Research Article

Psychopharmacological Activity of Chirata

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Received: 14-07-2023; **Revised:** 21-09-2023; **Accepted:** 27-09-2023; **Published on:** 15-10-2023.

ABSTRACT

The objective of the present study was to investigate the Psychopharmacological Activity of the ethanolic and methanolic extract of leaves of *Swertia chirayita* Roxb. ex Flem. in mice as a part of psychopharmacological screening of this plant, using different in vivo experimental models. The effects of the plant extract ethanolic and methanolic (250 mg/kg and 500 mg/kg respectively) on the central nervous system (CNS) was evaluated by forced swim test, plus maze method, Hole board test and spontaneous motor activity. The results revealed that the ethanolic extract of *Swertia chirayita* Roxb. ex Flem plant has showed better results as compared to methanolic extract. In the test studied for the Psychopharmacological Activity of ethanolic and methanolic extract of *Swertia chirayita* Roxb. ex Flem plant, using forced swim test, plus maze method, Hole board test and spontaneous motor activity it was revealed that the ethanolic extract at doses 250 mg/kg and 500mg/kg showed significantly ($p<0.05$). The overall results suggest that the ethanolic extract of *Swertia chirayita* Roxb. ex Flem plant contains flavonoids which may possess significant psychopharmacological activity.

Keywords: Psychopharmacological Activity, *Swertia chirayita*, Herbal Extract.

INTRODUCTION

The experimental and recreational use of organic medicines and drugs in the nineteenth century sprang up Psychopharmacology[1]. 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. Psychoactive drugs are typically utilized to alter mood or treat psychopathology. Depending on the influence on the central nervous system psychoactive drugs are categorized to alter mood or consciousness[2].

CNS depression is considered as an affective disorder characterized by change in mood, lack of interest in the surroundings, apathy, loss of energy, psychomotor retardation, melancholia as well as profound feelings of gloominess, despair and suicidal ideation. It has been estimated that the rate of prevalence of CNS depression is around 5% in the general population and is accepted to be heterogenous symptomatically, biologically and psychologically[3].

Anxiety is both a normal emotion and a psychiatric disorder. Anxiety is a feeling of apprehension or fear, combined with symptoms of sympathetic activity. It is a normal response to stress and becomes a clinical problem only if the anxiety becomes severe or persistent, and interferes with everyday performance. It has a lifetime prevalence of over 5% of the population[4]. It is typically associated with the former psychoneurotic disorders; hypothesis implicates over activity of adrenergic systems or irregular activity of serotonergic systems in the CNS and the symptoms of anxiety are commonly associated with depression[5]. In spite of the availability of CNS depressants and anxiolytic drugs, depression and anxiety continue to be a major medical problem[6]. Medicinal plants have been useful in the development of new drugs and continue to play an invaluable role in the drug discovery processes[7]. These herbs or plants are relatively cheap, available and their uses are dependent on ancestral experience[8].

In the present study, we selected a plant namely *Swertia chirayita* (Roxb. ex Flem.) Karsten, an annual or biennial herb, belongs to the family of Gentianaceae. It is known as Chirata in Hindi, Kiratatikta in Sanskrit, and in a trade known as Chiretta. The plant occurs sporadically in subtropical and temperate forests, in open forest margins, cool and moist places or in shady, moist slopes among tall grasses. It is reported as endangered due to loss of habitat and over-exploitation for medicinal uses[9]. This ethnomedicinal herb is known mostly for its bitter taste caused by the presence of different chemical constituents such as amarogentin, swerchirin, swertiamarin, and other bioactive compounds[10, 11].

Due to the huge demand for the herb, other species of Swertia and plants like Andrographis paniculata (green chirayita), Slevolgia orientalis, Exacum tetragonum, E. pedunculatum and E. bicolor, are adulterated/substituted in the trade [12, 13].

The present study was carried out to investigate the psychopharmacological screening of the ethanolic and methanolic extract of *Swertia chirayita* (Roxb. ex Flem.) plant in the Behavioral animal models for the assessment of psychopharmacological activity.

MATERIALS AND METHODS

Plant materials: The plant of *Swertia chirayita* was collected in the month of January from the local region of Lucknow; the plants were identified and authenticated by the taxonomist of CDRI, Lucknow and a voucher specimen were deposited CDRI, Lucknow. The collected leaves were cut into smaller pieces, dried under shade, pulverized and stored in a closed container until further use.

Preparation of extract: *Swertia chirayita* leaves was collected in local region then fresh leave wash and dry at room temperature; all leaves dry (after two weeks) & crushed with the help of in Mortars & Pestles; the powdered material used for extracted by Soxhlet apparatus using the solvents for Ethanol and Methanol successively the extraction process a (40-60 °C) temperature for 6 hours the concentrated product was collected and stored in refrigerator for further experimental analysis.[14, 15]

Drugs and Chemicals: Drugs and chemicals used in the study were obtained commercially and were of analytical grade. Phenobarbitone (Novartis India ltd., Hyderabad, India), Imipramine (Sigma, USA) ,DMSO and ethanol & methanol (Hi-pure Fine Chem Industries, Hyderabad, India).

Animals: Swiss albino male mice weighing 25-30 gms, were used for all sets of experiments in groups of six animals; they were maintained at controlled room temperature (25±2˚C) on 12-hour light/dark cycle and allowed free access to food and water. The experiments were performed after the experimental protocols approved by the Institutional Animal Ethics Committee and care of animals was taken as per CPCSEA guidelines; Animals were divided in to control group, standard group and extracts treated group and each group consists of 6 animals.

Preliminary Phytochemical Screening: The preliminary phytochemical investigations were carried out with the ethanolic and methanolic extract of *Swertia chirayita* (Roxb. ex Flem.) plant for qualitative identification of phytochemical constituents using standard conventional protocol. All the chemicals and reagents used were of analytical grade.[16]

Acute toxicity study: Acute toxicity study was performed for chloroform and methanol extracts of *Swertia chirayita* according to the acute toxic classic method as per guidelines prescribed by OECD and acute toxicity test aims at establishing the therapeutic index, i.e. the ratio between pharmacological effective dose and lethal dose on the same strain and species (LD_{50}/ED_{50}). 2000 mg/kg of extract was administered as per OECD guidelines per orally to 6 mice. Effects were observed on behavior for 72 hours; mice were examined for behavioral effects 45 minutes post administration of the extracts; no change in behavior or any abnormality in behavior was observed and no mortality was seen and thus it was concluded that chloroform and methanol extract of *Swertia chirayita* was nontoxic up to 2000 mg/kg doses; then 1/5th and 1/10th of the administered dose was selected for future studies as per OECD guidelines.[17, 18]

Psychopharmacological studies:

Treatment: Animals were divided into six (I-VI) groups for the assessment of both the plant extracts. Group I was a negative control; Group II was positive control; Groups III to IV received ethanol extract of *Swertia chirayita* at doses of 250 and 500 mg/kg p.o respectively. Group V to VI received methanolic extract of *Swertia chirayita* at doses of 250 and 500 mg/kg, p.o respectively.

Forced Swimming Test: The apparatus consisted of an opaque Plexiglas cylinder (50 cm high × 20 cm wide) filled with water at room temperature, to a depth of 30 cm; during the 6 min swimming test, immobility behavior was observed, defined as when the animal made no further attempts to escape except for the movements necessary to keep its head above the water and Reduction in immobility is considered as a behavioral profile consistent with an antidepressant like action.[19, 20]

Elevated Plus Maze: This apparatus consists of two open arms (50×10 cm) crossed with two closed arms (50×10×40cm); the arm was connected together with a central square (10×10 cm); the apparatus was elevated to a height of 70 cm in a dimly illuminated room; each mouse was placed individually at the center of the elevated maze, 45 minutes post administration of the extracts and the standard. The number of entries in the open and closed arm of the elevated maze during a period of 5 minutes and the duration of stay in the open and closed arm were noted; after each test, the maze was carefully cleaned up with a wet tissue paper (10% ethanol solution) entry into the arms was defined as the point when the animal places all four paws in the arm subsequently, the percentage of open arm entries (100 × open/total entries) and the percentage of time spent in the open arms (100 × open/open + enclosed) were calculated for each animal.[21, 22]

Head Dip Test Method: Exploratory behavior of mice in a novel environment was measured using a hole-board test (locally constructed); this method is used for measuring the response of the rat to an unfamiliar environment the apparatus consisted of a grey cardboard box (50×50×50 cm) with 18 equidistant holes 3 cm in diameter in the floor. 30 minutes after proposed treatment with std/samples, head-dipping behaviors were checked for 20 minutes.

CNS Inhibitory Activity: The actophotometer was switched on and the animals were placed individually in the activity cage for 10 min; standard, test and vehicle were injected in each animal of proposed groups and after 30 min each animal was tested for 10 min. The locomotor activity after treatment was noted.[23, 24]

Statistical Analysis: Results are represented as Mean ± SEM; The test extract, standard and control were analyzed with the help of one-way analysis of variance (ANOVA)

followed by Dunnett's Test and P values < 0.05 were considered as statistically significant.[25]

RESULTS AND DISCUSSION

Extraction of Drug: Percentage Yield of ethanol and extract *S. chirayita* is 9.67% and 8.47% respectively.

Phyto-chemical screening: Phytochemical studies revealed the presence of phenolics compound & flavonoids were noticed in ethanolic extract of S. chirayita leaves.

Psychopharmacological Activity: The results of the present investigation showed that the ethanol and methanol extracts of *Swertia chirayita* have some psychopharmacological activity.

Acute toxicity study: Assessment of acute toxicity is the first step in the toxicological investigation of an unknown substance The ethanol and methanol extracts of *Swertia chirayita* were well tolerated by mice and there were no signs of acute or delayed toxicity after oral administration Increasing doses up to 2000 mg/kg (p.o) were not lethal, the LD50 values for the extract was estimated to be higher than 2000 mg/kg for oral administration Thus, suggesting that this administration route is adequate and secure to produce its psychopharmacological effects.

Antidepressant Activity by Forced swim test: The ethanol and methanol extracts of *Swertia chirayita* (250 & 500 mg/kg) exerted increases in the immobility of mice following its administration to mice when compared with control group. These increases were significant (p<0.001) at lower dose (250 mg/kg) of the extract.

Table 1: Phyto-chemical screening of extracts

Plant constituents test/ reagent used	Ethanol	Methanol
Alkaloids		
Meyer's reagent	+	+
Dragendroff's reagent	+	+
Wagner's reagent	+	+
Hager's reagent	+	+
Flavonoids		
Alkaline reagent test	+	+
Zinc hydrochloride test	-	-
Anthraquinone Glycosides		
Test for hydroxyl	+	+
Bruntrager's test	+	+
Starch		
Starch (Amylum)	+	-
Proteins		
Hydrolysis test	-	-
Fats & Fixed Oils		
Fats & Fixed Oils	-	-

+ mean present, -mean absent

Table 2: Effect of *Swertia chirayita* extract on immobility time

Group	Treatment	Dose	Immobility period (Sec)
I	Control	-	146 ± 0.765
II	Standard (Imipramine)	15 mg/kg	105 ± 2.232
III	Ethanol Extract	250 mg/kg	108 ± 1.124**
IV	Ethanol Extract	500 mg/kg	123 ± 2.822*
V	Methanol Extract	250 mg/kg	109 ± 1.987**
VI	Methanol Extract	500 mg/kg	128 ± 1.765*

Values are expressed as mean ± SEM; n=6 in each group; *p<0.05; **p<0.01; ***p<0.001, significant as compared to control. Not significant – p>0.05.

Table 3: Effect of *Swertia chirayita* extract on % time spent in open arm of EPM

Group	Treatment	Dose	Time **spent in open arm**
I	Control	-	20.6 ± 0.754
II	Standard (Imipramine)	15 mg/kg	59 ± 0.213
III	Ethanol extract	250 mg/kg	39.8 ± 0.164**
IV	Ethanol Extract	500 mg/kg	51 ± 0.432*
V	Methanol Extract	250 mg/kg	35.9 ± 1.06**
VI	Methanol Extract	500 mg/kg	28 ± 1.05*

Values are expressed as mean ± SEM; n=6 in each group; *p<0.05; **p<0.01; ***p<0.001, significant as compared to control; Not significant – p>0.05.

Anxiolytic Activity by Elevated plus maze model: Anti-anxiety activity of chloroform and methanol extract of *Swertia chirayita* were evaluated employing a widely used model, elevated plus-maze the model was chosen as it is effective, inexpensive, simple, less time consuming, requires no preliminary training to the mice and does not cause much discomfort to the animals while handling 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 induces anxiety in the animals when placed on the elevated plus-maze the ultimate sign 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.

The ethanol extract of *Swertia chirayita* (250 and 500 mg/kg, p.o.) produced a significant (P<0.01) increase in % time spent in open arm of Elevated plus maze the methanol extract of *Swertia chirayita* (250 and 500 mg/kg, p.o.) produced a significant (P<0.05) increase in % time spent in open arm of Elevated plus maze and the ethanol extract of *Swertia chirayita* (250 and 500 mg/kg, p.o.) showed better anxiolytic activity than methanol extract.

Exploratory behavior pattern by Hole board test: Hole-Board test was a measure of exploratory behavior and an agent that decreases this behavior reveals sedative activity.

Table 4: Effect of *Swertia chirayita* extract on % open arm entries

Group	Treatment	Dose	% open arm entries
I	Control	-	7.68 ± 0.45
II	Standard (Imipramine)	15 mg/kg	32.8± 0.289
III	Ethanol extract	250 mg/kg	26.98± 0.142**
IV	Ethanol Extract	500 mg/kg	23.8 ± 0.326*
V	Methanol Extract	250 mg/kg	16.9 ± 0.26**
VI	Methanol Extract	500 mg/kg	15.7 ± 0.85*

Values are expressed as mean ± SEM; n=6 in each group; *p<0.05; **p<0.01; ***p<0.001, significant as compared to control. Not significant – p>0.05.

Table 5: Effect of *Swertia chirayita* extract on no. of head dips

Group	Treatment	Dose	no. of head dips
I	Control	-	27.62 ± 0.854
II	Standard (Imipramine)	15 mg/kg	11.6± 0.56
III	Ethanol extract	250 mg/kg	21.98± 0.76**
IV	Ethanol Extract	500 mg/kg	18.8 ± 0.532*
V	Methanol Extract	250 mg/kg	23.7 ± 0.32**
VI	Methanol Extract	500 mg/kg	19.8 ± 0.34*

Values are expressed as mean ± SEM; n=6 in each group; *p<0.05; **p<0.01; ***p<0.001, significant as compared to control. Not significant – p>0.05.

Table 6: Effect of *Swertia chirayita* extract on spontaneous locomotor activity

Group	Treatment	Dose	Locomotor activity
I	Control	-	143 ± 0.854
II	Standard (Imipramine)	15 mg/kg	31.6± 0.654
III	Ethanol extract	250 mg/kg	109.98± 0.455*
IV	Ethanol Extract	500 mg/kg	88.8 ± 0.554**
V	Methanol Extract	250 mg/kg	113.98 ± 0.32*
VI	Methanol Extract	500 mg/kg	89.9 ± 0.34**

Values are expressed as mean ± SEM; n=6 in each group; *p<0.05; **p<0.01; ***p<0.001, significant as compared to control Not significant – p>0.05.

The ethanol extract of *Swertia chirayita* (250 and 500 mg/kg, p.o.) produced a significant (P<0.01) and dose-dependent reduction of exploratory behavior in the hole board test and the methanol extract of *Swertia chirayita* (500 mg/kg, p.o.) produced a significant (P<0.01) reduction of exploratory behavior in the hole board test the methanol extract of *Swertia chirayita* (250 mg/kg, p.o.) does not produced (P>0.05) any reduction of exploratory behavior in the hole board test.

Spontaneous Motor Activity: Monitoring of locomotor activity of animal has been an important step in assessing effects of drugs on the CNS; The movement is a measure of the level of excitability of the CNS and its decrease may be intimately related to sedation resulting from the depression of the CNS the ethanol and methanol extracts of *Swertia chirayita* (250 and 500 mg/kg, p.o.) produced a significant ($P<0.05$, $P<0.01$) and dose-dependent decrease in spontaneous motor activity likewise, positive control Imipramine (15 mg/kg, p.o.) also produced significant reduction in spontaneous motor activity decrease in the spontaneous motor activity leads to sedation as a result of reduced excitability of the central nervous system.

CONCLUSION

Present work is an attempt to compile psychopharmacological work on *Swertia chirayita*. Toxicity studies were performed for different extract to assess their safety in mice. Methanol, and ethanol extract of both plants were found safe and did not cause any mortality at the dose of 2000 mg/kg body weight. Forced swim test, elevated plus maze model, head dip test and Immobility test were used to evaluate psychoharmacological activity of ethanol and methanol extracts of both plants. Antidepressant activity was evaluated by Forced Swim Test in which immobility time was noted. Anxiolytic activity was performed using elevated plus maze model. This model itself induces anxiety. The % open arm entries and % time spent in open arm was noted. The exploratory behavior was performed using hole board test apparatus and no. of head dipping was noted. The CNS inhibitory activity was done by using Actophotometer in which spontaneous motor activity count was noted. The extracts significantly decreased locomotor activity and increased immobility time suggesting depression and sedating potentials. Sedation may be due to interaction with benzodiazepines-like compounds. The ethanol extract of both the plants show better activity in all above mentioned model. The activity may be due to presence of terpenes, saponins, flavonoids and phenolics.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.