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Research Article

ANXIOLYTIC AND CNS DEPRESSANT–LIKE EFFECTS OF ETHANOLIC EXTRACT OF LEAVES OF *CLERODENDRUM VISCOSUM* IN RATS

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ABSTRACT

The present study was carried out to investigate the possible Anxiolytic and CNS depressant-like effects of ethanolic extract of leaves of *Clerodendrum viscosum* in Rats. The effects of the plant extract on anxiety was evaluated by elevated plus maze and hole board test and the Central Nervous System depressant activity was confirmed by forced swim test and diazepam-induced sleeping time in rats at the doses of 200 and 400 mg/Kg, p.o. Diazepam (1 mg/kg), i. p. was used as a standard except for diazepam-induced sleeping time in which diazepam (25 mg/kg), i. p. was used to induce sleep. The extract has shown significant effect on anxiety at doses of 200 and 400 mg/Kg, p.o. and potentiated the CNS depressant activity at doses, 200 and 400 mg/Kg p.o. The statistical analysis was done through one-way ANOVA followed by *post hoc* Dunnett's multiple comparison tests. In Conclusion, the results were indicative of the significant anxiolytic and CNS depressant effects of the ethanolic extract of leaves of *Clerodendrum viscosum* in rat models.

Keywords: Anxiolytic, Depressant, *Clerodendrum viscosum*, Diazepam, elevated plus maze, Hole board test.

INTRODUCTION

Anxiety and Depression are the most common stress-related mood disorders causing disability and premature death in more than 20% of the adult population[1]. The complexity of daily life in modern society leads anxiety and depression as the major illness among the various psychiatric disorders, worldwide[2]. Anxiety is related to the mechanism of GABAergic and serotoninergic neurotransmission in brain; whereas depression occurs due to the abnormality of certain monoamine neurotransmitters such as serotonin and noradrenaline in central nervous system[3]. Benzodiazepines are the drug of choice for anxiety and the amine uptake inhibitors and monoamine oxidase inhibitors are used for depression. Recently, antidepressants are found to be more efficacious in place of benzodiazepines in the treatment of anxiety and depressive disorders[4]. Herbal drugs play a major role as complementary medicines to benzodiazepines and other antidepressant drugs whose side effects are prominent with limited efficacy in controlling this mood disorders[5].

Clerodendrum viscosum Vent., is a well-known ethnopharmacological shrub belonging to the family Verbenaceae. It has a square, blackish stem with simple, opposite decussate, petiolate, hairy leaves and is 2-4 feet in height[6]. The plant is commonly known as saraswaty leaf with other names as Bhant in Hindi, Bhagri in Sanskrit, Bhandari in Marathi, Bhat and Ghetu in Bengali, Bhania in Oriya, Glory tree in English and Cheruteku in Tamil[7]. The shrub is found widely in West Bengal and is common throughout the plains of India[8]. Several species of *Clerodendrum* genus have been traditionally used for their antioxidant, analgesic, antimicrobial and hepatoprotective profile. Various parts of the plant are used for the treatment of diverse ailments such as wounds, vermifuge, inflammatory diseases, tumor, malaria, hyperglycemia and fever as also for snake bite[9]. The leaves are used for skin diseases and in smallpox. The plant is also useful in Indian Folk medicine to cure bronchitis, asthma, epilepsy, fever, blood disorders and burning sensation. The plant contains triterpenes, steroids and flavonoids as its major phytoconstituents[10]. The present study demonstrates the Anxiolytic and CNS Depressant activities of ethanolic extract of leaves of *Clerodendrum viscosum* in rat models.

MATERIALS AND METHODS

Collection of Plant Material

Fresh leaves of *Clerodendrum viscosum* were collected locally from Tamil Nadu, India and authenticated by DR. N. S. Jeganathan M. Pharm., Ph. D., Professor and HOD, Department of Pharmacognosy**,** Surya School of Pharmacy, Surya Group of Institutions, Vikravandi, Villupuram, Tamil Nadu. Leaves were separated from adulterants, shade dried and powdered coarsely.

Plant Extraction

The shade dried leaves of *Clerodendrum viscosum* were powdered using a mixer and the extract was prepared by macerating 500 g of the fresh leaves of *Clerodendrum viscosum* with 500 ml of ethanol for 7 days in a closed vessel (so as to avoid evaporation of ethanol) with occasional shaking. On 8th day, the liquid mixture was filtered and marc (the solid residue) was pressed to recover as much solution as possible. The liquid so obtained was mixed well and dried at 40°C. The dried extract obtained after complete evaporation of ethanol was collected and stored in an airtight container for further pharmacological studies[11].

Experimental Animals

Wistar rats of either sex, weighing 150-200 gm were used for the study. They were allowed food and water *ad libitum* up to the experimentation period. Prior to use, the rats were housed in polypropylene cages in group of six to eight animals under natural light-dark cycle. Each animal was used only once under standard laboratory conditions. All the observations were made at room temperature in a noiseless diffusely illuminated room and were made between 9.00 to 17.00 h in the experimental room. All experimental protocols were approved by Institutional Animals Ethics Committee (2009/PO/Re/S/18/CPCSEA) as per provisions of Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India.

Drugs and Chemicals

Diazepam (NEON Laboratories Ltd, Mumbai, India) was used as a reference standard in this study. Gum acacia in water (1% w/v) (M/S Hi-media, Mumbai, India) was used as a vehicle. All other chemicals used were of analytical grade.

Acute Toxicity Study

Acute oral toxicity study of ethanolic extract of *Clerodendrum viscosum* leaves (EECV) was performed according to the method described by an Organization for Economic Cooperation and Development Guideline (OECD) 423. Wistar albino rats (150-200 gm, n = 6) of either sex was selected by random sampling technique. The animals were kept fasting for 4 hours prior to experiment with free excess of water. The extracts of *Clerodendrum viscosum* (suspended with 0.5 % W/V, CMC) were administered orally at a dose of 5 mg/Kg body weight to separate group of rats and mortality was observed for 3 days. If mortality was observed in 4/6 or 6/6 animals, then the dose administered was considered as toxic dose. However, if the mortality was found in single rat out of 6 animals, then the dose was repeated with higher 50, 300, 500, 1000 and 2000 mg/Kg of body weight. Behavioral changes and mortality of experimental rats were observed for 24 hours. After that continued observation were composed to the 14^{th} day[12].

Anxiolytic Activity

Elevated Plus Maze Model

The elevated plus maze apparatus consisted of two open arms (30 × 5 cm) and two closed arms (30 × 5 × 20 cm) elevated to the height of 50 cm. The edges of each open arm are 0.5 cm in height to keep the rat from falling down and the edges of the closed arms are 15 cm in height. Four groups of rats (each containing 6 animals) were used for the study. Control group were treated with vehicle (10 ml/Kg, p.o.), test groups with EECV (200 and 400 mg/Kg, p.o.), respectively. Standard group of animals received, Diazepam (1 mg/kg, i. p.) 60 minutes before the experiment. During the study period each rat was placed at the center of maze with its head facing one of the open arms. The number of entries and the time spent in open and closed arms were recorded for next 10 minutes. The arm entries were counted when all the four paws were in the arm. After each trial, the apparatus was cleaned with hydrogen peroxide and dried with sponge, carefully[13].

Hole Board Test (HBT)

The test apparatus consisted of a wooden box (40 × 40 × 25 cm) with 16 holes, each of 3 cm in diameter. The apparatus was elevated to the height of 25 cm and the holes were evenly distributed on the base of the box. Rats (n = 6) were treated with EECV (200 and 400 mg/kg, p.o.) or vehicle (1 % gum acacia, 10.0 ml/kg, p. o.) one hour prior and Diazepam (1 mg/kg, i. p.) 30 min prior to the commencement of the experiment. The animals were placed in the apparatus and numbers of head dips were recorded for the period of next 5 min manually using stop watch[14].

CNS depressant Activity

Forced Swim Test (FST)

Forced swim test was carried out according to Porsolt *et al* (1977). Rats were placed in a glass cylinders (30 cm in height, 22.5 cm in diameter) containing water up to a height of 15 cm, maintained at room temperature. Each animal was trained to swim in water for 15 min., constituted the pre-test session. Twenty four hours later, the test animals were treated with EECV (200 and 400 mg/Kg, p.o.) or vehicle (control group) 60 min before and Diazepam (1 mg/kg, i. p.) for standard group, 30 min before the commencement of test session, during which the rats were again forced to swim in glass cylinder filled with water for a period of 6 min. Immobility time for each rat was recorded and the animal was assessed to be immobile when it remains floating motionless in water and struggling to keep its head above water[15,16].

Diazepam-Induced Sleeping Time

This is another test model to screen the CNS depressant activity of a medicinal plant. Three groups of rats (each containing 6 animals) were used to determine the effect of EECV on duration of diazepam-induced sleep test. Rats were subjected to pretreatment by treating the control group with 1 % gum acacia (10 ml/kg, p.o.) and the test groups with EECV (200 and 400 mg/Kg/p.o.), respectively. The pretreatment was carried out 30 min. prior to the treatment. In treatment, Diazepam (25 mg/Kg, i. p.) was administered to all groups. Each animal was observed for the duration of sleep and the readings were recorded as the time interval between the loss and regaining of righting reflex[17].

Statistical analysis

Data were expressed as mean ± S.E.M. The results obtained from the different test groups were compared against the control group by using analysis of variance (ANOVA) followed by a *post-hoc* Dunnett's multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULTS

Acute Toxicity Study

The ethanolic extract of leaves of *Clerodendrum viscosum* was evaluated for its acute toxicity in rats. No mortality was observed to a dose as high as 2000 mg/Kg, p.o. of EECV leaves and the results showed that the extract can be used safely in animals up to a dose of 2000 mg/Kg, p.o.

Anxiolytic Activity

Elevated Plus Maze Model

The result in Table 1 showed the effect of oral administration of EECV (200 and 400 mg/Kg) in Elevated plus maze model. The extract (200 and 400 mg/Kg, p.o.) and diazepam (1 mg/Kg, i. p.) significantly increased the mean number of entries and mean time spent in open arms and decreased the preference to the closed arms, when compared with control.

Table 1: Effect of ethanolic extract of leaves of *Clerodendrum viscosum* on Elevated plus maze model for Anxiolytic activity

Treatment	Mean No of Entries (s)		Mean time spent (s)	
	Open arm	Closed arm	Open arm	Closed arm
Control (Vehicle)	4.500 ± 0.5627	12.50 ± 1.258	52.50 ± 9.330	247.7 ± 14.44
Diazepam (1 mg/Kg, i. p.)	14.50 ± 1.522**	3.667 ± 0.4944**	199.3 ± 7.839**	71.50 ± 7.042**
EECV (200 mg/Kg, p. o.)	8.667 ± 0.8819*	7.833 ± 0.4773**	87.00 ± 6.148*	150.8 ± 7.859**
EECV (400 mg/Kg, p. o.)	10.00 ± 0.5774**	6.167 ± 1.046**	136.0 ± 9.564**	106.2 ± 5.425**

Values are expressed as mean ± S.E.M of six animals. Statistical significance was calculated by one-way ANOVA followed by Dunnett's test. *P < 0.05, **P < 0.01, was considered significant as compared with control group.

Table 2: Effect of ethanolic extract of leaves of *Clerodendrum viscosum* on Forced Swim Test

Treatment	Duration of Immobility (S)
Control (Vehicle)	81.17 ± 7.565
Diazepam (1 mg/kg, i.p.)	159.7 ± 10.31**
EECV (200 mg/Kg, p.o.)	121.0 ± 9.466*
EECV (400 mg/Kg, p.o.)	134.2 ± 8.526**

Values are expressed as mean ± S.E.M of six animals. Statistical significance was calculated by one-way ANOVA followed by Dunnett's test. *P < 0.05, **P < 0.01, were considered significant as compared with control group.

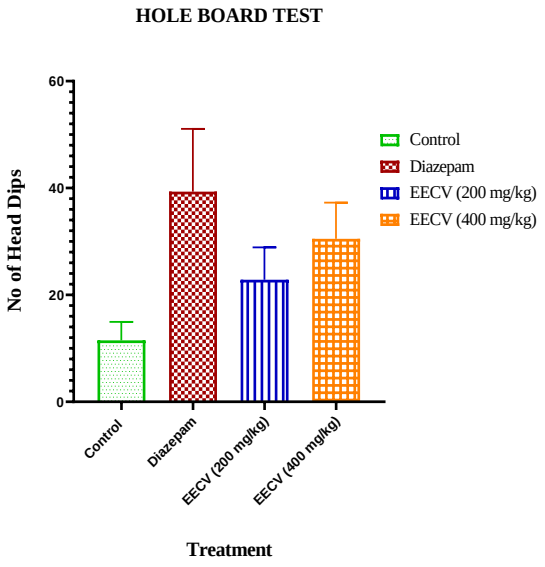

Figure 1: Graphical Representation of Effect of ethanolic extract of leaves of *Clerodendrum viscosum* on Hole Board Test in Rats

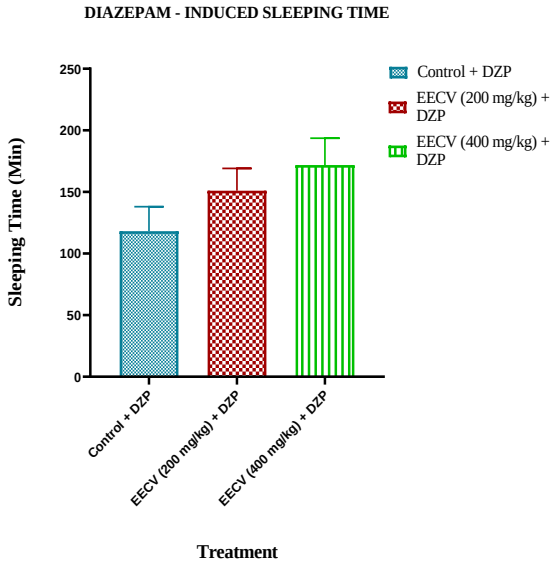

Figure 2: Graphical Representation of Effect of ethanolic extract of leaves of *Clerodendrum viscosum* on Diazepam-Induced Sleeping Time in Rats

Hole Board Test

The Figure 1 revealed that, in hole board test, the standard, Diazepam (1 mg/kg, i. p.) and the extract at a dose of 200 mg/kg, p. o. as well as 400 mg/kg, p.o. were found to increase the number of head dips, significantly, when compared with control.

CNS depressant Activity

Forced Swim Test

The ethanolic extract of leaves of *Clerodendrum viscosum* at doses 200 mg/Kg and 400 mg/Kg, p.o. is shown significant increase in immobility period in a dose dependent manner when compared to control. The results are shown in Table 2.

Diazepam-induced sleeping time

The extract potentiated diazepam-induced sleeping time in mice (Figure 2). The average sleeping time due to diazepam (25 mg/Kg, i. p.) alone was found to be 118.2 ± 8.118 in minutes. EECV (200 and 400 mg/Kg, p.o.) significantly potentiated the sleeping time induced by diazepam.

DISCUSSION

This study investigated the acute toxicity profile of EECV orally. The extract was evaluated for anxiolytic (Elevated plus maze model, Hole Board Test) and Antidepressant (Forced swim test, Diazepam-induced sleeping time) activities in rats. Acute toxicity was done according to OECD-423 guideline. No mortality was observed to a dose as high as 2000 mg/Kg, p.o. of EECV leaves and the result showed that the extract can be used safely in the animal up to the dose of 2000 mg/Kg. The *Clerodendrum*

viscosum leaf extracts were studied on elevated plus maze model and hole board test of anxiety. The extract at doses (200 and 400 mg/Kg, p.o.) increased the number of entries and time spent in open arm in elevated plus maze model and increased the number of head dips in hole board test, significantly, when compared with control. These findings revealed the anti-anxiety activity of EECV. The result that was obtained from anxiety - related behavior model in rats revealed that the EECV leaves possess an anxiolytic – like effect. Flavonoids are already reported as an anxiolytic agent through the γ-aminobutyric acid type A (GABA-A) receptors in the central nervous system (CNS)[18]. Hence the reported anxiolytic activity of the extract might be due to flavonoids.

The test models of depression (forced swim test and diazepam-induced sleeping time) were done for EECV leaves. Forced swim test was based on the observations that rats or mice when forced to swim in a restricted space from which there is no possibility of an escape eventually cease to struggle, surrounding themselves (despair or helplessness) to the experimental conditions. This state is considered to be as the state of depression[19]. The extract (200 and 400 mg/Kg, p.o.) is shown significant increase in immobility time in a dose dependent manner, compared to control. Diazepam induced sleep has been used to elucidate CNS active properties of drugs in animals. The *Clerodendrum viscosum* leaf extract potentiated the diazepam-induced sleeping time in mice. The average sleeping time due to diazepam (25 mg/Kg, i. p.) alone was found to be 118 in minutes. EECV (200 and 400 mg/Kg, p. o.) significantly potentiated the sleeping time, induced by diazepam (25 mg/Kg, i. p.). The potentiation of benzodiazepine induced sleep further suggests that the plant possess some sleep-inducing property.

The above anti-depressant screening models suggested the CNS-depressant activity of EECV leaves may be due to the increase in the concentration of GABA in brains[20]. GABA is known as an inhibitory neurotransmitter in a number of CNS pathways. Studies have also shown that GABA serves as a transmitter at about 30% of all the synapses in the CNS. The present study indicated that the leaf extract significantly increased brain GABA content in rats. According to a study conducted by Saad (1972), CNS-depressant drugs increased brain GABA content in rats[21], and these findings are in agreement with the above anti-anxiety studies that were shown that the anxiolytic like effect of EECV is mainly due to the presence of flavonoids since they act as a ligand for $GABA_A$ receptors and found to increase GABA content in brain. Further studies are required to explore the exact mechanism of action of the extract.

CONCLUSION

The experimental data thus obtained suggested that EECV possessed significant Anti-anxiety and CNS depressant-like effects in rats using experimental animal models. The activity may be due to the presence of flavonoids as its important phytoconstituent which may exhibits its action through $GABA_A$ receptors in CNS. Further detailed studies are required to isolate the active constituents that are present and to confirm their possible mechanism of action.

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