International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol. 3, No.2, pp 693-697, April-June 2011

Pharmacological Evaluation of *Gelsemium sempervirens* roots for CNS Depressant Activity

Kavita Gahlot*[1],M.Abid[1], Anupam Sharma[2]

[1]Department of Pharmacognosy and Phytochemistry, College of Pharmacy, IFTM, Moradabad,India

[2]University institute of Pharmaceutical sciences, Punjab University, Chandigarh,India.

Corres.author: kavita_pharma@yahoo.co.in, Tel: 09456099848

Abstract: The aim of the present study was to investigate CNS depressant activity of methanolic extract of *Gelsemium sempervirens* roots. *G. sempervirens* Ait (Loganiaceae) is used traditionally in the treatment of migraine, neuralgia, rheumatism, restlessness, mental irritability, insomnia and ovarian and uterine pain. The plant was found to contain alkaloids gelsemine, gelseminine, gelsemoidine and gelsevirine. Since roots of *Gelsemium sempervirens* are used as folk medicine in treatment of insomnia, we made an attempt to study its CNS depressant effect. The different activities studied were potentiation of pentobarbiton-induced sleep, test for locomotor activity, effect on muscle co-ordination, and antianxiety activities. The results of the study reflected that methanolic extract of the roots (100 mg/kg *p.o*) decreased locomotor activity, produced muscle relaxation and showed antianxiety activity. This substantiates the traditional use of roots of Gelsemium *sempervirens* for CNS depressant activity.
Key words: *Gelsemium sempervirens* sedative, muscle relaxant, antianxiety, CNS depressant.

Introduction

Advance in science and technology has contributed to an enormous improvement in the quality of life of humankind. However, modern life stress, associated trials and tribulation are responsible for the surge in incidence of variety of psychiatric disorders.

Path breaking research in psychopharmacology has flooded the market place with drugs for specification. For instance, benzodiazepines (diazepam, nitrazipam lorazipam and alprazolam etc) are the most frequently prescribed synthetic drugs for variety of condition particularly anxiety, depression, epilepsy and insomnia. But these psychoneural drugs have very serious side effects like chronic use of benzodiazepines causes deterioration of cognitive function, physical dependence and tolerance (1). In this context, a resurgence of interest in medicine from natural sources (mainly plant products) is seen and there is tremendous hope that drugs of plant origin will have significantly lesser side effects than that observed with synthetic drugs while having comparable efficacy.

G.sempervirens, commonly known as yellow jasmine, belongs to family Loganiaceae. It is a climbing shrub indigenous to the southern United States from Virginia to florida and Texas. The roots are upto 20 cm in length and 2 to8 mm thick, light brown, nearly smooth and wiry, fractured surface showing a broad, radiate, yellow wood and a thin bark. The root has been used in restlessness, mental irritability, insomnia associated with excitation, irritation of urinary tract, hyperemia and convulsions (2), in the treatment of migraine, neuralgia, rheumatism and in ovarian and uterine pain. The plant has also been used as analgesic, anodyne,

antispasmodic, CNS depressant and nervine tonic (3). Phytochemical reports on *G.sempervirens* indicate that the plant contains alkaloids gelsemine, gelseminine, gelsemoidine gelsevirine and 21-oxo gelsemine (4). Ethanolic extract of roots has been reported to increase the resistance of rabbits to pneumococcus toxin (5). The alkaloidal fraction possesses anticancer activity as evidenced by significant inhibition of hepatic carcinoma HepG2 cells in vitro (6). Sempervirine, isolated from *G.sempervirens* has been reported to possess vasoconstrictor action in the perfused isolated rabbit ear (7). Low doses of *G.sempervirens* and *Atropa belladonna* showed significant neurotropic and protective effects on behavioral and gastric alterations induced by experimental stress on mice(8). Based on the above information, we carryout pharmacological evaluation of *G.sempervirens* roots for CNS depressant activity.

Material and Methods

Plant material

The Plant *G.sempervirens* Ait. was procured from Rati Ram Nursery,Khurrampur,Saharanpur (U.P.) in the month of august 2004 .Roots were removed ,washed and dried in shade. Identification of the plant was confirmed through Forest Research Institute, Deharadun. A voucher specimen of the plant has been deposited in the Herbarium –cum –museum of the university institute of pharmaceutical sciences (U.I.P.S), Punjab University Chandigarh.

Preparation of extracts: The shade dried roots of the plants were powdered and were successfully extracted with Petroleum ether, chloroform, methanol and water separately in soxhlet apparatus. The liquid extracts were concentrated separately and dried under vacuum. The dried extracts were preserved in desiccators until further use.

Experimental animals

Swiss albino mice of either sex weighing between 12-35 g were used in the present study. The experimental protocol was approved by the Institutional Animal Ethics Committee. The animals were maintained under standard conditions in Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) approved Institutional Animal House.

Acute oral toxicity study

The acute oral toxicity study was performed according to the OPPTS (Office of Prevention, Pesticides and Toxic Substance) guidelines following Up and Down procedure (9).

Selection of the extract

All the extracts of *Gelsemium sempervirens* roots were evaluated for sedative-hypnotic activity in pentobarbitone induced sleep test. The extract, which potentiated the sedative-hypnotic activity of pentobarbitone, was chosen for further study (10).

Test for locomotor activity

The spontaneous locomotor activity of each mouse was recorded individually for 10 min using Actophotometer. Three doses of MEGS (50,100 and 150 mg/kg *p.o*) were administered 60 min before the test and chlorpromazine (3 mg/kg *i.p*), used as standard was given 30 min before the test. The control group was treated with 2%w/v Tween 80 orally, 60 min before test (11).

Muscle co-ordination test

This test was carried out using rotarod apparatus.

Rotarod

The rotarod apparatus consists of a metal rod (3 cm diameter) coated with rubber attached to a motor with the speed adjusted to 2 rotations per minute. The rod is 75 cm in length and is divided into 6 sections by metallic discs, allowing the simultaneous testing of 6 mice. The rod is in a height of about 50 cm above the tabletop in order to discourage the animals from jumping off the roller. Cages below the section serve to restrict the movements of the animals when they fall from the roller.

Swiss albino mice underwent a pretest on the apparatus. Only those animals, which had demonstrated their ability to remain on the revolving rod (20 rpm) for 5 min, were used for the test. The three doses of MEGS (50,100 and 150 mg/kg) were administered orally, the standard group was treated with diazepam (4 mg/kg) intraperitonially and control group received Tween 80 (2% w/v) orally. The test was carried out 30, 60 and 90 min after administration of drugs and vehicle (12).

Anti-anxiety activity

The anti-anxiety activity was evaluated using staircase test and elevated plus maize test

1. Staircase test

Staircase consists of five identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. The internal height of the walls is constant along whole length of the staircase. The drugs and treatments were same as mentioned under rotarod test. The animals were placed on the floor of the box with its back to the staircase. The number of steps climbed and the number of rears are counted over a 3 min period. A step is considered to be climbed only if the mouse had placed all four paws on the step. In order to simplify the observation, the

numbers of steps descended were not taken into account. After each step the box was cleaned in order to eliminate any olfactory cues, which might modify the behavior of the next animal (13).

2. Elevated plus maze

The apparatus consist of two open arms (5x10cm) and two closed arms (5x10x15cm) radiating from a platform (5x5cm) to form a plus-sign figure. The apparatus was situated 40 cm above the floor. The open-arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. The drugs and treatments were same as mentioned under Rotarod test.

The animal was placed at the center of the maze, facing one of the closed arms.

During 5 min test period the following measures are taken:

- The number of entries into open arms
- The number of entries into closed arms
- Time spent in the open arms

Arm entry was counted when the animal had placed all of its four paws on it. The procedure was conducted in a sound attenuated room, with observations made from an adjacent room (13).

Statistical analysis:

Results were expressed as Mean±SEM The differences between experimental groups were compared using one-way Analysis of Variance (ANOVA) followed by Dunnett's test and were considered statistically significant when $p<0.05$.

Table 1: Effect of MEGS in Pentobarbitone-induced sleep.

Pentobarbitone (40 mg/kg, I, p) 30 min Post treatment of the vehicle and drugs	*Onset of action (Min)*	*Duration of action (Min)*
Control (Vehicle 6ml/kg *p.o*)	5.66±0.49	132.66±0.16
Petroleum ether extract	5.45±0.58	125.45±0.39
Chloroform extract	5.83±0.16	118±14.00
methanolic extract	6.00±0.25	920.66±0.21**
Aqueous extract	5.32±0.13	135.42±0.57

All values are Mean±SEM**P<0.01when compared with control.

Table2: Effect of MEGS and diazepam in locomotor activity

Groups	**Actophotometer score in 10 min**.
Control (Vehicle 6ml/kg *p.o.*)	325.52±10.25
MEGS(50mg/kg *p.o*)	265.56±9.27
MEGS(100mg/kg *p.o*)	164.82±12.06
MEGS(150mg/kg *p.o*)	205.42±16.42
Chlorpromazine (3mg/kg *i.p.*)	140.16±14.12

Table 3: Effect of MEGS and diazepam in Rotarod test

Groups	**Time spent on revolving rod (sec)**
Control (Vehicle 6ml/kg *p.o.*)	325.00±24.82
MEGS(50mg/kg *p.o*)	295.00±32.45
MEGS(100mg/kg *p.o*)	197.5±40.58
MEGS(150mg/kg *p.o*)	245.5±25.52
Diazepam (4mg/kg *i.p.*)	104.6±2.75

Table 4: Effect of MEGS and diazepam in Stair case test

Groups	No. of climbing in 3 min	No. of rearing in 3 min.
Control(Vehicle 6ml/kg *p.o.*)	21.15±1.34	9.45±0.63
MEGS(50mg/kg *p.o*)	14.66±0.82	8.12±0.42
MEGS(100mg/kg *p.o*)	7.12±0.60	6.00±0.52
MEGS(150mg/kg *p.o*)	11.42±0.72	8.45±0.44
Diazepam (4mg/kg *i.p.*)	5.00±0.64	4.8±0.58

Table 5: Effect of MEGS and diazepam Elevated plus-maze test

Groups	No. of entries in open arms	Time spent in open arms (sec)
Control (Vehicle 6ml/kg *p.o.*)	2.6±0.51	3.05±0.30
MEGS(50mg/kg *p.o*)	4.8±0.66	6.05±0.77
MEGS(100mg/kg *p.o*)	7.0±0.71	12.22±1.05
MEGS(150mg/kg *p.o*)	5.8±0.78	9.89±1.21
Diazepam (4mg/kg *i.p.*)	6.8±0.80	12.17±0.62

Results

The preliminary phytochemical investigation of the MEGS revealed the presence of alkaloids, carbohydrates, steroids, tannins and proteins.

In acute oral toxicity study, MEGS produced death at doses of 2000mg/kg. MEGS was safe at a dose of 1000 mg/kg orally. Hence 50,100 and 150 mg/kg of MEGS were used for the study.

Pentobarbitone induced sleeping time

The MEGS (100mg/kg *p.o*) significantly increased the pentobarbitone induced sleeping time. No significant effect was observed with the other extracts (Table 1).

Test for locomotor activity

MEGS at dose of (100 mg/kg *p.o*) and Chlorpromazine (3 mg/kg *i.p*) decreased the locomotor activity significantly whereas; low dose and high dose of MEGS (50 and 150mg/kg *p.o*) did not show a significant reduction in the locomotor activity (Table 2).

Test for muscle coordination

Rotarod test

In this test, MEGS (100 mg/kg) significantly reduced the time spent by the animals on revolving rod when compared to control. The standard drug (diazepam) also showed significant effect when compared to control. While other doses of drug (50 and 100mg/kg) did not show any significant effect (Table 3).

Anti-anxiety activity

1. Staircase test

The statistical summary of the rearing and number of steps climbed is presented in Table 4. After 60 and 90 min of treatment, a reduction in anxiety-linked behavior was indicated by a reduction in number of rearing and sedation that was evaluated by number of steps climbed. High dose of MEGS (100 mg/kg *p.o*) and standard drug (diazepam 4 mg/kg, *i.p*) significantly reduced the number of rearing as well as the number of steps climbed. Other doses of MEGS (50 and 100mg/kg, p.o) did not produce a significant decrease in the number of rearing or the number of steps climbed.

2. Elevated plus maze*:*

High dose of MEGS (100 mg/kg *p.o*), significantly increased the time spent in open arms and number of entries into closed arms and open arms when compared with control. The standard drug (diazepam 4 mg/kg, i.p) showed a significant increase in the number of entries into closed arms and open arms and also significantly increased the time spent in open arms. Other doses of MEGS (50 and 100mg/kg, *p.o*) did not show any differences in activity compared to control (Table 5).

Discussion

The study reflected that MEGS (100 mg/kg *p.o*) possess sedative, antianxiety and muscle relaxant activity.

MEGS potentiated the sleep induced by pentobarbitone suggesting that it possess some sleep inducing property. The study on the spontaneous

motor activity showed that MEGS (100mg/kg *p.o*) decreased the frequency and the amplitude of movements. The reduction of the spontaneous motor activity could be attributed to the sedative effect of the extract

MEGS (100 mg/kg *p.o*) reduced the time spent on the revolving rod by mice in the rotarod test, a test mainly used to screen centrally acting muscle relaxants. This represented that MEGS may have muscle relaxant activity, which could be due to CNS depressant activity.

The mouse staircase was used for the assessment of anxiety (number of rearing) and sedation (number of steps ascended). Greater number of rear indicates anxiety like behavior and lesser number of steps ascended indicated increased sedation (13). The present investigation successfully detected the anxiolytic-like effects of MEGS and diazepam; both significantly decreased the number of rearing and number of steps ascended compare to control. This showed that MEGS has both anxiolytic and sedative properties.

Elevated plus-maze test is used to evaluate psychomotor performance and emotional aspects of rodents (14). The results showed that MEGS significantly increased the time spent on the open arms and decreased the number of entries into open and closed arms.

To conclude, the methanolic extract of roots of *G.sempervirens* possesses sedative, anti-anxiety and muscle relaxant properties. The result of the present study substantiates the traditional use of roots of *G.sempervirens* for the treatment of insomnia.

Acknowledgement

The authors are thankful to Dr.A.K.Ghosh, Director and Dr.R.M.Dubey, Managing Director, College of Pharmacy, IFTM, Moradabad, India, for providing facilities to carry out this work.

References

1. Dhawan K, Dhavan S, Chhabra S. Attenuation of benzodiazepine dependence in mice by a tri-substituted benzoflavone moiety of *Passiflora incarnata* Linneous: A non habit forming anxiolytic. *J Pharm Pharmceu Sci* 2003; 6(2): 215-222.

2.Felter H.W., Lolyed J.U.Kings American Dispensatory. *Gelsemium* (USP), 1898 ,Through www.ibilio.org/herbmed/kings/gelsemium.htm.

3. Chopra R.N., Posionous Plants of India, vol. 1 Council of Aggriculture Research, New Delhi, 1940.

4. Sayre L.E., A study of the more toxic alkaloids of gelsemium. Midland druggist and Pharmaceutical review, 1912, 45,439-441.

5. Baker W.F., *Gelsemium sempervirens* and *Bryonia alba* in influenza. Journal of the American Institute of Homoeopathy.1920, 12, 695-698.

6. Wang Y., Lin W.Y., Jiang M., Yin M., Gelsemium alkaloids extract on hepatic Carcinoma HepG2 cells in vitro. Journal of Chinese medicinal materials. 2001, 24, 579-81.

7. Rothlin E., Raymond H., Vascular action of sempervirine an oxygen free alkaloid of *Gelsemium sempervirens*. Comptes Rendus des séances de la science de biologic et de ses filiakes .1934,117,754-756.

8. Bousta D et al., Neurotropic, immunological and gastric effect on low doses of *Atropa belladonna* L., *Gelsemium sempervirens* and histamine in stressed mice .J Ethnopharnmacol, 2001, 74,205-215.

9. Health effects test guidelines. Acute oral toxicity (computer program) OPPTS 870-1100 United States office of prevention agency (7101). Available from: URL http://www.epa.gov/opts/home/guideline.htm.

10 Singh J, Khosla P, Srivastava PK. Nimodipine potentiats anesthetic effects of ethanol, Pentobarbitone and ketamine in rats. *Indian J Pharmacol* 2000; 32: 206-209.

11.Tatarezynska E, Klodzinska A, Stachowicz K, Chojnacka-Wojcik E. Effects of selective 5-HT_{1B} receptor against and antagonists in animal models of anxiety and depression. *Behav Pharmacol* 2004; 15(8): 523-534.

12. Allmark MG, Bachinski WM. A method of assay for curare using rats. *J Am Ph Ass* 1949; 38: 43-45.

13. Dehorah A, Gries, Geoge A, Condouris, Shey Z, Houpt M. Anxiolytic-like action in mice treated with nitrous oxide and oral triazolam or diazepam. *Life Sci* 2005;

14. Nogueira E, Vassilieff VS. Hypnotic, anticonvulsant and muscle relaxant effects of *Rubus lorasiliensis*. Involvement of $GABA_A$ system. *J Ethanopharmacol* 2000; 70: 275-280.

*****