Anti-ulcerogenic Lignans from *Taxus baccata* L.

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Four lignan type compounds, lariciresinol, taxiresinol, isolariciresinol and 3-demethyl-isolariciresinol, were isolated from the heartwood of *Taxus baccata* L. (Taxaceae) growing in Turkey through chromatographic techniques. *In vivo* anti-ulcerogenic potency of these compounds was investigated on ethanol-induced ulcerogenesis model in rats at two different doses, 50 and 100 mg/kg. All compounds were shown to possess significant anti-ulcerogenic activity at both doses. However, the effect of taxiresinol was the most prominent.

Key words: Taxus baccata, Lignans, Anti-ulcerogenic Activity

Introduction

Discovery and isolation of paclitaxel from the bark of the Pacific yew, *Taxus brevifolia*, and introduction in cancer chemotherapy has attracted scientists to investigate the constituents of other *Taxus* species worldwide. *Taxus baccata* L. (Taxaceae), English yew, is an evergreen and widespread shrub commonly used for ornamental landscaping. However, with the exception of the arillus part which is enveloping the seeds, all plant parts contain toxic taxine alkaloids (Wilson *et al.*, 2001) and have been implicated in many human and animal poisonings. Thus, due to the poisonous properties the plant has been rarely documented as folk medicine.

In historical documents from the Roman period, the plant was recommended to be used as an antimalarial and antirheumatic (Bryan-Brown, 1932; Appendino, 1993). In Ayurvedic medicine it was known indigenously as Talispatra, and is reported to be used as an emmenagoque, sedative, antispasmodic and aphrodisiac (Bryan-Brown, 1932; Shanker *et al.*, 2002) as well as against asthma (Singh, 1995). It was also listed in Avicenna's cardiac drugs, namely Zarnab (Tekol, 1989). Moreover, it is reported to be used as a sedative and stomachic (Baytop, 1999).

So far, the isolation of a large number of taxoids as well as lignans, flavonoids, steroids and sugar derivatives has been reported from different parts of various *Taxus* species (Baloglu and Kingston, 1999; Parmar *et al.*, 1999). In the course of our studies on bioactive constituents, the ethanolic extract of the heartwood of *T. baccata* afforded taxoids and lignans (Erdemoglu, 1999; Erdemoglu and Sener, 2000; Erdemoglu *et al.*, 2001, 2003).

In order to evaluate the afore-mentioned antirheumatic activity of the plant, *in vivo* anti-inflammatory and antinociceptive activity of the isolated compounds [four taxoids (taxusin, baccatin VI, baccatin III and 1β-hydroxybaccatin I) and five lignans (lariciresinol (**1**), taxiresinol (**2**), 3′-demethyl-isolariciresinol-9′-hydroxyisopropyl ether, isolariciresinol (**3**) and 3-demethyl-isolariciresinol (**4**) (Fig. 1)] were investigated and results were reported in a previous study (Kupeli *et al.*, 2003). All the compounds, both taxoids and lignans, were shown to possess significant antinociceptive activity against *p*-benzoquinone-induced abdominal stretching, while only lignan derivatives significantly inhibited carrageenan-induced hind paw edema in mice (Kupeli *et al.*, 2003).

Especially the anti-inflammatory and antinociceptive activities of the lignans which were investigated in that previous study, are worth further attention since they do not induce any gastric damage in mice compared to taxoids. Due to the gastric damage induced by current non-steroidal anti-inflammatory drugs (NSAIDs), agents with potent anti-inflammatory and antinociceptive activity without inducing gastric lesions would highly be appreciated (James and Hawkey, 2003). On the other hand, as reported above, *Taxus* species were also documented to be effective in gastric complaints and used as a stomachic in traditional medicine (Baytop, 1999). Consequently, this study is designed to investigate *in vivo* anti-ulcerogenic activity of lignans isolated from the chloroform-solu-

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ble portion of the ethanolic extract of the heartwood of *T. baccata.*

Materials and Methods

Plant material

Taxus baccata L. (Taxaceae) was collected from the vicinity of Camlihemsin, Rize, in June 1995. A voucher specimen (GUE 1560) is kept in the Herbarium of Faculty of Pharmacy, Gazi University.

Chemical procedures

General

Column chromatography (CC) was performed on silica gel (Kieselgel 60, 0.063–0.200 mm, Art. 7734, Merck) and Kieselgel 60 F_{254} (0.5 mm thickness, Art. 5554, Merck) was used for preparative TLC. Precoated TLC plates (Kieselgel 60 F_{254}) were employed for chromatographic analysis.

Extraction, isolation and purification of lignans

The dried and powdered heartwood (3078 g) was extracted with 95% EtOH at room temperature. The extract was concentrated and the concentrate (308.91 g) thus obtained was suspended in H_2O and extracted with $CHCl_3$. Evaporation of the $CHCl_3$ phase left a residue (63.54 g). A portion (49 g) of the $CHCl_3$ extract was chromatographed on silica gel eluting sequentially with increasing polarities of different solvents (hexane→acetone→$CHCl_3$→CH_3OH) to give seven main fractions (frs. I–VII). Each fraction was further purified by CC, preparative TLC or recrystallisation. Detailed isolation procedures of compounds **1**–**4** were described in our previous studies (Erdemoglu, 1999; Erdemoglu *et al.*, 2003).

Animals and test samples

Spraque-Dawley rats of either sex (125–220 g) purchased from the Animal Breeding Laboratories of Gülhane Military Academy of Medicine (Ankara) were used in biological tests. The animals were left 48 h for acclimatization at animal room conditions and were maintained at standard pellet diet and tap water *ad libitum.* The food was withdrawn 24 h before the experiment, but free access to water was allowed. To avoid coprophagy the rats were fasted in wire-bottomed cages. For each group 6 rats were used. The test samples were administered in 50 and 100 mg/kg of body weight doses to animals in 7.5 ml/kg volume as a suspension in 0.5% CMC/distilled water. The control group was given vehicle and received the same experimental handling as the test group. Misoprostol was used as reference drug in a 400 μg/kg dose.

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

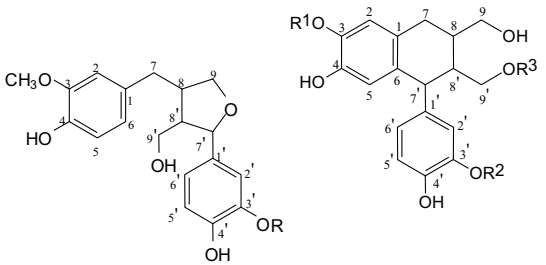
A test sample was administered orally to a group of six rats 15 min before the oral application of 96% EtOH (1 ml). 60 min later, the animals were sacrificed with an over-dose of ether. The stomachs were removed and inflated with 10 ml of formalin solution and immersed in the same solution to fix the outer layer of stomach. Each stomach was then opened along the greater curvature, rinsed with tap water to remove gastric contents and blood clots and examined under 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: [(UI control–UI treated)/UI control)] × 100.

Statistical analysis of data

Results were expressed as mean ± S. E. M. The statistical difference between the mean ulcer index of the treated group and that of the control was calculated by using ANOVA and Student Newman-Keuls multiple comparison tests.

Results and Discussion

As a common *in vivo* ulcerogenesis model with shorter processing time the EtOH-induced ulcerogenesis model in rats was used for the activity assessment. Dose-dependent anti-ulcerogenic effects of four lignans from the heartwood of *T. baccata* [lariciresinol (**1**), taxiresinol (3′-demethyl-lariciresinol) (**2**), isolariciresinol (**3**) and 3-demethyl-isolariciresinol (**4**); Fig. 1] on the EtOH-induced gastric lesions in rats are given in Tables I and II. All compounds showed statistically significant anti-ulcerogenic activity against ethanol-induced ulcer model, but taxiresinol was found to be more prominent (ulcer inhibition: 82.2% by 50 mg/kg and 85.3% by a 100 mg/kg dose). At a 100 mg/kg dose, one stomach out of 6 examined was completely protected from any visible gastric damage. Gastric protection provided by the administration of iso-



	R
1	CH_3
2	H

	R^1	R^2	R^3
3	CH_3	CH_3	H
4	H	CH_3	H

Fig. 1. Structures of investigated compounds; (–)-lariciresinol (**1**), (–)-taxiresinol (**2**), (–)-isolariciresinol (**3**), (–)-demethyl-isolariciresinol (**4**).

lariciresinol and lariciresinol was also found to be dose-dependent; at a 50 mg/kg dose 58.2% and 48.3%, while at a 100 mg/kg dose 80.3% and 76.6% ulcer inhibition was observed. On the other hand, the anti-ulcerogenic activity of 3-demethyl-isolariciresinol did not alter when administered to rats in both doses and provided between 37.0–37.7% inhibition. Meanwhile, the reference drug misoprostol (Fig. 2), a prostaglandin E_1 analogue, provided a full protection against any of the visible damage in the stomachs of all rats.

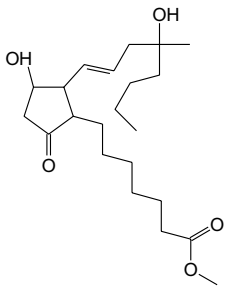


Fig. 2. Misoprostol, reference compound.

Although, lignans are known to possess a wide range of biological activities, including anti-cancer, antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory effects (MacRae and Towers, 1984; Arroo *et al.*, 2002), the anti-ulcer potency of lignans has not been much evaluated so far. A

Table I. Effects of the compounds against gastric lesions induced by EtOH in rats (50 mg/kg).

Material	Dose [mg/kg]	Ulcer Index (mean ± S. E. M.)	Prevention from ulcer[a]	Inhibition (%)
Control	–	182.8 ± 19.2	–	–
1	50	94.5 ± 30.8*	0/6	48.3
2	50	32.5 ± 12.0***	0/6	82.2
3	50	76.4 ± 32.4**	0/6	58.2
4	50	113.8 ± 12.8*	0/6	37.7
Misoprostol	0.4	0.0 ± 0.0***	6/6	100.0

* $p < 0.05$ significant from control; S. E. M., mean standard error.
** $p < 0.01$ significant from control; S. E. M., mean standard error.
*** $p < 0.001$ significant from control; S. E. M., mean standard error.
[a] Number of rats whose stomach were completely prevented from bleeding.

Table II. Effects of the compounds against gastric lesions induced by EtOH in rats (100 mg/kg).

Material	Dose [mg/kg]	Ulcer Index (mean ± S. E. M.)	Prevention from ulcer[a]	Inhibition (%)
Control	–	158.8 ± 13.5	–	–
1	100	37.1 ± 8.7***	0/6	76.6
2	100	23.4 ± 5.0***	1/6	85.3
3	100	31.3 ± 11.0***	1/6	80.3
4	100	100.0 ± 29.6*	0/6	37.0
Misoprostol	0.4	0.0 ± 0.0***	6/6	100.0

* $p < 0.05$ significant from control; S. E. M., mean standard error.
*** $p < 0.001$ significant from control; S. E. M., mean standard error.
[a] Number of rats whose stomach were completely prevented from bleeding.

dibenzocyclooctadiene type lignan, isoschizandrin, isolated from the fruits of *Schizandra chinensis* was reported to possess inhibitory effects on stress-induced gastric lesions at 100 mg/kg (Ikeya *et al.*, 1988) and a lignan constituent from *Mallotus anomalus* was also found to inhibit gastric acid secretion in mice (Akira *et al.*, 1991).

Taxiresinol (**2**) was shown to possess highest protection against gastric lesions in the present study. It was also reported as potent anti-inflammatory and antinociceptive principle of the plant. This compound showed 37.8% inhibition against *p*-benzoquinone-induced abdominal pain and 26.6% inhibition against carrageenan-induced paw edema in a 100 mg/kg dose (Kupeli *et al.*, 2003). Other two active lignans, lariciresinol (**1**) and isolarisiresinol (**3**), were also reported to possess significant anti-inflammatory (26.8% and 24.1% inhibition, respectively) and antinociceptive (42.7% and 31.3% inhibition, respectively) activity at the same dose levels.

Results of the present study have clearly demonstrated that lignan derivatives from *T. baccata* possess statistically significant anti-ulcerogenic activity which support the traditional utilization documented by Baytop (1999). Another important point is that potent anti-inflammatory, antinociceptive and anti-ulcerogenic activity of the lignans from *Taxus baccata* should be further evaluated to develop safe agents to introduce in modern therapy. Moreover further studies should be conducted to disclose the mode of activity of these effective lignans which might be helpful in understanding the possible roles of lignans in human physiology.

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