Gastric anti-ulcerative and anti-inflammatory activity of metyrosine in rats

Abdulmecit Albayrak¹, Beyzagul Polat¹, Elif Cadirci¹, Ahmet Hacımuftuoğlu¹, Zekai Halici¹, Mine Gulapoglu², Fatih Albayrak³, Halis Suleyman¹

¹Department of Pharmacology, ³Department of Gastroenterology, Faculty of Medicine, ²Department of Biochemistry, Faculty of Pharmacy, Ataturk University, 25240 Erzurum, Turkey

Correspondence: Halis Suleyman, e-mail: halis.suleyman@gmail.com

Abstract:

In this study, the anti-inflammatory and anti-ulcerative effects of metyrosine, a selective tyrosine hydroxylase enzyme inhibitor, were investigated in rats. For ulcer experiments, indomethacin-induced gastric ulcer tests and ethanol-induced gastric ulcer tests were used. For these experiments, rats were fasted for 24 h. Different doses of metyrosine and 25 mg/kg doses of ranitidine were administered to rats, followed by indomethacin at 25 mg/kg for the indomethacin-induced ulcer test, or 50% ethanol for the ethanol-induced test. Results have shown that at all of the doses used (50, 100 and 200 mg/kg), metyrosine had significant anti-ulcerative effects in both indomethacin and ethanol-induced ulcer tests. Metyrosine doses of 100 and 200 mg/kg (especially the 200 mg/kg dose) also inhibited carrageenan-induced paw inflammation even more effectively than indomethacin. In addition, to characterize the anti-inflammatory mechanism of metyrosine we investigated its effects on cyclooxygenase (COX) activity in inflammatory tissue (rat paw). The results showed that all doses of metyrosine significantly inhibited high COX-2 activity. The degree of COX-2 inhibition correlated with the increase in anti-inflammatory activity. In conclusion, we found that metyrosine has more anti-inflammatory effects than indomethacin and that these effects can be attributed to the selective inhibition of COX-2 enzymes by metyrosine. We also found that adrenalin levels are reduced upon metyrosine treatment, which may be the cause of the observed gastro-protective effects of this compound.

Key words: metyrosine, adrenalin, inflammation, ulcer, rat

Introduction

Inflammation is a physiopathological event that removes agents that damage tissue [24]. Inflammation is characterized by the presence of redness, heat, swelling, pain and loss of function [8, 13]. Typically, steroids and non-steroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of inflammation [4, 22]. Since NSAIDs are effective treatments for

redness, heat, swelling, pain and loss of function, they are the most common drugs used for inflammatory diseases [20]. However, NSAIDs can cause serious gastrointestinal (GI) side effects, ranging from significant blood loss to ulcer perforation [7, 32]. To prevent the toxic effects of NSAIDs on the GI, nitric oxide release NSAIDs and cyclooxygenase (COX-2) selective inhibitors have been developed [4, 26]. However, it is not known whether these drugs have any healing effects on gastric ulcers. Thus, research to identify an

anti-inflammatory agent with no ulcerative side effects is needed.

In 2002 it was reported that a NSAID, nimesulide, was an anti-ulcerative agent as potent as ranitidine in rats [25]. Interestingly, while nimesulide had anti-ulcerative effects in intact rats, it had ulcerative effects in adrenalectomized rats, pointing to the existence of anti-ulcerative factors originating from the adrenal glands [28]. Prednisolone, a glucocorticoid, is also ulcerative in intact rats and anti-ulcerative in adrenalectomized or metyrosine treated rats, indicating that a decrease in adrenalin levels is necessary for an anti-ulcerative effect [31]. In another study, the anti-inflammatory effects of NSAIDs were found to disappear in adrenalectomized rats [30]. In addition, NSAIDs have also been found to reduce adrenalin levels significantly [27]. These findings indicate that the level of adrenalin must be low for an anti-inflammatory effect to be observed. Furthermore, in our previous study nimesulide demonstrated anti-inflammatory and anti-ulcerative activities resulting from decreased adrenalin levels [31]. In light of these observations we suggest that drugs that decrease adrenalin levels may also have anti-inflammatory and anti-ulcerative effects. Here we sought to examine the anti-inflammatory and anti-ulcerative effects of metyrosine. Because metyrosine inhibits tyrosine hydroxylase activity, it can result in decreases of adrenalin levels by approximately 35–80% [14, 22]. For this reason, the aim of our study is to investigate the anti-inflammatory and anti-ulcerative effects of metyrosine in rats, and determine the correlation of these effects with the levels of COX enzymes.

Materials and Methods

Animals

A total of 90 male albino Wistar rats obtained from the Medical Experimental Research Centre at Atatürk University, weighing between 200 and 220 g, were used for these studies. The animals were fed in separate groups under normal conditions (22°C). Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and approved by the local animal care committee of Atatürk University.

Chemicals

For laboratory experimentation, indomethacin, ranitidine and sodium thiopental were obtained from Deva Holding-İstanbul, Fako-İstanbul and IE-Uluagay-İstanbul, respectively. Metyrosine and carrageenan were purchased from Merck Co., Inc. and Sigma Chemical.

Inflammatory paw edema tests in rats

The anti-inflammatory activity of metyrosine was investigated on carrageenan-induced inflammatory paw edema [19]. Metyrosine was injected intraperitoneally into groups of rats at doses of 50, 100 or 200 mg/kg. Distilled water was given to the control group at the same volume as the vehicle. One hour after injection, 0.1 ml of 1% carrageenan solution was injected into the footpad of each rat in all groups. Prior to carrageenan injection, the paw volume as far as the knee joint of each rat was measured with a pletysmometer. The increase in volume of paws with inflammation induced by carrageenan was measured at 1-h intervals over the next 4 h. The anti-inflammatory potential of metyrosine was compared with that of 25 mg/kg indomethacin.

Ulcer tests in rats

Indomethacin-induced ulcer test

The anti-ulcerative effects of metyrosine were investigated using an indomethacin-induced ulcer model in rats [11]. Metyrosine was administered intraperitoneally to the group of rats that were fasted for 24 h at doses of 50, 100 or 200 mg/kg. After 5 min, 25 mg/kg indomethacin was given to each rat in all groups by oral gavage. Distilled water was given to the control group at the same volume as the vehicle. Six hours after the indomethacin administration, all groups were sacrificed by a high dose (50 mg/kg) of thiopental anesthesia. The stomachs of the rats were removed, and the ulcerous regions were examined macroscopically. Ulcerative areas were measured using paper marked in square millimeters. The anti-ulcerative activity of metyrosine was evaluated by comparing the results obtained from the control and the ranitidine (50 mg/kg) treated groups.

Ethanol-induced ulcer test

The anti-ulcerative effects of metyrosine were also investigated using an ethanol-induced ulcer model in rats [6, 23]. Metyrosine was administrated intraperitoneally to rats fasted for 24 h at doses of 50, 100 or 200 mg/kg. After 30 min, 50% ethanol was given to each rat in all groups by oral gavage. Distilled water was given to the control group at the same volume as the vehicle. One hour after the ethanol administration, all groups were sacrificed by a high dose (50 mg/kg) of thiopental anesthesia. The stomachs of the rats were removed, and ulcerous regions were examined macroscopically. Ulcerative areas were measured on paper marked in square millimeters. The anti-ulcerative activity of metyrosine was evaluated by comparing the results obtained from the control and the ranitidine treated (50 mg/kg) groups.

Measurement of COX activity

COX activity in rat paw tissue was measured using a COX activity assay kit (Cayman, Ann Arbor, MI, USA). Paw tissue was collected free of paw membranes, and washed thoroughly with ice-cold Tris buffer, pH 7.4, containing 0.16 mg/ml of heparin (to remove any red blood cells and clots), and stored at -80°C until assayed. A sample of paw tissue was homogenized in 5 ml of cold buffer (0.1 M Tris-HCl, pH 7.8, containing 1 mM EDTA) per gram of tissue, and centrifuged at $10,000 \times g$ for 15 min. at 4°C . The supernatant was removed for assay measurements, and stored on ice. The protein concentration in the supernatant was measured by the Bradford method [1]. The COX kit measures the peroxidase activity of COX. The peroxidase activity is assayed colorimetrically by monitoring the appearance of oxidized N,N,N',N'-tetramethyl-*p*-phenylenediamine at 590 nm. COX-2 activity was measured using a potent COX-1-specific inhibitor, SC-560 (the reagent is ready to use as supplied). The results are given as unit per milligram of protein for COX-1 and COX-2 activity.

Statistical analysis

All data were subjected to one-way ANOVA using SPSS 13.0 software. Differences among groups were attained using the LSD option, and significance was declared at $p < 0.05$.

Results

Inflammatory paw edema test

As seen in Figure 1, while metyrosine reduced carrageenan inflammation at 50, 100 and 200 mg/kg doses (40% ($p < 0.01$), 67% ($p < 0.002$) and 87% ($p < 0.001$), respectively, at 4 h), indomethacin reduced the inflammation by 64% ($p < 0.001$). At the indicated doses, the increase in inflammatory paw volume in metyrosine treated rats was 0.29 ± 0.01 , 0.16 ± 0.01 , and 0.06 ± 0.01 ml, respectively, compared to control. In the indomethacin treated and control groups, this measurement was 0.17 ± 0.01 and 0.48 ± 0.03 , respectively (Tab. 1).

COX activity test

Carrageenan injection in rat paws significantly increased the COX-2 levels (108.5%, $p < 0.001$) when compared to intact rats. However, the increase in COX-1 levels (2.9%, $p > 0.05$) were not significant. In rats treated with 50, 100 and 200 mg/kg doses of metyrosine, no significant difference was observed in the COX-1 activity in inflammatory paw tissue when compared to the carrageenan only group. However, at the same doses of metyrosine, COX-2 activity was decreased by 29.5% ($p < 0.05$), 40.2% ($p < 0.05$) and 53.5% ($p < 0.05$), respectively, when compared to the carrageenan only group. Indomethacin administration

Fig. 1. Anti-inflammatory effect percentage of metyrosine in 50, 100 and 200 mg/kg doses and indomethacin in 25 mg/kg dose

Tab. 1. The effects of metyrosine and indomethacin on carrageenan-induced rat paw edema

Drugs	Dose (mg/kg)	Number of animals	Paw volume of rats (ml)		Increase in inflammatory paw volume (ml)
			Before inflammation	At the 4th hour of carrageenan injection	
Metyrosine	50	6	0.93	1.22	0.29 ± 0.01
Metyrosine	100	6	0.87	1.03	0.16 ± 0.01
Metyrosine	200	6	0.87	0.93	0.06 ± 0.01
Indomethacin	25	6	0.89	1.07	0.17 ± 0.01
Control	–	6	0.85	1.33	0.48 ± 0.03

Fig. 2. Carrageenan-induced COX-1 and COX-2 activity in rat paw following metyrosine or indomethacin treatment. * Means $p < 0.05$ is significant when compared to control

decreased both COX-1 (by 50.4%, $p < 0.05$) and COX-2 (by 34.4%, $p < 0.05$) activity significantly when compared to the carrageenan only group (Fig. 2).

Ulcer test

At doses of 50 and 100 mg/kg, metyrosine inhibited indomethacin induced ulcers by 13% ($p < 0.005$) and 64% ($p < 0.001$) compared to the control group. No ulcerative areas were noted for the group treated with 200 mg/kg metyrosine (Tab. 2). Metyrosine was also

Tab. 2. Effects of metyrosine on indomethacin-induced gastric ulcers in rats

Drugs	Dose (mg/kg)	Number of animals	Ulcer area (mm ²)	Antilulcer effect (%)	p
Metyrosine	50	6	25.0 ± 2.6	13	> 0.005
Metyrosine	100	6	10.3 ± 2.3	64	< 0.001
Metyrosine	200	6	0.0 ± 0.0	100	< 0.001
Ranitidine	50	6	2.2 ± 0.3	92	< 0.001
Control (indomethacin)	25	6	28.6 ± 3.8	–	–

Tab. 3. Effects of metyrosine on ethanol-induced gastric ulcers in rats

Drugs	Dose	Number of animals	Ulcer area (mm ²)	Antilulcer effect (%)	p
Metyrosine	50 (mg/kg)	6	18.0 ± 5.3	87.7	< 0.001
Metyrosine	100 (mg/kg)	6	9.2 ± 3.9	93.7	< 0.001
Metyrosine	200 (mg/kg)	6	6.6 ± 1.7	95.5	< 0.001
Ranitidine	50 (mg/kg)	6	2.5 ± 1.5	98.3	< 0.001
Control (ethanol)	50%	6	146.7 ± 25.2	–	–

found to have anti-ulcerative effects at all doses studied in the ethanol-induced gastric ulcer test. Anti-ulcerative activity was 87.7%, 93.7% and 95.5% for 50, 100 and 200 mg/kg doses of metyrosine, respectively, and 98.3% for ranitidine (Tab. 3).

Fig. 3. Ulcerative areas of metyrosine 50, 100 and 200 mg/kg vs. indomethacin control

Fig. 4. Ulcerative areas of metyrosine 200 mg/kg vs. ranitidine 50 mg/kg control in an indomethacin-induced ulcer test

Discussion

In this study, the anti-inflammatory and anti-ulcerative effects of metyrosine, a selective tyrosine hydroxylase enzyme inhibitor, were investigated. The results have shown that at all doses used (50, 100 and 200 mg/kg), metyrosine had a significant anti-inflammatory effect. Metyrosine doses of 100 and 200 mg/kg (especially the 200 mg/kg dose) inhibited carrageenan induced inflammation even more effectively than indomethacin. We were unable to find any scientific research on the anti-inflammatory or anti-ulcerative effects of metyrosine. Thus, the mechanism behind this anti-inflammatory effect is yet unknown.

As we mentioned before, in our previous studies we showed that reduction of adrenalin levels by nimesu-

lide or indomethacin plays a role in the anti-inflammatory mechanism of these two drugs. Indomethacin did not exert anti-inflammatory activity in either rats with low cortisol levels or in rats with low cortisone and adrenalin levels [27]. It has been determined that cortisol exerts potent anti-inflammatory effects *via* β -2 adrenergic receptors under adrenalin deficiency (in adrenalectomized rats) [30]. Metyrosine decreases adrenalin levels as a result of tyrosine hydroxylase enzyme inhibition [22]. Endogenous cortisol may contribute to the anti-inflammatory mechanism of metyrosine in cases of adrenalin deficiency that result from metyrosine application. These data are in line with previous literature.

To elucidate the anti-inflammatory mechanism of metyrosine, we investigated its effect on COX activity in inflammatory tissue. Our results showed that all doses of metyrosine used significantly inhibited high COX-2 activity. The degree of inhibition of COX-2 directly correlated with the increase in anti-inflammatory action. At an anti-inflammatory dose, indomethacin significantly reduces the activities of both COX-1 and COX-2. Additionally, our data suggest that metyrosine causes its anti-inflammatory effects by selectively inhibiting COX-2.

In our study, carrageenan was used to induce inflammation in the rat paw. This polysaccharide substance induces an inflammatory process that is composed of two phases: an early or first phase, and a late or second phase. It has been proposed that the early phase results from histamine, serotonin, and bradykinin liberation, while the late phase is associated with the formation of COX products [10, 19]. Following carrageenan induced inflammation, observation of the anti-inflammatory effects of metyrosine after 4 h (late phase) suggests that metyrosine exerts its inhibitory effect on COX activity.

It has been proposed that COX-2 is responsible for the anti-inflammatory effect of NSAIDs, while COX-1 inhibition is responsible for the GI side effects [17]. Therefore, attributing the gastro-protective effects of metyrosine to its selective inhibition of COX-2 is not substantiated by the data. Studies have shown that COX-2 has both physiological and patho-physiological roles. For this reason, it is inevitable that both selective and non-selective COX inhibitors will have negative side effects [2, 21]. Katori and Majima have shown that in gastric and other tissues, COX-2 is expressed even under normal physiological conditions [15]. Furthermore, nimesulide, which shows a slight

(5 fold) selectivity for COX-2 was able to prevent indomethacin ulcers, while rofecoxib, which is 800 times more selective, could not prevent ulcers [29]. In another study, Laudanno et al. have indicated that rofecoxib and celecoxib both increase indomethacin-induced ulcers [16]. This demonstrates that there is no direct correlation between the degree of COX inhibition and the GI damage caused by NSAIDs. It is known that the COX-1 products, prostaglandins (PGE₂, PGI₂), reduce acid secretion [5].

Experimentally, it can be shown that α -2b and α -2c adrenergic receptor subtypes are responsible for gastro-protection [9, 12]. Gastric secretion is inhibited by stimulation of the α -2 adrenergic receptor. This occurs when acetylcholine secretion is inhibited by activation of presynaptic α -2 receptors on the vagus nerve [9]. Because of this, attributing the side effects of classic NSAIDs on the GI system or the anti-ulcerative effects of metyrosine to only COX-1 activation or inhibition does not provide a sufficient explanation.

We suggest that metyrosine may exert its anti-ulcerative effects by reducing endogenous adrenalin levels in a similar manner to that of nimesulide. In previous studies metyrosine was shown to reduce circulating adrenalin levels significantly [22, 31]. As mentioned previously, NSAIDs create an anti-inflammatory effect by reducing adrenalin levels [27]. In addition, the role of α -2 adrenergic receptors in promoting anti-ulcerative effects has been previously demonstrated [31]. Thus, drugs that reduce adrenalin levels and block α -2 adrenergic receptors at the same time (such as indomethacin) may therefore have potent anti-inflammatory and ulcerative effects. The potent preventative activity of clonidine, an α -2 adrenergic receptor agonist [18], against indomethacin-induced gastric ulcers also demonstrates that indomethacin may create gastric ulcers by blocking α -2 adrenergic receptors. Studies should therefore be carried out to determine whether α -2 adrenergic receptor activation has a role in enhancing COX-1 activity. In another section of our study, we investigated the protective effects of metyrosine on ethanol-induced gastric ulcers. COX enzymes have a very limited role in the mechanism of formation of ethanol-induced gastric ulcers [3]. In our study metyrosine exerted anti-ulcerative effects as potent towards ethanol-induced gastric ulcers as towards indomethacin-induced gastric ulcers. From the evaluation of all of these data, we suggest that the role of metyrosine in decreasing adrenalin levels,

rather than its effects on COX levels play the primary role in the anti-ulcerative effects of this compound.

In conclusion, this study demonstrated that metyrosine has potent anti-inflammatory and anti-ulcerative effects. In addition, this study demonstrated that metyrosine decreased COX-2 levels significantly without affecting COX-1 levels. The selective reduction of COX-2 levels may be responsible for metyrosine's anti-inflammatory effect without any ulcerative side effects. This also can explain the anti-ulcerative effects of this compound. Metyrosine is known to be a potent inhibitor of circulating adrenalin. This reduction in circulating adrenalin levels may be the reason why metyrosine is able to exert both effects simultaneously. Future randomized experimental and clinical trials are required to verify this hypothesis.

References:

1. Bradford MM: Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding. *Anal Biochem*, 1976, 72, 248–254.
2. Brater DC: Renal effects of cyclooxygenase-2-selective inhibitors. *J Pain Symptom Manage*, 2002, 23, S15–S20.
3. Brzozowski T, Konturek PC, Konturek SJ, Brzozowska I, Pawlik T: Role of prostaglandins in gastroprotection and gastric adaptation. *J Physiol Pharmacol*, 2005, 56, Suppl 5, 33–55.
4. Burke A, Smyth E, FitzGerald GA: Analgesic-antipyretic agents: pharmacotherapy of gout. In: Goodman & Gilman's the Pharmacological Basis of Therapeutics. Ed. Brunton LL, The McGraw-Hill Companies, New York, 2006, 695–696.
5. Buttgerit F, Burmester GR, Simon LS: Gastrointestinal toxic side effects of nonsteroidal anti-inflammatory drugs and cyclooxygenase-2-specific inhibitors. *Am J Med*, 2001, 110, Suppl 1, 13–19.
6. Cadirci E, Suleyman H, Aksoy H, Halici Z, Ozgen U, Koc A, Ozturk N: Effects of *Onosma armeniacum* root extract on ethanol-induced oxidative stress in stomach tissue of rats. *Chem Biol Interact*, 2007, 170, 40–48.
7. Collier DS, Pain JA: Non-steroidal anti-inflammatory drugs and peptic ulcer perforation. *Gut*, 1985, 26, 359–363.
8. Dunne MW: Inflammation and repair. In: Pathophysiology. Ed. Porth CM, Lipincott, Philadelphia, 1990, 165–176.
9. Fulop K, Zadori Z, Ronai AZ, Gyires K: Characterisation of α ₂-adrenoceptor subtypes involved in gastric emptying, gastric motility and gastric mucosal defence. *Eur J Pharmacol*, 2005, 528, 150–157.
10. Gamache DA, Povlishock JT, Ellis EF: Carrageenan-induced brain inflammation – characterization of the model. *J Neurosurg*, 1986, 65, 679–685.

11. Guidobono F, Pagani F, Ticozzi C, Sibilia V, Pecile A, Netti C: Protection by amylin of gastric erosions induced by indomethacin or ethanol in rats. *Br J Pharmacol*, 1997 120, 581–586.
12. Gyires K, Mullner K, Furst S, Ronai AZ: Alpha-2 adrenergic and opioid receptor-mediated gastroprotection. *J Physiol (Paris)*, 2000, 94, 117–121.
13. Higgins AJ: The biology, pathophysiology and control of eicosanoids in inflammation. *J Vet Pharmacol Ther*, 1985, 8, 1–18.
14. Hoffman BB: Therapy of hypertension. In: Goodman & Gilman's the Pharmacological Basis of Therapeutics. Ed. Brunton LL. The McGraw-Hill Companies, New York, 2006, 845–897.
15. Katori M, Majima M: Cyclooxygenase-2: its rich diversity of roles and possible application of its selective inhibitors. *Inflamm Res*, 2000 49, 367–392.
16. Laudanno OM, Cesolari JA, Esnarriaga J, Rista L, Piombo G, Maglione C, Aramberry L et al.: Gastrointestinal damage induced by celecoxib and rofecoxib in rats. *Dig Dis Sci*, 2001 46, 779–784.
17. Laudanno OM, Cesolari JA, Esnarriaga J, San Miguel P, Bedini OA: In vivo selectivity of nonsteroidal antiinflammatory drugs and gastrointestinal ulcers in rats. *Dig Dis Sci*, 2000, 45, 1359–1365.
18. Luo GY, He Y, Tu LB: Effects of clonidine on experimental stomach ulcer in rats (in Chinese). *Zhongguo Yao Li Xue Bao*, 1990, 11, 457–459.
19. Marzocco S, Di Paola R, Serraino I, Sorrentino R, Meli R, Mattaceraso G, Cuzzocrea S et al.: Effect of methylguanidine in carrageenan-induced acute inflammation in the rats. *Eur J Pharmacol*, 2004, 484, 341–350.
20. Mitchell JA, Warner TD: Cyclo-oxygenase-2: pharmacology, physiology, biochemistry and relevance to NSAID therapy. *Br J Pharmacol*, 1999, 128, 1121–1132.
21. Mukherjee D: Selective cyclooxygenase-2 (COX-2) inhibitors and potential risk of cardiovascular events. *Biochem Pharmacol*, 2002, 63, 817–821.
22. Reams G, Bauer JH: Pharmacologic treatment of hypertension. In: Atlas of diseases of the kidney. Ed. Wilcox CS, Blackwell Science, Philadelphia, 1999, 28–30.
23. Schmidt KM, Klopfer FD: Ethanol and stomach ulcers: absence of influence in the albino rat. *Q J Stud Alcohol*, 1968, 29, 558–565.
24. Steinmeyer J: Pharmacological basis for the therapy of pain and inflammation with nonsteroidal anti-inflammatory drugs. *Arthritis Res*, 2000 2, 379–385.
25. Suleyman H, Akcay F, Altinkaynak K: The effect of nimesulide on the indomethacin- and ethanol-induced gastric ulcer in rats. *Pharmacol Res*, 2002, 45, 155–158.
26. Suleyman H, Cadirci E, Albayrak A, Halici Z: Nimesulide is a selective COX-2 inhibitory, atypical non-steroidal anti-inflammatory drug. *Curr Med Chem*, 2008, 15, 278–283.
27. Suleyman H, Cadirci E, Albayrak A, Halici Z, Polat B, Hacımuftuoğlu A, Alp HH: The reason of the aggravation of the diseases which are going with inflammation and ineffectiveness of NSAIDs on them in rainy weather. *Pharmacol Rep*, 2009, 61, 514–519.
28. Suleyman H, Demircan B, Gocer F, Halici Z, Hacımuftuoğlu A: Role of adrenal gland hormones in the mechanism of antiulcer action of nimesulide and ranitidine. *Pol J Pharmacol*, 2004, 56, 799–804.
29. Suleyman H, Demirezer LO, Kuruuzum-Uz A: Effects of *Rumex patientia* root extract on indomethacin and ethanol induced gastric damage in rats. *Pharmazie*, 2004, 59, 147–149.
30. Suleyman H, Halici Z, Cadirci E, Hacımuftuoğlu A, Bilen H: Indirect role of β_2 -adrenergic receptors in the mechanism of anti-inflammatory action of NSAIDs. *J Physiol Pharmacol*, 2008, 59, 661–672.
31. Suleyman H, Halici Z, Cadirci E, Hacımuftuoğlu A, Keles S, Gocer F: Indirect role of α_2 -adrenoreceptors in anti-ulcer effect mechanism of nimesulide in rats. *Naunyn Schmiedeberg's Arch Pharmacol*, 2007, 375, 189–198.
32. Wallace JL: Nonsteroidal anti-inflammatory drugs and gastroenteropathy: the second hundred years. *Gastroenterology*, 1997, 112, 1000–1016.

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