Department of Pharmacology[1], Faculty of Medicine, Atatürk University, Erzurum, and Department of Pharmacognosy[2], Faculty of Pharmacy, Hacettepe University, Ankara, Turkey

Effects of *Rumex patientia* root extract on indomethacine and ethanol induced gastric damage in rats

H. Süleyman[1], L. Ö. Demirezer[2], A. Kuruüzüm-Uz[2]

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Prof. Dr. L. Ömür Demirezer, Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara, Turkey
omurd@hacettepe.edu.tr

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The effects of an aqueous root extract from *Rumex patientia* (D-1) compared to COX-2 selective inhibitors on indomethacine and ethanol induced stomach ulcers were investigated. Adult male Wistar albino rats, weighing between 185–200 g were used. It was determined that D-1 does not show its gastroprotective activity via a COX enzyme in indomethacine induced ulcers. Antioxidant effects protect the gastrointestinal system. The effect of D-1 in ethanol induced ulcers may also be due to its antioxidant effect.

1. Introduction

The genus *Rumex* (Polygonaceae) is represented by 25 species in the flora of Turkey (Davis 1967; 1988). *Rumex patientia* has been investigated both from a phytochemical (Demirezer 2001a; 2001b; Kuruüzüm et al. 2001) and biological viewpoint; extracts have been screened for antiinflammatory (Süleyman et al. 1999; 2001a), analgesic and antipyretic (Süleyman et al. 2001b), gastroprotective (Süleyman et al. 2002), antioxidant (Çetinkaya et al. 2002), and antimicrobial (Demirezer et al. 1999) activities. The aim of this study was to investigate the effect of D-1 (aqueous extract of *Rumex patientia)*, on indomethacine and ethanol induced ulcer models in rats in comparison with nimesulide, rofecoxib and celecoxib, which are selective inhibitors of COX-2 (cyclooxygenase), and famotidine.

2. Investigations, results and discussion

This study aimed to determine, whether D-1 (the aqueous extract from the roots of *R. patientia*) possesses COX-2 selective inhibitor effects. The effects of D-1 were compared with the effects of COX-2 selective inhibitors such as rofecoxib, celecoxib, nimesulide. In addition, the effects of D-1 and famotidine on indomethacine and ethanol induced stomach ulcers in rats were compared.

Kataoka et al. (2000) reported that indomethacine increases stomach acid secretion, myeloperoxidase activity and stomach motility. In the same study it was shown that nimesulide and famotidine can contact there effects by decreasing stomach acid secretion, myeloperoxidase activity and stomach motility. Prostaglandins (PGE_2 and PGI_2) play an important role in stomach protection. They decrease stomach acid secretion, increase the thickness of the mucus sheet and improve blood flow through the mucosa. Buttgereit et al. (2001) claimed that the damaging effects of NSAIDs on the stomach result from inhibition of PG synthesis.

Warner et al. reported that the antiinflammatory effect of NSAIDs results from inhibition of COX-2, whereas inhibition of COX-1 is responsible for the gastrointestinal side effects (Derek et al. 2000; Warner et al. 1999). Previously, we reported that D-1, which has properties similar to NSAIDs, exhibited an antiinflammatory effect on various inflammation models and showed gastroprotective effects (Süleyman et al. 1999; 2001a). 150 and 500 mg/kg doses of D-1 and 100, 200, 300 mg/kg doses of nimesulide did not cause any damage on normal stomach tissue and the stress induced ulcer model in rats. Even at high doses (500 mg/kg) D-1 produced no damage to the gastric tissue. On the contrary, the ulcer area was reduced when compared with the control group (Süleyman et al. 2002). While COX nonselective NSAIDs, such as indomethacine, naproxen, ibuprofen and diclofenac, produced various, ulcer areas, COX-2 selective inhibitors reduced gastric damage (Bjarnason 1999).

The effects of D-1, nimesulide, rofecoxib, celecoxib and famotidine on indomethacine induced ulcer models were compared. In this study antiinflammatory doses of drugs were used. Significant hyperemia was seen only in rats receiving indomethacine (control group). Famotidine administered animals showed lighter hyperemia than D-1, rofecoxib and celecoxib administered rats. As shown in Table 1, the mean ulceration area is 22.1 ± 3.18 mm^2 in

Table 1: Effect of D-1, nimesulide, rofecoxib, celecoxib and famotidine on indomethacine induced ulcers

Samples	Dose (mg/kg)	Mean ulceration areas (mm^2)	Number of rats	P
D-1	150	19.1 ± 6.04	6	>0.05
Nimesulide	100	0	6	–
Rofecoxib	25	18.5 ± 5.24	6	>0.05
Celecoxib	100	19.6 ± 6.88	6	>0.05
Famotidine	40	0	6	–
Control	–	22.1 ± 3.18	6	–

Table 2: Effect of D-1, nimesulide, rofecoxib, celecoxib and famotidine on ethanol induced ulcers*

Samples	Dose (mg/kg)	Mean ulceration areas (mm^2)	Number of rats
D-1	150	68.1 ± 7.96	6
Nimesulide	100	46.3 ± 11.53	6
Rofecoxib	25	55.0 ± 10.37	6
Celecoxib	100	80.6 ± 7.03	6
Famotidine	40	61.3 ± 7.44	6
Control	–	152.8 ± 7.16	6

$p < 0.005$ (except control)

the stomach of animals treated with indomethacine (control group). The mean ulceration areas of animals treated with D-1, rofecoxib and celecoxib were are 19.1 ± 6.04, 18.5 ± 5.24 and $19.6 \pm 6.88\ mm^2$, respectively. In the D-1, group the indomethacine induced ulcer area was $3\ mm^2$ smaller than in the control group. Celecoxib and rofecoxib, which are COX-2 selective inhibitors, reduced the ulcer area by 2.5 and $3.6\ mm^2$, respectively. Their relative effects ranged as follows; nimesulide > rofecoxib > D-1 > celecoxib. The effect of D-1 is between rofecoxib and celecoxib. D-1 shows effects similar to celecoxib and rofecoxib on indomethacine induced ulcers. COX-2 selectivity of rofecoxib, celecoxib and nimesulide is 800, 375 and 5 times higher than COX-1 selectivity, respectively. While COX-2 selectivity of nimesulide disappears at high doses, this is not the case with celecoxib and rofecoxib. That is, celecoxib and rofecoxib inhibit COX-2 more than nimesulide and must therefore have a cytoprotective effect. Whereas COX-2 selectivities of rofecoxib and celecoxib are higher than that of nimesulide, they are not as effective as nimesulide in indomethacine induced ulcers.

It can be concluded that D-1 and COX-2 selective inhibitors do not produce their gastroprotective activity via a COX enzyme. Namely, there is no positive correlation between the degree of inhibition of COX and the Gastro intestinal system damage induced by NSAID.

Macroscopical investigations showed that the damage in ethanol induced stomach ulcers was observed in all animal groups. As shown in Table 2, the average ulcer area was $152.8 \pm 7.16\ mm^2$ in ethanol induced control group rats. The ulcer areas of D-1, nimesulide, rofecoxib, celecoxib and famotidine treated rats were 68.1 ± 7.96, 46.3 ± 11.53, 55 ± 10.37, 80.6 ± 7.03 and $61.3 \pm 7.44\ mm^2$, respectively.

D-1 and COX-2 selective inhibitors as well as famotidine are effective in ethanol induced ulcers. The relative efficacies of the drugs are: nimesulide > rofecoxib > famotidine > D-1 > celecoxib, respectively.

Formation of free radicals play roles in ethanol induced gastro intestinal damage (Smith et al. 1996). COX-2 selective inhibitors possess an antioxidant effect (Kotsinos et al. 1999). The efficacy of D-1 in ethanol induced ulcers can result from its antioxidant effect (Çetinkaya et al. 2002). Tannins may be responsible for the antioxidant effect of *R. patientia* (Demirezer et al. 2001b).

3. Experimental

3.1. Plant material

Rumex patientia (Polygonaceae) was collected from Niğde-Bor, Turkey (1050 m) in September 1999. A voucher specimen has been deposited in the herbarium of the Faculty of Pharmacy (HUEF-99003), Hacettepe University, Ankara, Turkey.

3.2. Extraction of the test sample

Five grams of material was extracted in a soxhlet apparatus with 100 ml water at 40 °C and the extract was lyophilized. The yield of extract (D-1) was 1.2 g, and this was kept in a dessicator.

3.3. Animals

72 adult male Wistar albino rats, weighing between 185–200 g from the experimental animal laboratory of Atatürk University, were used. Animals were fed under standard conditions.

3.4. Chemicals

Chemicals were obtained form Sigma.

3.5. Effect of D-1 and COX-2 selective inhibitors on indomethacine induced ulcers

The gastroprotective effects of D-1, COX-2 selective inhibitors, nimesulide, rofecoxib, celecoxib and famotidine on indomethacine induced ulcer models were investigated (Guidobono et al. 1997). The rats were separated in groups and placed in cages. 24 h before the assays, no food no drink except water was allowed. At the end of this period, D-1 (150 mg/kg), nimesulide (100 mg/kg) rofecoxib (25 mg/kg) and celecoxib (100 mg/kg) were administered p.o. gavage. For comparison of the gastroprotective effects of D-1 and COX-2 selective inhibitors, 40 mg/kg dose of famotidine was given p.o. A further group of animals (control group) received the same volume of distilled water. Five min after the administration of drugs, the animals received 25 mg/kg indomethacine p.o. After 6 h, the indomethacine administered animals were sacrified with thiopental sodium (50 mg/kg) and their stomachs were removed. The ulcerative zones were macroscopically evaluated to examine their numbers and areas.

3.6. Effect of D-1 and COX-2 selective inhibitors on ethanol-induced ulcers

The rats were not fed for 24 h. Then, D-1 (150 mg/kg), nimesulide (100 mg/kg), rofecoxib (25 mg/kg), celecoxib (100 mg/kg), and famotidine (40 mg/kg) were administered orally. A further group of animals (control group) received the same volume of distilled water. 30 min after the administration of the drug, the animals were given 1 ml ethanol (50%) p.o. gavage (Guidobono et al. 1997). One h after of ethanol administration, the animals were sacrified and their stomachs were removed. Ulcer areas were evaluated.

3.7. Statistical analysis

Values reported are mean ± SEM. The results were statistically evaluated with one-way analysis of variance, a p value of less than 0.05 was considered significant.

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