PRELIMINARY PHARMACOLOGICAL STUDIES ON EUGENIA UNIFLORA LEAVES: XANTHINE OXIDASE INHIBITORY ACTIVITY

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Summary

Eugenia uniflora is widely used in Paraguayan folk medicine. A hydroalcoholic extract of the leaves showed some central nervous system activity in hippocampal screening when given intraperitoneally, but little to no acute or subacute toxicity in doses up to 4200 mg/kg orally in BALB/c mice. The LD50 of the extract was 220 mg/kg i.p. in mice. A decoction or infusion of the leaves is recommended for treating gout by native herbalists. The known flavonoids quercitrin, quercetin, myricitrin and myricetin were found to be responsible for the xanthine oxidase inhibitory action of the plant extract.

Introduction

Several species belonging to the Myrtaceae family are used as medicinal plants in Paraguay. The most widely employed is the complex known under the common name of "Nangapiry". The herbalist recommends a water decoction of Nangapiry as a diuretic and antihypertensive, to lower cholesterol and uric acid, to lose weight, and to act as a digestive and astringent. Self-medication with the plant is very common. The crushed leaves are used as an additive in "maté" but used alone in "tereré". Eugenia uniflora L. has been identified as one of the species called Nangapiry in Paraguayan folklore.

The enzyme xanthine oxidase catalyses the oxidation of xanthine to hypoxanthine and finally to uric acid, the excess of which initiates the symptomatology of human gout. As Nangapiry is used to lower uric acid, specific xanthine oxidase inhibitor activity might be expected since the...
crude drug is considered to be clinically effective (Noro et al., 1983). After preliminary in vitro screening in this laboratory, the leaves of *E. uniflora* were found to possess an inhibitory effect on this enzyme.

**Methods**

**Plant material**

Leaves of *E. uniflora* were collected in Asunción on March 21 and on November 2, 1986. On the latter date, the tree was in the flowering and fruiting stage. Voucher specimens (Schmeda 775) were deposited at the Smithsonian Institution, Washington, DC, U.S.A., Instituto de Botánica del Nordeste, Corrientes, Argentina, Herbario de la Universidad Federal, Rio Grande do Sul, Brasil and at the Herbarium of the Facultad de Ciencias Químicas, Asunción, Paraguay. The plant was authenticated as *E. uniflora* by four independent taxonomists.

The air-dried material was ground and extracted three times with EtOH/H₂O (7:3) under reflux for 3 h. The extracts were filtered and concentrated under reduced pressure and then lyophilized. A total of 765 g of leaves, extracted with 3.7 l solvent each time, afforded 230 g of a greenish-brown powder.

**Assay of xanthine oxidase activity**

Xanthine oxidase derived from cow's milk was purchased from Boehringer Mannheim Co. Ltd. and xanthine was obtained from Nakarai Chemicals. Allopurinol from Sigma Chemical Co. was used as a standard inhibitor. The xanthine oxidase activities with xanthine as substrate were measured spectrophotometrically using the procedure reported by Iio et al. (1985).

**Hippocratic screening and *LD*₅₀ determination**

*E. uniflora* leaf extract was screened pharmacologically in mice using the hippocratic procedure (Malone, 1977). Eighty non-fasted male BALB c mice were divided into four groups of 20 and administered i.p. with 50, 100, 500 or 1100 mg/kg of the extract suspended in aqueous 0.25% agar solution. A 20 mice group was injected with the vehicle and used as a control. The mice were kept under observation for 14 days.

**Oral toxicity testing**

**Acute toxicity study**

Thirty non-fasted male BALB c mice, 11—12 g, were divided in three groups of ten. The members of group 1 were individually administered with 4200 mg/kg *E. uniflora* leaf extract p.o, while the group 2 members received
2100 mg/kg p.o. Group 3 was maintained as a control and administered an equivalent amount of vehicle. Subjects were observed over 6 days for any signs of toxicity and/or lethality.

**Subacute toxicity study**

Ten non-fasted BALB c mice, 24—26 g, were given 2000 mg/kg *E. uniflora* leaf extract once daily for 6 days. Subjects were watched over the entire duration of the experiment for signs of toxicity and/or lethality.

**Results**

**Hippocratic screening and LD₅₀ determination**

The leaf extract elicited a rapid-onset, dose-related decrease in motor activity, hind leg paralysis, enophthalmos, ear and tail cyanosis, piloerection, loss of screen grip reflex, and passivity to head tap and body grasp within 5 min of i.p. administration of 100—1100 mg/kg doses and within 20 min in animals receiving 50 mg/kg. There was a tendency for mice to group in the corners of the observation boxes. Death was seen within 1.5—20 hours for the two top doses, while animals from the two lower doses died 1—8 days after injection. Necropsy performed on animals expiring within the first 2 days and of survivors at +15 days indicated no gross pathological defects. The LD₅₀ was determined as 220 mg/kg i.p. (95% confidence limits 190—250 mg/kg).

**Oral toxicity study**

In the acute toxicity study, all 20 mice treated with the leaf extract appeared normal, exhibiting no symptoms of drug-induced toxicity. In the subacute toxicity study, the subjects appeared healthy, active and normal over the duration of the study except for two mice that died due to intestinal obstruction.

**Xanthine oxidation inhibition**

The crude leaf extract, when assayed for inhibitory effect against xanthine oxidase, showed an IC₅₀ of 22.0 μg/ml (95% confidence limits 12.6—31.4 μg/ml). The extract (120 g) was suspended in water and partitioned with hexane (9.1 g), CHCl₃ (3.7 g), EtOAc (6.2 g) and n-BuOH (18.5 g). As the suspension was extracted with hexane, a precipitate was formed; this was filtered off and dried (16.6 g). The aqueous phase was lyophilized yielding 58.2 g of a brownish powder. The fractions were concentrated in vacuo and assayed again for inhibitory effect on xanthine oxidase at 50 μg/ml, showing the following activities (% inhibition): hexane, 10; CHCl₃, 5; EtOAc, 79; n-BuOH, 77; precipitate, 72; aqueous, 48. The most active EtOAc extract was chromatographed on SiO₂ with an hexane/CHCl₃, CHCl₃/EtOH and EtOH/H₂O
gradient. The fractions were compared by TLC on silica gel and cellulose, and those with a similar TLC pattern were combined and assayed for inhibitory activity toward xanthine oxidase. Fractions 1—11 were devoid of activity, while fractions 12—13 (57%), 14—15 (80%), 16—17 (81%) and 18 (82%) proved to be active at 50 µg/ml. TLC and two-dimensional paper chromatography showed a main spot corresponding to a flavonol glycoside and a minor one to a closely related compound. The active constituents were identified as quercitrin (quercetin-3-O-rhamnoside) and myricitrin (myricetin-3-O-rhamnoside) using UV and 1H-NMR spectra (Mabry et al., 1970) and co-chromatography with standard samples. The precipitate formed on extracting the water-suspended total extract with hexane afforded after extensive chromatography quercitrin and quercetin as the major compounds as well as myricitrin and myricetin as the minor ones. The n-BuOH extract showed on two-dimensional paper chromatography the same flavonoid pattern as the ethyl acetate extract.

Discussion

According to literature data (Iio et al., 1985), myricetin, quercetin and quercitrin, the constituents of the active fractions of E. uniflora leaf extract, have been shown to be strong inhibitors toward xanthine oxidase at a 50 µM concentration, showing inhibitions of 96, 86 and 85% and an IC₅₀ of 2, 3 and 15 µM, respectively. In the present study, results obtained from oral toxicity testing showed that high doses of leaf extract were relatively safe in that the mice did not show symptoms of toxicity. The xanthine oxidase inhibitory activity of E. uniflora leaf extract establishes a pharmacological basis for the use of Nangapiry to lower uric acid in Paraguayan folk medicine.

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References