Antispasmodic and Spasmogenic Effects of *Scolymus hispanicus* and Taraxasteryl Acetate on Isolated Ileum Preparations

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Abstract: **Taraxasteryl acetate (1) was identified as the major triterpenoid of the ethanolic extract of the root bark of *Scolymus hispanicus* L. Antispasmodic and spasmogenic effects of the ethanolic extract, its fractions, taraxasteryl acetate, and a fluid extract of this plant were tested on isolated rat ileum preparations. The strong biphasic responses observed suggested a possible lithuretic activity of the compounds and the fractions tested.**

A fluid extract of the root bark of *Scolymus hispanicus* L. (Compositae) has been marketed in Turkey as a registered herbal pharmaceutical for its lithuretic effect under the name Lityazol Cemil® (read djemil). This medicine has been prescribed in Turkey to pass calculi for the last 60 years. The plant is distributed in almost all parts of Turkey except for the east and the southeast (1). It is locally known as Şevketi Bostan, Altın Dikeni, Akkız, or Sarıcakız and its fresh rootbark is used as vegetable in Aegean provinces of Turkey. The plant used in the preparation was initially misidentified as *Carduus marianus* L. (Compositae). Therefore, this name has appeared on the label of the medicine. Only recently has the correct name of the plant been established (2).

There are only a few publications on the chemistry and pharmacology of *Scolymus hispanicus*. From the aerial parts, flavonoids such as 6,8-di-*C*-glucosylapigenin, biorobin, trifolin, and saxifragin were isolated (3). The occurrence of rosmarinic acid, orientin, quercetin 5-glucoside, and isorhamnetin 3-galactoside has been reported from the petals (4). *n*-Nonacosane, α-amyrin, α-amyrin acetate, α-amyrin tetratriacontanoate, oleanolic acid, β-sitosterol, stigmasterol, fructose, galactose, and mannitol were isolated and characterised from the root bark (5, 6), and the absence of flavonoids and flavonolignans from the fruits was reported (7).

In a previous pharmacological study, no contracting or relaxing activity of the water and ethanolic extracts of the root bark of *Scolymus hispanicus* was observed on isolated rat stomach fundus, duodenum, and ileum muscles (8). In the only clinical study the registered fluid extract of the root bark of *Scolymus hispanicus* was found effective for passing calculi in 80% of the 162 patients (9).

Here, we report our findings on the isolation and identification of active constituents from the root bark of *Scolymus hispanicus*, and their antispasmodic and spasmogenic activities.

Materials and Methods

Scolymus hispanicus was collected from Balıkesir: Susurluk-Manyas regions on September 20, 1991. Voucher specimens are kept at the Herbarium of the Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey (ESSE 7050 and 9566).

Dried root bark of this plant was ground and refluxed with ethanol (70%) for 4 h. The extract was evaporated to dryness in vacuo, to yield 28.8% dry extract.

Lityazol Cemil® was manufactured in August 1991 by Cemil Şener Laboratory in Manisa, Turkey. The production procedure is as follows: Ground root bark of *Scolymus hispanicus* is wetted with ethyl alcohol (70%) for one day and then percolated in a conical percolator. The first 20 l of the percolate are kept aside and the percolation is continued until 80 l of percolate are collected. The 2nd percolate is evaporated until it acquires the consistency of honey. It is then mixed with the 1st percolate, flavoured with vanillin, and bottled. 300 kg plant material yields 300 kg of the fluid extract in 42 days. Some physical features of the final product are as follows: 3.25–4.25 cp, density 0.96–0.99, and pH 5.5–6.0.

For centrifugal thin layer chromatography, a Chromatotron Model 7924T (Harrison Research) was used. TLC densitometric studies were carried out using a high speed TLC scanner CS-920 (Shimadzu). ^{13}C-NMR spectra were obtained on a JEOL JNM-EX90A FT-NMR system. ^{1}H-NMR spectra were obtained on a Bruker DBX 400 NMR System. Both spectra were recorded in $CDCl_3$ solutions. Mass spectra were recorded on a VG PLATFORM mass spectrometer. Melting points were recorded using a Gallenkamp Melting Point Apparatus and are uncorrected. Optical rotations were recorded using an automatic polarimeter AA5 (Optical Activity).

For the separation of constituents the following route was followed: Ethanolic extract was dissolved in water and shaken with *n*-BuOH (1 : 1). The BuOH-fraction (200 mg) was separated on a Chromatotron using 1 mm-thick silica gel PF with petroleum ether at 2 ml/min flow rate. 5 ml fractions were collected. The fractions were checked by TLC using toluene : petroleum ether (3 : 1) system, and similar fractions were combined.

Fr. 45–49 yielded white star-shaped crystals (25 mg) with petroleum ether. This compound of the ethanolic extract was characterized as taraxasteryl acetate (**1**) by spectroscopic analyses and by comparison with an authentic sample.

Taraxasteryl acetate (**1**): m.p. 243 °C, $[\alpha]_D^{25}$: +86.2° (in $CHCl_3$), MS: 468 (M^+, $C_{32}H_{52}O_2$, 2%), m/z = 408 (M^+ – AcOH, 2), 365 (12), 229 (18), 190 (29), 189 (100), 109 (71), 95 (73). ^{1}H-NMR ($CDCl_3$): δ = 0.868–1.053 (7 × CH_3), 2.06 (3H, s, CH_3CO), 2.11 (1H, m, H-19), 2.21 and 2.46 (1H each, m, H-21), 4.48 (1H, m, H-3), 4.62 (2H, dd, H-30). Taraxasteryl acetate was refluxed with ethanolic KOH (5%) for 2 h to yield taraxasterol (**2**) (10).

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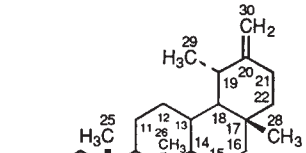
Fr. 52–54 yielded white needle crystals (15 mg) from absolute ethanol. The compound was proved to be taraxasterol (**2**) after spectroscopic analyses and by comparison with an authentic sample.

Taraxasterol (**2**): m.p. 214 °C, $[\alpha]_D^{25}$: +83.3° (in $CHCl_3$), ^{1}H-NMR ($CDCl_3$): δ = 0.865–1.040 (7 × CH_3), 2.17 (1H, m, H-19), 3.68 (1H, dd, H-3), 2.20 and 2.44 (1H each, m, H-21), 4.62 (2H, dd, H-30).

NMR values of taraxasterol and its acetate were compared with those reported in the recent literature (11–13). ^{1}H-NMR spectra of authentic taraxasterol and its acetate were found to be superimposable with the isolated materials.



1 R = Ac
2 R = H

Quantitative determination of the contents of taraxasteryl acetate in Lityazol Cemil® and the ethanolic extract was carried out by TLC-densitometry. 0.25 mm-thick silica gel G plates were used after 1 h activation at 105 °C. The plates were developed with hexane : $CHCl_3$ (3 : 2) system followed by drying at room temperature were sprayed with vanillin-sulphuric acid (VSA) reagent and heated at 110 °C for 4 min. The plates were cooled in the dark for 5 min and then subjected to TLC-densitometric analysis at 560 nm using EXT2 method. The results were expressed as mean of measurement using 10 TLC plates each containing two applications of each sample. The content of taraxasteryl acetate was determined as 0.15 % (± 0.014 ± SD) in the ethanolic extract and 0.01 % (± 0.010 ± SD) in the fluid extract.

Antispasmodic effects of the commercial fluid extract, ethanolic extract, butanolic fraction, water fraction, and taraxasteryl acetate were investigated on isolated rat ileum preparations. 150–200 g rats were killed by diethyl ether overdose. The ileum preparations (1.5 cm long) taken therefrom were suspended in the isolated organ bath filled with Krebs solution (given in mM/l, Na^+; 141.0, K^+; 5.9, Ca^{++}, 2.6, Mg^{++}; 1.2, Cl^-; 104.8, $H_2PO_4^-$; 2.2, HCO_3^-; 24.9, SO_4^-; 1.2, glucose; 10.0) and the load on the tissue was 2.0 g. The medium was aerated with a mixture of 95 % O_2 and 5 % CO_2, and the temperature was stabilized at 37 °C. Organs were incubated for 1 h with washing at every 15 min. The contraction and relaxation times of the preparations were 10 ± 1 seconds.

Spasmogenic effects of the materials were compared to the contractile effect of acetylcholine in rat ileum. Results were expressed as a percentage of maximal contractions induced by acetylcholine. Furthermore, the antispasmodic effect of taraxasteryl acetate was evaluated on the rat ileum precontracted with 1 × 10^{-6} M acetylcholine. In this case, results were calculated as percentage inhibition of acetylcholine induced contractions. Dose-response curves of acetylcholine were constructed in a 10^{-9}–10^{-5} M concentration range. Acetylcholine (1 μM) induced a rather stable tone. Student's t-test was used for statistical analysis.

Freeze-dried commercial extract, at the concentration of 1.2 mg/ml, was found to cause a relaxation of 9.4 ± 1.9 % $p < 0.001$, resulting in a decrease in intestinal motility. When ethanolic extract was introduced into the medium at the same concentration, biphasic responses were observed on ileum preparations. Compared to that induced by acetylcholine, ethanolic extract brought about an average of 8.7 ± 4.8 % $p < 0.001$ relaxation followed by 17.2 ± 8.2 % $p < 0.001$ contraction on the organ.

When 100 mg BuOH-fraction in DMSO were introduced into the medium in a manner to make the final bath concentration 1 mg/ml, again biphasic responses were observed on ileum preparations: 15.2 ± 1.8 % $p < 0.001$ contraction followed by 10.1 ± 3.7 % $p < 0.001$ relaxation. The effects of all the materials tested were dose-dependent. The effect of DMSO (0.01 ml/ml) on isolated rat ileum was also tested. No significant effect was observed. When water fraction was applied with final bath concentration of 1.2 mg/ml, no contracting or relaxing activity was observed on isolated rat ileum preparations.

When 0.1 ml of a 5 mg/ml solution of taraxasteryl acetate in DMSO was applied on isolated ileum preparation an obvious relaxation was observed on the organ. Therefore, the degree of inhibition induced by taraxasteryl acetate was measured on ileum preparations contracted by acetylcholine. To do this, two cumulative doses of taraxasteryl acetate (5.0 × 10^{-2} and 1.0 × 10^{-1} mg/ml) were given to the ileum precontracted with 1.0 × 10^{-6} M acetylcholine. Taraxasteryl acetate brought about biphasic responses. It induced 5.2 ± 1.5 % $p < 0.001$ contraction and inhibited the contraction induced by acetylcholine by 26.5 ± 5 % $p < 0.001$. On the other hand, taraxasteryl acetate with 1.0 × 10^{-1} mg/ml brought about the same degree of contraction, but inhibited the contraction induced by acetylcholine by 45.8 ± 4.2 % $p < 0.001$. Taraxasteryl acetate has previously been shown to have anti-inflammatory (14), antihepatotoxic and antihepatitis (10) activities.

The present study has aimed at investigating the effects of a commercial fluid extract, ethanolic extract from the root bark of *S. hispanicus*, BuOH-fraction, water fraction and taraxasteryl acetate isolated from BuOH-fraction were tested on isolated rat ileum preparations. The commercial extract relaxed the smooth muscle preparation. Ethanolic extract and BuOH-fraction showed biphasic effects, in other words, relaxation of the smooth muscle was immediately preceded by contraction. Stronger biphasic effect was observed with taraxasteryl acetate, the major triterpenoid of the BuOH-fraction. Taraxasteryl acetate induced a dose-dependent inhibition of contraction caused by acetyl choline. These results have suggested antispasmodic and spasmogenic activities due to ethanolic extract of the root bark of *Scolymus hispanicus* and taraxasteryl acetate. the biphasic effects observed may be inferred as an indication of a possible lithuretic effect. However, this point remains to be proven by experimental evidence.

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Hypoglycemic Effect of *trans*-Dehydrocrotonin, a Nor-Clerodane Diterpene from *Croton cajucara*

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Abstract: ***trans*-Dehydrocrotonin (*t*-DCTN), a 19-nor-clerodane diterpene isolated from the bark of *Croton cajucara* Benth. (Euphorbiaceae) demonstrated a significant hypoglycemic activity in alloxan-induced diabetic rats but not in normal rats, at oral doses of 25 and 50 mg/kg body weight. The drug also effectively lowered the blood sugar levels in glucose fed normal rats. The hypoglycemic effect of *t*-DCTN was almost comparable to that produced by glibenclamide (2 mg/kg), a clinically useful drug. The results indicate the antihyperglycemic potential of *t*-DCTN.**

Croton cajucara Benth. (Euphorbiaceae), commonly known as "Sacaca" is a medicinal plant largely grown in the Amazonian region of Brazil. Its leaves and bark are popularly used either in the form of infusions or pills to treat gastrointestinal, kidney, and liver disorders and in the control of high blood cholesterol (1, 2). A lipid lowering effect of its ethanolic extract has been reported previously in rats fed high fat diet but not in normal animals (3). *trans*-Dehydrocrotonin (*t*-DCTN), a 19-nor-clerodane diterpene (Fig. **1**), has been isolated from *C. cajucara* (4, 5) and its biological activity was studied. This compound exhibited an insect growth inhibitory property (6) and in animal models of inflammation and nociception it demonstrated anti-inflammatory and antinociceptive effects (7). Since no scientific study on the antidiabetic property of this plant has been reported, we have evaluated the effects of *t*-DCTN on blood glucose levels of alloxan-induced diabetic, glucose-fed hyperglycemic and normal rats using glibenclamide as a reference standard for comparison.

The fasting blood sugar levels in *t*-DCTN-treated normal and diabetic animals as compared to untreated and glibenclamide-treated groups of rats are shown in Table **1**. Alloxan injections were found to cause a five-fold elevation in the blood sugar levels compared to normal untreated rats. The oral medication with *t*-DCTN (25 and 50 mg/kg) when administered daily on 3 consecutive days, i.e., an hour before, and 24 and 48h after alloxan (preventive) or administered 48h after alloxan (established phase of diabetes), caused a significant ($P < 0.05$) decrease of blood sugar levels when compared to untreated diabetic controls. In contrast to the diabetic state,

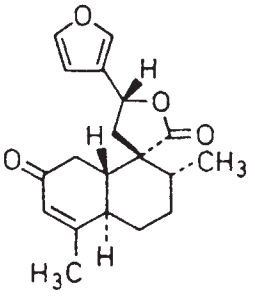

Fig. 1 Chemical structure of *trans*-dehydrocrotonin.