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Olive (*Olea europaea* L.) leaf extract elicits antinociceptive activity, potentiates morphine analgesia and suppresses morphine hyperalgesia in rats

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ABSTRACT

Aim of the study: Olive (*Olea europaea*) leaves are used as anti-rheumatic, anti-inflammatory, antinociceptive, antipyretic, vasodilatory, hypotensive, antidiuretic and hypoglycemic agents in traditional medicine. Recently, it has been shown that olive leaf extract (OLE) has calcium channel blocker property; however, its influences on nociceptive threshold and morphine effects have not yet been clarified.

Materials and methods: All experiments were carried out on male Wistar rats. The tail-flick, hot-plate and formalin tests were used to assess the effect of OLE on nociceptive threshold. To determine the effect of OLE on analgesic and hyperalgesic effects of morphine, OLE (6, 12 and 25 mg/kg i.p.) that had no significant nociceptive effect, was injected concomitant with morphine (5 mg/kg and 1 μg/kg i.p., respectively). The tail-flick test was used to assess the effect of OLE on anti- and pro-nociceptive effects of morphine.

Results: The data showed that OLE (50–200 mg/kg i.p.) could produce dose-dependent analgesic effect on tail-flick and hot-plate tests. Administration of 200 mg/kg OLE (i.p.) caused significant decrease in pain responses in the first and the second phases of formalin test. In addition, OLE could potentiate the antinociceptive effect of 5 mg/kg morphine and block low-dose morphine-induced hyperalgesia.

Conclusion: Our results indicate that olive leaf extract has analgesic property in several models of pain and useful influence on morphine analgesia in rats. Therefore, it can be used for the treatment and/or management of painful conditions.

1. Introduction

The management of pain is considered to be a major clinical problem. Opioids have been used for treating moderate to severe pain, but treatment with these drugs leads to the induction of side effects such as analgesic tolerance, physical dependence, emesis, constipation and drowsiness. Therefore, the finding of herbs that have analgesic property without hazardous side effects is essential skills for pain management.

The olive tree (*Olea europaea* L. [Family: Oleaceae]) has been cultivated in the Mediterranean for more than a thousand years. Not only the olive oil, but also the leaves have been used for medical purposes, and were introduced recently into the Pharmacopoea PhEur 5. In many countries, they are known as a folk remedy for hypertension and diabetes (Cherif et al., 1996). It has been traditionally used to cure rheumatic and neuralgic diseases in Lebenan (El Beyrouthy et al., 2008) and also to alleviate muscle and joint pain in some regions of Iran.

Olive leaf contains the active iridoid constituent oleuropein (chief constituent). Other secoiridoids include 11-demethyloleuropein, 7,11-dimethyl ester of oleoside, ligustroside, oleuroside, and un-conjugated secoiridoid aldehydes. Triterpenes and flavonoids, including luteolin, apigenin, rutin, and diosmetin, are also present. Oleasterol, leine, and glycoside oleoside have also found in the leaves (Briante et al., 2002).

It has been documented that olive leaf extract had Ca^{2+} channel-blocking activity (Gilani et al., 2005; Scheffler et al., 2008). Several investigators have reported that calcium ion has a physiological role in the regulation of pain sensitivity, and inhibition of calcium movement contributes to antinociception (Galeotti et al., 2004;

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Weiss and De Waard, 2006). Furthermore, L-type Ca^{2+} channel antagonists produce analgesia after peripheral and central administration (Miranda et al., 1993; Esmaeili-Mahani et al., 2006).

Due to the fact that calcium influx blockade is essential for normal opioid receptor signaling, Ca^{2+} channel antagonists have been shown to elevate antinociceptive effect of morphine (Fukuizumi et al., 2003; Esmaeili-Mahani et al., 2005) and suppress morphine-induced hyperalgesia (Esmaeili-Mahani et al., 2008).

Based on the facts that calcium influx is essential for pain perception and its blockade is necessity for normal opioid receptor signaling, the present study was designed to test the hypothesis that olive leaf extract could exert antinociceptive effects on chemical and thermal models of pain and influence morphine analgesic/hyperalgesic properties in rats.

2. Materials and methods

2.1. Animals

All experiments were carried out on male Wistar rats, weighing 200–250 g, that were housed four per cage under a 12 h light/dark cycle in a room with controlled temperature (22 ± 1 °C). Food and water were available *ad libitum*. Animals were handled daily (between 9:00 and 10:00 A.M) for 3 days, before the experiment day in order to adapt them to manipulation and minimize nonspecific stress responses. Rats were divided randomly into several experimental groups, each comprising 6–8 animals. All experiments followed the guidelines on ethical standards for investigation of experimental pain in animals (Zimmermann, 1983), and were approved by the Animal Experimentation Ethic Committee of Kerman Neuroscience Research Center (EC/KNRC/89-101-2).

2.2. Preparation of olive leaf extract

An ethanolic olive (*Olea europaea*; variety of *Sevillano*) leaf dry extract was prepared in Razi Herbal Medicines Research Center (Lorestan, Iran). There are different varieties of olive trees in some parts of Iran but the variety of *Sevillano* has the maximum oleuropein level (Hashemi et al., 2010). Olive leaves of Sevillano variety were collected from the Khoramabad Agricultural Research Orchard, Lorestan province, Iran, in August 2009. A sample was deposited at the herbarium of Lorestan Agricultural and Natural Resources Research Center with reference number 11505. Two hundred grams of the air-dried leaves was grinded into fine powder. The powder was extracted twice, on each occasion with 1 l of 80% ethyl alcohol. The collective ethanol extract was filtered, and the filtrate was concentrated to dryness under reduced pressure in a rotary evaporator and the resulting ethanol extract was freeze-dried. Quantification of some identified compounds of the extract using high performance liquid chromatography (HPLC) by our colleagues showed that oleuropein (356 mg/g), tyrosol (3.73), hydroxy tyrosol (4.89) and caffeic acid (49.41) were the main compositions of the olive leaf extract (Mohagheghi et al., 2010). The same extract was also used in this study.

2.3. Drugs

Aliquot portions of the crude olive leaf extract were weighed and dissolved in physiological saline plus dimethyl sulfoxide (DMSO) for use on each day of our experiments. The percentages of DMSO and saline in the final volume were 2% and 98%, respectively. Morphine hydrochloride (TEMAD, Iran) was dissolved in physiological saline. These drugs were given in the volume of 1 ml/kg (i.p.) and in a total volume of 10 μl (i.t.).

2.4. Intrathecal catheter implantation and drug delivery

Animals were anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg) i.p. An intrathecal catheter (PE-10) was implanted in each rat according to a previously published method (Yaksh and Rudy, 1976). Animals that exhibited neurological deficits (e.g., paralysis) after the catheter implantation or during drug delivery were excluded from the experiments.

2.5. Tail-flick test

Antinociception was assessed by tail-flick test (D'Amour and Smith, 1941). The tail-flick latency for each rat was determined three times and the mean was designated as baseline latency before drug injection. The intensity of the beam was adjusted to produce mean control reaction time between 2 and 4 s. The cut-off time was fixed at 10 s in order to avoid any damage to the tail. After determination of baseline latencies, rats received intraperitoneal injection of drugs, and the reaction latency was determined in different times after injection. The tail-flick latencies were converted to the percentage of antinociception according to the following formula:

$$\%\text{Antinociception (\%MPE)} = \frac{\text{reaction time of test} - \text{basal reaction time}}{\text{cut-off time} - \text{basal reaction time}}.$$

Hyperalgesia was assessed by a small modification in tail-flick test (Esmaeili-Mahani et al., 2007). The intensity of the beam was adjusted to produce a mean control reaction time between 4 and 6 s and the cut-off time was fixed at 15 s. In this manner, we were able to reveal potential, subtle alternations that may occur in basal thermal nociception. Experimentally induced decreases in control tail-flick latency provide an indication of hyperalgesic effect.

2.6. Hot-plate test

Rats were individually placed on a hot-plate maintained at 55 ± 0.2 °C and the time of licking of the hind paws or attempt to jump out of the beaker was recorded as the latency period. The cut-off time was 60 s to avoid tissue damage. Before drug administration, baseline latency was examined. The paw withdrawal latency was tested after drug administration. The maximum possible effect (MPE) was calculated as: MPE% = (latency after drug administration − baseline latency)/(60 − baseline latency) × 100.

2.7. Formalin test

The formalin test was carried out as described by Dubuisson and Dennis (1977). 10 μl of a 5% formalin solution in saline was injected in the dorsal surface of the left hind paw using a tuberculin syringe. Each animal was then placed in an observation chamber and monitored for 1 h. Severity of pain responses was recorded based on the following scale: (0) rats walked or stood firmly on injected paw; (1) the injected paw was favored or partially elevated; (2) the injected paw was clearly lifted off the floor; (3) the rats licked, chewed or shook the injected paw. This method of scoring allows a graded determination of responses thus showing finer degrees of antinociception as opposed to the method in which only the time the animal spent licking the injected paw is recorded. Antinociceptive effect was determined in two phases, an early acute phase and a late or tonic phase (0–5 and 15–60 min after formalin injection, respectively).