ANXIOLYTIC AND ANTIDEPRESSANT ACTIVITIES OF DIFFERENT EXTRACTS FROM
CITRUS PARADISI VAR. DUNCAN

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Abstract: The present study was designed to evaluate the anti-anxiety activity and anti-depressant activity of various extracts viz. petroleum ether, chloroform, methanol and water, of the leaves of Citrus paradisi var. duncan using elevated plus maze (EPM) model and forced swimming test (FST) respectively in Swiss albino mice. Albino mice were treated with different doses of the extracts (i.e. 100, 200 and 400 mg/kg, orally) and behavior was observed on the EPM and FST. Results showed that methanol extract at the dose of 100 mg/kg of the leaves of Citrus paradisi var. duncan markedly increased the average time spent in the open arms in EPM and methanol extract at the dose of 400 mg/kg showed a significant decrease in the time spent immobile by mice in FST. These results provide support for the potential anxiolytic and antidepressant activity of Citrus paradisi var. duncan.

Keywords: Anti-anxiety, anti-depressant, citrus paradisi var. duncan, elevated plus maze, forced swimming test.

INTRODUCTION

According to the World Health report [1], approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receives even the most basic treatment. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020 [2]. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models [3]. Anxiety, a state of excessive fear, is characterized by motor tension, sympathetic hyperactivity, apprehension and vigilance syndromes [4]. Anxiety may interfere with intelligence, psychomotor function and memory [5]. The benzodiazepines are considered the drug of choice in the treatment of anxiety. Unfortunately, there are several side effects [6-7]. Depression is the most prevalent mental disorder and depression is recognized to be symptomatically, psychologically and biologically heterogeneous [8-10]. The complexity of daily life in modern society frequently leads to varying degree of anxiety and depression. Mood, depression and anxiety disorders have been found to be associated with chronic pain among medical patients in both developed and developing countries [11-12]. These considerations implicate the search for new anxiolytic and antidepressant agents that have a fast onset of action present with less side effects and a wider safety margin. It has lead scientists to investigate plants, which are commonly employed in traditional and alternate system of medicine for sleep disorders and related diseases [13]. Various plants are being used in complementary and alternative medicines for management of anxiolytic Citrus fragrances have been particularly attributed with mood enhancing properties by aroma therapists. Volatile oils isolated from grapefruit (Citrus paradisi), lemon (Citrus limon), bergamot (Citrus bergamia), lime (Citrus aurantiifolia), mandarin (Citrus nobilis) and orange (Citrus aurantium) are often used in the treatment of anxiety [13-15]. A review of literature revealed that Citrus paradisi is a highly reputed plant, and has been widely employed in herbal medicine and aromatherapy [15] but no significant work has been carried out on the anxiolytic effects and antidepressant activity of the plant extracts. So, the present study was designed to evaluate the anti-anxiety activity and anti-depressant activity of different extracts of Citrus paradisi var. duncan.

MATERIALS AND METHODS

Plant material

The leaves of Citrus paradisi var. duncan were procured and identified from a cultivated source Punjab Agricultural University Regional Centre at Abobhar, Punjab, India in the month of March-April 2007.

Preparation of extracts

Leaves of Citrus paradisi var. duncan were dried in shade and powdered. The powdered leaves (100g) were subjected to successive solvent extraction by solvents in increasing order of polarity viz. petroleum ether (60 – 80 °C), chloroform and methanol and water. Before each extraction the powdered material was dried in hot air oven below 50 °C. Each extract was concentrated by distilling off the solvent and then evaporating to dryness on the water-bath. Extracts were weighed and percentage was calculated in terms of the air-dried weight of the plant material. The yield of the extract petroleum ether (60-80 °C), chloroform and methanol and water was 1.97%, 3.61%, 4.12%, 4.11% w/w respectively.

Test animals

The experimental animals [Swiss albino mice (20-25 gm) of either sex] were procured from the Animal House, Punjabi University, Patiala. The animals were given standard laboratory feed and water ad libitum. The experiments were performed between 8.00 am to 1.00 pm. The experiments were conducted in a sound proof laboratory. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee.

Anti-anxiety activity

Elevated plus maze model

The elevated plus-maze model is well established animal model for testing anxiolytic drugs [16]. The elevated plus-maze apparatus consist of two open arms (16 x 5 cm for mice and 50 x 10 cm for rats), two closed arms (16 x 5 x 12 cm for mice and 50 x 10 x 40 cm for rats), and an open roof with the entire maze elevated (25 cm for mice and 50 cm for rats) from the floor [17]. The animals were placed individually in the centre of the maze, head facing towards open arms and the stop watch was started and following parameters were noted for 5 min. a) First preference of mice to open and closed arm. b) Number of entries in open and closed arms (an arm entry defined as the entry of four paws into the arm). c) Average time each animal spends in each arm (average time = total duration in the arm/number of entries).

Treatments

Animals were divided into five (V) groups. Group I was a negative control and was given vehicle, consisting of simple syrup IP and carboxy methyl cellulose (20%), in a dose of 0.25 ml. Group II was a positive control and was given standard drug, diazepam (2mg/kg, orally), suspended in the vehicle. Group III-V were treated as test groups and were given petroleum ether (60-80°C), chloroform and methanol and water extracts of leaves of Citrus paradisi var. duncan at different doses viz. 100, 200 and 400mg/kg respectively.

All the test solutions, standard drug and control were administered orally 45 minutes prior to elevated plus maze test.

Anti-depressant activity

Forced swimming test

The FST is the most widely used pharmacological in vivo model for assessing antidepressant activity [18]. The swimming test includes two exposures to a water tank, spaced 1 day apart. For these experiments, the tank sizes were 22 cm in diameter and 40 cm in...
The forced swimming test is the most widely used tool for assessing antidepressant activity since their P values are insignificant.

RESULTS

The results obtained from the EPM model, indicates that methanol extract showed significant (p<0.05) anti depression activity as compared to diazepam. The average time spent in open arms increased from 7.73±0.613 (sec) in control to 21.264 ± 0.519 (sec) in methanol extract at a dose of 100mg/kg. The petroleum ether extract, chloroform extract and aqueous extract appeared to be devoid of anti anxiety activity since their P values are insignificant. Results obtained are presented in Table 1.

The results obtained from the FST, indicates that methanol extract showed significant (p<0.05) anti depression activity as compared to imipramine. Methanol extract at the dose of 400mg/kg showed a significant decrease in the time spent immobile by mice from 220.73 ± 1.941(sec) in control to 114.907 ± 3.102(sec). The petroleum ether extract, chloroform extract and aqueous extract appeared to be devoid of antidepressant activity since their P values are insignificant. Results obtained are presented in Table 2.

DISCUSSION

The fear due to height induces anxiety in the animals when placed on the EPM. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. Anxiolytic agents are expected to increase the motor activity, which is measured by the time spent by the animal in the open arms [23]. The forced swimming test is the most widely used tool for assessing antidepressant activity pre-clinically [28]. The widespread use of this simple model is mainly due to its ability to detect a broad spectrum of antidepressant agents. The test is based on the observation that rodents (rats and mice), following initial escape-oriented movements, develop an immobile posture when placed inside an inescapable cylinder with water. The immobility is thought to reflect either a failure of persistence in escape-directed behavior (i.e., despair behavior) or the development of a passive behavior, meaning the loss of the animal’s ability to cope with stressful stimuli [21]. The methanol extract of Citrus paradisi var. duncan (100mg/kg), markedly increased the percentage of average time spent by the animals in the open arms. The anxiolytic effect of the plant extract was more prominent at 100mg/kg and doses higher or lower than this did not show a consistent anxiolytic effects. The lack of significant anxiolytic effects at doses higher than 100mg/kg could be due to strong sedative properties of the plant extracts. Lower doses (less than 100mg/kg) of the plant extract did not show any significant anxiolytic effects. The methanol extract of Citrus paradisi var. duncan (400mg/kg) markedly showed a significant decrease in the time spent immobile by mice. The anxiolytic and antidepressant effects of methanolic extract of Citrus paradisi var. duncan may be related to their flavonoid content. However, further studies are required to identify the phytoconstituent responsible for the observed anxiolytic and anti-depressant effect of methanol extract at dose 100 mg/kg and 400mg/kg orally respectively and to explain mechanism.

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