Innovations in Pharmaceuticals and Pharmacotherapy

www.innpharmacotherapy.com

eISSN: 2321–323X
pISSN: 2395-0781

Research article

Antidepressant activity of "Cissampelos pareria" extract in mice

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Abstract

Depression is a wide spread psychiatric disorder affecting around 5% of the population and it is difficult to predict the patient response to any given treatment. The present study aimed at studying the pharmacological activity of *Cissampelos pareria* leaves that contain bis benzylisoquinoline alkaloids (BIA), tetrandin, bebriberin, and flavons that are known for their anti anxiolytic activity. These alkaloids act by inhibiting mono amino oxidase (MAO), the enzyme responsible for metabolism of monoamine neurotransmitters. The present study was under taken to evaluate the anti depressant activity of "cissampelos pareria" extract in mice at doses of 100 and 200 mg/kg p.o., against standard Imiparamine (10mg/kg) and control (1% gum acacia). Tail suspension test and forced swim test was conducted and duration of immobility was recorded, in both of these tests carried, *Cissampelos Pareria* (200mg/kg p.o.) produced significant antidepressant activity compared with the standard Imipramine, which was evident from the decreased immobility when extract was used.

Keywords: forced swim test, tail suspension test, *Cissampelos Pareria.*

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1. Introduction

Depression is a common mental disorder, characterized by sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, feelings of tiredness and poor concentration [1]. More than just sadness in response to life's struggles and setbacks, depression changes how you think, feel, and function in daily activities. It can tire or deplete you and interfere with your ability to work, study, eat, sleep, and enjoy your life. Severe depression can be intense and unrelenting.

While some people describe depression as sadness or "living in a black hole," others don't feel much at all. They feel lifeless, empty, and apathetic, or men in particular may even feel angry and restless. No matter how you experience depression, left untreated it can become increasingly debilitating. In addition to medication, there are now lifestyle changes that are proving just as effective in relieving mild to moderate forms of depression [2-4].

Major depression is a serious disorder of enormous sociological and clinical relevance. The discovery of antidepressant drugs in the 1950s led to the first biochemical hypothesis of depression, which suggested that impairment in central monoaminergic function was the major reason underlying the disorder [2-9]. Basic research in all fields of neuroscience (including genetics) and the discovery of new antidepressant drugs have revolutionized our understanding of the mechanisms underlying depression and drug action. Depression could thus result from an inability to make the appropriate adaptive responses to stress or other aversive stimuli, and antidepressants may act by correcting this dysfunction or by directly inducing the appropriate adaptive responses.

2. Materials and methods

Material: Imipramine and N-Hexane was purchased from Sigma Aldrich, Acacia was purchased from Parchem.

Plant profile

Cissampelos pareria is a woody climbing vine with leaves up to one foot long and it belongs to the family menispermaceae and genus Cissampelos. It is found

throughout the tropical region of India and Bangladesh The plants whole vine, seed, bark and leaf have medicinal effect. The leaves of the plant as shown in Fig.1. contain alkaloids like tetrandine which has analgesic effect and has recently been shown to have antitumor, antilukaemic properties as well. The roots and stem contain the bisbenzylisoquinoline alkaloids which are proven to have anti-inflammatory property. Traditionally Cissampelos pareria has been used for numerous medicinal purposes like fever, diarrhea and infections. Cissampelos pareriais thought to be responsible for many of its properties like antioxidant, anti inflammatory. It is commonly referred to as midwives' herb and has been traditionally used to combat women's ailments such as preventing miscarriages, uterine hemorrhages and stimulation of lactation. Leaves are used as an Antiseptic against inflammation and can be put on wounds in order to heal sores [1-4]. Immunomodulation using medicinal plants especially rasayana drugs can provide an alternative to conventional chemotherapy for a variety of diseases especially when the host defense mechanism has to be activated under the condition of impaired immune response when a selective immune suppression is desired *in situ* actions like auto immune disorders. Indian medicinal plants are a rich source of substance which are claimed to induce innate immunity the non specific immunomodulation of essentially granulocytes, macrophages, natural killer cells and complement functions**.**

Fig.1. Cissampelos pareria leaves

Collection of plant material

The leaves of Cissampelos pareria were collected from Bobbili, Vizianagaram located in Andhrapradesh, India on Nov 2014 It was authenticated by Dr. M.Venkiah, Associated professor, Department of Botany Andhra University Visakhapatnam. The botanical nomenclature of the plant was duly identified by using standard flora and also cross-checked with Herbarium records. The materials were shade dried for 10 days and pulverized.

Preparation of extract

The leaves of *Cissampelos pareria* were collected and dried in shade (25°c) by the air drying method for 7 days and then they were grinded with electrical grinder. The dried materials under go by Soxhelation process with n-hexane, Aqueous in 300g/Lit for 72 hr. The obtained extract was dried under vacuum using rotary evaporator and stored in an air tight container at room temperature.

Animals:

Albino mice of either sex weighing between 20-30gm were used in this study. All the animals were acclimatized in the quarantine room in Andhra University Animal house for 7 days and housed in groups of 5 under standard husbandry conditions like room temperature 23±2°C, relative humidity 30-70% and light/ dark cycle of 12 hours. All the animals were fed with standard food and water. All the experimental protocols were approved by Institutional Animal Ethical Committee (IAEC) and conducted as per the rules and regulations in accordance to the guidelines of CPCSEA.

All animals were fasted 3h prior to oral administration of vehicle/standard/test compounds during the experiment were carried out during the light period (9:00 to 17:30h) to avoid circadian rhythm.

Methods

The Aqueous and n-hexane extract of leaves and shoots of *Cisampelos pareria* investigated for its Pharmacological activities which include-Acute oral Toxicity and Antidepressant activity

Pharmacological Activities

Acute Toxicity Study

Determination of Maximum Tolerance Dose: OECD guidelines (425) state that, before establishing pharmacological activity of the new chemical Entity (NCE), it is mandatory to establish Maximum Tolerated Dose (MTD) in mice. The purpose of the citing study was to allow selection of appropriate starting dose for the main study and the starting dose selected from the fixed dose levels of 5, 50, 100, 300, 1,000 and 2,000 mg/kg.

Animal Study

Five mice (2males and 3 females) were taken for each dose. Mice were fasted prior to oral administration for 3-4h but water is continued with ad labitum. The scheduled doses of the test substance were given orally to the animals according to individual weights of rats. The animals were observed for behavioral changes at 0.5h, 1h, 2h and 4h after oral administration. The mortality was recorded till 14 days. After completion of 14 days, animals were sacrificed to ascertain the absorption of the concentration of the test compound and the changes in the vital organ due to the test compound. $1/10^{th}$ and $1/20^{th}$ of Maximum Tolerance Dose (2000mg/kg; p.o) were selected for the pharmacological study.

***In Vivo* Pharmacological Models in Mice**

Two familiar tests Forced swim test, Tail suspension tests were carried on mice. Animals were divided into 4 groups of 5 animals in each, weighing between 20-25 gms. The extracts of both AECP and nHECP are given to the animals. In addition to the test (AECP & nECP- high and low doses), control and standard (Imipramine) selected

Table1. Treatments Vs. Groups

Category	Treatment	Dose of the drug P.O.
Group I	Control-Gum Acacia	1 % Gum Acacia
Group II	Standard-Imipramine	10 mg/kg
Group III	Test-ECP-Low Dose	100 mg/kg
Group IV	Test ECP-High Dose	200 mg/kg
Group V	Test-nECP-Low Dose	100 mg/kg
Group VI	Test nECP-High Dose	200 mg/kg

Tail suspension test

The tail suspension method used in this study was similar to those described by steru et al., (1985). Treatment was given 6 min prior to study as described by study design. Mice were suspended on the edge of the table, 50cm above the floor, with the help of adhesive tape placed approximately 1cm from the tip of tail. The total duration of immobility induced by tail suspension was recorded during 6 min of the 10 min period. Animal was considered to be immobile when it did not show any movement of the body, hanging passively and motionless completely [4].

Forced swim test of aqueous extract

On the first of the two test days, all test animals were placed in a vertical Plexiglas cylinder (height: 45 cm, diameter: 19 cm) with 23°C tap water to the level that made rats impossible for to reach the bottom with hind paws (28–30 cm). The animals were removed from the water after 10 min, and dried before being returned to their home cages. On the next day, the procedure was repeated with two main differences: the animals were removed from the water after 5 min, instead of 10 min which was done before; Three different behaviors were noted first is immobility – according to the previous literature of Porsolt et al., a rat is judged to be immobile when it floated passively, making mere small movements to keeping nose above the surface; second is climbing (or thrashing) – upward-directed movements with its forepaws along the edges; third is swimming – active movements (usually horizontal) with forward movement. Diving and face shaking behaviors were not considered[4].The time (*t*) spent in immobility and climbing was measured; the time spent swimming was calculated: *t* swimming = 5 − (*t* climbing + *t* immobility). Additionally, the latency to the first bout of immobility was determined: period of time since the beginning of the rat mobilization in the water until the first episode of immobility.

Acute oral toxicity study

The both extracts of leaves and shoots of *Cissampelos pareria* was administered orally to different groups of mice at different dose levels to test the acute oral toxicity.

Statistical Analysis

All the values were expressed as Mean ± S.E.M. the results were analyzed statistically by one-way ANOVA followed by Dunett Multiple comparison test, $P<0.05$ was considered significant.

3. Result

Preliminary phytochemical screening

The AECP, nHECP of Cissampelos pareria leaves was subjected for phytochecimal screening and found that carbohydrates, glycosides, alkaloids and steroids, tannins, saponins were present. The results were shown in table no.2.

Table 2. Phytochemical tests for AECP, nHECP, extract of cissampelos parerialeaves (+) indicates presence:(-) indicates absence

S.N	Phytochemical Constituents	Aqueous	nHexane
1.	Test for carbohydrates		
	Molisch`s test	+	+
	Fehling`s test	+	-
	Barfoed`s test	-	-
	Benedict`s test	+	-
2.	Test for Alkaloids		
	Dragendroff`s test	+	-
	Wagner`s test	-	+
	Mayer`s test	+	+
	Hager`s test	+	+
3.	Test for Anthraquinone glycosides	-	-
4.	Test for steroids		
	Salkowski test	+	+
	Libermann Burched test	+	+
5.	Test for flavonids	+	+
	Shinoda test		
6.	Test for saponins		
	Foam test	+	+
7.	Test for tannins	+	+

8.	Test for glycosides	+	+
9.	Test for triterpinoids	-	-
10.	Test for Glucosides	+	+

Acute oral toxicity study

The both extracts of leaves and shoots of *Cissampelos pareria* was administered orally to different groups of mice at different dose levels and found to be safe even up to the dose level of 2000 mg/kg and did not produce any mortality or toxic symptoms. For the present study $1/10^{th}$ and $1/20^{th}$ (i.e.100 & 200mg/kg) of maximum Tolerance Doses (2000mgkg; p.o) were selected.

Table 3. Effect of Cissampelos Pareria on immobility time in Forced swim test and Tail Suspensiontest Each value represents Mean ± S.E.M., n=6. $^{**}p< 0.05$ compared with control

	Treatment	Forced Swim test Duration of Immobility (Sec)	Tail Suspension test Duration of Immobility (Sec)
Group I	Control-Gum Acacia	140 ± 6.2	149 ± 5.2
Group II	Standard-Imipramine	60 ± 1.65**	90 ± 4.32**
Group III	Test-AECP-Low Dose	63± 1.56**	91 ± 4.25**
Group IV	Test AECP-High Dose	48 ± 1.36**	82 ± 4.65**
Group V	Test-nECP-Low Dose	46 ± 1.33**	86 ± 1.32**
Group VI	Test-nECP-High	40 ± 1.33**	72 ± 1.33**

4. Discussion

Depression is a heterogeneous mood disorder characterized with regular negative moods, decreased physical activity, feelings of helplessness and is caused by decreased brain levels of monoamines like noradrenaline, dopamine and serotonin. Therefore, drugs restoring the reduced levels of these monoamines in the brain either by inhibiting monoamine oxidase or by inhibiting reuptake of these neurotransmitters might be fruitful in the treatment of depression that has been classified and treated in a verity of ways. Although a number of synthetic drugs are being used as standard treatment for clinically depressed patients, they have adverse effects that can compromise the therapeutic treatment [15]. Thus, it is worthwhile to look for antidepressants from plants with proven advantage and favourable benefits-to-risk ratio.

On the basis of the above information, both aqueous and non aqueous (nHexane) leaf and shoot extract of *Cissampelos pareria* were selected for evaluating its antidepressant activity due to its traditional use in treatment of depression.

In Acute Oral Toxicity study, both AECP and nHECP did not show any lethal effect even up to the doses of 2000mg/kg, po and test doses of 100 & 200mg/kg, po were used for the Pharmacological activity.

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 behaviours. The two most widely used animal models for antidepressant screening are the forced swimming and tail suspension tests. These tests are quite sensitive and relatively specific to all major classes of antidepressants. In TST, immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. Similarly in the FST, mice are forced to swim in restricted space from which they cannot escape. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression. It has been seen that the TST is less stressful and has higher pharmacological sensitivity than FST [Santosh P et al., 2011].

Results shown that the administration of nHECP produced more activity compared with AECP in both forced swim test and Tail suspension test. In the present study, from the results given in Table 3 it is clear that there is significant difference between control and all other groups. The HECP (200mg/kg, po) produced significant anti depressant effect than AECP and their efficacies were found to be comparable to Imipramine (10mg/kg, po).

From all the above, the Anti depressant activity of nHexane extract of leaf of *Cissampelos pareria* was found to be significant at 200mg/kg, po. The flavanoid components of nHECP might be interacting with 5-HT in mediating the anti depressant effect of. *Cissampelos pareria*

Conclusion

The AECP and nHECP contains carbohydrates, alkaloids, flavanoids, steroids, glycosides, saponins, amino acids, gums and mucilage. From the above findings, the Anti depressant activity of nHECP and AECP found significant at 200 mg/kg, p.o. In Forced swim test, Tail suspension test shortening of immobility

time in the forced swimming and tail suspension tests indicating nHECP acting either by enhancement of central 5-HT and catecholamine neurotransmission. However, more extensive Pharmacological studies of this plant are required for complete understanding of the Anti depressant activity of nHexane extract of leaf extract of Cissampelos pareria.

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