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Influence of Herb *Filipendula ulmaria* (L.) Maxim Tincture on Pro-/antioxidant Status in Gastric Tissue with Indomethacin-induced Gastric Ulcer in Rats

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Oxygen-generated free radicals play an important role in the pathogenesis of gastric lesions induced by various agents, including nonsteroidal anti-inflammatory drugs. Natural flavonoids present in plants are known for scavenging effects on reactive oxygen radicals. The **objective** of our study was investigation of the influence of *Filipendula ulmaria* (L.) Maxim tincture (FU) on the pro/antioxidant status of gastric tissue *in vivo* and *in vitro* in rats after indomethacin-induced ulceration. **Materials and methods**. We examined pro-/antioxidant status *in vivo* and *in vitro* in the gastric tissue of 28 female Wistar rats weighing 200–240 g. Among them 18 rats were with indomethacin-induced gastric ulcer and 10 rats were control. A FU tincture of was given *per os* to one group of rats with the above-mentioned gastric ulcers. For evaluation of the pro/oxidant status of gastric tissue, we determined the activity of superoxide dismutase (SOD), catalase, malondialdehide and the total antioxidant activity. **Results**. Administration of indomethacin *per os* induced significant changes in the pro/antioxidant parameters of serum and gastric tissue in rats. The results showed a significant increase of total antioxidant activity, a lowered intensity of lipid peroxidation (MDA content) in *in vivo* and *in vitro* experiments and an increased SOD content *in vivo* in indomethacin-induced ulcerative gastric tissue in rats after administration of FU tincture. **Conclusions**. The data of experiment *in vivo* revealed positive influence of *Filipendula ulmaria* (L.) Maxim tincture on the pro-/antioxidant status in gastric tissue of rats. The inhibition of reactive oxygen species by tincture of FU may affect ulcer formation after indomethacin administration.

Key words: *Filipendula ulmaria* (L.) Maxim, pro-/antioxidant status, indomethacin, ulcer, flavonoids, rats

INTRODUCTION

Nonsteroidal anti-inflammatory drugs are widely used in clinical pathological conditions (*i.e.* in rheumatoid arthritis, osteoarthritis, acute pain), but the major limitation of their clinical application is serious gastrointestinal side effects (erosions, ulcers, bleeding). There are several causes to evoke formation of mucosal disturbances (increased formation of reactive oxygen species, inhibition of prostaglandin synthesis, etc.). It is known that oxygen-generated free radicals play an important role in the pathogenesis of acute gastric lesions induced by various agents in experiment – ethanol (1), cold-restraint stress (2), as well as by nonsteroidal antiinflammatory drugs (3). The most potent anti-inflammatory activity is generated by indomethacin, which is commonly used in clinical practice.

From the ancient times herbal tinctures have been used for a wide variety of pathological conditions, but the mechanisms of many of their different effects are not completely clear. In folk medicine, many herbs were used to treat various pathological conditions of gastrointestinal tract, including ulcers. Natural flavonoids present in plants are known for their significant scavenging properties on

reactive oxygen radicals *in vivo* and *in vitro*, affecting various steps of ulcer heeling (4). *Filipendula ulmaria* (L.) Maxim (FU) is widely spread in Lithuania. The aqueous extract of the herb is used in folk medicine to treat a variety of gastrointestinal disorders (5). The chemical constituents of this herb are well known (6). In spite of numerous compounds there are some prominent groups of active phytochemicals:

– salicylic acid and its derivates (salicylaldehyde, methylsalicylate, ethylsalicylate, methoxybenzaldehyde and others),

– flavonoids (spireozide, rutine, quercetine, hyperoside, avicularine);

– tannins;

– polycarboxylic acids, vitamin C, traces of cumarine, yellow pigments.

The aim of our study was to test the influence of *Filipendula ulmaria* (L.) Maxim tincture on the pro-/antioxidant status of gastric tissue with indomethacin-induced ulcers in rats.

MATERIALS AND METHODS

For our study, we took a herb tincture produced from *Filipendula ulmaria* (L.) *Maxim* (1:5), a registered drug for treating cold and influenza.

We examined 28 female Wistar rats weighing 200–240 g, 2–3 months of age, and divided them into three groups. Rats of the first group with induced gastric ulcers (UL) received indomethacin, rats of the second group (UL + FU) received the FU tincture two days before and during the administration of indomethacin, and the third group was control. Each group of rats consisted of 9–10 of animals. The animals were kept in large plastic cages, five rats in each, at artificial lighting, at 12:12 light/dark periods and maintained on a constant diet – standard rat chow and water *ad libitum*.

Indomethacin was administered *per os* to rats to induce gastric ulcers (7). The control group of rats received water *per os*. The FU tincture was given *per os*. The dose of FU, 0.5 ml · kg^{-1} of weight, was chosen empirically from a series of dilutions. The whole experiment was performed *in vivo* and *in vitro*. *In vivo*, the FU tincture was administered to rats *per os* every day for five days. *In vitro*, the FU tincture was added to the gastric tissue homogenate from all groups of animals. In the course of ulcer induction, the schedule of indomethacin administration was as follows: the first day – the animals were allowed free access to food and water and given indomethacin *per os* in a dose of 30 mg/kg; the second day – in the morning the animals were allowed free access to food and water and were given indomethacin *per os* in a dose of 30 mg/kg. In the afternoon, the animals were deprived of food but allowed free access to water. After 12 h of fasting, the animals were given a 60 mg/kg indomethacin dose *per os* and after 24 h were killed by decapitation in a room separated from the other rats. The stomachs were removed, rinsed by injecting cold physiological solution and opened along the greater curvature. Samples of gastric mucous membrane were immediately placed on ice, frozen to –20° and kept for 3–7 days. Samples of tissues were homogenised in phosphate buffer (pH 7.4; 10%) with a glass homogenizer and centrifuged at 13000 rpm for 15 min at 4° C. For incubation, the homogenate (0.25 ml) was supplemented with FU tincture (0.1 ml), dry substances 8 mg/ml.

Lipid peroxidation assay. Lipid peroxidation was induced by adding 50 µl (0.05 mM) of $FeSO_4$ and ascorbic acid. After a 60 min incubation at 37 °C, thiobarbituric reactive substances were measured as described by V. B. Gavrilov et al. (8); the method is still used now (9).

Catalase activity detection. Samples of gastric mucous membrane were homogenised in TRIS-HCl buffer (0.05 M; pH 7.8; 10%) and the incubation was performed in the same way. Catalase activity was measured as described by N. A. Koroliuk et al. (10).

Total antioxidant activity. Total antioxidant activity after homogenization and incubation was measured according to the method described by L. P. Galaktionova et al. (11).

Superoxide dismutase activity after homogenization in phosphate buffer (0.15 M; pH 7.8) and incubation were measured as described by S. Csovari et al. (12).

In serum, the above-mentioned indices were measured according to methods described by the mentioned authors.

Methods for investigation of indomethacin-induced gastric ulcers were approved by Lithuanian Ethic Committee for Laboratory Animal Use prior to commencement of the experiment.

The statistical analysis of experimental data was carried out by using Statistica software, Student's test and the probability level $p < 0.05$ were considered as significant.

RESULTS

Administration of three doses of indomethacin produced severe changes in te pro-/antioxidant parameters of serum and gastric tissue in rats. The reduction rate of total antioxidant activity was 32.00 ± 3.61, malondialdehide (MDA) 6.29 ± 0.37 nmol/ml, catalase and superoxide dismutase (SOD) were 87.73 ± ± 2.82 nmol/l·min and 126.33 ± 13.06 UA/ml respec-

tively in the control group. Administration of indomethacin significantly reduced total antioxidant activity and catalase activity in the serum of rats with induced ulcer.

Oral pretreatment of rats with tincture of *Filipendula ulmaria* (L.) Maxim showed no remarkable changes in the test indices of serum as compared to the ulcer group (Table 1).

Other tendencies and changes of the above-mentioned pro-/antioxidant indices were seen in gastric mucous membrane in all groups of animals. The level of total antioxidant activity in gastric mucous membrane was the lowest in the control group (Table 2). Induction of gastric ulcers by indomethacin increased total antioxidant activity, and the effect of the herbal tincture was similar. The testing of total antioxidant activity in the ulcerated gastric mucous membrance *in vitro*, incubation with ethanol and appropriate buffer exerted a insignificant effect on the results as compared to that of the herbal tincture, which significantly increased total antioxidant activity in tissue samples (Table 3).

The induction of ulcer did not affect SOD levels in gastric tissue, but remarkably increased the activity of SOD in gastric tissue of rats pretreated with FU tincture (Table 2).

Incubation with herbal tincture in all groups remarkably reduced the SOD levels as compared to ethanol or appropriate buffer. There were no differences in SOD activity, interaction with ethanol or appropriate buffer in gastric tissue (Table 4).

Catalase activity was higher in the control group, while indomethacin reduced catalase activity in both study groups.

Pretreatment with the herbal tincture in the ulcer group did not affect activity in comparison to the other groups (Table 2).

Table 1. **Influence of FU tincture on indices of pro-/antioxidant system in serum of rats with indomethacin-induced ulcer (mean ± SD)**

Groups	UL n = 9	UL + FU n = 9	Control n = 10
AOA, % of reduction	–9.09 ± 9.95[1]	–12.38 ± 4.22	32.00 ± 3.61
MDA, nmol/ml	5.49 ± 0.26	5.41 ± 0.23	6.29 ± 0.37
Catalase, nmol/l·min	26.95 ± 2.28[1]	24.78 ± 2.32	87.73 ± 2.82
SOD, UA/ml of serum	85.59 ± 9.20[2]	67.49 ± 11.28	126.33 ± 13.06

[1] $p < 0.05$, [2] $p = 0.08$ (UL group *versus* control group).

Table 2. **Influence of FU tincture on indices of pro-/antioxidant system in gastric tissue of rats with indomethacin-induced ulcer (mean ± SD)**

Groups	UL n = 9	UL + FU n = 9	Control n = 10
AOA, % of reduction	34.54 ± 7.29	43.33 ± 6.13*	27.65 ± 4.57
MDA, nmol/ml	3.33 ± 0.11	1.09 ± 0.15**	4,01 ± 0.23
Catalase, nmol/l·min	89.88 ± 2.77[1]	88.27 ± 2.05	104.24 ± 0.74
SOD, UA/ml of serum	114.07 ± 6.33	147.61 ± 12.00**	110.80 ± 5.33

* $p < 0.05$ (UL + FU group *versus* control group of rats);
** $p < 0.05$ (group UL + FU *vias* group UL);
[1] $p < 0.05$ (UL group *versus* control group of rats).

Table 3. **Influence of FU tincture on total antioxidant activity in gastric tissue homogenate with indomethacin-induced ulcer during incubation *in vitro* (mean ± SD)**

Groups / % of reduction	UL n = 9	UL+FU n = 9	Control n = 10
With FU tincture, dose 0.8 mg	64.41 ±2.41[n]	68.93 ± 1.58 [n]	61.23 ± 4.49 [n]
With 40° ethanol	31.44 ±7.73	38.58 ± 5.41	25.51 ± 4.60
With buffer solution	29.98 ± 5.23	35.50 ± 5.88	30.92 ± 4.08

[n] $p < 0.05$ (samples of incubation with FU tincture or ethanol *versus* incubation with buffer solution).

Table 4. **Influence of FU tincture on SOD content in homogenate of gastric tissue with indomethacin-induced ulcer during incubation *in vitro* (mean ± SD)**

Groups / Vv/ml of homogenate	UL n = 9	UL+FU n = 9	Control n = 10
With FU tincture, dose 0.8 mg	83.82 ± 7.78	77.02 ± 8.51[1]	99.16 ± 0.95[1]
With 40° ethanol	118.62 ± 14.45[1]	104.48 ± 7.08	113.97 ± 8.66
With buffer solution	96.18 ± 13.43	108.82 ± 9.48	119.88 ± 9.80

[1] $p < 0.05$ (samples of incubation with FU tincture or ethanol *versus* incubation with buffer solution).

The herbal tincture *in vitro* as well as in untreated ulcers extremely diminished catalase activity in all tissue homogenate samples of all groups of rats. Incubation with ethanol and an appropriate buffer in all groups showed the same proportional rates of catalase activity as in gastric tissue without incubation (Table 5).

The end product of lipid peroxidation MDA in the control group was not significantly higher than in the ulcer group of rats. The herbal tincture pretreatment of rats with ulcer significantly reduced MDA levels, indicating a lower intensity of lipid peroxidation in ulcerogenic gastric tissue (Table 2).

Incubation of homogenized tissue samples of all groups of rats showed the same proportional rates of MDA content as compared to essential gastric tissue, indicating a significantly lower MDA content in rats with induced ulcers pretreated with herbal tincture (Table 6).

Table 5. **Influence of FU tincture on catalase content in gastric tissue homogenate with indomethacin-induced ulcer during incubation *in vitro* (mean ± SD)**

Groups / Nmol/l·min	UL n = 9	UL+FU n = 9	Control n = 10
With FU tincture, dose 0.8 mg	18.29 ± 0.71[1]	20.16 ± 0.60[1]	18.99 ± 0.73[1]
With 40° ethanol	61.37 ± 4.11[1]	63.43 ± 3.73	91.34 ± 1.62[1]
With buffer solution	51.55 ± 2.85	59.24 ± 3.50	78.02 ± 2.00

[1] $p < 0.05$ (samples of incubation with FU tincture or ethanol *versus* incubation with buffer solution).

Table 6. **Influence of FU tincture on MDA content in gastric tissue homogenate with indomethacin-induced ulcer during incubation *in vitro* (mean ± SD)**

Groups / nmol/ml	UL n = 9	UL+FU n = 9	Control n = 10
With FU tincture, dose 0.8 mg	3.73 ± 0.09	1.39 ± 0.20*	3.87 ± 0.06
With 40° ethanol	–	1.53 ± 0.27	3.88 ± 0.15
With buffer solution	–	1.24 ± 0.17	–

* $p < 0.05$ (group UL + FU *versus* group UL).

DISCUSSION

It is known that in *in vivo* experimental models of gastric ulcer in rats, reactive oxygen species play a significant role among other mechanisms of gastric ulcer induction (inhibition of prostaglandin synthesis, hypersecretion of acid, etc.) (13). Indomethacin in three doses (total amount 120 mg/kg) during three days induced significant changes in the parameters of the pro-/antioxidant status of gastric tissue and serum, indicating a direct influence on systemic changes and ulcer formation. More evidently in serum was reduced the level of total antioxidant activity and catalase and less evidently the level of superoxide dismutase, indicating the action of various free oxygen radicals (superoxide, hydroxyl radicals). It is known that *Filipendula ulmaria* in folk medicine is used to control problems associated with the gastrointestinal tract, particularly for diarrhoea, heartburn (14). FU contains chemicals known as tannins, which have a drying effect and may decrease congestion and mucus. Among the chemicals contained in FU are salicylates, contributing to the pharmacological activity of FU. Other compounds include flavonoids, various other phenols. Barnaulov et al. (15) found an unexpected ability of FU to prevent ulcers in laboratory animals. Salicylates (*i.e.* salicin) having the anti-inflammatory properties (like aspirin or other NSAIDS) may cause gastric ulcers. We tested the tincture of FU to prevent formation of ulcer induced by indomethacin, expecting antiradical activity. We found that significantly increased total antioxidant activity *in vivo* and decreased MDA administering the both tincture *in vivo* and *in vitro*. There were no statistical significant changes in serum indices in the ulcer and the FU treated groups, which indicated no toxical influence on the process of ulceration. There were no differences in total damaged areas between these groups (data not shown). It is known that in indomethacin-induced ulcers a significant damaging role belongs to increased levels of end products of lipid peroxidation (MDA) (16, 17), which aggravate mucosal injury, despite changes in SOD or other enzymes. In our experiment, despite ulcer formation, the tincture *per os* notably suppressed MDA formation, suggesting the antioxidant activity against ROS. With this idea agrees the fact of SOD changes. Our results revealed that in rats with ulcer and treated with FU tincture, the SOD level increased as compared to the ulcer group, indicating a reduced ROS formation. The same effects of another herbal mixture were found by Shin H. M. (18) who investigated the influence of a popular herbal medicine formula on indomethacin-induced gastric mucosal lesions. These results showed that in rats orally pretreated with a herbal formula, mucosal

SOD and CAT activities were increased. In our case, the catalase activity was the same, giving no evidence for the interactions between the tincture and catalase *in vivo*. Despite SOD and MDA changes, the role of the tincture in ROS formation reveals an enhanced total antioxidant activity in homogenate, which was highest in ulcerated rats treated with FU tincture. A comparison of the influence of FU tincture and of ethanol and vehicle solution on tissue homogenate during incubation revealed similar tendencies of changes in all groups of experimental animals, indicating a stronger *per os* influence of FU on ROS in gastric tissue than *in vitro*. Our results indicate a reduced SOD and catalase activity *in vitro*, suggesting that FU tincture may affect SOD and catalase by directly inhibiting their activity. Lichtenberger (19) focuses attention on the fact that NSAIDs can injure gastric mucosa by the mechanism other than COX inhibition. NSAIDs can directly attenuate the surface hydrophobic barrier of gastric mucosa due to their ability to bind phospholipids. The salicilates (constituents of FU), like other NSAIDs, may affect the gastric mucous barrier by destroying the phospholipid layer. In our experiment, we have not investigated the influence of FU tincture on the gastric mucous barrier.

In conclusion, the present study has revealed the ability of *Filipendula ulmaria* tincture to influence the pro-/antioxidant status in gastric tissue in indomethacin-induced gastric ulcers, indicating a beneficial therapeutic effect of the tincture in cases of gastric disorders induced by NSAIDs.

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References

1. Salim AS. Removing oxygen-derived free radicals stimulates healing of ethanol induced erosive gastritis in rat. Digestion 1990; 47: 24–8.
2. Das D, Banerjee RK. Effect of stress on the antioxidant enzymes and ulceration. Mol Cell Biochem 1993; 125(2): 115–25.
3. Hassan A, Martin E, Puig-Parellada P. Role of antioxidants in gastric mucosal damage induced by indomethacin in rats. Methods Find Exp Clin Pharmacol 1998; 20(10): 849–54.
4. Martin MJ, La-Casa C, Alarcon-de-la-Lastra C, Cabesa J, Villegas I, Motilva V. Anti-oxidant mechanisms involved in gastroprotective effects of quercetin. Z Naturforsch 1998; 53(1–2): 82–8.
5. Vasiliauskas J. Augalai ir sveikata. Vilnius: Mokslas, 1991: 264–66.
6. Keturkienë A, Leonavièiûtë L, Vaitkienë D. *Filipendula Ulmaria* (L.) Maxim tincture toxicity studies in rats. Baltic J Lab Anim Sci 2002 12; (1–2): 62–7.
7. Vasiliauskas A, Keturkienë A, Vaitkienë D, Leonavièienë L. An improved model of gastric mucosal lesions in rats and their therapy with herbal tincture. Baltic J Lab Anim Sci 2002; 12(1–2): 57–61.
8. Gavrilov VB, Gavrilova AP, Mazhul LM. Analysis of methods for detection of lipid peroxidation products in blood serum using thiobarbituric acid test. Vopr Med Khim 1987; 1: 118–22 (in Russian).
9. Arutiunian AV, Dubinina EE, Zibina NN. Methods for evaluation of organism free radical oxidation and antioxidant system. St. Petersburg 2000: 49–51 (in Russian).
10. Korolyuk MA, Ivanova LI, Mayorova IG, Tokarev VE. Method for detection of catalase activity. Lab Delo 1988; 1: 16–19 (in Russian).
11. Galaktionova LP, Molchanov AV, Yelchaninova SA, Varshavsky BY. Lipid peroxidation in patients with gastroduodenal ulcer. Clin Lab Diag 1998; 6: 10–4.
12. Csovari S, Andyal T, Strenger J. Blood antioxidative parameters and their diagnostic value in elderly patients. Lab Delo 1991; 10: 9–13.
13. Fukumura D, Kurose I, Miura S, Tsuchiya M, Ishii H. Oxidative stress in gastric mucosal injury: role of platelet-activating factor-activated granulocytes. J Gastroenterol 1995; 30(5): 565–71.
14. Blumenthal M, Gruenwald J, Hall T, Rister RS, eds. The Complete German Commission E Monographs. Austin, Texas: American Botanical Council, 1998.
15. Barnaulov OD, Denisenko PP, Anti-ulcer action of a decoction of the flowers of the dropwort, *Filipendula ulmaria* (L.) Maxim. Farmakologia i Toksikologia 1980; 43: 700–5.
16. Smith GS, Mercer DW, Cross JM, Barreto JC, Miller TA. Gastric injury induced by ethanol and ischemia-reperfusion in the rat. Differing roles for lipid peroxidation and oxygen radicals. Dig Dis Sci 1996, 41: 1157–64.
17. Tanaka J, Yuda Y. Lipid peroxidation in gastric mucosal lesions induced by indomethacin in rat. Biol Pharm Bull 1996; 19: 716–20.
18. Shin HM. Anti-oxidative herbs and indomethacin-induced rat gastric mucosal lesions: protection by GamiHyangsa-Yukgunja. Am J Clin Med 2001; 29: 101–9.
19. Lichtenberger LM. Where is the evidence that cyclooxygenase inhibition is the primary cause of nonsteroidal anti-inflammatory drug (NSAID)-induced gastrointestinal injury? Topical injury revised. Biochem Pharmacol 2001; 61: 631–7.

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***FILIPENDULA ULMARIA* (L.) MAXIM TINKTÛROS POVEIKIS ÞIURKIØ SKRANDÞIO AUDINIO PROOKSIDANTINEI IR ANTIOKSIDANTINEI BÛKLEI INDOMETACINO SUKELTOS OPALIGËS METU**

S a n t r a u k a

Reaktyvûs deguonies junginiai dalyvauja ávairiuose patologiniuose procesuose. Þinoma, kad laboratorinëms þiurkëms indometacinu sukëlus skrandþio gleivinës opà padaugëja

deguonies laisvøjø radikalø. Sumaþinti laisvøjø radikalø kieká gali ávairios medþiagos, taip pat augaluose esantys flavonoidai. Liaudies medicinoje pelkinë vingiorykðtë vartojama sergant ávairiomis virðkinimo trakto ligomis. **Darbo tikslas** – nustatyti pelkinës vingiorykðtës poveiká *in vivo* ir *in vitro* þiurkiø skrandþio gleivinës prooksidantinei ir antioksidantinei bûklei, indometacinu sukëlus opaligæ. **Metodai**. Tyrimo metu *per os* þiurkëms taikyta patentuota pelkinës vingiorykðtës spiritinë tinktûra (1:5). Tirtos 28 Wistar þiurkiø 200–240 g svorio patelës. Dviem grupëms po 9 þiurkes buvo sukelta opaligë indometacinu; ið jø viena grupë, likus dviem dienoms iki indometacino skyrimo, gavo *per os* vingiorykðtës tinktûros (dozë 0,5 ml/kg^{-1}). Treèia grupë – kontrolinë (10 þiurkiø). Visø grupiø þiurkëms skrandþio gleivinës prooksidantinë ir antioksidantinë bûklë tirta nustatant superoksido dismutazës, katalazës fermentø aktyvumà, lipidø peroksidacijos galutiná produktà – malondialdehidà ir bendrà antioksidantiná aktyvumà. **Rezultatai**. Eksperimentu nustatyta, kad, vartojant pelkiniø vingiorykðèiø tinktûrà *per os* statistiðkai patikimai padidëjo bendras skrandþio gleivinës antioksidantinis aktyvumas (36%), superoksido dismutazës kiekis (25%), sumaþëjo malondialdehido kiekis (73%) i*n vivo*. *In vitro* tyrimai parodë, kad pelkiniø vingiorykðèiø tinktûra patikimai padidino bendrà antioksidantiná aktyvumà, sumaþino SOD, katalazës kieká skrandþio audinio homogenate, taèiau nepaveikë lipidø peroksidacijos. **Išvados**. Eksperimento rezultatai patvirtina teigiamà pelkiniø vingiorykðèiø tinktûros poveiká prooksidantiniams ir antioksidantiniams procesams skrandþio audinyje tiriant *in vivo*. Manoma, kad tai susijæ su deguonies reaktyviø junginiø maþëjimu skrandþio gleivinëje indometacino sukeltos opaligës metu.