinių savybių gali paveikti prooksidacinius procesus pažeistoje gleivinėje.

Praktinė darbo reikšmė:
Vaistiniai augalai yra vertinga žaliava augalinių preparatų gamybai ir jų taikymas gastroenterologinių ligų klinikinėje praktikoje, kaip papildas šalia pagrindinės medikamentinės terapijos, leistų pagerinti šių patologijų gydymo galimybes. Remiantis pelkinės vingiorykštės ir paprastojo kaštono tinktūrų *in vivo* ir *in vitro* eksperimentinės ir klinikinės medžiagos tyrimų rezultatais, parodančiais šių augalų tinktūrose esančių biologiškai aktyvių medžiagų teigiamą, antioksidacinį poveikį pažeistos gleivinės oksidacinės sistemos grandims, galima išplėsti minėtų augalų tinktūrų vartojimo galimybes sergantiesiems gastroduodeninės srities ligomis.

Ginamieji teiginiai:
1. Eksperimentinė skrandžio gleivinės opaligė keičia oksidacinės sistemos būklę tiek fiziologinėmis sąlygomis, tiek ir sisteminio susirgimo metu.
2. Skirtingas oksidacinės sistemos pažeidimo laipsnis atspindi ūmią ar gyjančią opinio pažeidimo stadiją gastroduodeninės srities gleivinėje.
3. Opaligės metu paprastojo kaštono (*Aesculus hippocastanum (L.)*) ir pelkinės vingiorykštės (*Filipendula ulmaria* (L.) Maxim) tinktūros teigiamai veikia gleivinės oksidacinę sistemą, ryškiausiai mažindamos prooksidacinių reakcijų intensyvumą.

Tyrimo medžiaga:
Eksperimentinėje dalyje atlikti tyrimai su 95 Wistar žiurkėmis, adaptuojant indometacino opaligės modelį (15 žiurkių), ištiriant skrandžio gleivinės homogenate antioksidacinės sistemos rodiklius sveikoms ir adjuvantiniu artritu sergančioms žiurkėms, sukeliant skrandžio opą indometacinu (80 žiurkių).
Klinikinėje darbo dalyje ištirti 129 ligoniai, kuriems ezofagogastroduodenofibroskopo pagalba paimta biopsinė medžiaga ištirta histologiškai pagal Sydney rekomendacijas. 35 ligoniams nustatytas lėtinis gastritas, 14 - skrandžio opa, 8 - skrandžio opa gijimo fazėje, 18 - dvylikapirštės opa, 19 - dvylikapirštės opa gijimo fazėje. Iš jų, 92 ligoniams ištirtas

vandenilio peroksido kiekis gastroduodeninės zonos gleivinėje Be to, ištirti 35 kontrolinės grupės asmenys.

Tyrimo metodai:
Maloninio dialdehido (MDA), katalazės (KAT), superoksido dismutazės (SOD), bendro antioksidantinio aktyvumo (*in vivo* ir *in vitro*) bei vandenilio peroksido kiekio (*in vitro*) nustatymas.

2. REZULTATAI

Vertinant makroskopinius pokyčius žiurkių skrandžio gleivinėje, sukėlus opaligę indometacinu, stebime sumažėjusį bendrą ir antrum dalies skrandžio gleivinės plotą lyginant su kontroline grupe, apskaičiuotas pažeidimo indeksas yra 5,11. Gydant žiurkes vingiorykštės žiedų tinktūra, pažeidimo indeksas sumažėjo 3% (4,96). Eksperimentinės opaligės metu mažėja antioksidacinių rodiklių aktyvumas kraujo serume. Vingiorykštės tinktūra nežymiai veikia lipidų peroksidacijos galinį produktą MDA ir KAT, reikšmingai sumažindama SOD aktyvumą, tačiau 36,2% sustiprindama AOA. Žiurkių su eksperimentine opalige pro/antioksidantinės sistemos rodikliai skrandžio audinio homogenatuose inkubuojant juos vingiorykštės žiedų tinktūra mažėja, tačiau stipriai padidėja bendras antioksidantinis aktyvumas (AOA). Gydant eksperimentinę opaligę vingiorykštės tinktūra per os ryškiai sumažėja oksidacinio pažeidimo rodiklio MDA kiekis skrandžio audinio homogenatuose. Kaštonų žiedų tinktūra teigiamai veikė pro/antioksidantinės sistemos rodiklius kraujo serume, ypač reikšmingai padidindama katalazės aktyvumą. Gydant eksperimentinę opaligę kaštono žiedų tinktūra *per os* ryškiai sumažėja oksidacinio pažeidimo rodiklio MDA kiekis skrandžio audinio homogenatuose, ir nežymiai išauga AOA, o inkubacijos juos kaštono žiedų tinktūra metu stebėtas kiek didesnis MDA kiekis ir sumažėjęs katalazės aktyvumas gleivinės audinyje.

Skrandžio gleivinės pažeidimo plotas sukėlus opaligę AA sergančioms žiurkėms buvo 53,2% didesnis negu sveikoms (atitinkamai 51,85 mm^2 ir 33,84 mm^2).
Stipresnis skrandžio gleivinės pažeidimas žiurkėms su AA ir indometacinu sukelta opa, rodo, kad egzistuoja ryšys tarp sisteminio uždegimo ir padidėjusio skrandžio gleivinės jautrumo indometacinui. Po gydymo vingiorykštės tinktūra skrandžio gleivinės pažeidimo indeksas sumažėjo 11,5%. Eksperimentinė opaligė, sukelta žiurkėms su AA, ypač stipriai lemia antioksidacinio fermento KAT ir AOA sumažėjimą kraujo serume.

Po gydymo vingiorykštės tinktūra AOA ir SOD aktyvumas reikšmingai padidėja.
Ryškiausi pokyčiai gauti tiriant KAT kiekį skrandžio audinio homogenatuose, inkubuotuose vingiorykštės tinktūra, kur stebėtas ryškus šio fermento sumažėjimas
Reikšmingas SOD aktyvumo sumažėjimas po homogenatų inkubacijos vingiorykštės tinktūra aptiktas tik žiurkėms su AA ir eksperimentine opalige, o AOA skrandžio gleivinės homogenatuose reikšmingai padidėjo tiek pastariesiems, tiek ir kontroliniams gyvūnams su AA. Vertinant skrandžio audinio homogenatų inkubaciją kaštono žiedų tinktūromis, ryškiausi pokyčiai aptikti tiriant KAT kiekį, kuris reikšmingai sumažėjo.

Tiriant ligonius, didžiausias infekuotumas *H. pylori* rastas sergantiems skrandžio opa. Gyjant skrandžio opai, 37,5% asmenų *H. pylori* infekcija neaptikta, o negausus infekuotumas stebėtas 50% ligonių.

Ryškiausias ir statistiškai reikšmingas MDA padidėjimas viršijantis 64,9% sveikų asmenų lygį rastas sergantiems dvylikapirštės žarnos opalige.

AOA sergant gastroduodeninės srities ligomis statistiškai reikšmingai mažėjo visose pacientų grupėse, o ryškiausias sumažėjimas aptiktas ligoniams, sergantiems dvylikapirštės žarnos opalige.

Nepakitusioje skrandžio gleivinėje H_2O_2 kiekis 5 kartus mažesnis nei dvylikapirštės žarnos gleivinėje. Lėtinio gastrito ir gyjančios skrandžio opos atvejais jo kiekis skrandžio gleivinės bioptatuose reikšmingai padidėja daugiau kaip 2 kartus, lyginant su kontrole, o skrandžio opos metu jis reikšmingai išauga 40,4% .

Dvylikapirštės žarnos patologijos atveju H_2O_2 kiekis skrandžio gleivinėje yra mažesnis, lyginant su normalia kontrolinės grupės gleivine. Reikšmingas sumažėjimas stebimas sergant ūmios fazės dvylikapirštės žarnos opa.

Tiek lėtinio gastrito, tiek ir skrandžio opaligės metu H_2O_2 kiekis reikšmingai sumažėja dvylikapirštės žarnos gleivinėje, nestebint jokių matomų pokyčių joje.

Nors vertinant H_2O_2 kiekį skrandžio gleivinėje įvairių gastroduodeninės srities patologijų atvejais tarp *H. pylori* neigiamų ir *H. pylori* teigiamų grupių statistiškai reikšmingų skirtumų neaptikta, tačiau didesni jo kiekiai nustatyti *H. pylori* infekuotiems ligoniams, sergantiems lėtiniu gastritu (padidėjo 36,8 %), skrandžio opa (100%), gyjančia dvylikapirštės žarnos opa (36,7%), bei kontrolinės grupės asmenims, kur padidėjimas siekė 15,9%.

Kadangi tinktūrų gamybai buvo naudojamas 40º etanolis, ištyrėme, kokią įtaką šio tirpiklio panaudojimas turi H_2O_2 kiekiui skrandžio ir 12-pirštės žarnos gleivinės audinio homogenatuose

Etanolio tirpalo pridėjimas reikšmingos įtakos H_2O_2 kiekiui skrandžio gleivinėje, sergant visomis tirtomis ligomis, neturėjo, išskyrus sergančius skrandžio opa, kur etanolis sumažino H_2O_2 kiekį .

Dvylikapirštės žarnos gleivinės audinio homogenatuose reikšmingas skirtumas tarp jo kiekio gryname homogenate ir homogenate su etanoliu rastas tik ligoniams su gyjančia skrandžio opa, kur etanolis sumažino H_2O_2 kiekį 49,7%.

Vingiorykštės tinktūra ryškiau negu kaštono žiedų tinktūra mažino H_2O_2 kiekį. Reikšmingi skirtumai tarp naudotų tinktūrų gauti skrandžio audinio gleivinės homogenatuose, paimtuose iš ligonių, sergančių skrandžio opa, dvylikapirštės žarnos opa ir gyjančia skrandžio opa.

Mažesnis H_2O_2 kiekis po inkubacijos vingiorykštės tinktūra rastas ir 12-pirštės žarnos gleivinės audinio homogenatuose, tačiau reikšmingų skirtumų tarp tinktūrų poveikio neaptikta. Tik ligoniams su gyjančia skrandžio opa, tiek skrandžio, tiek ir dvylikapirštės žarnos gleivinės audinio homogenatuose kaštono tinktūra stipriau negu vingiorykštės slopino H_2O_2 kiekį.

Lyginant H_2O_2 kiekį inkubuotuose augalinėmis tinktūromis homogenatuose su nepakitusios gleivinės H_2O_2 kiekiu, jis statistiškai reikšmingai sumažėjo dvylikapirštės žarnos opa sergančiųjų skrandžio gleivinės audinio homogenatuose, inkubuotuose vingiorykštės tinktūra, o abi tinktūros statistiškai reikšmingai mažino H_2O_2 kiekį dvylikapirštės žarnos audinio homogenatuose, paimtuose iš ligonių, sergančių lėtiniu gastritu.

3. IŠVADOS:

1. Sukeliant eksperimentinę opaligę indometacinu, ryškiai sumažėja antioksidantinio fermento katalazės aktyvumas ir bendras antioksidantinis aktyvumas kraujo serume ir superoksido dismutazės aktyvumas skrandžio gleivinės homogenatuose.
2. Vingiorykštės žiedų tinktūra sumažina skrandžio gleivinės pažeidimus, didina kraujo serume bendrą antioksidantinį aktyvumą, o skrandžio gleivinės homogenatuose, inkubuotuose ja, ryškiai mažina maloninio dialdehido koncentraciją ir didino bendrą antioksidacinį aktyvumą.
3. Eksperimentinės opaligės metu kaštono žiedų tinktūra padidina katalazės aktyvumą kraujo serume, o skrandžio audinio homogenatuose ryškiai mažina maloninio dialdehido kiekį.
4. Sergant gastroduodeninės srities ligomis didėja maloninio dialdehido koncentracija kraujuje ir vandenilio peroksido kiekis skrandžio gleivinėje. Šis padidėjimas susijęs su *H. pylori* infekuotumu sergantiems lėtiniu gastritu ir skrandžio opa.
5. Vingiorykštės tinktūra ryškiausiai mažina vandenilio peroksido kiekį skrandžio gleivinės homogenatuose ligoniams, sergantiems skrandžio ir dvylikapirštės žarnos opomis, o dvylikapirštės žarnos gleivinės homogenatuose, sergantiems lėtiniu gastritu, skrandžio opomis ir gyjančiomis dvylikapirštės žarnos opomis, tačiau didina esant ūmiai dvylikapirštės žarnos opai.
6. Kaštono žiedų tinktūra stipriau negu vingiorykštės slopina vandenilio peroksido kiekį skrandžio ir dvylikapirštės žarnos gleivinės audinio homogenatuose ligoniams su gyjančia skrandžio opa, o dvylikapirštės žarnos gleivinės homogenatuose - sergantiems lėtiniu gastritu, skrandžio opomis ir didina sergant dvylikapirštės žarnos opalige.