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Anti-inflammatory Activity of a Flavonol Glycoside from *Tephrosia Spinosa*

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Abstract – A rare flavonol glycoside identified as eupalitin-3-*O*-β-D-glucoside (I) was isolated from *Tephrosia spinosa* (Leguminosae) and its anti-inflammatory activity was evaluated against carrageenin induced paw edema. The compound exhibited significant activity when compared to the standard drug indomethacin.
Keywords – *Tephrosia spinosa*, Leguminosae, Flavonol glycoside, Eupalitin-3-*O*-β-D-glucoside, Anti-inflammatory activity.

Introduction

The genus *Tephrosia* is a rich source of flavonoids. Earlier, we reported the isolation and identification of some novel flavonoids from *T. spinosa* (Venkata Rao and Rajendra Prasad, 1992a; Venkata Rao and Rajendra Prasad, 1993b). The various extracts and compounds isolated from different species of *Tephrosia* showed interesting biological activities (Jagat *et al.*, 1976; Sree Rama Murthy *et al.*, 1986; Leng *et al.*, 1997a; Michael *et al.*, 1995; Mitra *et al.*, 1998; Sanchez *et al.*, 2000; Leng *et al.*, 2000b; Gokhale and Saraf, 2000a; Gokhale and Saraf, 2000b; Mohammad *et al.*, 2001; Deshpande *et al.*, 2003; Dae *et al.*, 2003). The methanolic extract of the aerial parts of *T. spinosa*, when subjected to column chromatography, yielded a rare flavonol glycoside (I), identified as eupalitin-3-*O*-β-D-glucoside. This is the first report of its occurrence in the genus *Tephrosia*. The compound was evaluated for its anti-inflammatory activity.

Experimental

Instruments and reagents – IR spectra were recorded with Perkin-Elmer 881 on KBr disc. ^{1}H and ^{13}C-NMR spectra were recorded with a Bruker DRX 300 MHz NMR spectrometer in DMSO-d_6 using TMS as internal standard. Electron Impact Mass spectra (EI-MS) were obtained on an Autospec Instrument. All the other materials used for this experiment are of analytical grade. Carrageenan (Hi-Media), Sodium CMC (E. Merck) and Saline (Core Health Care) were purchased from the local supplier. Indomethacin was a gift sample from Jagsonpal, New Delhi.

Plant material – The whole plant was collected near Bobbilli, Vijayanagaram District, A.P., India in the month of July 2001 and verified by Dr. M. Venkaiah, Andhra University. A voucher specimen of this plant has been deposited at the Herbarium, Department of Botany, Andhra University, Visakhapatnam, India.

Extraction and isolation – The air-dried powdered aerial parts (0.5 kg) of *Tephrosia spinosa* were successively extracted with chloroform and methanol under reflux. The resultant extracts were concentrated under reduced pressure to afford 10.0 g and 15.0 g of the residues respectively.

The methanolic extract was chromatographed on a silica gel column (5×120 cm) eluting with a gradient of *n*-hexane–EtOAc and followed by EtOAc-MeOH to afford compound I (95 mg; 90-10, EtOAc-MeOH) along with a known compound Spinoflavanone B (II) (Venkata Rao and Rajendra Prasad, 1993b). Compound I was previously reported from *Birickellia scoparia* (Li Rongzhi *et al.*, 1986) and *Sesuvium portulacastum* (Ravi *et al.*, 1982).

Eupalitin-3-*O*-β-D-glucoside (I) – Yellow amorphous powder from methanol, m.p. 220-222°C, [á]$_D$: + 61°, IR ν_{max} (KBr) cm^{-1}: 3400, 3100, 1660, 1060, 1120. ^{1}H-NMR (300MHz, DMSO-d_6) δ_H (ppm): 12.59 (1H, *s*, 5-OH), 8.15 (2H, *d*, *J* = 6Hz, H-2' & 6'), 6.89 (2H, *d*, *J* = 6Hz, H-3'& 5'), 6.88 (1H, *s*, H-8), 6.93 (1H, *s*, C-4'-OH, D_2O exchangeable), 5.49 (1H, *d*, *J* = 6Hz, H-1"), 2.54-3.10 (*m*, H-2" to H-6"). ^{13}C-NMR (75MHz, DMSO-d_6) δ_C (ppm): 178.0 (C-4), 160.2 (C-4'), 158.8 (C-7), 156.9 (C-2), 151.9 (C-5), 151.8 (C-8a), 133.4 (C-3), 131.8 (C-6), 131.2 (C-2'

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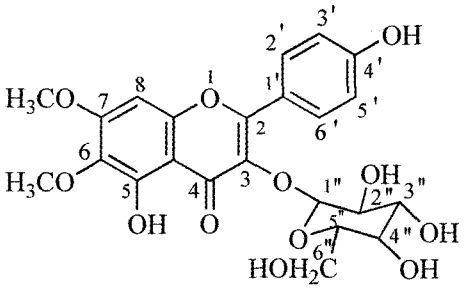

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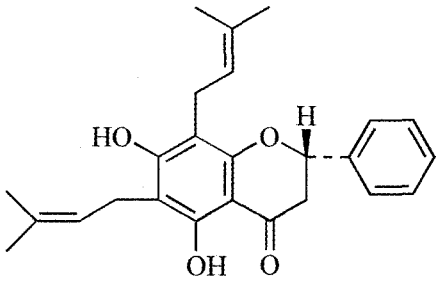

II

& 6'), 121.0 (C-1'), 115.3 (C-3' & 5'), 105.4 (C-4a), 101.7 (C-1"), 91.4 (C-8), 56.5 (OMe-C-6), 60.1 (OMe-C-7). EI-MS *m/z* (rel. int., %): 492 (60), 330 (100), 165 (30).

Anti-inflammatory activity – Sprague-Dawley rats of either sex weighing 200-250 gm were used for the experiment. Animals were maintained on 12 hr light/dark cycle at approximately 30°C. The animals were divided into four groups of six animals each. All the animals were fasted for overnight and allowed water *ad libitum*. Group I was administered with 1% sodium CMC. The same was used for suspending the test compound and the standard drug. Group II was administered with 15 mg/kg body weight of the compound, group III was administered with 30 mg/kg body weight of the compound and group IV was administered with 4 mg/kg body weight of indomethacin. All the doses were administered orally. One hour after treatment, 0.1 ml of 1% carrageenan in saline (0.9% NaCl) solution (Winter *et al.*, 1962) was injected subcutaneously into the sub plantar tissue of the left hind foot in all four groups. The thickness of left paw of each rat, lower and upper surface was measured using Zeitlin's constant load lever consisting of a graduated micrometer combined with a constant loaded lever system to magnify the small changes in paw thickness during the course of the experiment (Al-Hoboubi and Zeitlin, 1983). The paw thickness was determined at 0, 1, 2, 3, 4, 5, 6hrs after induction of inflammation (Duwiejua *et al.*, 1994).

$$\% \text{ increase in paw thickness} = \frac{Y_t - Y_o}{Y_o}$$

where

Y_t = paw thickness at the time t hrs (after injection)

Y_0 = paw thickness at the time '0'hrs (after injection)

The percent increase in paw thickness during 6 hrs was determined. The percent inhibition of paw edema thickness is calculated using the formula

$$\text{Percent Inhibition} = 100\left[1-\frac{Y_t}{Y_c}\right]$$

where

Y_t = average increase in paw thickness in group tested with test compound

Y_c = average increase in paw thickness in control

Statistical analysis – The results are expressed as mean ± S.E.M. Dunnet's *t*-test was used to verify the statistical significance. P values < 0.05 were considered as significant.

Results and Discussion

A chromatographic separation of the methanolic extract of the aerial parts led to the isolation of eupalitin-3-*O*-β-D-glucoside (I) along with a known compound, spinoflavanone B (II) which was confirmed by the NMR experiments and published data.

Eupalitin-3-*O*-β-D-glucoside obtained from the aerial parts of *T. spinosa* was tested for its anti-inflammatory activity in carrageenan induced pedal inflammation in rats and the results are given in Table-I.

Carrageenin-induced hind paw edema is the standard experimental model of acute inflammation (Agarwal and Rangari, 2003; Thangam and Dhananjayan, 2003; Kapil and Sarma, 1995). Measurement of paw thickness is a standard method for evaluating anti-inflammatory activity of drugs/extracts/compounds (Al-Hoboubi and Zeitlin, 1983; Duwiejua *et al.*, 1994). In carrageenin treated control group rats significant percent increase in paw oedema of 48.9 ± 3.28 is observed at 0.5 hr and is increased to 59.09 ± 4.17 after 1 hr. In case of eupalitin-3-*O*-β-D-glucoside treated groups (15 mg/kg and 30 mg/kg), the percent increase is 16.95 ± 2.85 and 8.35 ± 2.84 at 0.5 hr. It is significantly less with the two groups when compared with control group throughout the experimental schedule ($P < 0.001$) where as in case of indomethacin treated groups percent increase is 11.57 ± 3.18 at 0.5 hr and significantly less 3.57 ± 2.41 ($P < 0.001$) at 1 hr (Table 1). Indomethacin produced significant percent inhibition of 76.34 at 0.5 hr and maximum percent inhibition of 93.96 at 1 hr. In case of eupalitin-3-*O*-β-D-glucoside treated rats, significant percent inhibition in paw oedema was observed which was 65.34 and 82.92 at 0.5 hr with doses of 15 and 30 mg/kg body weight. Maximum percent

Table 1. Effect of eupalitin-3-O-β-D-glucoside on percent increase of carrageenin induced paw edema in rats

Treatments	Time (hrs)						
	0.5	1	2	3	4	5	6 hrs
Control	48.9 ± 3.28	59.09 ± 4.17	56.05 ± 5.09	47.06 ± 3.95	50.74 ± 4.58	56.86 ± 2.78	58.90 ± 2.36
Indomethacin 4 mg/kg	11.57 ± 3.18*	3.57 ± 2.41*	12.08 ± 4.20*	7.76 ± 1.85*	7.81 ± 2.62*	8.76 ± 3.94*	9.10 ± 3.70*
Eupalitin-3-*O*-β-D-glucoside 15 mg/kg	16.95 ± 2.85*	13.20 ± 3.82*	15.15 ± 4.16*	19.50 ± 3.47*	15.60 ± 2.25*	13.40 ± 2.90*	19.54 ± 3.66*
Eupalitin-3-*O*-β-D-glucoside 30mg/kg	8.35 ± 2.84*	5.08 ± 4.05*	11.35 ± 3.29*	7.10 ± 1.38*	10.15 ± 2.97*	8.95 ± 3.49*	12.50 ± 2.08*

*P < 0.001 when compared with control group

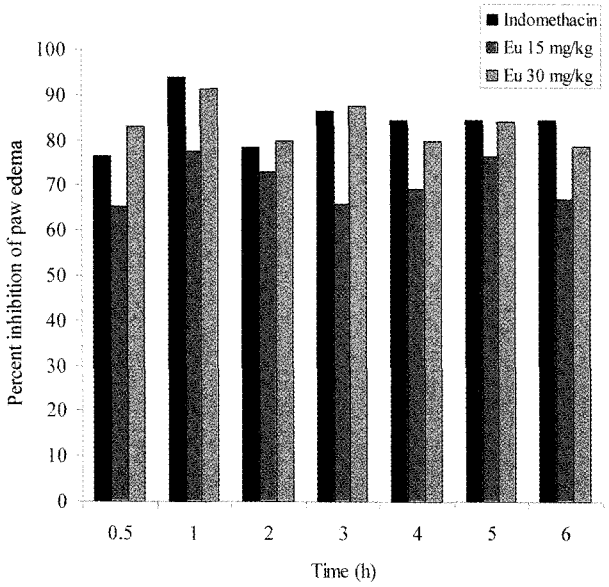

Fig. 1. Effect of eupalitin-3-O-b-D-glucoside on percent inhibition of carrageenin induced paw edema in rats.

inhibition observed was 77.66 and 91.40 at 1 hr. Results are shown as Fig. 1. The anti-inflammatory effect produced by eupalitin-3-*O*-β-D-glucoside was dose dependant. The results clearly indicate that the anti-inflammatory effect of compound I at a dose of 30 mg/kg was maximum from 0.5 h and the activity is comparable to the standard drug indomethacin ($P < 0.001$).

The present study revealed that compound I produced a significant percentage inhibition against carrageenin-induced edema. It has shown significant anti-inflammatory activity and comparable with that of standard drug at 30 mg/kg dose. The edema suppressant activity exhibited by the compound may be due to the inhibitory effects on the release of histamine, 5-hydroxytryptamine and kinin like substances, which are reported to be released from the mast cell degradation during the first hour of carrageenan induced artificial paw edema (Vinegar *et al.*, 1969). However there is a need to investigate the anti-inflammatory activity in other transudative and exudative experimental models of inflammation before concluding about the efficacy of eupalitin-3-*O*-β-D-glucoside. The mechanism of action of this compound against inflammation also has to be investigated further. Its usefulness clinically in rheumatoid arthritis and other non-dermatological inflammatory disorders has to be evaluated.

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