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Research Article

PSYCHOPHARMACOLOGICAL SCREENING OF CYLISTA SCARIOSA ROXB. LEAVES IN MICE

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ABSTRACT

The aqueous and ethanolic exrracts of Cylista scariosa Roxb. leaves were evaluated for its psychopharmacological activities in several experimental models using swiss albino mice. Use of the elevated plus-maze test revealed that aqueous (100 mg/kg and 200 mg/kg, i.p) and ethanolic (50, 100 and 200 mg/kg, i.p) extracts of Cylista scariosa and standard drug diazepam (1 mg/kg, i.p) increased the percentage of time and entries spent in open arms. Chronic treatment with the aqueous (100 mg/kg and 200 mg/kg, i.p) and ethanolic (50, 100 and 200 mg/kg, i.p) extracts of Cylista scariosa leaves, were able to decrease the immobility time of mice to forced swim tests and the effects were comparable to that of standard drug imipramine (15 mg/kg, i.p). The mice treated intraperitoneally with reserpine (5 mg/kg) were submitted to increased catalepsy and tremors time. Treatment with aqueous (100 mg/kg and 200 mg/kg, i.p) and ethanolic (50, 100 and 200 mg/kg, i.p) extracts of Cylista scariosa leaves antagonized the reserpine induced catalepsy and tremors time these effects comparable to standard drug Levodopa (150 mg/kg, i.p). All these results were compared with respective controls for the evaluation of significance. The presence of flavonoids, glycosids, tannins and L-dopa in the aqueous and ethanolic extract of Cylista scariosa leaves might be responsible for anxiolytic, antidepressant and antiparkinsonian activity of Cylista scariosa extracts. Cylista scariosa extracts may have potential therapeutic value for the management of psychopharmacological disorders.

Keywords: Cylista scariosa, Psychopharmacology, Antianxiety, Antidepressant, Antiparkinsonian.

INTRODUCTION

According to the World Health report approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receives even the most basic treatment [1]. 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]. Mental ailments are heterogeneous diseases and will probably require a selected arsenal of drugs with different modes of action for successful treatment of their various manifestations [4]. Anxiety disorders are among the most prevalent of all psychological problems worldwide [5].

In present era, a sudden holocaust of mental disorders, and recognition of severe side effects and addiction liabilities associated with long term administration of widely prescribed synthetic drugs have aroused the attention of researchers towards natural resources. Plants like *Valeriana officinalis*, *Nardostachys jatamansi*, *Withania somnifera* and *Panax qinseng* have been used extensively in various traditional systems of therapy because of their adaptogenic and psychotropic properties. Inclusion of these well established CNS affecting plants in the arsenal of modern therapeutics has revived the faith of researchers in the plants [6].

Despite the development of new molecules for pharmacotherapy of depression, it is unfortunatethat this disorder goes undiagnosed and untreated in many patients. Reserpine induces symptoms resembling those of Parkinson's disease in humans [7,8] and signs of similar motor disturbance in laboratory animals [9, 10]. In the mice, reserpine induces rigidity of skeletal muscles [11,12] as well as tremor, postural flexion,hypokinesia and several other signs of motor disturbance [13, 14]. Although the currently prescribed molecules provide some improvement in the clinical condition of patients, it is at a cost of having to bear the burden of their adverse effects [15, 16]. Ayurveda, the Indian traditional system of medicine, mentions a number of single and compound drug formulations of plant origin that are used in the treatment of psychiatric disorders [15,17].

Cylista scariosa Roxb., (Syn: Paracalyx scariosus), commonly known as Nadinishpara (Sanskrit), Kalawel (Hindi), Ranghevada (Marathi), belongs to family Fabaceae s. A survey of ethanopharmacologic records reveals that the plant has been traditionally used in the treatment of fracture, stomachache; the root is astringent and is given in the form of a decoction in dysentery and leucorrhoea, snake bite and venereal disease. The traditional healers of Chhattisgarh use this plant in treating different types of cancer with some herbal combination [19, 20, 21].

The leaves of Cylista scariosa have not been phytochemically investigated, so far. It contains new prenylated dihydroflavonol, scariosin and a new prenylated flavonol, isorhynchospermin along with kaempferol, quercetin, kaempferol-3-O-rutinoside, rutin L-dopa, phenols, proteins, fatty acids, amino acids and minerals [22,23]. An exhausted literature survey on *Cylista scariosa* revealed that sporadic pharmacological reports are available on this plant as a anticonvulsant activity against metrazol seizure [18]. As *Cylista scariosa* has been used traditionally for the treatment of various ailments, this plant holds great potential for in depth phytochemical and pharmacological evaluations. Despite a long history of use of *Cylista scariosa* as a traditional medicine for the treatment of various ailments, especially in CNS disorders, the plant has never been subjected to other CNS activity studies. Thus, it was considered worthwhile to subject *Cylista scariosa* to anti-anxiety, anti-depressant and anti-parkinsonian screening studies.

MATERIALS AND METHODS

Plant material

The plant *Cylista scariosa* Roxb. leaves were collected from Bhandara District, in December 2012. The plant was botanically identified and authenticated by Prof. (Dr.) S. M. Bhuskute, Department of Botany, Bhawabhuti Mahavidyalaya, Amgaon, Dist. Gondia (M. S.) India. Specimen of *Cylista scariosa* Roxb. leaves is available for further references. The specimen voucher no is 6190 B.

Animals

The albino mice (Swiss strain) weighing 22-28 g, bred in the animal house of Institute of Pharmaceutical Education and Research (IPER), Wardha, were procured. The animals were housed in polypropylene cages at a temperature of 22±2º C with relative humidity of 40-60% and 12 hrs. light dark cycle. Animals were fed with a balanced diet and water *ad libitum* during the complete experimental period. All animal experiments were approved by the Institutional Animal Ethical Committee (Registration No. 535/02/a/CPCSEA/Jan2002) of Institute of Pharmaceutical Education and Research (IPER), Wardha, India.

Solvents

Petroleum ether (60°–80°C), chloroform and ethanol, all of LR grade, distilled under normal atmospheric pressure were employed for extraction of the plant material.

Preparation of extracts, and their phytochemical screening

Dried, coarsely powdered leaves of *Cylista scariosa* (500 g) were successively extracted with petroleum ether, chloroform and ethanol using a Soxhlet apparatus. The marc was air dried and mark obtained from ethanol extraction was air dried and macerated with distilled water at room temperature and the extract was dried at 50 ºC after the maceration process and get water extract. All the four extracts were dissolved in respective solvents, and were screened for different classes of phytoconstituents [24].

Dose preparation and administration of extracts and standard drugs

All the extracts were suspended in carboxy methyl cellulose (CMC), 0.5% solution and administered intra peritoneal route (i.p). The doses for aqueous and ethanolic extracts were 50 mg/kg, 100 mg/kg and 200 mg/kg. These extracts were dissolved in 0.5 % solution of CMC. Diazepam was used as standard for antianxiety screening given at a dose of (1 mg/kg, i.p) [25] dissolved in saline solution and this solution was ultrasonicated. Imipramine was used as standard for antidepressant screening given at (15 mg/kg, i.p) [25] and Levodopa was used as standard antiparkinsonian drug given at (150 mg/kg, i.p) [26] were also dissolved in saline solution. Reserpine (5 mg/kg, i.p) [26] dissolved in 0.1 % glacial acetic acid solution.

Vehicle and standard

Distilled water + CMC (0.5%) was used as vehicle for preparing the suspension of various test doses of different extract s. Diazepam (Plethicopharma, Indore), imipramine (Zim Laboratories, Nagpur), Levodopa (Taj Pharmaceuticals, Mumbai) and reserpine (HiMedia Laboratories Ltd, Mumbai), were used as standard drug.

Elevated plus maze (EPM) model of anxiety

The plus-maze apparatus consisting of two open arms (16 × 5cm) and two closed arms (16 × 5 × 12 cm) having an open roof, with the plus-maze elevated (25 cm) from the floor was used to observe anxiolytic behaviour in animals[27, 28]. Each mouse was placed at the centre of the elevated plus maze with its head facing the open arms. During this 5 minutes experiment, the behavior of the mouse was recorded as: (a) the number of entries into the open arms, (b) average time spent by the mouse in the open arms (average time = total time spent in open arms/number of entries in arms). Extracts of *Cylista scariosa* were administered intra peritoneal route using an insulin syringe. The dose administration schedule was so adjusted that each mouse was having its turn on the elevated plus-maze apparatus 45 minutes 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 in voke anxiety in the animals.

% Time Spent In Open Arm (Epm)

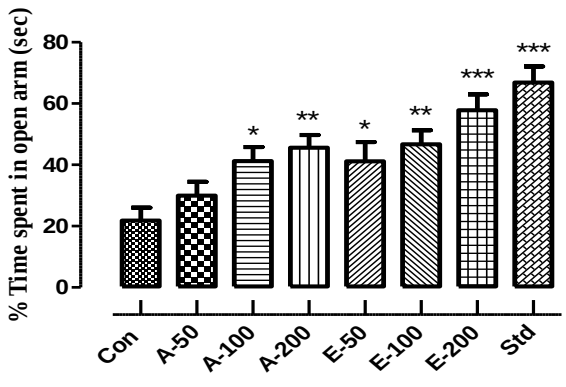

Fig. 1: Effects of aqueous and ethanolic extracts of *Cylista scariosa* on % time spent in open arm.

No. Of entries in open arm (epm)

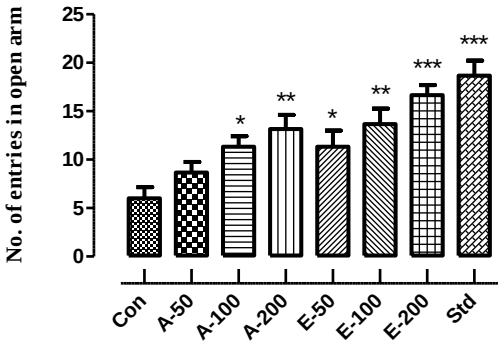

Fig.2: Effects of aqueous and ethanolic extracts of *Ccylista scariosa* on No. of entries in open arm.

Forced swimming test (FST) [29]

This test was performed as described by Porsolt et al. (1977) with slight modifications. The FST is the most widely used pharmacological in vivo model for assessing antidepressant activity. The development of immobility when the mice are placed in an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behavior. The apparatus consisted of a clear plexi glass cylinder (25 cm high × 12 cm diameter) filled to a 15 cm depth with water (24 ± 1 ◦C). In the pre-test session, every animal was placed individually into the cylinder for 15 min, 24 hr prior to the 5 min swimming test. *Cylista scariosa* leaves extract, Imipramine and distilled water were administered three times: immediately after the initial 15 min pre-test, 18 and 1 hr prior to the swimming test. During the test session a trained observer registered the immobility time, considered to be when the mouse made no further attempts to escape, apart from the movements necessary to keep its head above the water.

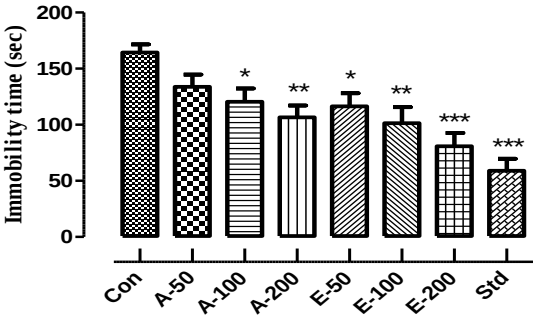

Fig.3: Effects of aqueous and ethanolic extracts of *Cylista scariosa* on immobility time (sec).

Catalepsy bar test

Bar test determinations were carried out by gently placing mice forepaw over a horizontal bar, fixed at a height of 10 cm with heads of animals towards upward on an inclined surface at an angle of 60 ° with the hind limbs abducted. A horizontal glass bar having (2 mm diameter) elevated 4.5 cm above the observation floor. The length of time during which the animal retained this position was recorded by measuring the time from the placement of the rat until removal of one of its forepaws. Testing was performed 30 minutes post injection and the time to withdrawal of legs by the rats was measured

Statistics

Data were analyzed using Prism 5 for Windows (version 5.03). Result were expressed as Mean ± SEM. One-way analysis of variance (ANOVA), Dunnet's T test & Tukeys multiple comparison test were used to test the significance of the difference between the variables in various groups. The p values of less than 0.05 were considered to be statistically significant.

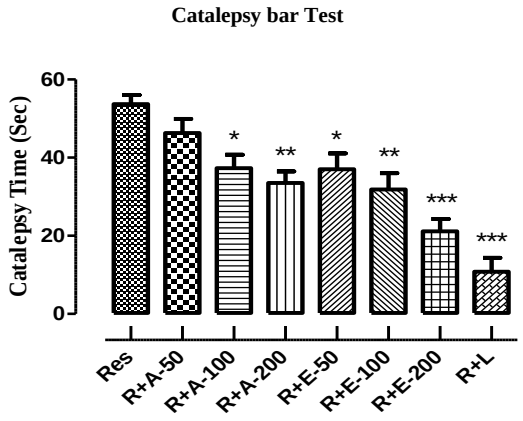

Fig. no. 4 Effects of aqueous and ethanolic extracts of *Cylista scariosa* on Catalepsy

RESULTS AND DISCUSSION

Anti-anxiety activity of various extracts of *Cylista scariosa* leaves was evaluated employing a widely used model, elevated plus-maze. The model is principally based on the observations that the exposure of animals to an elevated and open maze results in approach–avoidance conflict which is manifested as an exploratory-cum-fear drive. The fear due to height (acrophobia) induces anxiety in the animals when placed on the elevated plus-maze. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in motor activity, which is measured by the time spent by the animal in the open arms. On the other hand, several plants increase the exploration of open arms in the elevated plus-maze test and are used to diminish anxiety in folk medicine. Dried aqueous and ethanol extracts of *Cylista scariosa* leaves, separately suspended in a suitable vehicle, were administered intraperitoneally to mice, and the activity was compared with that observed in the control group as well as with the group treated with the standard anxiolytic drug diazepam. Complete manifestation of anxiety in mice of the control group is evident from the minimum mean time spent in the open arms of elevated plus-maze by these animals. Diazepam is used as a standard anxiolytic and has been frequently employed in behavioural pharmacology as a reference compound to potentially anxiolytic-acting substances[30, 31, 32]. In the present study, a single acute administration of diazepam led to "anxiolytic" behaviour compared to the controls, diazepam increased the numbers of entrances into the open arms and prolonged the stay on these arms in addition to a reduction of the parameters for a risk assessment[33, 34]. Treatment with different doses of aqueous extracts of *Cylista scariosa* leaves (100, 200 mg/kg, i.p.) resulted in a significant increase in percent time spent and entries into open arms (*p < 0.05 and **p < 0.01) compared to control. Similarly treatment with different doses of ethanolic extracts of *Cylista scariosa* leaves (50,100 and 200 mg/kg, i.p.) resulted in significant increase percent time spent and entries into open arm (*p < 0.05, **p < 0.01 and ***p < 0.001) compared to control. Among the extracts tested, maximum anxiolytic activity was observed in the ethanol at the dose of 200 mg/kg which was at par with that of diazepam as is evident from statistical equivalence between the results of this dose and that manifested by diazepam. However, the activity decreased at higher doses, which might be due to sedation.

Table 1: Yield of various extracts of *Cylista scariosa* leaves.

Sr. No	Solvent	Extraction process	% Yield
1	Petroleum ether (60-80 ºC)	Soxhlation	6.41 %
2	Chloroform	Soxhlation	8.83 %
3	Ethanol	Soxhlation	16.0 %
4	Water	Maceration	11.25 %

Table 2: Results of phytochemical screening of various extracts of *Cylista scariosa* leaves.

Sr. No	Plant constituent	Test/ Reagent	Petroleum extract	eth.	Chloroform extract	Ethanol extract	Water extract
1	Sterol	Salkowaski	+		+	-	-
2	Fatty acid	Spot test	+		+	-	-
3	Glycoside	Kellerkillani	+		+	+	+
4	Tannins	FeCl3	-		-	+	+
5	Flavonoids	Shinoda	-		-	+	+
		FeCl3	-		-	+	+
6	Saponin	Foam test	-		+	-	-
7	Amino acid	Millon's test	-		-	-	+

+: present, -: absent

On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviors [29]. In the present study we have evaluated the antidepressant activity of *Cylista scariosa* leaves in FST, mice are forced to swim in a restricted space from which they cannot escape and are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. Treatment with different doses of aqueous extracts of *Cylista scariosa* leaves (100, 200 mg/kg, i.p.) resulted in a significant decrease in immobility time (*p < 0.05 and **p < 0.01) compared to control. Similarly treatment with different doses of ethanolic extracts of *Cylista scariosa* leaves (50,100 and 200 mg/kg, i.p.) resulted in significant decrease in immobility time (*p < 0.05, **p < 0.01 and ***p < 0.001) compared to control. Regarding the medical treatment of psychiatric disorders, the results obtained in this work became important because not only anxiolytic effects were observed; antidepressant activity was also shown. Results showed that the administration of the *Cylista scariosa* leaves extract produced a diminution of immobility time of mice exposed to the forced swimming test. These behavioral effects were similar to that found by other authors after treating mice with classical antidepressant drugs as imipramine. The assumption of contrasting pathophysiology responsible for parkinsonian-like and tardive dyskinesia, namely, striatal dopamine deficiency and overactivity, respectively, leads one to expect that these two conditions might be mutually exclusive and therefore not found in same patient. The administration of reserpine has been shown to induce all these symptoms of PD in mice (catalepsy and tremor), as well as dyskinetic perioral movements. The animals treated with this monoamine-depleting drug exhibited a significant increase in catalepsy and tremors time[26]. The plant *Cylista scariosa* antagonized the reserpine treated mice for antiparkinsonian activity. Treatment with different doses of aqueous extracts of *Cylista scariosa* leaves (100, 200 mg/kg, i.p.) resulted in a significant decrease in catalepsy and tremor (*p < 0.05 and **p < 0.01) compared to control. Similarly treatment with different doses of ethanolic extracts of *Cylista scariosa* leaves (50,100 and 200 mg/kg, i.p.) resulted in significant decrease in catalepsy, grooming and tremor (*p < 0.05, **p < 0.01 and ***p < 0.001) compared to control. Results of this study suggest that both aqueous and ethanolic extracts of *Cylista scariosa* leaves produced antianxiety, antidepressant and antiparkinson's - like effects in mice in EPM, FST and catalepsy bar test. The efficacy of the extracts was comparable to that of standard drugs diazepam, imipramine and levodopa. Phytochemical screening showed presence of flavonoids, glycosides, amino acids and tannins present in aqueous and ethanolic extracts of *Cylista scariosa* leaves. The ethanolic extract show more potential effect as compared to aqueous extract because of the presence of total extract of plant constituent i.e., both non-polar and polar components. It also contains the aglycon (non-sugar) part of flavonoids which is not present in aqueous extract.

In conclusion, our results suggest that both aqueous and ethanolic extracts of *Cylista scariosa* leaves possess antianxiety, antidepressant and antiparkinsonian-like effects using various behavioral models in mice. This study supports the usefulness of animal models of anxiety (EPM), depression (FST) and Parkinson (Reserpine induced catalepsy) to screen the psychopharmacological activity. Thus, *Cylista scariosa* leaves extracts may have potential therapeutic value for the management of psychopharmacological disorders.

CONCLUSION

From the results of the study it was concluded that both aqueous and ethanolic extracts of *Cylista scariosa* leaves possess antianxiety, antidepressant and antiparkinsonian-like effects using various behavioral models in mice.

This study supports the usefulness of animal models of anxiety (EPM), depression (FST) and Parkinson (Reserpine induced catalepsy) to screen the psychopharmacological activity.

Cylista scariosa extracts may have potential therapeutic value for the management of psychopharmacological disorders.

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CONFLICT OF INTEREST

None.

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