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Original article

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

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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 antihelminthic, 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,

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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 sound-proof 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 ($p < 0.05$).

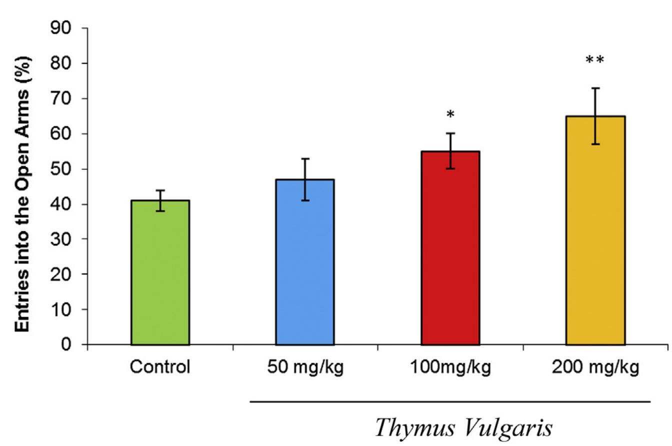

Fig. 1. Effects of *T. vulgaris* extract (50 mg/kg, 100 mg/kg, and 200 mg/kg) on the percentage of entries into the open arms of the EPM during the 10-minute test session ($n = 10$). Data are expressed as mean ± SEM. Comparisons were made using ANOVA followed by *post hoc* Tukey's multiple comparison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

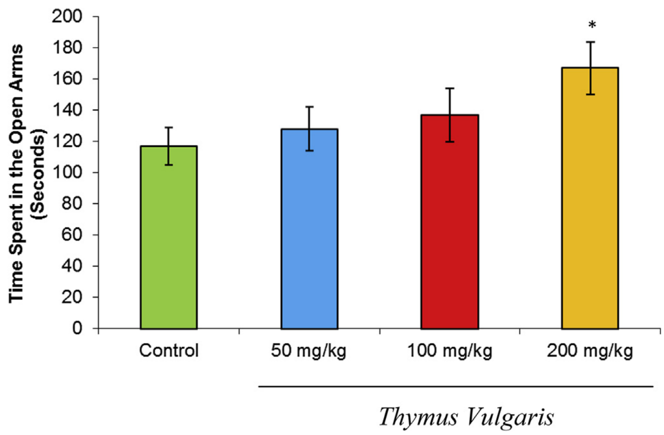

Fig. 2. Effects of *T. vulgaris* extract (50 mg/kg, 100 mg/kg, and 200 mg/kg) on the time spent in the open arms during the 10-minute test session ($n = 10$). $^{*}p < 0.05$. $^{**}p < 0.01$.

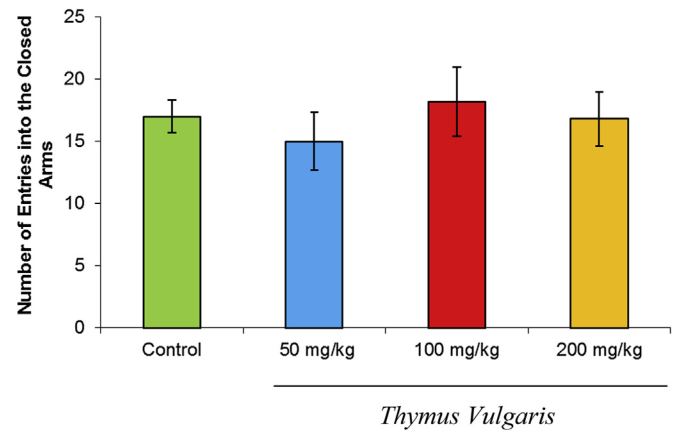

Fig. 4. Effects of *T. vulgaris* extract (50 mg/kg, 100 mg/kg, and 200 mg/kg) on the number of closed-arm entries during the 10-minute test session ($n = 10$).

The total distance covered by the *T. vulgaris* extract-treated rats during the 10-minute test was not significantly ($p > 0.05$) different from controls (Fig. 3). The number of entries into the closed arms also was not significantly different between the *T. vulgaris*-treated group and the control group (Fig. 4).

4. Discussion

Plants were used for medicinal purposes long before recorded history, and their utilization in medication is still well disseminated around the world.[23] Many plants exert recognized medicinal effects on the central nervous system, and are able to act on chronic conditions such as anxiety and depression that do not respond well to conventional therapeutic treatments.[24] Various types of herbal medicines have been used as anxiolytics in different parts of the world.[3] On the basis of these considerations, the purpose of this study was to characterize the anxiolytic-like activity of the hydroalcoholic extract prepared from *T. vulgaris* (百里香 bǎi lǐ xiāng). The results of the present study demonstrated that the extract of *T. vulgaris* increased the percentage of both the entries and the time spent in the open arms of the maze. Therefore, the extract was able to produce anxiolytic effect in rats after the 1-week oral administration. The effect of *T. vulgaris* was not induced by changes in motor activity at these doses, because the total distance covered by the rats was not altered. An increase in the time and proportion of the entries into the open arms lacking a changed locomotor activity is confirmed as a potent sign of an anxiolytic substance effect.[15]

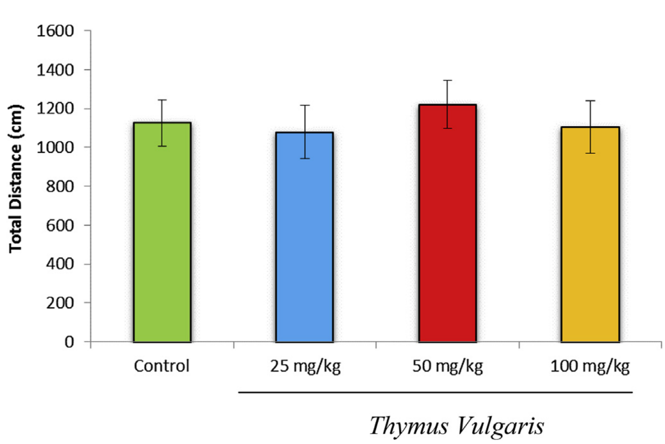

Fig. 3. Effects of *T. vulgaris* extract (50 mg/kg, 100 mg/kg, and 200 mg/kg) on the total distance covered by the rats during the 10-minute test session ($n = 10$ in each group).

Many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at a single target site or at multiple target sites.[25] *T. vulgaris* is a well-known herb that is widely cultivated in many regions of the world.[13,26] *T. vulgaris* is used mainly as a food seasoning, but also as a source of essential oils that are used in perfumery and as a worming and bactericidal agent in medicine.[27] Additionally, thyme is known to contain a high concentration of phenolic compounds, such as thymol and carvacrol, which are found in its essential oils. Fecka and Turek[28] found phenolic compounds in wild thyme, with caffeic acid and rosmarinic acid derivatives being the most important of these. Several authors already reported that flavonoid groups exhibited a wide range of biological activities, such as antioxidant, anti-inflammatory, antimicrobial, antiangiogenic, anticancer, and anti-allergic effects.[29–31] Reports indicate that the volatile oils of thyme are among the main essential oils used in the food industry and in cosmetics as preservatives and antioxidants.[32] *T. vulgaris* essential oil is a mixture of monoterpenes. The main compounds of this oil are the natural terpenoid thymol and its phenol isomer carvacrol,[33] which have antioxidative, antimicrobial, antitussive, expectorant, antispasmodic, and antibacterial effects.[34,35] Accordingly, it has been reported that ethanol thyme extract may be used as a natural antioxidant to prolong the stability of oils.[36] In addition, it could be concluded that the essential oil of *T. vulgaris* has a potential antioxidant activity and a protective effect against toxicity of aflatoxins, and this protection is dose dependent.[37]

Extensive research has been conducted to reveal multiple neural substrates and mechanisms that contribute to the etiology of depression and anxiety, among which the imbalance between oxidation and the antioxidant defense system has gained attention.[38] Some studies have demonstrated the role of oxidative stress in anxiety of rodents.[39,40] Furthermore, it has recently been reported that two agents that induce oxidative stress, L-buthionine-(S,R)-sulfoximine and xanthine plus xanthine oxidase (X + XO), cause increased anxiety-like behavior in rats.[41] Moreover, increasing evidence suggests that the impairment of antioxidant defense and neuronal cell death are important in the process of emotional disorders, such as depression and anxiety.[38] Accordingly, Masood et al[42] reported that the induction of oxidative stress in mouse hypothalamus occurs in parallel with anxiety. Consumption of diets with high levels of sucrose was reported to increase the oxidation of proteins in the frontal cortex and to cause anxiety in rats. Increased anxiety has been correlated positively with the increase of reactive oxygen species levels. In another study, oxidative stress in the hippocampus of adult rats was reported to be anxiogenic.[43] Interestingly, the induction of oxidative stress by a nonpharmacological method also leads to anxiety-like behavior in rats.[40] Moreover, the increase in anxiety-like behavior is reversed by treatment with the antioxidant tempol, suggesting direct involvement of oxidative stress in mediating anxiety-like behavior in rats.[41] Tempol is a water-soluble and cell-membrane-permeable molecule,[44] with demonstrated antioxidant activity in various biological systems.[45] Moreover, it has been shown that oxidative stress (L-buthionine-(S,R)-sulfoximine)-induced anxiety-like behavior was prevented with antioxidant tempol treatment in rats.[40] The antioxidant effect of plant extracts *in vitro* is probably caused by their ability to act as reducing agents and free radical scavengers or as quenchers of singlet oxygen formation. Some authors ascertained the fact that phenolic compounds were able to chelate metal ions. Melidou et al[46] found that intracellular binding of iron is responsible for the protection offered by flavonoids against H_2O_2-induced DNA damage. On the other hand, complexation of plant extracts with metal ions results in a significant reversal from antioxidant to pro-oxidant properties for the resulting complexes.[47]

It has been reported that thyme oils find wide use in dietary supplementations due to their antioxidant property.[48] Several researchers have evaluated the antioxidant properties of extracts from different herbs and spices in lipid systems.[49] Phenolic monoterpenes in thyme, thymol, and carvacrol are the primary compounds that have been reported to possess a high antioxidant activity.[32,50] Youdim and Deans[51] measured changes in antioxidant enzyme activity of different organs during the lifetime of rats. They found out that dietary supplementation of *T. vulgaris* reduced the unfavorable age-related decline in activities of superoxide dismutase in the liver and heart of old rats. These results highlighted the benefit of *T. vulgaris* as a dietary antioxidant. It is very interesting that even the drying methods of plants used for preparation of extracts could be responsible for the content of phenolics and flavonoids, as well as for the antioxidant activity of extracts.[52]

In the present study, *T. vulgaris* extract was found to decrease the level of anxiety in animals. *T. vulgaris* oil also contains bioactive monoterpenes such as linalool.[13] According to this finding, linalool inhalation has been shown to reduce anxiety.[53] The presence of linalool and linalyl acetate in the plant extract supports the claim that the extract has sedative effect.[54] In another study, it has been shown that kaempferol induces anxiolytic activities in the EPM test in mice.[55] In addition, it has been shown that carvacrol presents anxiolytic effects in the plus maze test, which are not influenced by the locomotor activity in the open-field test.[56] It is possible that these components play essential roles in the anxiolytic properties of *T. vulgaris* in the EPM test.

As mentioned previously, thyme contains thymol and carvacrol compounds.[32,50] It has been reported that thymol exhibits significant anticonvulsant and antiepileptogenic properties.[57] The similarity of action of thymol and GABA suggests that this terpenoid acts centrally by mimicking or facilitating GABA action.[58] Furthermore, it is known that thymol acts as a positive modulator of the GABA (A) receptor.[59] Moreover, it has been shown that the anxiolytic-like effect of carvacrol in mice is involvement with GABAergic transmission.[56] GABA is widely known to be involved in the etiology of anxiety, hence the short-term effectiveness of diazepam, a GABA agonist, in relieving anxiety.[60]

5. Conclusion

In conclusion, our results demonstrate that oral administration of *T. vulgaris* (百里香 bǎi lǐ xiāng) extract may have an anxiolytic profile in rats. The presence of polyphenols, flavonoids, and essential oil in the extract of *T. vulgaris* reinforces the anxiolytic effects of this plant observed in this study. Possibly, the anxiolytic activity observed in this work was not only dependent on the flavonoid or essential oil content, but also related to other substances with antioxidant activity. Further studies would be necessary to evaluate the contribution of other substances to the activity shown, as it still remains to be determined which components were responsible for these effects.

Conflict of interest

There is no conflict of interest.

Acknowledgments

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