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Original article

Effects of *Stachys tibetica* essential oil in anxiety

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Abstract

Ethnopharmacological relevance: *Stachys tibetica* (Lamiaceae) is an important medicinal plant in the folk medicine of Ladakh, India and Tibet for the treatment of various mental disorders.

Aim of the study: Present study is aimed to characterize the essential oil of *S. tibetica* and its anxiolytic properties.

Materials and methods: The oil was extracted from the plant by hydrodistillation method. The isolated oil (0.7%) was subjected to GC–MS for identification of its constituents and evaluated for anxiolytic effects by employing the social interaction, hole-board, elevated plus maze and light/dark arena tests in rats.

Results: GC–MS of *S. tibetica* essential oil (SEO) indicated the presence of 33 constituents. Aciphyllene (66.415%), fenchyl alcohol (8.897%), α-pinene (8.188%), caryophyllene oxide (4.648%), menthol (1.69%) and geraniol (1.315%) are the major constituents of SEO. All the anxiolytic studies were conducted at doses of 25 and 50 mg/kg body weight. In the social interaction test, SEO decreased aggressive behaviors of the animals (albino rats) while the same significantly increased social interaction time of the high light, familiar as well as unfamiliar test conditions. In the hole-board test, SEO significantly increased head-dipping counts and its duration. The SEO significantly increased number of entries and time spent in open arms on the elevated plus maze test while as in the light/dark arena test, SEO showed an increase in number of crossings and time spent in light arena.

Conclusion: Results indicate that SEO has a wide range of anxiolytic properties and pave a way for new drug search for anxiety.

Keywords: *Stachys tibetica* oil; Social interaction test; Hole-board test of exploration; Elevated plus maze test; Light and dark arena

Introduction

The use of medicinal plants for the treatment of human diseases has increased considerably worldwide. Evaluation of the effects of these plants on organs and systems has contributed to the development of the scientific basis for their therapeutic application and also has enriched considerably the therapeutic arsenal for the treatment of a number of diseases [1]. Anxiolytic drugs are among the most frequently prescribed drugs as the disease is highly prevalent in the society. Existing anxiolytic agents are associated with several limitations such as sedation, addiction with benzodiazepines, tachycardia, insomnia, decreased libido and ineffectiveness (delayed but sustained) with fluoxetine [2] an antidepressant having selective serotonin reuptake inhibitor (SSRI) property and being extensively used in patients of generalized anxiety disorder including social anxiety. These are some of the factors that led to the interest in using alternative remedies. Several indigenous drugs are being evaluated because of their easy availability, lack of adverse effects and cost-effectiveness. Traditional medicines are used by about 60% of the world population in rural areas in the developing countries as well as in the developed countries where use of modern medicine predominates [3]. Though the use of herbal medicine is steadily increasing in western world [4], the major hindrance in the amalgamation of herbal medicine into medical practice is the lack of sufficient scientific and clinical data and better understanding of efficacy and safety of the herbal products [5]. The historical use of such medicine provides the source to study the specific plant species with potential to be used in a particular disease.

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Plants belonging to the family Lamiaceae are rich in essential oils. Previous studies have shown that a number of plants from this family like *Salvia officinalis*, *Salvia elegans*, *Salvia reuterana*, and *Scutellaria baicalensis* have shown anti-anxiety activity [6]. *Salvia sclarea* and Lavender also have shown anti-anxiety activity and the activity has been attributed to their essential oils [7]. Moreover another species of stachys namely *Stachys lavandulifolia* has shown anti-anxiety activity because of its essential oil [8].

Stachys L. (Lamiaceae) is a large genus of herbs and shrubs comprising 300 species distributed in temperate and tropical regions of the world, with the exception of Australasia [9]. Plants of this genus have been used in folk medicine for centuries to treat genital tumors, sclerosis of the spleen, inflammatory diseases, cough and ulcers [10]. *Stachys tibetica* is an herb distributed in tropical and subtropical regions of the world. The herb is distributed in Tibet, China, India, etc. In India, it is found in cold desert region of Ladakh valley and in the mountains of Himachal Pradesh. The traditional practitioners in Ladakh region of India use the drug for the treatment of various mental disorders and phobias. In traditional practice, the whole plant is boiled and made into a decoction. A tea cup of the decoction is given twice a day to treat fever [11].

Different drugs, known for their sedative properties at high doses, such as minor tranquillizers, also exhibit an anticonflict effect. Angelica and *Ducrosia anethifolia* essential oil were previously evaluated for anxiolytic activity by oral route in murine tests [12,13]. In this study, a possible anxiolytic property of *S. tibetica* essential oil was investigated using murine tests for anxiety: social interaction test, hole-board test, elevated plus maze test and light–dark arena test in rats. The elevated plus maze is one of the most extensively used models for the investigation of drug effects on anxiety-related behavior in laboratory rodents. It is based on the aversion of rodents for open spaces and anxiolytics have been found to increase the proportion of time spent on the open arms [14]. The light/dark test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors, that is, novel environment and light [15]. Anxiolytics have been found to increase locomotion and time spent in the light zone, whereas anxiogenics decrease them [16]. In the social interaction test, anxiolytic decreases aggressive behaviors and increases social interaction time of the high light, unfamiliar test condition and also prolong social interaction time of the high light, familiar test condition. In the hole-board test, it increases head-dipping counts and its duration. Because the above tests have been validated pharmacologically, behaviorally and physiologically as models of experimental anxiety, we used them to validate the putative anxiolytic effect of stachys essential oil and diazepam treatment was included as a positive control.

Materials and methods

Plant material

The plant specimen of *S. tibetica* Vatke was collected from Kargil Valley of Ladakh, Jammu & Kashmir, India. The plant was identified and authenticated by Dr. Zulfiqar Ali Bhat, from Department of Pharmaceutical Sciences, University of Kashmir, Srinagar 190006, India (Voucher specimen number – KUST01).

Extraction of stachys oil

100 g of *S. tibetica* drug was subjected to hydrodistillation in a Clevenger apparatus according to the method recommended in British Pharmacopoeia (1988) [17] and 0.7 ml of the oil (0.7%, v/w) was collected.

Gas chromatography–mass spectrometry analysis of the essential oil of S. tibetica

About 1 μL of aliquot of oil sample, appropriately diluted in hexane, was subjected to gas chromatography–mass spectrometry (GC–MS) analysis. The GC–MS analysis was performed using a Varian GC–MS series 3800 with a VF-5MS column (60 cm × 0.25 mm; film thickness, 0.25 μm). The column temperature was kept at 60 °C for 3 min programmed to 280 °C at a rate of 3 °C/min and kept constant at 250 °C for 1 min. Flow rate of helium as a carrier gas was 1 mL/min. The sample was analyzed twice.

Animals

Albino rats (Wistar Strain) of either sex weighing 150–200 g respectively were used for studies. The albino rats were obtained from animal house of Indian Institute of Integrative Medicine – Jammu, Jammu and Kashmir, India. They were housed in polypropylene cages with standard pellet chow and water ad libitum. In social interaction test, six pairs of rats were used in HU and HF behavioral study and in all other experimental sets, 10 rats were used for each dosage. This Institution is approved for carrying out animal studies (Approval No. 801/03/ca/CPCSEA) and the protocol for the present study was approved by Institutional Animal Ethical Committee [Approval No. F-IAEC (Pharm. Sc.) APPROVAL/2011/01].

Acute toxicity study: (OECD guidelines-425, 2001)

Acute toxicity study was conducted as per the internationally accepted protocol drawn under the OECD guidelines 425 (OECD, 2001). Overnight fasted, healthy rats ($n = 6$) were administered orally the stachys essential oil in the doses of 100, 200, 400, 800, 1600 mg/kg body weight and observed continuously for 4 h and 24 h for any abnormality and mortality. Stachys oil at a dose level of 1600 mg/kg was found safe. 25 and 50 mg/kg were selected as the study dose of stachys oil.

Drugs

Diazepam was obtained from Ranbaxy Lab. Ltd., HPSIDC-Baddi, Solan (India). Sodium carboxy methyl cellulose was purchased from CDH-Laboratory Reagent Pvt. Ltd., Post Box No. 7138, New Delhi 110002 (India). Diazepam and stachys essential oil (SEO) were both suspended in a 1% sodium carboxy methyl cellulose solution. All drugs were prepared immediately before use and were given orally. Control mice received 1%