Anti-Inflammatory and Antinociceptive Potential of Major Phenolics from *Verbascum salviifolium* Boiss.

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The potential effects of flavonoids, phenylethanoid and neolignan glycosides from the aerial parts of *Verbascum salviifolium* Boiss. were studied in the *p*-benzoquinone-induced writhing reflex, for the assessment of the antinociceptive activity, and in carrageenan- and PGE1-induced hind paw edema and 12-*O*-tetradecanoyl-13-acetate (TPA)-induced ear edema models in mice, for the assessment of the anti-inflammatory activity. Through bioassay-guided fractionation and isolation procedures ten compounds from the aqueous extract of the plant, luteolin 7-*O*-glucoside (**1**), luteolin 3′-*O*-glucoside (**2**), apigenin 7-*O*-glucoside (**3**), chrysoeriol 7-*O*-glucoside (**4**), *β*-hydroxyacteoside (**5**), martynoside (**6**), forsythoside B (**7**), angoroside A (**8**), dehydrodiconiferyl alcohol-9′-*O*-*β*-D-glucopyranoside (**9**) and dehydrodiconiferyl alcohol-9-*O*-*β*-D-glucopyranoside (**10**), were isolated and their structures were elucidated by spectral techniques**.** Results have shown that **1**, **2**, **3** and **5** significantly inhibited carrageenan-induced paw edema at a 200 mg/kg dose, while **1**, **2** and **5** also displayed anti-inflammatory activity against the PGE1-induced hind paw edema model. However, all the compounds showed no effect in the TPA-induced ear edema model. The compounds **1** and **2** also exhibited significant antinociceptive activity.

Key words: Verbascum salviifolium, Anti-Inflammatory Activity, Antinociceptive Activity

Introduction

Despite progress within medicinal research during the past decades, the treatment of many serious diseases remains problematic (Bohlin, 1995). Chronic inflammatory diseases remain one of the world's major health problems (Yesilada *et al.*, 1997). Currently, both steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of inflammatory disorders. Steroids have an obvious role in the treatment of inflammatory diseases, but, due to rate limiting toxicities, can only be prescribed over short periods except for very severe cases where the risks are acceptable. Prolonged use of NSAIDs is also associated with severe side effects, notably gastrointestinal haemorrhage (Miller, 1983; Robert *et al.*, 1979). The recently developed cyclooxygenase-2 (COX-2)-selective drugs introduced into therapy, however, do not seem to be free of risks (Wallace *et al.*, 1998). Consequently, there is a need to develop new anti-inflammatory agents with minimum side effects.

The genus *Verbascum* (Scrophulariaceae) is represented by 228 species in the flora of Turkey and 185 species are recorded as endemic. *Verbascum salviifolium* is a perennial and eglandular herb growing up to 30–60 cm on steppes and dry slopes at elevations of 300–1400 m, and it is one of the endemic species in Anatolia (Huber-Morath, 1978). The leaves, flowers and whole aerial parts of *Verbascum* L. species have been used in traditional Turkish medicine to treat respiratory problems in particular to ease cough in bronchitis as well as eczema and other types of inflammatory skin conditions (Baytop, 1999). They have also been widely utilized as a folk medicine to have a soothing and anti-inflammatory effect on the urinary tract (Turker and Camper, 2002).

Phytochemical studies on *Verbascum salviifolium* have revealed the presence of phenolic com-

pounds. They were reported to possess radical scavenging activity on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical in a TLC-autographic assay (Akdemir *et al*., 2003, 2004). These phenolic compounds are also of particular interest because of their important roles in cardiovascular disease, aging, cancer and inflammatory disorders.

In the current study, we aimed to evaluate the anti-inflammatory and antinociceptive activities of the isolated components obtained through bioassay-guided procedures from *Verbascum salviifolium* by using carrageenan- and PGE1-induced hind paw edema and 12-*O*-tetradecanoyl-13-acetate (TPA)-induced ear edema as well as *p*-benzoquinone-induced writhing models in mice.

Material and Methods

Plant material

Verbascum salviifolium Boiss. (Scrophulariaceae) was collected at Burdur, Yesilova, southwest of Burdur Lake, 880 m, in June 2002. A specimen of the original collection can be found in the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (I. I. Tatli !, HUEF 02003).

Bioassay-guided fractionation and isolation of the compounds

Chromatographic separation and isolation of the active constituents

The air-dried and powdered aerial parts of *Verbascum salviifolium* (339.08 g) were extracted twice with MeOH (2×2000 ml) at 40 °C. After evaporation of the combined extract *in vacuo*, 40.84 g of MeOH extract were obtained. The crude extract was dissolved in water and partitioned in $CHCl_3$. The lyophilized H_2O phase (29.49 g) was fractionated over a polyamide column (VLC, 250 g), eluting with H_2O (400 ml) and gradient MeOH/H_2O mixtures (25–100%) to afford 4 main fractions (Frs. A–D; Fr. A: 855.1 mg; Fr. B: 1.8 g; Fr. C: 484.1 mg; Fr. D: 91.8 mg) and 2 flavonoid glucosides [luteolin 7-*O*-glucoside (**1**): 59.5 mg; luteolin 3′-*O*-glucoside (**2**): 20.4 mg]. Fraction A (855.1 mg) was subjected to LiChroprep C_{18} VLC. Employment of H_2O/MeOH (0–75% MeOH) and MeOH afforded 5 fractions (Frs. A1–A5). Purification of Fr. A3 (244.8 mg) by C_{18} MPLC (20–70% MeOH) furnished 2 fractions (Frs. A3*a* and A3*b*). Fraction A3*b* (106.1 mg) was rechromatographed on a silica gel column ($CHCl_3$/MeOH, 90:10 to 80:20 v/v) to obtain angoroside A (**8**, 5.4 mg). Fraction B (1.8 g) was likewise subjected to C_{18} MPLC using stepwise gradients of MeOH (0–70%) in H_2O to yield forsythoside B (**7**, 53.3 mg) and an additional fraction B3. Fraction B3 (271.0 mg) was purified by silica gel CC using gradient $CHCl_3$/MeOH mixtures (90:10 to 85:15 v/v) to afford a mixture of dehydrodiconiferyl alcohol-9′-*O*-*β*-D-glucopyranoside (**9**) and dehydrodiconiferyl alcohol-9-*O*-*β*-D-glucopyranoside (**10**) (14 mg). Repeated chromatography of Fr. C (484.1 mg) using a similar method (C_{18} MPLC; 20–70% MeOH) gave *β*-hydroxyacteoside (**5**, 12.4 mg) and martynoside (**6**, 15.0 mg). Fraction D (91.8 mg) was rechromatographed on a polyamide column eluting with $CHCl_3$/MeOH/ethylmethylketone/acetone (3:2:0.5:0.5 v/v) to afford chrysoeriol 7-*O*-glucoside (**4**, 5.6 mg) and apigenin 7-*O*-glucoside (**3**, 4.3 mg).

Structure elucidation of the compounds **1**–**10**

Structure elucidation of the isolated compounds **1**–**10** was carried out by spectral techniques; UV, IR, 1D- and 2D-NMR and mass spectroscopy (HR-ESIMS) detailed data were recently submitted elsewhere (Akdemir *et al*., 2003, 2004). The structures of compounds **1**–**10** were as follows (Fig. 1): Luteolin 7-*O*-glucoside (**1**), luteolin 3′-*O*-glucoside (**2**), apigenin 7-*O*-glucoside (**3**), chrysoeriol 7-*O*-glucoside (**4**), *β*-hydroxyacteoside (**5**), martynoside (**6**), forsythoside B (**7**), angoroside A (**8**), dehydrodiconiferyl alcohol-9′-*O*-*β*-D-glucopyranoside (**9**) and dehydrodiconiferyl alcohol-9-*O*-*β*-D-glucopyranoside (**10**).

Chemicals

The following solvents and chemicals were purchased and used as received: Carrageenan (Sigma, St. Louis, Missouri, USA; Art. No. C-1013), PGE1 (Fluka Chemie AG; Art. No. 82475), 12-*O*-tetradecanoyl-13-acetate (TPA) (Sigma-Aldrich; Art. No. 014K0720), *p*-benzoquinone (PBQ) (Merck; Art. No. S 31445 028), indomethacin (Bayer AG), acetylsalicylic acid (ASA) (Bayer AG), sodium carboxymethylcellulose (Aldrich), polyamide (ICN), silica gel (Merck), LiChroprep C_{18} (Merck), methanol (Merck), chloroform (Merck).

Pharmacological procedures

Animals

Male Swiss albino mice (20–25 g) were purchased from the animal breeding laboratories of

Refik Saydam Central Institute of Health, Ankara, Turkey. The animals left for 2 d for acclimatization to animal room conditions were maintained on standard pellet diet and water *ad libitum*. The food was withdrawn on the day 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.

Preparation of test samples for bioassay

After suspending in a mixture of distilled H_2O and 0.5% sodium carboxymethyl cellulose (CMC), test samples were given orally to the test animals The control group animals received the same experimental handling as those of the test groups except the drug treatment was replaced with appropriate volumes of dosing vehicle. Indomethacin (10 mg/kg and 0.5 mg/ear) and acetylsalicylic acid (100 and 200 mg/kg) in 0.5% CMC were used as reference drugs.

Anti-inflammatory activity

Carrageenan-induced hind paw edema model

The carrageenan-induced hind paw edema model was used for the determination of the anti-inflammatory activity (Yesilada and Kupeli, 2002). 60 min after the oral administration of a test sample or dosing vehicle, each mouse was injected with a freshly prepared suspension of carrageenan (0.5 mg/25 μl) in physiological saline (154 nM NaCl) into the subplantar tissue of the right hind paw. As the control, 25 μl saline solution was injected into the left hind paw. Paw edema was then measured every 90 min during 6 h after induction of inflammation. The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with those of a control group and analyzed by using statistical methods. Indomethacin (10 mg/kg) was used as the reference drug.

PGE1-induced hind paw edema model

The PGE1-induced hind paw edema model used for the determination of the anti-inflammatory activity was evaluated as given by Kasahara *et al.* (1985). 60 min after the oral administration of a test sample or dosing vehicle, each mouse was injected with a freshly prepared suspension of PGE1 (1 μl) in Tyrode's solution into the subplantar tissue of the right hind paw. As the control, 5 μl Tyrode's solution were injected into the left hind paw. Paw edema was measured every 15 min during a period of 75 min after the induction of inflammation. The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge calipers (Ozaki Co.). Mean values of the treated groups were compared with those of the control group and analyzed by using statistical methods. Indomethacin (10 mg/kg) was used as reference drug.

TPA-induced mouse ear edema

Each mouse received 2.5 μg of TPA dissolved in 20 μl of 70% EtOH (De Young *et al.*, 1989). This was applied by an automatic pipette in 20 μl volumes to both anterior and posterior surfaces of the right ear. Simultaneously the left ear (control) received the same volume of solvent (70% EtOH). Indomethacin (0.5 mg/ear) was used as reference drug. For the evaluation of the activity, two different ways were followed:

1. The thickness of each ear was measured 4 h after induction of inflammation using a gauge calipers (Ozaki Co.). The edema was expressed as the difference between the thickness of the right and left ears due to TPA application, and consequently inhibition percentage was expressed as the reduction of thickness with respect to the control group.

2. 4 h after administration the animals were killed under deep ether anesthesia. Discs of 6 mm diameter were removed from each ear and weighed in balance. The swelling was estimated as the difference in weight between the punches from right and left ears and expressed as an increase in the ear thickness.

Antinociceptive activity

The *p*-benzoquinone-induced abdominal constriction test (Okun *et al.*, 1963) was performed on mice for the determination of the antinociceptive activity. According to the method evaluated, 60 min after the oral administration of a test sample, the mice were intraperitoneally injected with 0.01 ml/g body weight of 2.5% (w/v) *p*-benzoquinone solution in distilled water. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for the observation, and the total number of the abdominal contractions (writhing movements) was counted for the following 15 min, starting 5 min after the PBQ injection. The data represent the average of

the total number of writhes observed. Antinociceptive activity was then expressed as the percentage change from writhing controls. Acetylsalicylic acid at 100 and 200 mg/kg doses was used as the reference drug in this test.

Acute toxicity

Animals employed in the carrageenan-induced paw edema experiment were observed during 48 h, and morbidity or mortality was recorded, if happens, for each group at the end of the observation period.

Gastric-ulcerogenic effect

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

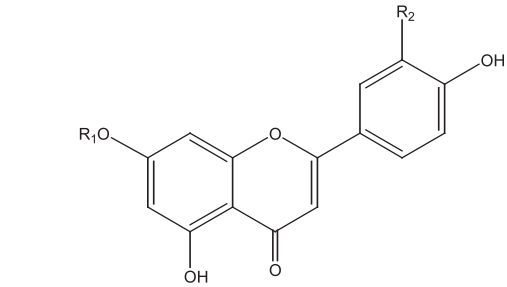
Statistical analysis of data

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

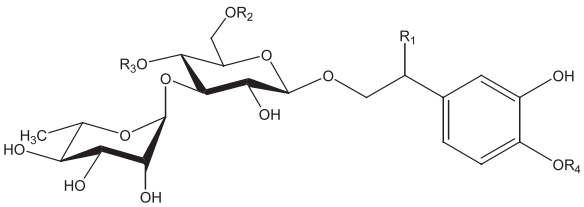
Result and Discussion

Major constituents from the aqueous extract of *Verbascum salviifolium* aerial parts were investigated for their *in vivo* antinociceptive and anti-inflammatory effects. The highest activities were observed at a dose of 200 mg/kg in both, therefore, in the following studies the compounds were administered at that dose.

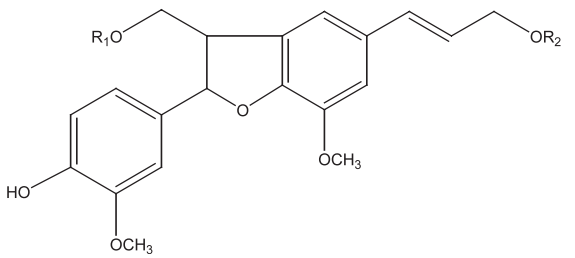
Carrageenan-induced hind paw edema in mice is a biphasic event. The early phase (90–180 min) of inflammation is due to the release of histamine, serotonin and similar substances. While the later phase (270–360 min) is associated with the activation of kinin-like substances, *i.e.* prostaglandins, proteases and lysosome (Olajide *et al.*, 1999). As shown in Table I, luteolin 7-*O*-glucoside (**1**), luteolin 3′-*O*-glucoside (**2**), apigenin 7-*O*-glucoside (**3**) and *β*-hydroxyacteoside (**5**) (Fig. 1) were found to be active particularly in the later phase (270–360 min) of this edema model.



	R^1	R^2
1	*β*-glucopyranose	OH
2	H	*O*-*β*-glucopyranose
3	*β*-glucopyranose	H
4	*β*-glucopyranose	OCH_3



	R^1	R^2	R^3	R^4
5	OH	H	caffeoyl	H
6	H	H	feruloyl	CH_3
7	H	apiose	caffeoyl	H
8	H	arabinose	caffeoyl	H



	R^1	R^2
9	H	*β*-glucopyranose
10	*β*-glucopyranose	H

Fig. 1. Isolated compounds from *Verbascum salviifolium* Boiss.: luteolin 7-*O*-glucoside (**1**), luteolin 3′-*O*-glucoside (**2**), apigenin 7-*O*-glucoside (**3**), chrysoeriol 7-*O*-glucoside (**4**), *β*-hydroxyacteoside (**5**), martynoside (**6**), forsythoside (**7**), angoroside A (**8**), dehydrodiconiferyl alcohol-9′-*O*-*β*-D-glucopyranoside (**9**) and dehydrodiconiferyl alcohol-9-*O*-*β*-D-glucopyranoside (**10**).

As a matter of fact, a similar activity pattern was observed for the PGE1-induced hind paw edema model. As shown in Table II, **1**, **2** as well as **5** exhibited significant inhibition, ranging between 5.9–26.8%, 2.5–22.5% and 2.5–25.8%, respectively. These results have indicated that the anti-

Table I. Effect of isolated compounds on carrageenan-induced hind paw edema in mice.

Test sample	Dose [mg/kg]	Swelling thickness [$\cdot 10^{-2}$ mm] ± SEM (inhibition, %)			
		90 min	180 min	270 min	360 min
Control		35.9 ± 4.7	43.2 ± 4.1	49.5 ± 3.7	54.2 ± 3.9
1	200	33.5 ± 3.4 (6.7)	38.9 ± 3.1 (9.9)	37.9 ± 3.2 (23.4)*	40.1 ± 3.0 (26.0)**
2	200	31.4 ± 3.0 (12.5)	40.4 ± 3.8 (6.5)	42.1 ± 3.3 (14.9)	41.4 ± 2.8 (23.6)*
3	200	34.7 ± 3.1 (3.3)	40.1 ± 3.4 (7.2)	39.5 ± 3.2 (20.2)	42.1 ± 2.9 (22.3)*
4	200	36.3 ± 2.8	44.2 ± 3.4	47.1 ± 3.1 (4.8)	44.5 ± 3.5 (17.9)
5	200	33.1 ± 2.9 (7.8)	38.9 ± 3.4 (9.9)	38.3 ± 3.1 (22.6)*	40.9 ± 2.7 (24.5)*
6	200	37.4 ± 4.0	46.9 ± 5.4	51.3 ± 3.9	60.1 ± 6.2
7	200	36.3 ± 5.2	44.6 ± 4.8	48.9 ± 4.1 (1.2)	52.5 ± 3.3 (3.1)
8	200	39.4 ± 3.6	45.3 ± 3.2	52.1 ± 3.4	56.8 ± 3.1
9, **10**	200	37.1 ± 4.2	44.3 ± 3.8	51.5 ± 3.1	56.2 ± 3.4
Indomethacin	10	25.6 ± 3.8 (28.7)*	27.1 ± 3.2 (37.3)**	28.6 ± 4.1 (42.2)**	35.4 ± 3.1 (34.7)**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. SEM, standard error mean.

Table II. Effect of isolated compounds against PGE1-induced paw edema in mice.

Test sample	Dose [mg/kg]	Swelling thickness [$\cdot 10^{-2}$ mm] ± SEM (inhibition, %)					
		0 min	15 min	30 min	45 min	60 min	75 min
Control		1.3 ± 0.9	16.2 ± 1.1	23.5 ± 1.9	30.2 ± 1.7	25.8 ± 1.6	13.6 ± 1.5
1	200	1.2 ± 0.7 (7.7)	15.2 ± 1.3 (6.2)	19.0 ± 1.5 (19.1)	22.1 ± 1.2 (26.8)**	20.5 ± 1.4 (20.5)*	12.8 ± 1.5 (5.9)
2	200	1.3 ± 1.0	15.8 ± 1.7 (2.5)	19.4 ± 1.4 (17.4)	23.4 ± 1.2 (22.5)*	21.7 ± 1.1 (15.9)	13.1 ± 1.4 (3.7)
3	200	1.2 ± 0.8 (7.7)	16.9 ± 1.4	21.2 ± 1.1 (9.8)	26.1 ± 1.3 (13.6)	21.3 ± 1.1 (17.4)	13.1 ± 1.0 (3.7)
4	200	1.3 ± 1.0	16.7 ± 1.5	24.2 ± 1.4	28.5 ± 1.1 (5.6)	23.7 ± 1.3 (8.1)	13.9 ± 1.1
5	200	1.4 ± 0.7	15.8 ± 1.1 (2.5)	19.7 ± 1.3 (16.2)	22.4 ± 1.0 (25.8)*	19.5 ± 1.3 (24.4)*	12.1 ± 1.1 (11.0)
6	200	1.5 ± 1.1	18.6 ± 1.3	25.2 ± 1.7	33.4 ± 1.4	27.9 ± 1.5	15.8 ± 1.3
7	200	1.2 ± 0.7 (7.7)	16.9 ± 1.2	24.1 ± 1.1	28.2 ± 1.4 (6.6)	24.2 ± 1.2 (6.2)	13.9 ± 1.3
8	200	1.4 ± 0.8	17.1 ± 1.3	26.9 ± 1.7	32.2 ± 1.4	26.5 ± 1.1	14.9 ± 1.3
9, **10**	200	1.3 ± 0.6	18.4 ± 1.4	24.3 ± 1.5	30.7 ± 1.2	27.9 ± 1.4	13.7 ± 1.1
Indomethacin	10	1.3 ± 0.6	11.5 ± 1.3 (29.0)**	17.2 ± 1.4 (27.4)**	19.3 ± 1.1 (36.1)***	18.5 ± 1.2 (28.3)**	12.4 ± 1.3 (8.8)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. SEM, standard error mean.

inflammatory activity of these compounds might possibly be through the inhibition of inflammatory prostanoids.

Table III shows the results of activity induced by topical administration of the isolated compounds on ear edema provoked by local application of TPA. The majority of the activities of this phorbol ester appears to involve or depend on arachidonic acid release and metabolism, which may occur simultaneously with the interaction of TPA with a receptor site on protein kinase C (Rao *et al.*, 1993). However, none of the compounds showed a noteworthy activity against the TPA-induced ear edema model (Table III).

As shown in Table IV, luteolin 7-*O*-glucoside (**1**) and luteolin 3′-*O*-glucoside (**2**) were found to exhibit significant inhibitory activity on wriths due to antinociceptive activity.

Test sample	Dose [mg/ear]	Swelling thickness [μm] ± SEM	Inhibition (%)	Weight edema [mg] ± SEM	Inhibition (%)
Control		241.3 ± 32.9		31.5 ± 5.9	
1	0.5	201.1 ± 27.6	16.7	24.5 ± 7.9	22.2
2	0.5	209.3 ± 38.1	13.3	26.7 ± 5.4	15.2
3	0.5	235.1 ± 28.6	2.6	27.3 ± 6.2	13.3
4	0.5	244.9 ± 34.1	–	36.1 ± 4.7	–
5	0.5	254.3 ± 29.9	–	34.5 ± 6.5	–
6	0.5	260.3 ± 34.7	–	34.8 ± 5.2	–
7	0.5	226.9 ± 27.6	5.9	29.1 ± 5.1	7.6
8	0.5	254.2 ± 27.5	–	35.1 ± 7.4	–
9, **10**	0.5	262.4 ± 21.3	–	34.5 ± 6.2	–
Indomethacin	0.5	78.5 ± 23.8	67.5***	15.1 ± 3.2	52.1***

Table III. Effect of isolated compounds against TPA-induced ear edema in mice measured as swelling thickness and weight of edema.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. SEM, standard error mean.

Test sample	Dose [mg/kg]	Number of writhings ± SEM	Inhibitory ratio (%)	Ratio of ulceration[a]
Control		39.8 ± 5.7		0/6
1	200	30.1 ± 3.2	24.3*	0/6
2	200	29.7 ± 2.9	25.4*	0/6
3	200	37.8 ± 3.2	5.0	0/6
4	200	39.9 ± 6.1	–	0/6
5	200	31.2 ± 3.1	21.6	0/6
6	200	43.2 ± 5.4	–	0/6
7	200	37.2 ± 5.4	6.5	0/6
8	200	45.8 ± 5.1	–	0/6
9, **10**	200	41.8 ± 4.8	–	0/6
ASA	100	22.3 ± 1.8	43.9***	3/6
	200	19.7 ± 1.9	50.5***	5/6

Table IV. Effect of isolated compounds against *p*-benzoquinone-induced writhings in mice.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. SEM, standard error mean.

[a] Number of stomachs in experimental animals with induced gastric lesions.

In a previous study, Lu *et al.* (2002) reported that apigenin 7-*O*-glucoside (**3**) and luteolin 7-*O*-glucoside (**1**) significantly suppressed the fMLP-induced superoxide generation in a concentration-dependent manner when preincubated with neutrophils. **1** and **3** also suppressed the arachidonic acid-induced and PMA-induced superoxide generation in a concentration-dependent manner, and higher activity was observed for **3** than for **1** (Lu *et al.*, 2002). On the other hand, the inhibitory effect of **3** on the skin inflammation induced by different generators of reactive oxygen species and free radicals, *i.e.* glucose-oxidase (hydrogen peroxide), xanthine-oxidase/hypoxanthine (superoxide anion radical), and cumene hydroperoxide (peroxyl radical), was studied. The results indicated that its antioxidant properties contribute to the anti-inflammatory effect in this model system (Fuchs and Milbradt, 1993). Odontuya *et al.* (2005) reported that luteolin derivatives exhibit a high inhibitory activity against both thromboxane and leukotriene synthesis, important mediators of the inflammatory pathway. Results of the present study also supported the suggested structural requirements for the anti-inflammatory and antinociceptive activities of flavon glycosides, *i.e.* luteolin 7-*O*-glucoside (**1**), luteolin 3′-*O*-glucoside (**2**) and apigenin 7-*O*-glucoside (**3**), by Odontuya *et al.* (2005). The presence of *ortho*-dihydroxy groups at the B-ring could significantly contribute to the anti-inflammatory and antinociceptive activities of flavonoids. While introduction of a methoxy function at C-3′, as it occurs in chrysoeriol 7-*O*-glucoside (**4**), decreases the topical activity (Odontuya *et al.*, 2005). In the present study, our results supported the previous findings, additionally, in a reference survey, no reports relating to the antinociceptive activities of luteolin 7-*O*-glucoside have been found so far. This study is the first to demonstrate that luteolin 7-*O*-glucoside possesses a significant antinociceptive activity. Furthermore, to the best of our knowledge, this is the first report

on luteolin 3′-*O*-glucoside exhibiting anti-inflammatory and antinociceptive activities.

Schapoval *et al.* (1998) reported that verbascoside showed inhibitory effects on histamine- and bradykinin-induced contractions of guinea-pig ileum as well as exhibited antinociceptive activity measured by the hot-plate test. To the best of our knowledge, this is the first report on the structurally related phenylethanoid β-hydroxyacteoside exhibiting anti-inflammatory activity. This activity might be attributable to the existence of a hydroxy function at β position of the aglycone (Jimenez and Riguera, 1994). Further studies are required in order to reveal the possible pharmacokinetic factors which contribute to the activity of that compound.

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Akdemir Z. S., Tatli I. I., Bedir E., and Khan I. A. (2003), Antioxidant flavonoids from *Verbascum salviifolium* Boiss. J. Pharm. Sci. Ankara (FABAD) **28**, 71–75.

Akdemir Z. S., Tatli I. I., Bedir E., and Khan I. A. (2004), Neolignan and phenylethanoid glycosides from *Verbascum salviifolium* Boiss. Turk. J. Chem. **28**, 621–628.

Baytop T. (1999), Therapy with Medicinal Plants in Turkey, Past and Present. Nobel Publishing House, Istanbul, Turkey, pp. 334–335.

Bohlin L. (1995), Structure-activity studies of natural products with anti-inflammatory effects. In: Phytochemistry of Plants Used in Traditional Medicine (Hostettman K., ed.). Clarendon Press, Oxford, pp. 137–161.

De Young L. M., Kheifets J. B., Ballaron S. J., and Young J. M. (1989), Edema and cell infiltration in the phorbol ester treated mouse ear are temporally separate and can be differently modulated by pharmacologic agents. Agents Action **26**, 335–341.

Fuchs J. and Milbradt R. (1993), Skin antiinflammatory activity of apigenin-7-glucoside in rats. Arzneim. Forsch./Drug Res. **43**, 370–372.

Huber-Morath A. (1978), '*Verbascum*'. In: Flora of Turkey and the East Aegean Islands, Vol. 6 (Davis P. H., ed.). University Press, Edinburgh, pp. 461–603.

Jimenez C. and Riguera R. (1994), Phenylethanoid glycosides in plants: structure and biological activity. Nat. Prod. Rep. **11**, 591–606.

Kasahara Y., Hikino H., Tsurufiji S., Watanabe M., and Ohuchi K. (1985), Studies on the constitutents of *Ephedra*. 18. Antiinflammatory actions of ephedrines in acute inflammations. Planta Med. **51**, 325–331.

Lu J., Feng X., Sun Q., Lu H., Manabe M., Sugahara K., Ma D., Sagara Y., and Kodama H. (2002), Effect of six flavonoid compounds from *Ixeris sonchifolia* on stimulus-induced superoxide generation and tyrosyl phosphorylation in human neutrophils. Clin. Chim. Acta **316**, 95–99.

Miller T. A. (1983), Protective effects of prostaglandins against gastric mucosal damage: current knowledge and proposed mechanisms. Am. J. Physiol. **245**, G601–G623.

Odontuya G., Hoult J. R. S., and Houghton P. J. (2005), Structure-activity relationship for antiinflammatory effect of luteolin and its derived glycosides. Phytother. Res. **19**, 782–786.

Okun R., Liddon S. C., and Lasagnal L. (1963), The effect of aggregation, electric shock and adrenergic bloking drugs on inhibition of the "writhing syndrome". J. Pharm. Exp. Therap. **139**, 107–109.

Olajide O. A., Makinde M. J., and Awe S. O. (1999), Effects of the aqueous extract of *Bridelia ferruginea* stem bark on carrageenan-induced oedema and granuloma tissue formation in rats and mice. J. Ethnopharmacol. **66**, 113–117.

Rao T. S., Currie J. L., Shaffer A. F., and Isakson P. C. (1993), Comparative evaluation of arachidonic acid (AA)- and tetradecanoylphorbol acetate (TPA)-induced dermal inflammation. Inflammation **17**, 723–741.

Robert A., Hanchar A. J., Lancaster C., and Nezamis J. E. (1979), Prostacyclin inhibits enteropooling and diarrhea. In: Prostacyclin (Vane J. R. and Bergström S., eds.). Raven Press, New York, pp. 147–158.

Schapoval E. E. S., Winter de Vargas M. R., Chaves C. G., Bridi R., Zuanazzi J. A., and Henriques A. T. (1998), Antiinflammatory and antinociceptive activities of extracts and isolated compounds from *Stachytarpheta cayennensis*. J. Ethnopharmacol. **60**, 53–59.

Turker A. and Camper N. D. (2002), Biological activity of common mullein, a medicinal plant. J. Ethnopharmacol. **82**, 117–125.

Wallace J. L., Bak A., McKnigt W., Asfaha S., Sharkey K. A., and Mac-Naughton W. K. (1998), Cyclooxygenase-1 contributes to inflammatory responses in rats and mice: implications for gastrointestinal toxicity. Gastroenterology **115**, 101–109.

Yesilada E. and Kupeli E. (2002), *Berberis crataegina* DC. root exhibits potent anti-inflammatory, analgesic and febrifuge effects in mice and rats. J. Ethnopharmacol. **79**, 237–248.

Yesilada E., Ustun O., Sezik E., Takaishi Y., Ono Y., and Honda G. (1997), Inhibitory effects of Turkish folk remedies on inflammtory cytokines: interleukin-1α, interleukin-1β and tumor necrosis factor-α. J. Ethnopharmacol. **58**, 59–73.