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Cognition Enhancing and Anti-Psychotic Effect of Hydro-Alcoholic Leaves Extract of *Cissampelos pareira L.*

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Abstract

Aim: The present study was performed to explore effects of leaves hydro-alcoholic extract of *Cissampelos pareira L* as cognitive enhancer and as antipsychotic. **Methods:** The leaves powder of *C. pareira* was extracted using hydro alcoholic solvents. Acute oral toxicity study was performed for obtained crude extract as per OECD guideline 423. Based on which three doses were selected for evaluation of cognitive enhancing using morris water maze model and anti-psychotic activity using cook's pole climbing test. The data of the study was analysed using Graph pad prism version 3 software. **Results:** Acute oral toxicity study of hydro alcoholic leaves extract of *C. pareira* revealed that the extract displayed no sign of toxicity in mice up to dose 2000 mg/kg, based on which three doses viz 100, 200 and 400 mg/kg of *C. pareira* hydro alcoholic extract (CPHE) was selected. In Morris water maze model, mice of group CPHE 100, 200 and 400 mg/kg displayed cognitive enhancing activity in dose dependant manner. While, in cook's pole climbing test, mice of group CPHE 400 mg/kg displayed anti-psychotic effect, whereas CPHE 100 and 200 mg/kg did not display anti-psychotic effect. **Conclusion:** Based on the results of the study, it can be concluded that treatment with hydro alcoholic extract of leaves of *C. pareira* justifies its traditional uses as memory enhancer and anti-psychotic. However, further detailed study is required to warrant its use in condition of AD associated with psychosis.

Keywords

Cissampelos pareira, cook's pole climbing test, Morris water maze test, neuroprotection.

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INTRODUCTION

Alzheimer's disease (AD), the commonest form of dementia, characterized by progressive loss of memory, decline of cognition, impairment of daily activities, psychological and behavioural disturbances, impose major health threat. It has been reported that more than 115 million people are going to be affected by dementia, by 2050. [1, 2] Alzheimer's disease [Alzheimer's disease with psychosis (AD + P)] is frequently associated with hallucinations and delusions. Delusions of persecution, abandonment, dead people still living, infidelity are common delution in patients with AD. Misidentification delusions such as non-identification of self's home or family members, or thinking of family member's to be imposter is also common with patient of AD. However, the delusions in AD are not as complex as with schizophrenia. When AD is associated with psychosis, there is co-occurrence of behavioural disturbances such as aggression and agitation.

Current available treatment for AD with psychosis is associated with limited efficacy and higher toxicity. For instance, Haloperidol, a conventional antipsychotic, has been reported to have mild to moderate efficacy in AD with psychosis however, it is associated with some serious side effects such as parkinsonism, tardive dyskinesia, and akathisia. [3] Therefore, there is constant search of potential treatment option, both synthetic and natural for AD and psychosis. In recent years, researchers are more inclined toward exploring traditional and complementary medicines to achieve medical benefits related to several disease including neurological disorders.

Cissampelos pareira (C.pareira), Menispermaceae, is a plant of great significance and has been traditionally used in alleviating anxiety, depression and other brain related disorders. The leaves of the plants are rich in several beneficial phytoconstituents such as alkaloids, flavonoids, phenolic compounds etc. [4,5]. Leaves of *C.pareira* are rich in alkaloids such as magnoflorine, Laudanosine, nuciferine dicentrine,; and flavonoids such as quercetin 3-O-sophoroside, eriodictyl-7-O-beta-D-glucoside, naringenin 7-OD-glucoside, baicalein-7-O-glucoside, and, galangin-7-glucoside [6].

Therefore, based on its traditional uses and phytoconstituents present in its leaves, the present study was undertaken to evaluate the effect of leaf extract of *C.pareira* on cognition and psychosis.

MATERIALS AND METHODS

Plant material:

Fresh leaves of *C. pareira* were identified and collected from the Udaipur region, Rajasthan, and authenticated from Bilwal Medchem and Research Laboratory pvt. Ltd., Reengus Rajasthan. A voucher specimen (voucher no. BSI/ AZRC / I.12012 / Tech. / 2019 (PI. ID.) 549) of the plant was deposited in the herbarium laboratory. *C. pareira* leaves were cleaned and reduced into small parts and kept under shade until dried completely followed by its pulverization into coarse powder. The powdered material was stored in air-tight container.

Drugs and Chemicals:

Solvents used for extraction were laboratory reagent (LR) grade and were purchased from Loba Chemie Ltd. Mumbai. fluoxetine (Flunil 20mg/5ml Suspension) was procured from Intas Pharmaceutical Ltd., Ahmedabad, Gujarat India. The suspensions of fluoxetine tablets were made into distilled water using 2 % Tween-80 as a suspending agent. Tween 80 used was pharma grade and procured from S.D. Fine Ltd., Mumbai. Test drug were formulated as suspension in distilled water using Tween-80 as suspending agent.

Experimental animals

Swiss mice (20-30 g) of either sex were procured and housed in the animal house of Bilwal Medchem and Research Laboratory Pvt. Ltd., Reengus Industrial Area (RIICO), Sikar, (Raj.) under standard conditions of temperature (25 ±2°C), relative humidity (45-55%) and 12 hour dark/light cycles and were provided with a standard diet (standard pellets, Hafed, Rohatak India) and water *ad libitum*. The animal experiments were in compliance with Animal Ethical Committee, Committee for the Purpose of Control And Supervision of Experiments on Animals (CPCSEA) and were approved by Institutional Animal Ethics Committee (IAEC) of Bilwal Medchem and Research Laboratory Pvt. Ltd., SKS Reengus Industrial Area,Reengus Sikar, (Raj.) (Ethical committee IAEC reg.no.2005/PO/RcBT/S/18/CPCSEA).

Extraction of leaves of *C.pareira*

250g coarse leaves powdered of *C.pareira* were defatted with 6000 ml petroleum ether (60-80^{0}C) using Soxhlet apparatus. The obtained dried marc was 240 gm in weight that was used for further extraction with hydro alcoholic solvent, ethanol: water (7:3). The dried marc obtained was packed in soxhlet apparatus and extracted with 1000 ml of hydro alcoholic solvent. Extraction was continued until a drop of solvent from the siphon tube become clear. The extract obtained, was filtered and collected. The filtered extract was evaporated in rotary evaporator and dried in oven to remove remaining moisture. The crude extract thus obtained was weighed and kept in a sealed container until further use [7].

Acute oral toxicity study

Organisation of Economic Co-operation and development (OECD) guideline 423 for testing of chemical was used for carrying acute oral toxicity of the crude extract. Swiss male mice were divided into six groups containing three animals (n=3) in each group. Test extract was prepared as a suspension by triturating with 2% Tween-80. The different doses of test extract solution of (1, 10, 100, 500, 1000 and 2000 mg/kg b.wt.) were given orally. Animals were observed at regular time intervals at least once during the first 30 minutes of initial dosing during the first 24 hours (with special attention given during first 4 hours), and daily then after for total 14days for any sign of acute toxicity. [8]

Experimental group: For anti-depressant study swiss mice of either sex were divided into five groups containing 6 animals in each group:

Group 1: Normal control group of vehicles (NCGV): Distilled water, 1ml/100 gm, p.o

Group 2: Positive control group of Fluoxetine (PCGF): fluoxetine 10 mg/kg, p.o
Group 3: Low dose of *C.pareira* hydro-alcoholic extract group: CPHE 100 mg/kg, p.o
Group 4: Medium dose of *C.pareira* hydro-alcoholic extract group: CPHE 200 mg/kg, p.o
Group 5**:** High dose of *C.pareira* hydro-alcoholic extract group: CPHE 400 mg/kg, p.o

Morris water maze test for cognitive enhancing activity

The water maze test consisted of a circular tank with 100 cm diameter and a wall of 20 cm above the water level. A circular platform (9 cm diameter, covered with white linen material for grip) was hidden 2 cm below the water level. The water was made opaque using titanium dioxide suspension and is kept at about 23°C during the experiment. The pool was divided into four quadrants (NE, NW, SE, and SW) by two imaginary lines crossing the centre of the pool. For each animal, the invisible platform was placed in the centre of one of the quadrants and remained there for a training period of 4 days. Each mouse was gently placed in the water facing the wall of the pool from one of the four starting points (N, E, S, or W) along the perimeter of the pool, and the animal was allowed to swim until it climbed onto the platform. The mice were transferred from their housing facility to the behaviour room. All the mice were kept in an area where they cannot see the pool or spatial cues. All the mice were adjusted to the new environment for at least 30 min before testing. Mouse was transferred from cage to water maze by lifting the mouse by the base of the tail and mouse were gently placed into the water, facing the edge of the pool. If the mouse found the platform before the 60 sec cut-off time, then mouse was allowed to stay on the platform for 5 seconds and then returned it to its home cage. If the mouse did not find the platform, then the mouse was placed on the platform and allowed it to stay there for 20 seconds before returning it to its home cage. Mice were trained for three consecutive days; each mice were received 4 consecutive trials per day with an inter-trial interval of 6–10 min. Each trial was started from one of four assigned polar positions with a different sequence each day. Different groups of mice received their respective treatment. Thirty min after treatment as per their groups the escape latency to find the platform were measured (escape latency was time difference between the time of placement of the mice in the water to the time of finding the platform) in each group of mice. If the animal failed to find the platform in any trial within 3 min it was placed again for 10 second. The animals became anxious when put in to the water for water maze test, as per the previous memory of 4 consecutive trials the retention time and escape latency to find the platform was a measure of cognitive function of the mice. [9, 10]

Pole climbing test for anti-psychotic activity

Cook's pole climbing apparatus use to study cognitive function, mainly a response to conditioned stimuli during learning and its retention. The apparatus had an experimental chamber (25 × 25 × 25 cm^3) with the floor grid in a soundproof enclosure. Scrambled shock (6mA) was delivered to the grid floor of the chamber composed of stainless-steel rods. A pole, 2.5 cm in diameter, hangs inside the chamber through a hole in the upper centre of the chamber. The study mouse was placed in the chamber and allowed to explore the chamber for 45 seconds. Conditioned stimulus (CS) i.e. buzzer signal was turned on and unconditioned stimulus (US) i.e. electric shock delivered through grid floor for 45 Sec. Animal learned to associate the buzzer with the impending foot shock and was capable of avoiding the foot shock by climbing the pole after buzzer signal. Avoidance response was defined as climbing reaction time 10 Sec. Every mouse was subjected to maximum 05 trials on 1st day, and 24 hrs later, mouse was subjected to relearning trials (2nd day 3 trials and on 3rd day one trial) and transfer latency was noted to check the retention of Conditioned Avoidance Response (CAR) and escape response. Animals were screened by using this model and those who demonstrated at least one escape response either on day one or two were included in the study. Pole climbing test on trained mice were performed to study anterograde amnesia of fluoxetine and other treatments as per study design. Every mouse was subjected to maximum 05 trials on each day to responding Conditioned Avoidance Response (CAR) and perfect for it if the response was less than 2 seconds. Animals were screened by using this model and those pass this trial were included in the study. Different groups of mice were treated with their respective treatments. Thirty min after treatment as per their groups the latency to climb the pole were measured in each group of mice. [9,11]

Statistical analysis

All the data were analysed using Graph pad prism version 3; Values are expressed as Mean ± SEM and analysed by one-way analysis of variance (ANOVA), followed by Dunnett's test of multiple comparison Results are considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Acute oral toxicity

Oral administration of the hydro-alcoholic extract of leaves of *C.pareira* up to 2000 mg/kg, B.W did not produced any toxic effect. Based on which, three doses of *C.pareira* hydro-alcoholic extract (CPHE) were selected randomly viz CPHE 100 mg/kg, CPHE 200 mg/kg, CPHE, 400 mg/kg.

Morris water maze test for cognitive enhancing activity

Figure 1 and Table 1 depicts the cognitive enhancing effect of CPHE at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg BW on spatial memory in memory deficit mice induced by fluoxetine. The results of PCGF group of mice showed that fluoxetine that was an antidepressant drug produced showed no significant changes of both escape latency and retention time. These findings indicated that antidepressant drug impaired cognitive function of mice and produced no effect on the mentioned parameters. Mice which were exposed to CPHE 100 mg/kg, CPHE 200 mg/kg and CPHE 400 mg/kg treatments significantly decreased escape latency and enhanced retention time as compared to PCGF group. These changes indicated that CPHE extracts at all dose levels (100, 200, 400) improve cognitive function of mice. CPHE 100 mg/kg, CPHE 200 mg/kg and CPHE 400 mg/kg showed not only cognitive enhancing effect but also neuroprotective effect in mice. Consequently, CPHE at all dose levels could prevent the neurodegeneration and cognitive deficit in mice.

Figure 1: Effect of various treatments on the escape latency and retention time evaluated by using Morris water maze test on cognitive function (learning and memory) in mice.

Table 1: Extent of cognitive function (learning and memory) after the treatment of hydro alcoholic extract of leaves of *C. pareira* in water maze test of mice

S. No.	Group	Dose. (mg/kg)	Escape latency time (Seconds)	Retention time (Seconds)
1	NCGV	1.0ml/kg	5.34± 2.77*	23.64± 2.77*
2	PCGF	10mg/kg	16.76 ± 1.80	8.96 ± 2.68
3	CPHE 100	100 mg/kg	5.66 ± 1.02*	23.66 ± 1.42*
4	CPHE 200	200 mg/kg	5.83 ± 0.79*	25.34± 1.37*
5	CPHE 400	400 mg/kg	6.45± 0.96*	26.15± 1.49*

All data were expressed as mean ± SEM and analysed by one-way analysis of variance (ANOVA), followed by Dunnett's test of multiple comparison. *p<0.05 represents statistical significance, where other treatment group was compared with PCGF group (p< 0.05).

These findings concluded that antidepressant drug impaired cognitive function of mice and produced no effect on the mentioned parameters. Mice which were exposed to CPHE 100 mg/kg, CPHE 200 mg/kg and CPHE 400 mg/kg treatments significantly decreased escape latency and enhanced retention time as compared to PCGF group. These changes concluded that CPHE extracts at all dose levels (100, 200, and 400 mg/kg) improve cognitive function of mice. Consequently, CPHE at all dose levels could prevent the neurodegeneration and cognitive deficit in mice. Quercetin is a natural flavonoid that is abundantly found in fruits and vegetables and has been shown to possess multiple forms of desirable biological activities including anti-inflammatory, antioxidant and neuroprotective properties. As per the scientific study conducted by Khan A *et. al*. (2018). [12, 13] quercetin treatment significantly reversed the lipopolysaccharide (LPS) induced synaptic loss in the cortex and hippocampus of the adult mouse brain and improved the memory performance of the LPS treated mice. Therefore, cognitive enhancing effect of CPHE seems mostly likely to be mediated through antioxidant and

neuroprotective properties of quercetin which is also a phytoconstituent of *C. pareira*. [14, 15]

Pole climbing test for anti-psychotic activity

As depicted in the results in Figure 2 and table 2, fluoxetine treatment remarkably enhanced the time to climb the pole in mice compared to NCGV group of mice in cook's pole climbing test. Furthermore, administration of CPHE 400 mg/kg in mice significantly enhanced the time to climb the pole as compared to NCGV group of mice. Moreover, CPHE 400 mg/kg treatment significantly increased the time latency to climb the polein cook's pole climbing test of mice as compared to the NCGV group of mice. It indicates that CPHE 400 mg/kg possess antipsychotic effect in mice of cook's pole climbing test. However, at the low dose levels below 400 mg/kg (CPHE 100 mg/kg and CPHE 200 mg/kg) of CPHE, there were no significant difference in time latency to climb the pole in cook's pole climbing test of mice were observed compared to NCGV group. It indicates that these doses of CPHE did not displayed antipsychotic effect.

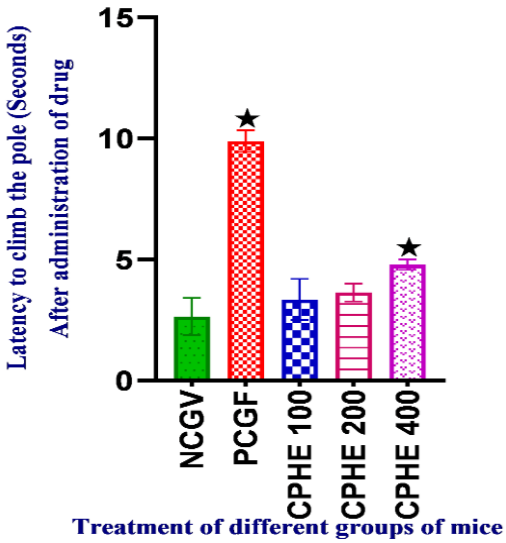

Figure 2: Antipsychotic activity of hydro alcoholic extract of leaves of *C. pareira* in mice by cook's pole climbing test

Table 2: Antipsychotic activity of hydro alcoholic extract of leaves of *C. pareira* in mice by cook's pole climbing test.

S. No.	Group	Dose. (mg/kg)	Latency to climb the pole (Seconds)
1	NCGV	1.0ml/kg	2.64± 0.77
2	PCGF	10mg/kg	9.89± 0.45*
3	CPHE 100	100 mg/kg	3.34± 0.85
4	CPHE 200	200 mg/kg	3.64± 0.37
5	CPHE 400	400 mg/kg	4.78± 0.21*

All data were expressed as mean ± SEM and analysed by one-way analysis of variance (ANOVA), followed by Dunnett's test were used for comparison. *p<0.05 represents statistical significance, where other treatment group was compared with PCGF group (p< 0.05)

In cook's pole climbing test for evaluation of antipsychotic activity in trained mice CPHE 400 treatment remarkably enhanced the time to climb the pole and significantly increased the time latency to climb the pole compared to the NCGV group of mice. However, at the low dose levels below 400 mg/kg (CPHE 100 mg/kg and CPHE 200 mg/kg) of CPHE, there were no significant difference were observed in time latency to climb the pole in cook's pole climbing test of mice. It concluded that CPHE 400 mg/kg possess antipsychotic effect in mice at higher dose level of cook's pole climbing test. Restoring glutamatergic neurotransmission in the treatment of schizophrenia is the therapeutic approach taken by Fan *et. al*. (2018). They used quercetin, a natural flavonoid that acts as a negative allosteric modulator for GABAA receptors, to reduce inhibition in the prefrontal cortex and potentiate glutamatergic transmission, effectively alleviating the positive symptom of hyperactive mice. [16]

Consequently, antipsychotic effect of CPHE seems mostly likely to be mediated through negative allosteric modulator of GABAA receptors by quercetin which is also a phytoconstituent of *C. pareira.* [14, 15]

CONCLUSION

Based on the results obtained from the study it can be concluded that CPHE at all doses acted as cognitive enhancer in dose dependant manner, while for anti-psychotic activity, CPHE 400 mg/kg was effective and not CPHE 100 and 200 mg/kg. The findings of the present study ascertain the traditional uses of leaves of *C.pareira* in treatment of CNS disorders. However, further detailed investigation is required to ascertain its use in psychosis associated with AD.

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