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Original article

Study of the effect of extract of *Thymus vulgaris* on anxiety in male rats

Alireza Komaki*, Faeghe Hoseini, Siamak Shahidi, Negar Baharlouei

Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

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ABSTRACT

There is some evidence in traditional medicine for the effectiveness of *Thymus vulgaris* (百里香 bǎi lǐ xiāng) in the treatment of anxiety in humans. The elevated plus-maze (EPM) has broadly been used to investigate anxiolytic and anxiogenic compounds. The present study investigated the effects of extract of *T. vulgaris* on rat behavior in the EPM. In the present study, the data were obtained from male Wistar rats. Animals were divided into four groups: saline group and *T. vulgaris* groups (50 mg/kg, 100 mg/kg, and 200 mg/kg infusion for 7 days by feeding). During the test period, the total distance covered by animals, the number of open- and closed-arm entries, and the time spent in open and closed arms of the EPM were recorded. *T. vulgaris* increased open-arm exploration and open-arm entry in the EPM, whereas extract of this plant has no effects on the total distance covered by animals and the number of closed-arm entries. The results of the present experiment indicate that *T. vulgaris* may have an anxiolytic profile in rat behavior in the EPM test, which is not influenced by the locomotor activity. Further research is required to determine the mechanisms by which *T. vulgaris* extract exerts an anxiolytic effect in rats.

1. Introduction

Anxiety disorders are among the most common psychiatric illnesses.[1] Anxiety is characterized by a diffuse, unpleasant, vague sense of apprehension. It is often accompanied by autonomic symptoms, such as headache, perspiration, palpitations, tightness in the chest, and mild stomach discomfort.[2] Benzodiazepines are the major class of compounds used to treat anxiety, and they have remained the most commonly prescribed drugs for anxiety.[3] However, the realization that benzodiazepines present a narrow safety margin between the anxiolytic effect and those causing unwanted side effects has prompted many researchers to evaluate new compounds in the hope that other anxiolytic drugs will have less undesirable effects.[3]

Medicinal plants have been used from ancient times for their medicinal values as well as to impart flavor to food.[4] Plants have been used in the management of illnesses since antiquity and has continuously grown over time as complementary medicine, because they are readily and cheaply available healthcare alternatives.[5] Nowadays, there is a growing interest in the use of crude extracts and dry powder samples of medicinal and aromatic plants and for the development and preparation of alternative traditional medicine and food additives.[6,7] Drugs derived from traditional herbs may have possible therapeutic relevance in the treatment of anxiety. Research has been conducted to investigate natural anxiolytic agents in the search for an alternative.[3]

Approximately 150 species of *Thymus* are abundantly found, mainly in Asia, Africa, and North America. Recently, its range has been widely been extended to the Iberian Peninsula, with most of the species being endemic.[4] *Thymus vulgaris* L. (百里香 bǎi lǐ xiāng; Lamiaceae) is a medicinal plant belonging to the Lamiaceae family.[8,9] In folk medicine, some *Thymus* spp. are used for their anthelminthic, expectorant, antiseptic, antispasmodic, antimicrobial, antifungal, antioxidative, antivirotic, carminative, sedative, and diaphoretic effects. They are usually administered by infusion or are used externally in baths to cure rheumatic and skin diseases.[8,10] Thyme contains high concentrations of phenols, including thymol (12–61%), carvacrol (0.4–20.6%), 1,8-cineole (0.2–14.2%), q-cymene (9.1–22.2%), linalool (2.2–4.8%), borneol (0.6–7.5%), a-pinene (0.9–6.6%), and camphor (0–7.3%). Carvacrol and thymol are the main phenolic components that are primarily responsible for its antioxidative activity.[11] In addition, thyme oil is widely used in phytotherapy, most notably to treat and offer protection from acne,

* Corresponding author. Neurophysiology Research Center, Department of Physiology, Hamadan University of Medical Sciences, Shahid Fahmideh Street, Hamadan 65178-3-8736, Iran.
E-mail address: alirezakomaki@gmail.com (A. Komaki).
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hypertension, infections, and cancers.[12] The oil contains bioactive monoterpenes such as thymol, carvacrol, and linalool.[13]

There are a variety of animal tests for the investigation of anxiolytic effects of substances.[14] The elevated plus-maze (EPM), a well-established animal test causing a fear status by comprehensible stimuli and the use of innate behavior of animals, is one of the most widely used models to assess anxiety in small rodents, and is a validated and reliable test for detecting both anxiolytic- and anxiogenic-like effects of agents.[15,16] In this animal model, an anxiolytic effect is evaluated by the relation of the entries into the open arms to the total entries and the time spent on the open arms of the EPM, in comparison with the same parameters of the control group. An increase of the time and proportion of the entries into the open arms without a changed locomotor activity is regarded as a powerful marker for an anxiolytic substance effect.[17] Locomotor activity of the animals was assessed by measuring the total distance travelled by them.[18,19] There are no published reports in the literature about the effect of the extract of *T. vulgaris* on anxiety. On the basis of these considerations, this study was designed to characterize the anxiolytic-like activity of extract prepared from *T. vulgaris* leaves, using an EPM test.

2. Materials and methods

2.1. Preparation of plant extract

Leaves of *T. vulgaris* (百里香 bǎi lǐ xiāng) were collected in spring and identified at the Botanic Institute of this University. The plant material was dried at 40°C with air circulation, ground, and extracted with 70% ethanol by percolation at room temperature. The extract was then taken to the laboratory for the process of evaporation. The evaporation process involved complete removal of ethanol and water used for the extraction. The extracts were dried at 40°C under vacuum and finally freeze dried.[20] Pharmacological assays were carried out with aqueous suspensions of the dried extract. The doses are expressed as milligrams of dried extract per kilogram of rat body weight. The extracts were redissolved in their solvents prior to each individual experiment.[21]

2.2. Animals

Male Wistar rats, weighing 230–250 g, were transported from the animal house to a room adjacent to the test laboratory 72 hours prior to the test. They were housed in groups of five per cage under a 12:12 dark/light cycle (lights on at 07:00 AM) at 22 ± 2°C and given free access to food and water. Rats were randomly assigned to different treatment groups ($n = 10$). Animals were tested under the same experimental conditions. All experiments were carried out in a quiet room under controlled light conditions between 11:00 AM and 3:00 PM. Behavioral observations were conducted in soundproof rooms at the same period of the day to reduce the confounding influence of diurnal variation on spontaneous behavior. Each animal was tested only once. All research and animal care procedures were approved by the Veterinary Ethics Committee of the Hamadan University of Medical Science, and were performed in accordance with international standards of animal welfare recommended by the Society for Neuroscience (Handbook for the Use of Animals in Neuroscience Research, 1997). The minimum number of animals and the minimum duration of observation required to obtain consistent data were used.

2.3. EPM test

The EPM design was similar to that originally described by Lister.[22] In brief, the apparatus was composed of two open (50 cm × 10 cm × 1 cm) and two enclosed (50 cm × 10 cm × 50 cm) arms, which were radiated from a central platform (10 cm × 10 cm) to form a plus sign. A slightly raised edge on the open arms (1 cm) provided an additional grip for the animals. The plus-maze was elevated to a height of 50 cm above the floor level by a single central support. *T. vulgaris* extract were administered orally in three doses (50 mg/kg, 100 mg/kg, and 200 mg/kg infusion for 7 days by feeding). Then animal behavior in the EPM were videotaped for 10 minutes and saved on a computer.

The number of entries into and the time spent in each of the two types of arms were counted during a 10-minute test period. The open-arm entries and open-arm time were used as indices of anxiety, and the number of entries into the closed arms as an indicator of the reduction of spontaneous locomotion in rats. A rat was considered to have entered an arm when all its four paws were on that arm.

2.4. Statistical analysis

Results are expressed as mean ± standard error of the mean. The difference between the means was determined by one-way analysis of variance, followed by Tukey *post hoc* analysis. In all cases, differences were considered significant if $p < 0.05$.

3. Results

The effects of different doses of hydroalcoholic extract of *T. vulgaris* (百里香 bǎi lǐ xiāng) on the percentage of entries into the open arms are shown in Fig. 1. One-way analysis of variance indicated that, compared with the control group, extract of *T. vulgaris* caused an increase in the percentage of entries into the open arms. Tukey-post-test analysis showed that *T. vulgaris* exhibited a significant increase in the percentage of entries into the open arms at concentrations of 100 mg/kg ($p < 0.05$) and 200 mg/kg ($p < 0.01$), but not at 50 mg/kg, in comparison with the control group.

The effects of the different doses of *T. vulgaris* extract on the duration of time spent in the open arms are shown in Fig. 2. One-way analysis of variance indicated that, compared with the control group, the *T. vulgaris* extract-treated groups spent more time in the open arms. Tukey-post-test analysis showed that the extract-treated groups spent more time in the open arms at the dose of 200 mg/kg ($p < 0.05$).

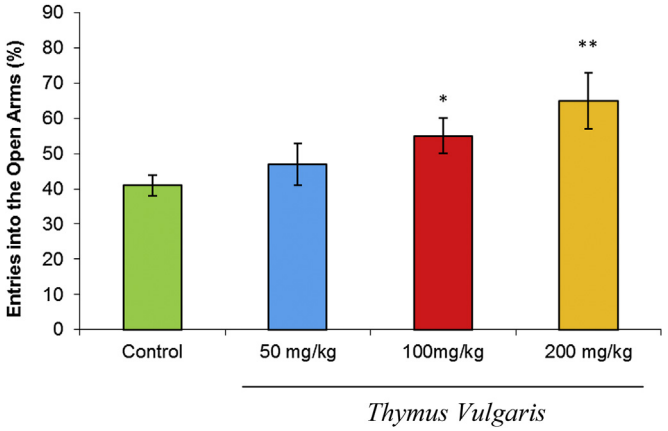

Fig. 1. Effects of *T. vulgaris* extract (50 mg/kg, 100 mg/kg, and 200 mg/kg) on the percentage of entries into the open arms of the EPM during the 10-minute test session ($n = 10$). Data are expressed as mean ± SEM. Comparisons were made using ANOVA followed by *post hoc* Tukey's multiple comparison test. $^{*}p < 0.05$. $^{**}p < 0.01$. ANOVA = analysis of variance; EPM = elevated plus-maze; SEM = standard error of the mean.