Evaluation of *in vivo* Biological Activity Profile of Isoorientin

Esra Küpeli, Mustafa Aslan, İlhan Gürbüz, and Erdem Yesilada*

Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, Etiler, 06330, Ankara, Turkey. Fax: +90-312-2235018. E-mail: yesilada@gazi.edu.tr

* Author for correspondence and reprint requests

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Anti-nociceptive, anti-inflammatory and gastroprotective activities of the known *C*-glycosyl flavonoid, isoorientin, were studied in rats and mice. For the anti-nociceptive activity assessment the *p*-benzoquinone-induced writing test, for the anti-inflammatory activity the carrageenan-induced hind paw edema model in mice, and for the gastroprotective activity the EtOH-induced ulcerogenesis model in rats were used. Isoorientin was shown to possess significant anti-nociceptive and anti-inflammatory activities at 15 mg/kg and 30 mg/kg doses, without inducing any apparent acute toxicity as well as gastric damage. However, the compound did not possess any significant gastroprotective activity against EtOH-induced ulcerogenesis.

Key words: Anti-inflammatory Activity, Anti-nociceptive Activity, Gastroprotective Effect, Isoorientin

Introduction

Isoorientin is a common *C*-glycosyl flavone, luteolin-6-C glucoside, and has been reported to be present in many different plant species such as *Gentiana*, *Rumex, Swertia* and *Vitex* species (Harborne, 1994).

During our studies on a Turkish folk medicine, *Gentiana olivieri* Griseb. (Gentianaceae), isoorientin was isolated as the active anti-hepatotoxic component from the flowering herbs through *in vivo* bioassay-guided fractionation procedures against carbon tetrachloride-induced hepatic damage (Deliorman-Orhan *et al.*, 2003). In a following bioassay-guided activity assessment study on the same plant material the same compound was again isolated as the active hypoglycaemic component (Sezik *et al.*, 2004). In a reference survey, several other biological activities were attributed to isoorientin including potent antioxidant (Ko *et al.*, 1998), antimicrobial (Afifi *et al.*, 1999), myolitic activity on smooth muscle-containing preparations from the rat and guinea pig uterus (Afifi *et al.*, 1999). Therefore, we decided to conduct further pharmacological studies in order to reveal the biological activity profile of isoorientin. The present study deals with the evaluation of anti-nociceptive, anti-inflammatory and anti-ulcerogenic activity of isoorientin using *in vivo* test models in mice and rats.

Materials and Methods

Chemical procedures

Isoorientin was isolated from the flowering herbs of *Gentiana olivieri* Griseb. (Gentianaceae) and extraction and isolation procedures as well as structure elucidation and spectral specifications were described in a previous report in detail (Deliorman-Orhan *et al.*, 2003).

Test animals

Male Swiss albino mice (20–25 g) were purchased from the animal breeding laboratories of Refik Saydam Central Institute of Health (Ankara, Turkey) and Spraque-Dawley rats of either sex (140–200 g) from the Animal Breeding Laboratories of Gülhane Military Academy of Medicine (Ankara, Turkey). The animals left at least 2 d for acclimatization to animal room conditions were maintained on standard pellet diet and water *ad libitum.* The food was withdrawn 24 h before the experiment, but free access to water was allowed. A minimum of six animals was used in each group. Throughout the experiments, animals were processed according to the suggested ethical guidelines for the care of laboratory animals as well as the rules of the Gazi University Animal Ethic Committee. To avoid coprophagy the rats were fasted in wire-bottomed cages for the EtOH-induced ulcerogenesis.

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Preparation of test samples for bioassay

Test samples were given per os to test animals after suspending in 0.5% carboxymethyl cellulose (CMC)/distilled water. The test samples were administered in 5 ml/kg volume. The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced by appropriate volumes of the dosing vehicle. Misoprostol (0.4 mg/kg), indomethacin (10 mg/kg) or acetylsalicylic acid (ASA) (200 mg/kg) in 0.5% CMC were used as reference drugs.

p-Benzoquinone-induced abdominal constriction test in mice (Okun *et al.*, 1963)

60 min after the oral administration of test samples, the mice were intraperitoneally injected with 0.1 ml/10 g body weight of 2.5% (v/v) *p*-benzoquinone (PBQ; Merck) solution in distilled H_2O. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting on the 5th min after the PBQ injection. The data represent the average of the total number of writhes observed. The anti-nociceptive activity was expressed as percentage change from writhing controls. 200 mg/kg ASA was used as reference drug.

Carrageenan-induced hind paw edema (Yesilada and Küpeli, 2002)

60 min after the oral administration of test sample or dosing vehicle each mouse was injected with a freshly prepared (0.5 mg/25 μl) suspension of carrageenan (Sigma, St. Louis, Missouri, USA) in physiological saline (154 nM NaCl) into subplantar tissue of the right hind paw. As the control, 25 μl saline solution was injected into that of the left hind paw. Paw edema was measured every 90 min during 6 h after induction of inflammation. The difference in footpad thickness was measured by a gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. Indomethacin (10 mg/kg) was used as a reference drug.

Effects on ethanol-induced ulcerogenesis (Robert *et al.*, 1979)

The test sample was administered orally 15 min before the oral application of 96% EtOH (1 ml) to a group of six rats. 60 min later, the animals were sacrificed with an over-dose of ether. The stomachs were removed, inflated with 10 ml of formalin solution and immersed in the same solution to fix the outer layer of the stomach. Each stomach was then opened along the greater curvature, rinsed with tap water to remove gastric contents and blood clots and examined under a dissecting microscope (20 × 6.3 x) to assess the formation of ulcers. The sum of length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the percentage of inhibition was calculated by the following formula:

$$\text{Inhibition (\%)} = [(\text{UI control} - \text{UI treated}) / \text{UI control}] \times 100\%.$$

Acute toxicity

Animals employed in the carrageenan-induced paw edema experiment were observed during 24 h for behavioral changes and the mortality was recorded, if happens, for each group at the end of the observation period.

Gastric-ulcerogenic effect

After the analgesic activity experiment mice were killed under deep ether anesthesia and stomachs were removed. Then the abdomen of each mouse was opened through the great curvature and examined under a dissecting microscope for lesions or bleedings.

Statistical analysis of data

Data obtained from animal experiments were expressed as mean standard error (± S. E. M.). Statistical differences between the treatments and the control were evaluated by ANOVA and Students-Newman-Keuls post-hoc tests. $p < 0.05$ was considered to be significant [* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$].

Results and Discussion

Carrageenan-induced inflammation is a common *in vivo* model for determination of active nonsteroidal anti-inflammatory agents (Ismail *et al.*, 1997). Paw edema induced by carrageenan

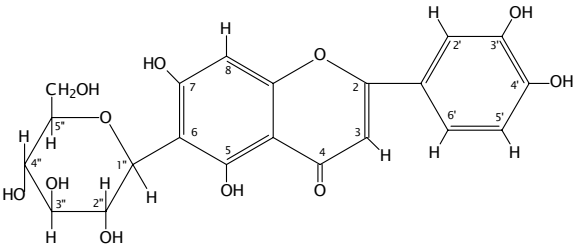

Fig. 1. Structure of isoorientin.

is a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The edema produced at the peak (3 h) is thought to be due to the release of kinin-like substances, especially bradykinin. The second phase of edema is due to the release of prostaglandins, protease and lysosome. The second phase is sensitive to most clinically effective anti-inflammatory drugs (Olajide *et al.*, 1999).

Isoorientin (Fig. 1) induced inhibition between 28–37% and 36–43% against carrageenan-induced inflammation at 15 and 30 mg/kg doses, respectively (Table I), and was found to act in both phases of acute inflammation. The most remarkable point was the anti-inflammatory activity of isoorientin. It was found equal to that of indomethacin (10 mg/kg), exerted 30–40% activity, almost in very close dose levels. Maybe the more important point was isoorientin did not induce any visible gastric damage (Table IIa). Due to the high gastric lesion risk of nonsteroidal anti-inflammatory agents, *i.e.* indomethacin and acetylsalicylic acid, this is a very important peculiarity. On the other hand, isoorientin was also found to possess significant anti-nociceptive activity, although not potent as acetylsalicylic acid (Table IIa).

Since isoorientin did not induce any gastric lesion during anti-inflammatory and anti-nociceptive tests in contrast to nonsteroidal anti-inflammatory agents, we also investigated the gastroprotective effect of the compound against EtOH-induced ulcerogenesis in rats. As shown in Table IIb, although a 26% protection was observed for isoorientin; this value was not statistically significant.

During our study on a Turkish folk medicine, *Gentiana olivieri* Griseb., isoorientin was isolated from the flowering herbs through bioassay-guided procedures (Deliorman-Orhan *et al.*, 2003). Through subacute administration a potent hepatoprotective effect was determined against carbon tetrachloride-induced hepatic damage as evidenced by biochemical [plasma and hepatic tissue malondialdehyde formation, liver tissue glutathion level as well as plasma transaminase enzyme levels (aspartate transferase and alanine transferase)] and histopathological techniques. The same compound was also isolated as the potent hypoglycaemic component in streptozotocin-induced diabetic rats as well as antihyperlipidemic from the same plant (Sezik *et al.*, 2004).

In addition to these previously reported potent activities of isoorientin, results of the present study have shown that it possesses significant anti-nociceptive and anti-inflammatory activities as potent as that of known nonsteroidal anti-inflammatory agents without inducing any gastric lesion. Since many of the known anti-inflammatory and anti-nociceptive agents frequently employed in current therapy have been reported to possess

Table I. Effects of isoorientin isolated from *G. olivieri* against carrageenan-induced paw edema in mice.

Material	Dose [mg/kg]	Swelling thickness [x 10^{-2} mm] ± S. E. M. (% inhibition)			
		90 min	180 min	270 min	360 min
Control		50.0 ± 3.9	56.8 ± 3.9	64.2 ± 4.8	74.2 ± 4.4
Isoorientin	15	35.8 ± 4.7 (28.4)*	40.0 ± 4.5 (29.6)**	44.3 ± 4.6 (30.9)**	46.7 ± 4.3 (37.1)***
Isoorientin	30	31.8 ± 3.2 (36.4)*	36.0 ± 3.4 (36.6)**	38.0 ± 2.7 (40.8)***	42.0 ± 2.8 (43.4)***
Indomethacin	10	34.8 ± 2.9 (30.4)*	38.2 ± 2.5 (32.7)**	42.0 ± 3.0 (34.6)	44.3 ± 3.9 (40.3)***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ significant from control.
S. E. M., mean standard error.

Table II. Effect of isoorientin isolated from *G. olivieri* against *p*-benzoquinone-induced writhings in mice (a) and EtOH-induced ulcerogenesis in rats (b).

(a) *p-Benzoquinone-induced writhings in mice*

Test sample	Dose [mg/kg]	Number of writhings ± S. E. M.	Ratio of ulceration	Inhibitory ratio (%)
Control		45.2 ± 4.4	0/6	
Isoorientin	15	31.3 ± 2.0	0/6	30.8**
Isoorientin	30	27.3 ± 1.7	0/6	39.6***
Acetylsalicylic acid	200	18.3 ± 1.7	4/6	59.5***

(b) *EtOH-induced ulcerogenesis*

Test sample	Dose [mg/kg]	Ulcer index (mean ± S. E. M.)	Prevention from ulcer[a]	Inhibition (%)
Control		152.3 ± 10.7	0/6	
Isoorientin	15	112.8 ± 27.6	1/6	25.9
Misoprostol[b]	0.4	0.0 ± 0.0***	6/6	100.0

** $p < 0.01$; *** $p < 0.001$ significant from control.
S. E. M., mean standard error.
[a] Number of rats whose stomachs were completely prevented from bleeding.
[b] Supplied from G. D. Searle & Co. Ltd. (England).

harmful effects on gastric (Pisegna, 2003) and hepatic tissues (Bray *et al.*, 1992), the relevant activities of isoorientin might be of importance. Due to such a broad and potent activity profile of isoorientin further studies should be addressed to evaluate it as a safe medicine for human health.

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