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ORIGINAL ARTICLE

Antianxiety Activity of Methanol Extract of *Gelsemium sempervirens* (Linn.)Ait.

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Background: Despite significant advances in understanding and management of neuropsychiatric disorders during past few decades, anxiety and depression, still remains the leading cause of deaths, primarily for want of effective and safe treatment of these ailments. Approximately, two third of the anxious or depressed patients respond to the currently available treatment but the magnitude of improvement is still disappointing

Purpose of Study: The aim of the present study was to investigate the antianxiety activity of *Gelsemium sempervirens* (Linn.) Ait. Various doses (50,100, 150, 200mg/kg) of plant extracts viz., of petroleum ether, chloroform, methanol and water were administered orally to Swiss Albino Mice before evaluating their behavioural pattern. Diazepam (2.5 mg/kg) was used as standard drug.

Result: The methanol extract of *G. sempervirens* (150 mg/kg) increased the mean time spent, mean number of arms entries in the open arms of elevated plus maze (EPM) and decreased the mean time spent in the closed arms. The locomotor activity of methanol extract was not affected to the same extent as observed for diazepam.

Conclusion: The results suggested that methanol extract of *G. sempervirens* possess anxiolytic effects with no sedative activity when compared to diazepam.

Key words: Anxiolytic, Elevated Plus Maze Test, Gelsemium sempervirens, Yellow Jasmine

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Neuropsychiatric disorders and debilitating mental illnesses are mainly responsible for global burden of disease with about 28% aggregate in low, middle and high-income countries (Zwanzger *et al* 2007; Christmas, 2006). According to World Health Organization (WHO), anxiety and related disorders will become the second leading cause of disability in both developed and developing countries by the year 2020 (Kim et al., 2007; Rybnikova *et al*., 2007; Reynolds, 2003). Benzodiazepines have been extensively used for the last 40 years to treat several forms of anxiety (Jordan *et al*., 1996; Rickels and Schweizer, 1997). Although these compounds have well known benefits but their side

effects are prominent, including sedation, muscle relaxation, ethanol potentiation, anterograde amnesia and seriousness of anxiety disorders, the researchers working in the field of development of pharmacotherapeutic agents have endeavored in the recent past to develop some synthetic as well as natural products which have potential for the management of such diseases (Barbotte *et al*., 2001).

Gelsemium sempervirens Aiton (Syn. *Gelsemium nitidum*) belonging to family Loganiaceae is commonly known as Yellow jasmine. *Gelsemium sempervirens* Aiton (Syn. *Gelsemium nitidum*) belonging to family Loganiaceae is commonly known as Yellow jasmine. The roots and rhizomes of *G. sempervirens* have been used traditionally in mental irritability, insomnia, headache, irritation of the urinary tract and in hyperaemia. Roots and rhizomes of *G. sempervirens* have been reported to be used in the treatment of migraine, neuralgia, rheumatism, hysteria, dysmenorrhoea, bronchitis, ovarian and uterine pain (Chopra, 1940; Grieve, 1994).

Phytochemical reports on *G. sempervirens* indicate that the plant contains alkaloids viz., gelsemine (Sayre, 1911), gelseminine (Chou, 1931), gelsemiodine (Ferrerio, M., 1945) and gelsemicine (Janot *et al*., 1951) 21-oxo gelsemine, gelsevirine (Nikiforov et al., 1974; Wenkert *et al*., 1972); iridiods viz., gelsemide, gelsemide-7-glucoside, gelsemiol, gelsemiol 1,3-glucoside and 9-hydroxysemperoside (Jensen *et al*., 1987) and steroids; pregna-4,16-diene-3,20 dione and 12β-hydroxyl pregna 4,16-diene-3,20-dione (Nikiforov *et al*., 1974).

G. sempervirens has been used in the treatment of influenza symptoms (Vorrhees and Nachmann, 2002). Low doses of *G. sempervirens* showed significant neurotropic and protective effects on behavioural and gastric alterations induced by foot shock stress in mice (Bousta et al., 2001). Sempervirine nitrate, isolated from *G. sempervirens*, exhibited antimitotic activity in mice bearing several types of tumors (Bassleer *et al*., 1985).

G. sempervirens has also been used as a homoeopathic medicine in the CNS disorders. In search of alternative, more specific and relatively cost effective natural anxiolytic agents, the aim of the present study was to investigate the antianxiety activity of various extracts from roots and rhizome of *G. sempervirens*.

MATERIALS AND METHODS

Plant material

Dried roots and rhizomes of *G. sempervirens* (Plate 1) were procured from a commercial source, Himalaya Herbs, Saharanpur (U.P.). The identity of roots and rhizomes of *G. sempervirens* was confirmed through National Institute of Science Communication and Information Resources (NISCAIR), Delhi. Voucher specimen (NISCAIR/RHMD/Consult/2008-09/1146/178) was deposited in NISCAIR, Delhi for further reference.

Preparation of Plant Extract

Air dried and powdered (#10) roots and rhizomes of *G. sempervirens* (5 kg) were successively and exhaustively extracted with petroleum ether (60-80^{o} C), chloroform and methanol in soxhlet apparatus. The marc was dried and further extracted warm distilled water and filtered. The extracts were evaporated to dryness *in vacuo* and weighed.

Phytochemical Screening

Standard screening tests for various constituents were carried out on the extracts. The extracts were screened for the presence of alkaloids, steroids, tannins, protein and glycosides using conventional protocol (Fransworth, 1966). The chloroform and

methanol extracts tested positive for alkaloids and steroids. All the extracts gave negative test for anthraquinones glycosides, saponins, flavonoids and carbohydrates.

Animals

Adult Swiss Albino mice of either sex (18-25 g) procured from Haryana Agricultural University, Hissar were housed in Animal House of Guru Gobind Singh College of Pharmacy, Yamunanagar (Haryana) in groups of 5-8 at constant room temperature 25 °± 1° C and 45-55% humidity with a 12 h light/dark cycle. The mice had a free access to food and water. They were transferred to laboratory at least 1 h before the start of each experiment. All the experiments were performed during the daytime between (8:00-5:00 pm). The protocols for the experimental animals were duly approved by College's Animal Ethical Committee (Reg.No.873/ac/05/CPCSEA).

Acute Toxicity

The median lethal dose of methanol extractive was determined by administering varying doses (100, 200, 400, 800, 1000 mg/kg, i.p) to groups of mice (n = 5) and the approximate LD_{50} was determined after 24 h. (Ghosh, 1984).

Treatments

The petroleum ether, chloroform, methanol and water extracts were administered to mice at various dose levels (50, 100, 150, 200 mg/kg) by oral route. Diazepam was used as standard anxiolytic at a dose of 2.5 mg/kg, The test material were suspended in a vehicle that consisted of 0.9% NaCl containing 0.2% Tween 80.

Pharmacological Experiments

Elevated Plus Maze test (EPM)

The plus-maze apparatus consisting of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof, with the plus-maze elevated (25 cm) from the floor was used to observe anxiolytic behaviour in animals (Pellow *et al*., 1986). Each mouse was placed at the centre of the elevated plus maze with its head facing the open arms. During the 5 min. experiment, the behavior of the mouse was recorded as: (a) the number of entries into the open arms and (b) average time spent by the mouse in the open arms (average time = total time spent in open arms/number of entries of arms). Test substance were administered orally using a tuberculin syringe fitted with oral canula. Dose administration schedule was so adjusted that each mouse was having its turn on the EPM apparatus, 45 min after the administration of the dose. During the entire experiment, the animals were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of plus-maze, could invoke anxiety in the animals.

Locomotor Activity

The locomotor activity of plant extracts of *G. sempervirens* was measured with Photoactometer (Vogel and Vogel 1998). Five mice were used for each treatment group. The control, standard drug Diazepam (2.5 mg/kg i.p.) and test compounds were administered orally to each mouse 30 min. before the start of experiment. The each mouse was placed in the activity cage. The locomotor activity was measured for 5 min for each group. The treatments were randomized throughout the day, between 09:00 and 14:00 h to control for diurnal variations in activity.

Statistical analysis

The data are expressed as Mean ± SEM for each group. A one way analysis of variance (ANOVA) followed by Studentized Tukey's test for multiple comparisons were used. The test doses were compared with control and standard drug diazepam. Effects or differences were considered significant at $p \leq 0.05$.

RESULTS AND DISSCUSSION

The percentage yield (w/w) of petroleum ether, chloroform, methanol and water extracts were found to be 0.62%, 1.4%, 14,72%, 3.02% and 3.01% resp. The approximate LD_{50} of methanol extractive of *G. sempervirens* was 1000 mg/kg.

In the present study we used the EPM model of anxiety to evaluate the anxiolytic effects of various extract of *G. sempervirens*. As expected, diazepam produced significant increase in open arm entries and mean time spent in open arms. Petroleum ether, chloroform, methanol and water extracts of *G. sempervirens* exhibited antianxiety activity based on EPM (Table 1). The methanol extract of *G. sempervirens* at the dose of 150 mg/kg exhibited significant increase of open arms entries and mean time spent in the open arms. These increases were accompanied by statistically significant change in motor activity. Therefore the behavioural alternations induced by methanol extract of *G. sempervirens* in EPM are consistent with an anxiolytic similar to diazepam. Since other plant extract viz., petroleum ether, chloroform and water extract didn't produce meaningful effects on EPM, it was eliminated from further pharmacological investigation in this study. Methanol extractives (150 mg/kg) had no change in locomotor than diazepam thus has a better profile for anxiolytic agents (Table 2). Literature revealed that alkaloids, iridoids and steroids are the active constituents in *G. sempervirens* (Janot, *et al*., 1951; Nikiforov, *et al*., 1974; Wang *et al*., 2001).

Table 1 Antianxiety effects of *Gelsemium sempervirens* roots and rhizomes on EPM

S.No.	Treatment	Dose (mg/kg)	Mean entries in open arms	Mean time spent open arms (sec)
1	Control	Vehicle	0.4 ± 0.22	1.8 ± 1.01
2	Diazepam	02.05.12	6.8±0.34	15.0 ± 0.66
3	Petroleum Ether extract	50	1.0±0.40	3.9 ± 1.58*
		100	2.4±0.22*	5.6 ± 0.36*
		150	1.6 ± 0.22*	5.1 ± 0.69*
		200	0.2 ± 0.18	0.8 ± 0.73
4	Chloroform extract	50	1.1 ± 0.40*	3.1 ± 1.24*
		100	3.4 ± 0.22*a	4.9 ± 0.81*
		150	2.4 ± 0.36*	4.8 ± 1.01*
		200	1.8 ± 0.44	2.5 ± 0.59*
5	Methanol extract	50	3.4 ± 0.22*a	6.2 ± 0.78*
		100	6.2 ± 0.60*a	10.9 ± 1.68*a
		150	5.4 ± 0.54*a	12.4 ± 0.89*a
		200	3.8 ± 0.34*a	7.4 ± 1.10*a
6	Water extract	50	0.4 ± 0.36	0.9 ± 0.82
		100	2.2 ± 0.18*	3.6 ± 0.35*
		150	2.6 ± 0.46*	4.4 ± 0.48*
		200	1.2 ± 0.34*	3.2 ± 0.78*

Values are expressed as mean ± SEM. ANOVA followed by Studentized Tukey's Test showed a significant treatment effect.
* $p \leq 0.05$ versus control, [a] $p \leq 0.05$ versus diazepam.

Table 2 Effect of methanol extract of *G. sempervirens* roots on locomotor activity

Treatments	Dose(mg/kg)	Locomotor activity(Min.)
Control vehicle	10 ml/kg	98 ± 1.11
Diazepam	2.5 mg/kg i.p.	46 ± 1.9*
Methanol extract	50	83 ± 1.12
	100	87 ± 0.30
	150	94 ± 0.80
	200	101 ± 2.67

Values are expressed as mean ± SEM. Locomotor activity was measured for 5 min.

In our phytochemical analysis, alkaloids, steroids and proteins are present in the methanol extract of *G. sempervirens*. Therefore, it may be concluded that antianxiety activity may be attributed to the individual or combined effects of the above mentioned group of constituents of the plant.

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