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Anti-inflammatory and antinociceptive potential of *Maclura pomifera* (Rafin.) Schneider fruit extracts and its major isoflavonoids, scandenone and auriculasin

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Abstract

The aqueous, ethanolic and chloroform extracts and two prenylated isoflavones: scandenone (**I**) and auriculasin (**II**), isolated from the fruits of *Maclura pomifera* (Rafin.) Schneider, were investigated for their in vivo anti-inflammatory and antinociceptive activity. For the anti-inflammatory activity, both carrageenan-induced hind paw edema and 12-*O*-tetradecanoyl-13-acetate (TPA)-induced mouse ear edema models and for the antinociceptive activity, *p*-benzoquinone-induced abdominal constriction test were used. Scandenone, the chloroform and the ethanolic extracts were shown to possess antinociceptive activity and anti-inflammatory activity on carrageenan-induced hind paw edema model at 100 mg/kg dose. The same compound and the extract were also found to be highly active in (TPA)-induced mouse ear edema model whereas auriculasin and the H_2O extract showed to be inactive in all of the assays.

Keywords: Anti-inflammatory activity; Antinociceptive activity; 12-*O*-tetradecanoyl-13-acetate (TPA); *Maclura pomifera*; Scandenone; Auriculasin; Prenylated isoflavone

1. Introduction

Maclura pomifera (Rafin.) Schneider (*syn. Maclura aurantiaca* Nutt., *Ioxylon pomiferum* Raf., *Toxylon pomiferum* Raf. ex Sarg.), a member of Moraceae family, is a widely cultivated hardwood tree in Turkey for ornamental purposes. The plant, known as osage orange, horse apple, mock orange or hedge apple, is in fact native to the north America and of interest from fruit to root. The bark of the roots is orange-colored and furnishes a yellow dye (Wolfrom et al., 1963; Wolfrom and Bhat, 1965).

Various parts of the *Maclura* species are used in folkloric medicine worldwide. Decoction prepared from the roots of *Maclura pomifera* is used for the treatment of sore eyes by Comanche Indians in the North America (Carlson and Volney, 1940). The sap of the plant is used for the treatment of tooth pain and the barks and leaves for uterine haemorrhage in Bolivia (Bourdy et al., 2004). While the bark of *Maclura tinctoria* has been reported to be used against toothache by Kaiowa and Guarani indigenous people living in the Caarapo Reserve in Brazil as well as the in the other parts of Amazon region, it was also recorded to be used in southern Ghana for dental health (Elvin-Lewis et al., 1980; Elvin-Lewis and Lewis, 1983; Bueno et al., 2005). *Maclura tinctoria*, known as "insira or insira amarilla" in the Amazons, was also recorded to treat cough, gout, pharyngitis, rheumatism, sore throat, and syphilis by Iberoamericans (Rutter, 1990). *Maclura aurantiaca* is used for cardiovascular ailments in Azerbaijan (Yakupoglu and Adisoglu, 1991). Several biological activities of *Maclura pomifera* and its components including antibacterial, antifungal, antiviral, cytotoxic, antitumor, estrogenic and antimalarial activities have been so far reported (Peterson and Brockemeyer, 1953; Jones and Soderberg, 1979; Mahmoud, 1981; Voynova et al., 1991; Maier et al., 1995; Bunyapraphatsara et al., 2000; Hay et al., 2004). Besides, various phytochemical studies carried out on *Maclura pomifera* showing that the plant contains lectins, triterpenes, xanthones and flavone-type compounds (Wagner and Harris, 1952; Ulevitch et al., 1974; Gearien and Klein, 1975;

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Gerber, 1986; Young et al., 1991, 1995; Lee et al., 1998; Marek et al., 2003). Strong antioxidant capacity of *Maclura pomifera* has been attributed to flavonoid type components in particular isoflavones, osajin and pomiferin (Tsao et al., 2003; Vesela et al., 2004) or a dihydroxychalcon derivative in *Maclura tinctoria* (Cioffi et al., 2003).

On the other hand, a number of isoflavones have been so far reported to display anti-inflammatory activities. Among them, 2′-hydroxygenistein, daidzein and 2′-hydroxydaidzein, isolated from *Crotolaria pallida*, had strong in vitro anti-inflammatory effect via inhibiting the release of β-glucuronidase and lysozyme from rat neutrophils (Ko et al., 2004). In the same study, daidzein was also found to display remarkable inhibitory effect on superoxide anion generation in rat neurophils. In another study, dietary isoflavonoid intake was reported to prevent the inflammation-associated induction of metallothioneninin in the intestine in mice and it was stated that isoflavones suppress the intestinal response to inflammation by modulating the action of interleukin-6, the pro-inflammatory cytokine (Paradkar et al., 2004). Dijsselbloem et al. (2004) emphasized the possible role of isoflavones in cancer chemoprevention and NF-kappa B-related inflammatory disorders.

Therefore, the present study is aimed to investigate the possible anti-inflammatory and antinociceptive effects of the aqueous, ethanolic and chloroform extracts from *Maclura pomifera* and isolated prenylated isoflavonoids, scandenone (**I**) and auriculasin (**II**), on the above-mentioned folkloric utilizations using in vivo experimental models, i.e., carrageenan-induced hind paw edema and 12-*O*-tetradecanoyl-13-acetate (TPA)-induced mouse ear edema models for anti-inflammatory activity and *p*-benzoquinone-induced abdominal constriction test for antinociceptive activity assessment.

2. Materials and methods

2.1. Plant material

Fruits of the plant were collected from the garden of Ankara University, Tandogan, Ankara in December, 2004. Authenticated voucher specimen encoded as GUE 2234 is deposited in the Herbarium of Faculty of Pharmacy, Gazi University, Ankara, Turkey.

2.2. Preparation of plant extracts

Maclura pomifera fruits were rinsed, dried at room temperature for 1 day, and then chopped into little pieces. A 100 g of fresh material was extracted with chloroform at room temperature for 2 days (2 × 400 ml). The combined chloroform extract was evaporated to dryness in vacuo to give [$CHCl_3$ extract] (yield: 4.03%). Another 100 g of fresh material was extracted with ethanol 90% at room temperature for 2 days (2 × 400 ml). The combined ethanolic extract was evaporated to dryness in vacuo to give [EtOH extract] (yield: 9.11%). Additional batch of fresh material (100 g) was extracted with distilled water at room temperature for 2 days (2 × 400 ml). The combined aqueous extract was lyophilized to give the [H_2O extract] (yield: 7.17%).

2.3. Extraction and isolation of flavonoids (*I*) and (*II*)

About 25 g of the ground fruit was first defatted with light petroleum and then extracted with chloroform. The chloroform extract was concentrated under reduced pressure and applied to preparative TLC (Si 60) using toluene:pyridine:formic acid (36:9:5) as a mobile system, leading to isolation of two compounds. (**I**) (R_f: 0.61; 0.89 g) was crystallized from chloroform; (**II**) (R_f: 0.50; 0.74 g) was obtained by crystallization first with xylol and then methanol.

Structures were elucidated by using several spectral techniques as two prenylated-isoflavone derivatives, scandenone (**I**) and auriculasin (**II**). UV spectra were recorded on Beckman DU 680 UV–vis Spectrophotometer, mass spectra were recorded on a V6-platform II, LC–MS spectrometer, ^1H, ^{13}C NMR spectra were obtained using Bruker GmbH DPX 400 (400 MHz), FT NMR spectrometer (Toker and Erdogan, 1998).

2.4. Animals

Male Swiss albino mice (20–25 g) were purchased from the animal breeding laboratories of Refik Saydam Central Institute of Health (Ankara, Turkey). The animals left for 2 days for acclimatization to animal room conditions were maintained on standard pellet diet and water ad libitum. The food was withdrawn on the day before the experiment, but allowed free access of water. A minimum of six animals was used in each group. Throughout the experiments, animals were processed according to the suggested ethical guidelines for the care of laboratory animals.

2.5. Preparation of test samples for bioassay

Test samples were given orally to test animals after suspending in a mixture of distilled H_2O and 0.5% sodium carboxymethyl cellulose (CMC). The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indomethacin (10 mg/kg) or acetyl salicylic acid (ASA) (100 and 200 mg/kg) in 0.5% CMC was used as reference drug.

2.6. Antinociceptive activity

p-Benzoquinone-induced abdominal constriction test (Okun et al., 1963) was performed on mice for determination of antinociceptive activity. According to the method, 60 min after the oral administration of test samples, the mice were intraperitonally injected with 0.1 ml/10 g body weight of 2.5% (w/v) *p*-benzoquinone (PBQ; Merck) solution in distilled H_2O. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting on the fifth minute after the PBQ injection. The data represent average of the total number of writhes observed. The antinociceptive activity was expressed as percentage change from writhing controls. Aspirin