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Original article

Preclinical evidence of the anxiolytic and sedative-like activities of *Tagetes erecta* L. reinforces its ethnobotanical approach

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ABSTRACT

Morelos State is one of the regions of Mexico where several plant species are used in traditional medicine. Species from *Tagetes* genus (Asteraceae) are reported as useful in infusion to treat stomachache and intestinal diseases, but also as tranquilizers. In this study, medicinal uses of *T. erecta* including its depressant effect on the central nervous system (CNS) were explored by interviewing healers and merchants of local markets of Morelos State, and by investigation of the phytochemical and pharmacological tranquilizing properties. Specific anxiolytic and/or sedative-like responses of different doses of *T. erecta* (10, 30 and 100 or 300 mg/kg, i.p.) were investigated using experimental models in mice such as: open-field, exploration cylinder, hole-board, and the barbituric-induced hypnosis potentiation. The possible anxiolytic mechanism of action was assessed in the presence of WAY100635 (0.32 mg/kg, i.p.) and flumazenil (10 mg/kg, i.p.), antagonists of 5-HT_{1A} and GABA/BDZs receptors, respectively. Individual flavonoids reported in this species were also evaluated in these experimental models. As a result of this study, healers and merchants from ten local regions of Morelos State recommended *T. erecta* flowers as an infusion or as a tincture for several culture-bound syndromes associated with CNS, among others. Anxiolytic and sedative-like activities of the *T. erecta* aqueous and organic polar extracts were corroborated in these models associated to a participation of rutin, kaempferol, quercetin, kaempferitrin, and β-sitosterol constituents; where 5-HT_{1A}, but not BDZs, receptors were involved as anxiolytic mechanism of action. These data support the anxiolytic and sedative-like properties of *T. erecta* in traditional medicine by involving mainly serotonergic neurotransmission because of the presence in part of flavonoids and the terpenoid β-sitosterol.

1. Introduction

At global level there is a high occurrence of mental disorders, where anxiety is one of the most prevalent [1]. Around the world, medicinal plants, mainly those aromatics, are commonly used because of their tranquilizer properties; it is the case of *Tagetes* genus [2–4]. This genus belongs to the tribe Tageteae, which refers 56 species distributed among the North and South America. Approximately 24–30 species of them are in Mexico, considered a country center of high biodiversity [5]. As aromatic plants, *Tagetes* species are used as ceremonial, ornamental, in production of essential oils [6], as natural larvicide [7], insecticide [8], and antibacterial [9,10]. In addition, it is used in respiratory inflammation, as antioxidant [11], and cytotoxic [12,13]; as well as against wrinkles [14] and for its antidiabetic and antilipidemic potential [15].

Tagetes erecta L. is commonly known as *cempasúchil* or marigold [16,17]. It is an appreciated species of Mexico because of the use in traditional ceremonies or rituals, such as in "día de muertos" (day of the dead) or to clean house from bad spirits. Furthermore, it is known in India and used for worship like *Vat-savitri vrat* [18] or in Nepal especially used during festivals [19], like in Tihar festival.

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Recently, it has been preliminary explored its activity in central nervous system (CNS) as analgesic [20] and antidepressant [21]; however, it has not been investigated at all its anxiolytic and/or sedative-like effects.

Phytochemical studies have reported the presence of essential oils [22], terpenes like β-sitosterol and lupeol, and alkaloids [23,24]. It has also been reported the presence of flavonoids such as kaempferol, isorhamnetin, quercetin, patuletin, quercetagetin, myricetin and luteolin [25–28], and recently an m-coumaric acid [29].

In order to recognize the potential use of *T. erecta* as an anxiolytic medicinal plant, in this study we integrated information from an ethnobotanical, and pharmacological study recognizing its anxiolytic and/or sedative-like activity. It was applied open interviews about the uses of marigold conducted to hear the voice of the healers and merchants using medicinal plants of the State of Morelos, Mexico. Once the interviews were performed, data from pharmacological evaluation in experimental models were obtained from preparation of aqueous and organic extracts, and analyzing the possible anti-anxiety mechanism of action by exploring the participation of GABA/BDZ and 5-HT_{1A} receptors and the effect of individual compounds as possible bioactive metabolites.

2. Material and methods

2.1. Ethnobotanical survey

To know the general medicinal uses of *T. erecta,* personal interviews were applied to eight healers and twenty-five merchants of medicinal plants in the localities of Alpuyeca, Amatlán of Quetzalcóatl, Cuernavaca, Cuautla, Temixco, Tepoztlan, Tetela del Volcán, Tlayapacan, Yautepec and Yecapixtla in Morelos State, México.

For the interviews, healers were chosen because they are specialists in the diagnosis of cultural diseases and in the use and management of vegetal species as alternative therapy of anxiety or for "nervios". Direct interviews were also made to merchants since they are considered qualified employees for sale of medicinal plants. Questions included in questionnaires were associated to terms of cultural filiations equivalent to anxiety as follows: What is the condition of "nervios"?, What plants are used for the treatment of "nervios"? When the interviewee did not mention *T. erecta* we asked: What are marigold uses?

Answers were gathered by writing or in some cases audio-recording the interviews. It is important to mention that healers and merchants gave their consent for the use of ethnobotanical information after briefly explaining the project. The analysis of the interviews was only qualitative.

2.2. Plant material

Flowers of *T. erecta* were collected in Huertas of San Pedro, municipality of Huitzilac, Morelos, Mexico, near to the forest of oak (19° 00′ N and 99° 15′ W, altitude of 2250 m) in November 03, 2012. The species identification was confirmed by the taxonomist M.Sc. Abigail Aguilar Contreras. A voucher specimen was deposited in the Herbarium of the Instituto Mexicano del Seguro Social in Mexico City (No. IMSSM-15987).

2.3. Extracts preparation

Fresh material was dried at room temperature and under shade conditions. Flowers were weighed (80 g) and finely cut for processing by maceration in ethanol (1.2 L) a room temperature. The liquid extract was obtained every 72 h for three times and the solvent completely removed in a rotatory evaporator (Büchi R-210, Switzerland) under reduced pressure for three times. The *T. erecta* ethanol crude extract yielded 10.65 g (13.31% d.w). A sample of this crude extract (1 g) was percolated on silica gel (182 g) to obtain three different fractions employing solvents in the increased polarity as follows: hexane (500 mL), ethyl acetate (750 mL) and ethanol (600 mL) to yield 0.02 g (2% d.w.), 0. 41 g (41% d.w.) and 0.39 (39% d.w.), respectively.

For the *T. erecta* aqueous extract, flowers were infusing (39 g in 800 mL of distilled water) by inserting them into a baine-Marie for 1 h. Subsequently, the liquid was frozen in Florentine flasks with liquid nitrogen to be placed in a Labconco freeze dry/SHELL to obtain after 12 h of process, an aqueous dry extract which yielded 5.61 g (14.39% d.w.).

2.4. Pharmacological approach

2.4.1. Animals

Male Swiss Webster mice (25–30 g) were used in the pharmacological evaluation. Animals were provided by Instituto Nacional de Psiquiatría "Ramón de la Fuente Muñiz", they were kept at a controlled temperature of (22° ± 1 °C) with light/dark cycle of 12 h and fed *ad libitum* with standard water and food. The acute toxicity test and the anxiolytic and sedative-like exploration were carried out in the Institute following the specifications issued by the Committee of Ethics and Research of that institution with the approval of the project number NC-123280.0, as well as according to the Official Mexican Norm for the care and handling animal (NOM-062-ZOO-1999), and the international rules of care and use for laboratory animals.

2.4.2. Drugs

Kaempferol, kaempferitrin, quercetin, rutin, β-sitosterol, WAY100635, flumazenil, Tween 80, dimethyl sulfoxide (DMSO) were purchased at Sigma (St. Louis MO, USA). Diazepam (DZP) and sodium pentobarbital (SP) were adquired from Psicofarma S.A. de C.V. and PISA Farmacéutica (Mexico, City), respectively.

All the extracts (10–100 or 300 mg/kg), fractions (10–100 or 300 mg/kg) and pure compounds (3 or 10 mg/kg) were used in fresh preparation and intraperitoneally (i.p.) administered at a volumen of 0.1 mL/10 g body weight. Vehicle consisted in 0.5% tween 80 in saline solution (s.s. 0.9% NaCl) or distilled water depending of their solubility. DZP was suspended in 0.5% tween 80 and SP in s.s. Silica gel 60 was purchased from Merck, S.A. de C.V. Individual flavonoids kaempferol, kaempferitrin, quercetin, rutin, and the terpene β-sitosterol were used in the anxiolytic and sedative evaluation as possible bioactive compounds.

2.4.3. Acute toxicity

The aqueous and ethanol extracts were given in a preliminary maximal dosage of 2000 mg/kg, i.p. according to the OECD [30]. After administration mice were kept under observation for the following 14 days to register mortality. Survived mice were sacrificed in a CO_2 gas chamber to determine macroscopic alterations of tissues. If mice showed death at this highest dosage, then dose was reduced to 1000 and 100 mg/kg. Lethal dose fifty (LD_{50}) was calculated by i.p. route using a modified method reported by Lorke [31] where a minimum of experimental animals is required to calculate LD_{50}-value as parameter of acute toxicity.

2.4.4. Pharmacological evaluation

All mice were adapted to the experimental handling for three days prior to the pharmacological tests. For each treatment the animals were separated into groups of at least six mice. The tests were conducted 60 min after administration in mice receiving aqueous (10, 30, 100 mg/kg, i.p.) or organic (10, 30, 100, 300 mg/kg,