Evaluation of Neuropharmacological Effects of Ethanolic Extract of *Clitorea Ternatea* Flowers

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Summary

Anxiety is the most prevalent psychiatric illness in the general community. Anxiety affects one-eighth of the total population worldwide and has become an important area of research in psychopharmacology during this decade, although many drugs are available in allopathic system of medicine to treat anxiety disorders; they produce various systemic side effects or exhibit tolerance upon chronic use. The fact that benzodiazepines (BZDs), the major class of compounds used in anxiety have a narrow safety margin has prompted researchers to evaluate new compounds in the hope of identifying other anxiolytic drugs with fewer unwanted side effects[1]. The present study aims to study the effect of ethanolic extracts of flowers of c.ternatea on different paradigm for studying anxiety in rats. Administration of diazepam (2mg/Kg i.p) results in a significant increase in the time spent in the open arms and a reduction in time spent in closed arms. Whereas ethanolic extract of c.ternatea significantly increased the number and duration of head poking in the hole-board test. At a dose of 200mg/Kg i.p the extract significantly increased both the time spent in and the entries into the open arm by rats. Further, in the open field test, the extract significantly increased assisted rearing. In the light/dark paradigm the extract produced significant increase in time spent in the lighted box.

Keywords: Clitorea ternatea, Neuropharmacological, CNS depressant, neurotoxicity, memory impairment.

Introduction

Clitorea ternatea (CT) belonging to family Fabaceae is commonly called "gokarn". The plant is vigorous, herbaceous perennial legume. The leaves are compound and pinnate. The flowers have two sets of colours pale yellow bassaly and dark blue terminal [2]. The leaves and the roots of CT have been used in traditional medicine as diuretic, antidiabetic, cathartic and aperients[13]. Leaf infusion is used as anti-venom for snake bite. Phytochemical reports on DC indicate that the plant contains flavonoids, tannins, triterpenes, anthrocyanin, carbohydrates, glycosides, fixed oil, volatile oil and other constituents.

No major investigative reports were found pertaining to its CNS activity; therefore, we undertook the present study to determine the anxiolytic potential of CT by using mice as the animal model for anxiety based on exploratory behaviour.

Materials and Methods

Collection and extract preparation

The plant material (flowers) was collected from Berhampur, Orissa and was authenticated in the Department of Botany, Berhampur University, Berhampur. Fresh flowers were washed, shade dried and crushed to a coarse powder. The powdered plant material was defatted using petroleum ether (60^0-80^0 C) using Soxhlet extractor. The marc was further extracted by ethanol to obtain the extract. The extract was filtered and evaporated to dryness under reduced pressure on a rotary evaporator. The yield of ethanolic extract of CTF was found to be 2.2% w/w. Before use, the extract was dissolved in distilled water for intraperitoneal (i.p) administration.

Phytochemical Screening

Phytochemical investigation of the extract for the presence of phenolic flavonoids, tannins, triterpenes, anthrocyanins, anthroqinones, and sterols was carried out using the methods according to standard protocol [3,4] . The presence of alkaloids and saponins was also ascertained.

Animals

Albino Rats(150-180)g were used for the study. The animals were housed in colony cages and maintained under standard environmental conditions: 25 ± 2^0C temperature, 12;12h light:dark cycle, and 45-55% relative humidity, with free access to food and water *ad libitum*. The animals were fasted overnight and during the experiment. All experiments were carried out during the light period (08.00-16.00h). the institutional Animal Ethical Committee approved the protocol of the study.

The animals were divided into five groups, each containing six rats. The groups of rats were assigned to receive one of the following (i) vehicle (distilled water 0.1ml/Kg)
(ii) diazepam (1mg/Kg) (iii) CT (50mg/Kg, i.p) (iv) CT (100mg/Kg, i.p.) and CT (200mg/kg, i.p); this group pattern was used to assess the behavioural parameters.

Drugs and chemicals

Diazepam was used as the standard anxiolytic agent. Petroleum ether (60^0-80^0C) and ethanol were purchased locally and were of analytical grade. Distilled water was used as vehicle.

Neuropharmacological Tests

Test for Alertness: Hole-board test:

This test was done using Hole-Board. The hole-board apparatus consisted of a 0.8 m^3 wooden board with 16 holes (5cm in diameter). The rat was placed at the corner of the board and was allowed to move freely. First two minutes were allowed for adaptation and the number of head dippings in next four minutes was counted [5].

Test for locomotor activity:

The locomotor activity was measured by using an actophotometer (Inco, Ambala, India). It consists of a cage which is 40 cm long and 40X40X40 cm and has a wire mess at the bottom. Six lights and six photo cells are placed in the outer periphery of the bottom in such a way that a single rat blocks only one beam. Photo cells are activated when the rays of light fall on photocells. The beam of light is cut as and when animal crosses the light beam, number of cut offs were recorded for 10 minutes [6,17].

Elevated plus-maze test (EPM) :

The EPM consisted of two open arms (50X10 cm) crossed with two closed arms (50x10x40cm). The arms were connected together with a central square of 10 cm. The apparatus was elevated to a height of 50cm in a dimly illuminated room[12,15,16]. Rats were treated with the CAE (50, 100, 200 mg/kg, i.p.), diazepam (1mg/kg i.p), or vehicle 30 min before being placed individually in the centre of the EPM, facing a closed arm. The time spent in both the open arms was recorded for 5min. The number of entries into the open and closed arms was also counted during the test. An entry was defined as having all four paws within the arm[14]

Tail Suspension test (TST):

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. [7]. Rats were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility was recorded during the next 4 min of a total 6 min test [1,6,7].

Motor Co-ordination Test (Rota Rod Test):

Motor Co-ordination test was conducted using a Rota rod apparatus (Inco Ambala, India). The animals were placed on the moving rod prior to the treatment and the rats that stayed on the rod without falling for 120 seconds were choosen for the study. The fall of time of animals before and after the extract was noted [8,9].

Phytochemical Analysis

Phytochemical screening of CTE extract revealed the presence of Glycosides, steroids, saponins, carbohydrates, fixed oil, volatile oil and flavonoids.

Statistical Analysis:

Results are expressed as mean ± S.E.M. The statistical analysis of data was done using the one-way analysis of variance (ANOVA) followed by Dunnet's 'T' test, in all tests the criteria for statistical significance was $p<0.05$.

Results and Discussion

The results of the hole-board test are summarized in (Fig.1). A significant ($p<0.01$) increase in exploratory behaviour was observed at all dose levels and followed a dose dependent increase in comparison to control vehicle group.

Ethanolic extract of CT in a dose of 800 mg/Kg did not cause any mortality in groups of mice during the 24 h period after oral administration. CT extracts at doses of 100, 200 mg/Kg produced significant ($p<0.01$) and dose dependent decrease in locomotor activity in comparison to control vehicle group (Fig.2). They showed a decrease, however in the time spent in closed arms of elevated plus maze as summarized in Fig.3.
In the present study, rats that received diazepam showed a significant increase in the time spent and the rears in open arms and the percentile ratio of open arms are summarized in Fig.4. The CT flower extract 100mg/Kg and 200mg /Kg significantly increased the time spent in open arm. The results of the present study indicate that the crude alcoholic extract of the CT flower extract produced a significant decrease in spontaneous motor activity. Alteration of general behaviour is a good index of CNS depressant activity [10]. which could be attributed to the sedative effect of the extract.

Results of tail suspension test (Fig 5) revealed that there was a significant ($p<0.01$) and dose dependent increase in the immobility time at 100 mg/Kg and 200 mg/Kg doses treated groups in comparison to control vehicle group.

Rotarod test revealed a significant loss of muscular coordination and the poor performance in the tail suspension test in 100 mg/Kg and 200 mg/Kg doses (Fig 6). This test is mainly used to screen centrally acting muscle relaxant [11], which could be due to loss of muscular strength. CNS depressant action may be due to the phytochemicals present of in the crude extract of CT.

The CT flower extract possessed CNS depressant activity as indicated by the significantly reduced, motor coordination, spontaneous motor activity, and increased immobility time in tail suspension test and forced swimming test indicated by CNS depressant effects.

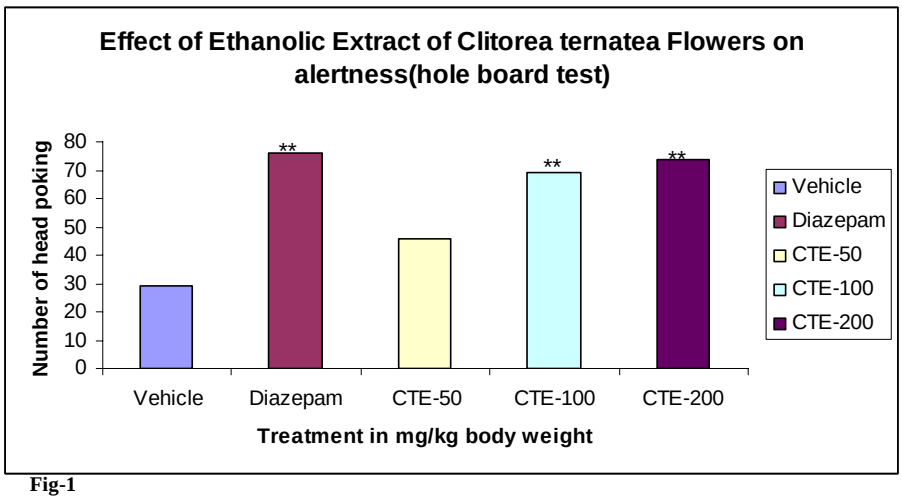

Fig-1
values are expressed as Mean±SEM of 6 animals,** p<0.01Vs Control Group

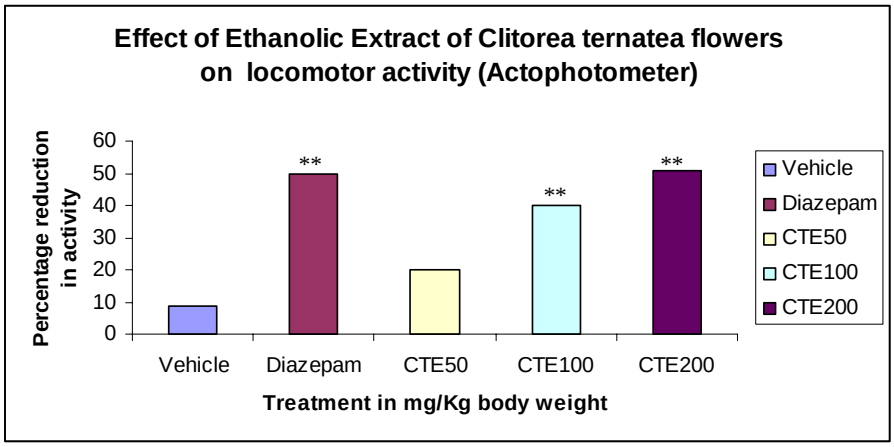

Fig-2
values are expressed as Mean±SEM of 6 animals,** p<0.01Vs Control Group

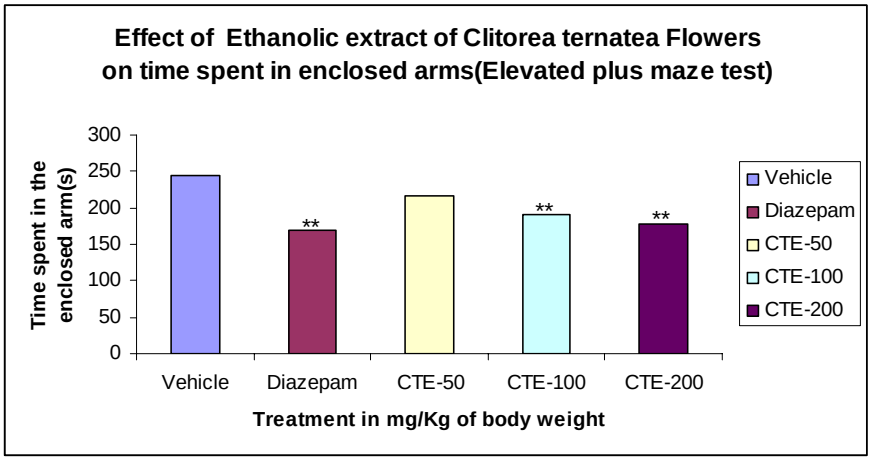

Fig-3
values are expressed as Mean±SEM of 6 animals,** p<0.01Vs Control Group

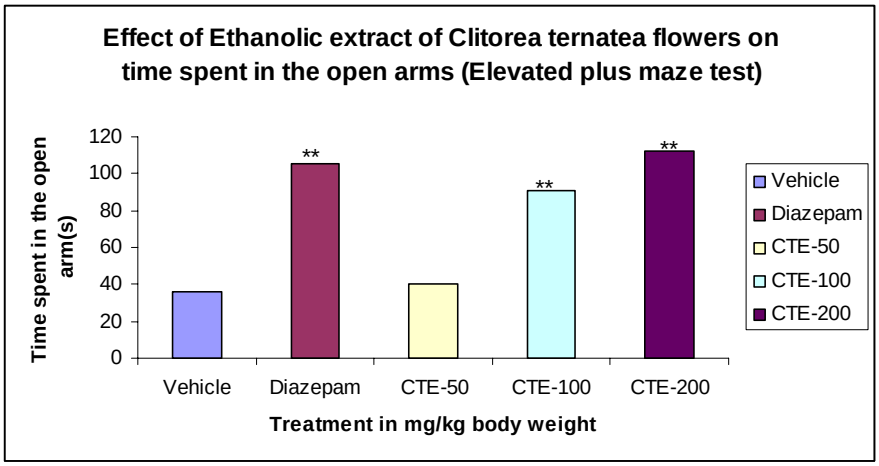

Fig-4
values are expressed as Mean±SEM of 6 animals,** p<0.01Vs Control Group

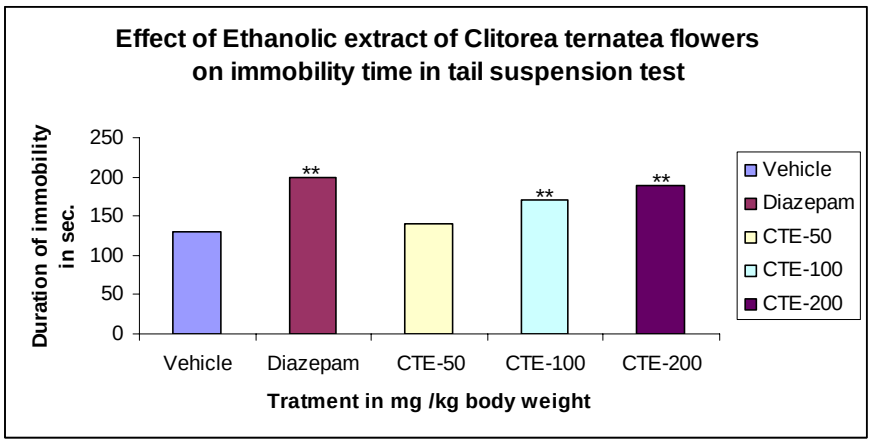

Fig-5
values are expressed as Mean±SEM of 6 animals,** p<0.01Vs Control Group

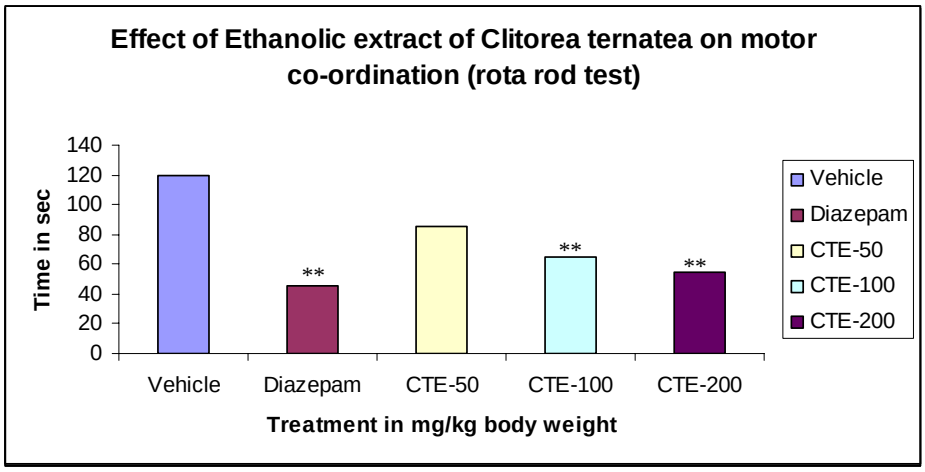

Fig-6
values are expressed as Mean±SEM of 6 animals,** p<0.01Vs Control Group

Conclusion

The neuropharmacological investigations on the alcoholic extract of *Clitorea ternatea* flower indicate that the flower may have active principles with CNS depressant activity[18,19].

The results obtained in this elevated plus maze and rota rod study suggest that the extract of the flowers of *C.ternatea* possesses anxiolytic and muscle-relaxant activities, which is possibly mediated through the $GABA_A$-BZD mechanism. Thus *C. ternatea* has a potential clinical application in the management of anxiety and muscle tension disorders. Further investigation of the mechanism(s) of action of the plant extract, as well as the active substances responsible for its biological actions, is necessary.

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