

RESEARCH ON THE TURKISH MEDICINAL PLANT

Ecbalium elaterium

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In Turkey, the fresh fruit juice of Ecbalium elaterium (Cucurbitaceae) is directly applied into the nostrils for the treatment of sinusitis. Clinical tests on voluntary patients revealed that the healing rate of double-high dosage application is higher than that of the single-low dosage (71.0 and 56.6%, respectively). In addition, total relief from the main symptoms is observed in 20% of the patients, although the sinuses are not completely drained off on x-ray examination. As the next step, the active anti-inflammatory principle which might be responsible for its activity is isolated through the fraction of the fruit juice with solvent extraction and preparative TLC. For the fractionation process, the effects of the fractions on increased vascular permeability induced by AcOH in mice are used and the structure of the active anti-inflammatory principle thus obtained is elucidated as cucurbitacin B. Further studies are conducted to evaluate the anti-inflammatory activity of cucurbitacin B using serotonin- and bradykinin-induced edemas in mice. On the other hand, cucurbitacin B content in the juice is determined as 2.48% by HPLC.

Fresh fruit juice of *Ecbalium elaterium* (L.) A. Rich (Cucurbitaceae), squirting cucumber, is widely used for the treatment of sinusitis in folk medicine in Anatolia. The juice of the mature fruit is directly applied into the nostrils of the patient, but this application often causes severe congestion of the upper respiratory tract, due to the irritant nature.

For the determination of an effective and nontoxic dose of the drug, a clinical study is performed on voluntary patients. After physical and radiographical consultation, 49 voluntary patients were selected for the survey. Patients were divided into groups and properly diluted juice was applied into the nostrils in different doses, mainly single-low dose application and double-high dose application groups of treatment. Ten days after the application physical and radiographical consultations are repeated.

Results of the clinical tests showed that, in double-high dose application of the diluted juice, a high recovery is observed: in 71% of the patients total relief is observed from the main symptoms especially that of obstructive aeration. On the other hand, in 20% of the patients, although the radiographical findings did not improve, physiological relief from the symptoms is observed [1].

As the second step in this study, the active anti-inflammatory principle is investigated by testing the activity of fractions obtained from fruit juice on the increased vascular permeability induced by HOAc in mice as an index of anti-inflammatory activity. Through the multi-step fractionation of fruit juice, an active anti-inflammatory principle is isolated and its structure is elucidated as cucurbitacin B, a well-known triterpenoid.

Cucurbitacin B also showed a dose-dependent and significant inhibition against serotonin- and bradykinin-induced edemas in mice. On the other hand, the cucurbitacin B content of freeze-dried fruit juice is determined as 2.48% by HPLC.

EXPERIMENTAL

Extraction and Fractionation of Material. Green mature fruits of *E. elaterium* are collected and squeezed to obtain juice, then filtered through muslin and freeze-dried to give powdered material. A part of the freeze-dried juice (33.8 g) is dissolved in H₂O and the H₂O-insoluble fraction is filtered. The H₂O-soluble fraction is extracted with chloroform, ethylacetate,

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and n-butanol satd. with H₂O, successively. Each extract is evaporated to dryness under reduced pressure. The remaining aqueous solution is also dried by freeze-drying. Each extract is tested for its activity on the increased vascular permeability induced by HOAc in mice as an index of anti-inflammatory activity.

Isolation of Active Principle. Active chloroform extract is fractionated by preparative TLC on silica gel, EtOAc–MeOH–H₂O (10:1:3, upper layer), and grouped into three zones. The nonpolar upper zone is further fractionated on preparative TLC with CHCl₃–MeOH (11:0.4), likewise. The active zone is then chromatographed on silica gel column and eluted with CHCl₃ and CHCl₃–MeOH (100:1), respectively. The final purification of the active principle is performed by preparative TLC on silica gel, Et₂O–C₆H₆ (8:2) and crystallized from MeOH.

Structure Elucidation of Isolate. The physical data for the isolate (IR, UV, MS, ¹H-NMR) are identical with previously published values for cucurbitacin B: mp 182-184°C; [α]³¹ + 75.9° (c = 0.98, EtOH).

Quantitative Analysis of Cucurbitacin B. Conditions used for reversed phase HPLC are as follows: column TSK Gel ODS 120A (Toyo Soda), 150 × 4.6 mm; flow rate 1.0 ml/min; detection, absorption at 230 nm; solvent system, acetonitrile/H₂O (2.3).

Assays for Anti-Inflammatory Activity. 1) Effect of the test samples on the increased vascular permeability induced by HOAc in male mice is determined according to the Whittle method with some modifications [2].

2) Effect of cucurbitacin B against serotonin-induced hind paw edema in mice is tested by using the method Kasahara et al. with some modifications [3].

3) Effect of active principle, cucurbitacin B, on bradykinin-induced hind paw edema model is tested by using a modified method of Kasahara et al. [3].

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