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Antinociceptive Effect of Two Flavonoids from *Aloysia Triphylla L.*

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Abstract

Aloysia triphylla, known in Jordan as Mellisa, is a plant that belongs to the Verbenaceae. This plant has been used in herbal medicine as sedative agent and helps to counter depression. Phytochemical analysis of the ground aerial parts of *Aloysia triphylla* resulted in the isolation of two known compounds: artemitin and hesperidin. The two compounds were assessed for antinociceptive activities in mice, using the classical *in vivo* model of pain, the hot plate test. Artemitin and hesperidin (given i.p.) increased significantly ($P<0.05$) the pain latency of nociceptive response in dose dependent manner. The ED50 values were 1.6 $x10^{-3}$ mg/kg for artemitin (n=6) and 3.2 x 10^{-1} mg/kg for hesperidin (n=6). The present data indicate that the two flavonoids (artemitin and hesperidin) possess significant antinociceptive effects in mice which seems to justify the traditional analgesic use of *Aloysia triphylla.*

الملخص

تنتمي نبتة (Aloysia triphylla) المعروفة في الاردن بأسم المليسا الى العائلة الفربينية (المينائية). تستخدم هذة النبتة في الطب الشعبي كعامل مسكن ومقلل للاكتئاب. أدى التحليل الكيميائي للأجزاء العلوية لنبتة المليسه إلى عزل مركبين معروفين تم عزلهم للمرة الأولى من هذه النبتة وهم الأرتيميتين والهيسبيردين. لقد تم دراسة التأثير المسكن لهذه المركبات على الالم النسيجي باستخدام فحص الصفيحة الساخنة، لقد أحدث إعطاء هذين المركبين عن طريق التجويف البطني زيادة في فترة تحمل الألم عند الحيوانات وكانت الزيادة معتمدة على التركيز. ان التراكيز المحدثة لـ 50% من الاستجابة القصوى لمركب الارتيميتين والهيسبيرودين كانت 1.6×10^{-3} ملغم/كغ و 3.2×10^{-1} ملغم/كغ على التوالي. أشارة هذة الدراسة الى ان هذان المركبان يمتلكان تأثيراً مسكناً في الفئران والذي يبرر استخدام هذه النبتة في الطب الشعبي كمادة مسكنة.

Keywords: *Aloysia triphylla;* Artemitin; Hesperidin; Antinociceptive; Rat.

1. Introduction

Aloysia triphylla (Verbenaceae) is a perennial, bushy plant, originally from South America, and cultivated in various areas in the Middle East. *Aloysia triphylla* has long been used in traditional medicine. *Aloysia triphylla* has been reported to have a gentle sedative action and helps to counter depression (Guerrera et al., 1995: Chevallier, 1996; Pascual et al., 2001). An infusion of aerial parts of *Aloysia triphylla* is used as antipyretic, antispasmodic and diuretic agent (Guerrera et al., 1995; Ragone et al., 2007)). The plant has tonic effect upon the nervous system and has reputation for soothing abdominal discomfort (Guerrera et al., 1995). The plant has been found to possess antioxidant effect (Valentão et al., 2002). Phytochemical study of the plant revealed the presence of ganial, neral ,pinene, caryophyllene, limonene, curcumene, camphor, and luteolin 7-diglucuronide (Kim NS and Lee DS; 2004; Carnat, et al., 1995). Large amount of polyphenolic compounds were also isolated (Carnat, et al., 1999).

Despite of the traditional use of *Aloysia triphylla* as an analgesic, no systemic studies concerning the antinociceptive effects are available. In the present study, we are reporting the antinociceptive effects of two flavonoids (artemitin and hesperidin) which were isolated from the *Aloysia triphylla*.

2. Materials and Methods

2.1. Plant

Aerial parts of *Aloysia triphylla* (Verbenaceae) were collected from Hashemite university medicinal plant garden, Zarka, Jordan by (E.Y.Q) in April. The plant material was identified and authenticated taxonomically at the Hashemite university herbarium by a plant taxonomist.

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A voucher specimen (HU # 237) was deposited at the Hashemite university herbarium for future reference.

2.2. Extraction and isolation

The dried and finely powdered whole plant of *Aloysia triphylla* (4.0 kg) was exhaustively extracted with ethanol. The combined ethanol extracts were filtered and evaporated in vacuum to give a residue. The residue was later suspended in H_2O and fractionated with petroleum ether, ethyl acetate and n-butanol. The ethyl acetate extract was applied to a silica gel column with chloroform-ethyl acetate step gradients and finally purified on a Sephadex LH-20 column eluting with chloroform-methanol (1:1,v/v) to afford compound (1) (102 mg). The n-butanol extract was applied to RP-C18 column, using water-methanol step gradients and finally chromatographed repeatedly on a Sephadex LH-20 column eluted with methanol to yield compound (2) (120 mg). The structures of compound 1 and 2 were elucidated as artemitin and hesperidin, respectively (Figure 1). ^{1}H NMR was used to assign the structures of the two compounds.

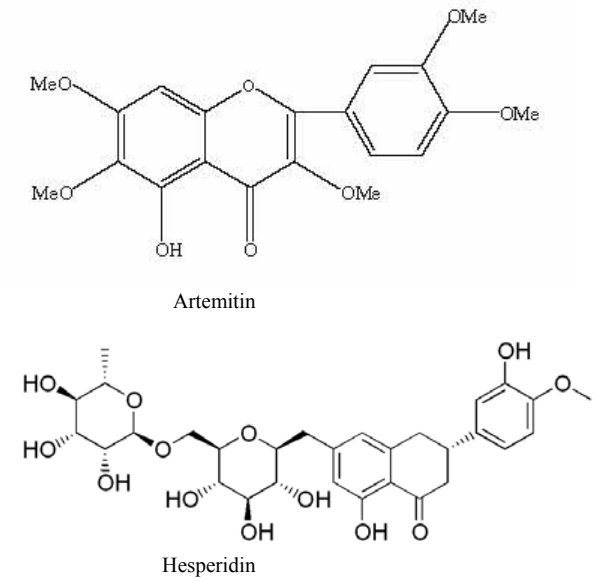

Figure 1. Chemical structure of artemitin and hesperidin.

2.3. Animals

Male mice (29-33 g), housed at 22-25ºC under a 12-h light/12-dark cycle and with access to food and water *ad libitum*, were used in the present study. The experiments were carried out in accordance with the current guidelines for the care of laboratory animals at Hashemite University and in accordance with the Ethical Guidelines for the Investigation of Experimental Pain in Conscious Animals (Zimmermann, 1983).

2.4. Hot-plate test

The hot plate test was assessed by using groups of male mice, each of 6 animals. The temperature of a hot plate was maintained at 50 ± 1ºC. Latency to a discomfort reaction (licking paws) was determined in seconds before and 60 min after intraperitoneal administration artemitin and hesperdin ($3x10^{-4}$- 10^1mg/kg). The cut-off time was 60s. The prolongation of the latency times was compared to the values of the control and used for statistical comparison. Baseline was considered as the mean of three readings of the reaction time obtained before administration of artemitin and hesperdin and was defined as the normal reaction time of animals to this temperature. Change in latency period (% of basal)) was calculated by the formula: (A-B/B) X 100, where A is the mean of three readings of reaction time after treatment taken within 5-7 minutes; B is the mean of three readings of reaction time obtained before treatment.

2.5. Statistical analysis

The values were expressed as the mean ± SEM. Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's test for multiple comparisons. Differences were considered significant when $P < 0.05$. ED_{50} was obtained by the best visual fit from the plot of the individual experiments.

3. Results and discussion

Phytochemical investigation of the aerial parts of *Aloysia triphylla* has led to the isolation of two compounds artemitin (102mg) and hesperidin (120 mg) (figure 1). Identification was on basis of ^{1}H NMR by comparison with data reported previously (Abu Zarga et al., 1995; Garg et al., 2001).

Artemitin and hesperdin significantly increased the time the animals took to raise their hind paw from the hotplate in a dose dependent manner (Figure 2 and 3). The ED50 values were 1.6 $x10^{-3}$ mg/kg and 3.2 x 10^{-1} mg/kg for artemitin and hesperidin, respectively. No mortality was observed during 48hr after drug administration.

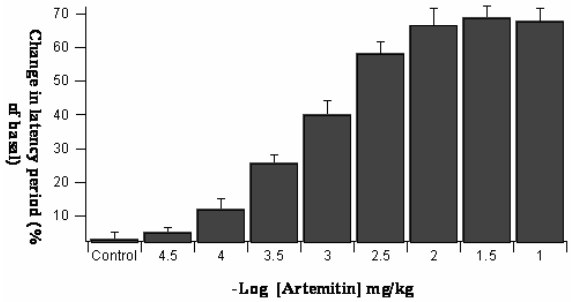

Figure 2. Effect of artemitin on the latency of mice submitted to the hotplate test.

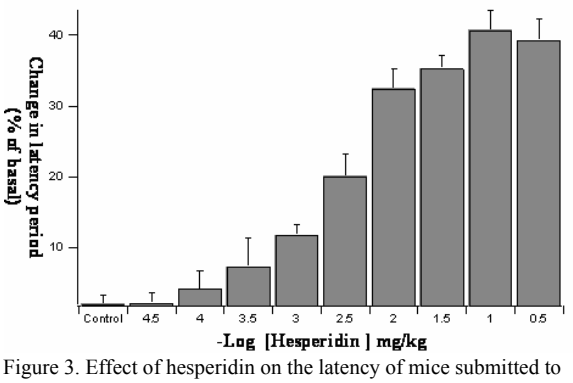

Figure 3. Effect of hesperidin on the latency of mice submitted to the hotplate test.

Artemitin is a bioflavonoid, which has been reported as a potential anticancer (Li et al., 2005) and chemopreventive and chemotherapeutic agent (Ko et al.,

2000). It has been claimed that it has an anti-inflammatory effect (Sertié et al., 1990). However, other studies showed that such claim is not justified (Bayeux et al., 2002). Additionally, artemitin was found to induce relaxation in smooth muscle (Abu Zarga et al., 1995). To the best of our knowledge, this the first report to show that artemitin possess an antinocicpetive activity. The mechanism by which artemitin induced an antinociceptive effects need further studies to be elucidated.

Hesperidin is a bioflavonoid, which has been reported to possess a wide range of pharmacological properties. It has been reported to have significant anti-inflammatory and analgesic effects (Galati et al., 1994). Several mechanisms have been suggested to explain such activity including: inhibition of histamine release (Emim et al., 1994); and inhibition of eicosanoid synthesis (Jean and Bodinier, 1994). Additionally, hesperidin was found to have central nervous system depressant effects (Marder et al., 2003). Recently, Loscalzo et al., (2008) showed that the effects of hesperidin were fully blocked by the nonselective opioid antagonist naltrexone, which may implicate opioid receptors on the antinociceptive effects of hesperidin.

In conclusion, results obtained from the present study indicate that the two flavonoids (artemitin and hesperidin) possess significant antinociceptive effects in mice which seem to justify the traditional analgesic use of *Aloysia triphylla.*

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