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Original article

Antinociceptive activity of *Euadenia trifoliolata* (Schum. & Thonn.) Oliv. leaves and roots in mice

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ABSTRACT

The leaves and roots of *Euadenia trifoliolata* are used in Nigeria traditional medicine for the treatment of ear ache, head ache and inflammation. The aim of the study was to evaluate the antinociceptive activity of ethanolic extract of the leaves (EL) and roots (ER) of *E. trifoliolata* in mice. Oral toxicity testing was performed using OECD guidelines. Antinociceptive effect was studied in mice using acetic acid-induced writhing, formalin, tail immersion and hot plate tests. Total polyphenolic contents were determined using standard methods. No mortality was recorded 24 h after oral administration of both EL and ER up to 5000 mg/kg. At the dose of 50, 100 and 200 mg/kg, administration of EL and ER resulted in significant reduction in the number of writhes compared to control. The percentage inhibition of writhings was calculated as 35.67%, 46.71% and 67.94% (EL) and 55.41%, 57.32% and 72.61% (ER), respectively. In hot plate test, EL and ER showed statistically significant antinociceptive effect, although low percentage inhibition (<50%) was recorded for ER at all the doses tested. Only EL (100 and 200 mg/kg) significantly ($p < 0.001$) increased the reaction time in tail immersion test. Both extracts significantly ($p < 0.001$) reduced the licking time in both phases of formalin test compared to control. The content of total phenolic, flavonoid and proanthocyanidin varies between the two extracts and may be the basis of the observed antinociceptive effect. The results indicate antinociceptive activity for the leaves and roots of *E. trifoliolata*, with the extract of the leaves showing better activity.

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1. Introduction

Over the years, natural products have contributed enormously to the development of important therapeutic drugs used in modern medicine and one of the most important analgesic drugs employed in clinical practice today continues to be the alkaloid morphine.[1] In spite of this advancement, many traditional medicinal plants have not been scientifically evaluated in order to provide evidence of their efficacy. One of such plants is *Euadenia trifoliolata*.

E. trifoliolata (Schum. & Thonn.) Oliv. (Capparaceae) is a leafy shrub that grows up to 4 m high and is found in the dense forest in Nigeria, Gabon, Ghana and Cameroon.[2] The leaves are trifoliate, on 6 inch long or longer petioles. The leaflets are elliptical, with the central one narrowed below and the lateral leaves more or less ovate-elliptical and oblique at the base.[3]

The leaves are eaten as potherb, and a decoction of leaves is taken as a tonic and anti-anaemic. The decoction of the leaves and roots or root sap is given in nasal instillation for headache and earache and in the treatment of inflammation.[2,4] The roots emit a strong smell and are also used for the treatment of chest, kidney and general pains. Some other uses of the plant include its use in the treatment of chronic wound[5] and as mild aphrodisiac.[6]

The search for literature on this species, to the best of our knowledge, yielded no previous pharmacological and chemical reports. Moreover, we found no relevant literature substantiating the use of the plant in the management of pain. The purpose of the present study, therefore, is to evaluate the antinociceptive activity of the ethanolic extract of *E. trifoliolata* leaves and roots using different models of pain in mice.

2. Materials and methods

2.1. Plant materials

Fresh leaves (EL) and roots (ER) of *E. trifoliolata* were collected from Abatadu village, in Ikire, Osun state, Nigeria (7°30′N 4°30′E) in

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February, 2013. The plant was botanically identified and authenticated by Mr. T.K. Odewo, Herbarium unit, Department of Botany, University of Lagos, Nigeria. A voucher specimen was made and deposited at the Herbarium with voucher specimen number, LUH 5617. The leaves and roots were cleaned, air dried for 14 days and crushed into coarse powder using a grinder (Christy and Morris Limited, England).

2.2. Preparation of extracts

The extract of EL and ER was prepared by macerating 300 g and 400 g of the dried powdered samples in 1 L and 1.5 L of absolute ethanol respectively, at room temperature for 48 h. Each extract was then filtered using Whatman's filter paper and evaporated *in vacuo* at 40 °C using the rotary evaporator (Buchi, England). The yield of the extracts was 5.36% and 4.16% for EL and ER, respectively.

2.3. Phytochemical study

2.3.1. Preliminary phytochemical screening

Qualitative phytochemical screening was carried out to test for the presence of phytochemical constituents (alkaloids, tannins, saponins, anthraquinones, glycosides, flavonoids, phenols and terpenoids) using standard procedures.[7]

2.3.2. Determination of total phenolic content

Total phenol was evaluated using Folin Ciocalteu reagent.[8] The extracts (1 mg/ml, 1 ml) were mixed with 2.5 ml of Folin Ciocalteu's reagent and 2 ml aqueous Na_2CO_3 (75 g/L) solution. The mixtures were allowed to stand for 30 min, centrifuged and absorbance was recorded at 765 nm using a Pg instruments T80 UV–Vis spectrophotometer. The standard curve was prepared using gallic acid (0.01–0.05 mg/ml)) in methanol. The curve was established by plotting absorbance against concentration (mg/ml) ($y = 19.063x + 0.23642$; $R^2 = 0.9853$). Total phenol content was expressed in terms of gallic acid equivalent (GAE)/g of dried extract.

2.3.3. Determination of total flavonoid content

Total flavonoid content was determined by the $AlCl_3$ method, using quercetin as standard.[8] The test samples were dissolved in methanol. The sample solution (1 ml) was mixed with 1 ml of $AlCl_3$ (2%). After 10 min of incubation at ambient temperature, the absorbance of the supernatant was measured at 435 nm. The total flavonoid content was expressed as quercetin equivalent (QE)/g of dried extract. For the quercetin, the curve was established by plotting absorbance against concentration (mg/ml) ($y = 19.397x - 0.1196$; $R^2 = 0.9665$).

2.3.4. Determination of total proanthocyanidin content

Proanthocyanidin content was determined using the method of Sun et al.[9] The extracts (0.5 ml, 1 mg/ml) were mixed with 3 ml of 4% vanillin–methanol solution and 1.5 ml hydrochloric acid and the mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm and the result presented as catechin (CE) equivalent/g of dried extract. The standard curve was prepared using catechin (0.01–0.05 mg/ml)) in methanol. The curve was established by plotting absorbance against concentration (mg/ml) ($y = 4.92x + 0.1369$; $R^2 = 0.9825$).

2.4. Animals

Albino mice (15–30 g) of male sex were used in this study. They were purchased from a private firm (Korede Farm Ltd, Ikeja, Lagos) and maintained for two weeks in the Animal House of College of Medicine, University of Lagos, Nigeria. The animals were fed with standard mouse cubes (Livestock Feed PLC, Ikeja, Lagos, Nigeria), given water ad libitum and maintained under well-ventilated conditions of 12 h light cycle. The experimental procedures used in this study conform to the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research.[10] The experiments were performed with the permission of the Institutional Animal Ethical Committee of University of Lagos (CM/COM/08/VOL.XXV).

2.5. Acute toxicity

Toxicity studies were performed for EL and ER using Organization for Economic Cooperation and Development (OECD) guidelines-420: acute oral toxicity-fixed dose procedure.[11] The animals were fasted overnight before the start of the experiment. The animals were divided into four groups of five animals and doses of extracts starting from 500, 1000, 2000 and increasing up to 5,000 mg/kg body weight were given, and signs and symptoms of toxicity were observed for the first four hours and for mortality for 24 h and further for seven days.

2.6. Evaluation of antinociceptive activity

2.6.1. Acetic acid-induced writhing test

The acetic acid-induced abdominal writhing test was performed according to the procedures described previously[12] with slight modification. One hour prior to injection of 0.6% acetic acid (10 ml/kg, i.p.), mice received EL or ER (50, 100 and 200 mg/kg, p.o.), 1% Tween 20 or acetylsalicylic acid (ASA, 100 mg/kg, p.o.). Each animal was placed in a transparent observation cage and the number of writhes per mouse was counted for 30 min. The writhing activity consists of a contraction of the abdominal muscles together with a stretching of the hind limbs. The percentage of inhibition was calculated using as follows:

$$\%\ \text{Inhibition} = \frac{(\text{mean of control} - \text{mean of treated})}{(\text{mean of control})} \times 100$$

2.6.2. Hot plate test

Pain reflexes in response to a thermal stimulus were performed at a fixed temperature of 55 ± 0.5 °C and has described by Ibrahim et al.[13] Five groups of six mice fasted overnight were used in this experiment. Pre-treatment reaction for each mouse was determined after which treatment was carried out as follows: distilled water (10 ml/kg, p.o.), morphine (10 mg/kg, s.c.) and extracts (50, 100 and 200 mg/kg). The reaction time (hind paw licking or jumping) of each mouse was then determined 60 min post-treatment. Post-treatment cut-off time of 20 s was used. Antinociceptive response expressed as percent inhibition was calculated as follows:

$$\%\ \text{Inhibition} = \frac{(\text{post-treatment latency} - \text{pre-treatment latency})}{(\text{cut-off time} - \text{pre-treatment latency})} \times 100$$

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