Original Article

Evaluation of Centella Asiatica for its Neuropharmacology

1. Rafi Akhtar Sultan 2. Rahila Najam 3. Iqbal Azhar 4. Bushra Riaz 5. Abeer Adil 6. Zafar Alam Mahmood

1. Ph.D. Student, of Pharmacognosy 2. Assoc. Prof. of Pharmacology 3. Assoc. Prof. of Pharmacognosy 4. M.S. Student of Pharmacology 5. M.S. Student of Pharmacology, Faculty of Pharmacy, University of Karachi 6. Country Manager Pakistan, Gulf and Middle East Region, Colorcon Limited, UK.

ABSTRACT

Objective: To determine the effect of Centella Asiatica on Neuro pharmacological activities as memory, behavior (anxiety, depression).

Study Design: Experimental Study.

Place of study: This study was conducted in the department of Pharmacology, Faculty of Pharmacy, University of Karachi, Karachi from 15th March 2011 to 30th April, 2011.

Materials and Methods: Albino mice and albino rats were used. Animals were divided into control and treated groups (10 animals each). Neuro pharmacological parameters were assessed using standard techniques as Stationary rod activity, Swimming induced depression (FST), Open field, Light and dark box test and water maze model. Control group was maintained on distilled water and treated group was fed with 8.3 mg/kg Centella asiatica for 10 days. Observations were taken on 1st, 5th and 10th days.

Results: The results showed decline in the elapsed time taken by animal to reach the platform in Stationary rod and water maze model, significantly enhanced struggling time in FST, decreased number of peripheral square crosses but relatively increased central square crosses on 10th day in open field test and increased time spent in light box in Light and dark box model.

Conclusion: It can be concluded that Centella asiatica enhances memory and show antidepressant activity on acute administration while chronic use results in anxiolytic behavior.

Key Words: Centella asiatica, Cognition, Anxiolytic, Parkinsonism, Alzheimer's disease.

INTRODUCTION

Pakistan is renowned for treating different illnesses with medicinal plants especially the Unani system of medicine which has been established since Indus valley civilization [1]. One of the medicinal plant Centella asiatica belongs to the family Umbelliferae (synonymous known as Apiaceae) is distributed widely in South America and Asia especially in the damp and marshy places throughout India. The plant is richly employed in the Ayurvedic and unani systems of medicine in different forms either as whole plant, fresh leaves or in extract form [2]. The local names of plant are Vallarai in Tamil, Mandukaparni in Sanskrit, Indian Pennywort in English [3] and the Barhami boti in Urdu[4]. Centella asiatica is reported to be used as a brain tonic for enhancing memory. One such formulation of this plant is Gotu kola which is claimed to alleviate anxiety, insomnia and improve overall brain function. Centella asiatica is also the ingredient of herbal medicine mixture Medhya Rasayana. Centella asiatica is effective in improving general mental ability and cognition in mentally retarded children and in people with cognitive dysfunction[5,6,7,8]. Centella asiatica also possess antioxidant properties, capable of protecting brain against age related oxidative damage[9]. Studies also revealed that the administration of Centella asiatica is associated with the protection of brain against neurodegenerative disorders as Parkinsonism and Alzheimer's disease[10,11]. In Alzheimer's disease animal model the use of Centella asiatica extract showed decline in amyloid beta levels in hippocampus region of brain[11]. The fresh leaf extract of this plant proved efficacious in improving learning and memory[12,5,6,7]. In the study including neonatal rats (during growth spurt period), the use of fresh leaf juice of Centella asiatica resulted in increased memory [13] but according to another research Centella asiatica is involved in the retention of a learnt task for a longer period but did not accelerated the learning process as expected[14]. Different clinical studies revealed that Centella asiatica increases the level of neurotransmitters involved in learning and memory like Ach, noradrenaline, 5HT, dopamine, GABA[15,16,17,18].

MATERIALS AND METHODS

Ethanolic extract of plant: The plant material was collected from Tehsil Kahuta district Rawalpindi, Pakistan and identified by qualified taxonomist, the plant material was dried under shade and soaked in ethanol for two weeks at room temperature. After two weeks the ethanolic extract was filtered and dried by using rotary evaporator.

Animals: Albino mice (avg wt = 24 g) and albino rats (avg wt =250g) were used for the experiments and they

were fed with commercial diets. Following preliminary experiments, an optimum dose of 0.2mg was arrived for mice and 2mg for rats. The standard dose was 8.3mg/kg[19]. Extract was administered orally for 10 days.

Effects of centella asiatica on neuropharmacological activities in mice and rats: The effects of Centella asiatica on a number of neuropharmacological activities were assessed using standard techniques as described below:

Stationary rod activity: Mice were given a short training period (2 or 3 trials), before treatment. This pre-treatment training ensures mice ability to walk across a horizontal steel rod (5/8″ in diameter and approx 2 ft long) positioned 18″ at a height above the surface of the table. Mice were placed on the mid-point of the rod individually and were forced to walk towards a platform at either end of the rod[20].

Swimming induced depression: The test method used for assessing antidepressant activity was discovered by Porsolt and coworkers in 1977. The FST apparatus consists of clear water bath (20cm height × 12cm diameter) and filled with 15cm deep water. The mice were allowed to swim in a FST apparatus for 10min after that period they were returned to their home cages. On fourth day chronic mild stress 1 (CMS-1) was applied to mice i.e. tilting of cage more than 30 degree from horizontal for 48 hours. On seventh day animal were exposed to CMS-2 i.e. 200ml of water was poured on the sawdust bedding of mice home cage for 24hours [21] and on ninth day CMS-3 was applied i.e. deprivation of animals from food for 24 hours [22]. At the end of 10th day animals were allowed to swim again for 5 min and struggling time was noted. The same procedure was adopted for control and treated mice.

Open field: Open Field Method was essentially as described by Haleem and coworkers [23]. The activities were scored by counting the number of squares crossed by individual mouse during a 10 minute period [24].

Light and dark test: The apparatus comprises of a box with two compartments (20 × 20 cm). One compartment is illuminated with light and the other is kept dark. Individual animal is placed in the center of the illuminated compartments; facing one of the dark places as well the number of entries in each space and time spent in light and dark compartment is recorded for 10 minutes respectively [25].

Water maze test for rats: Water maze was developed by Richard Morris at the University of St Andrews in Scotland [26, 27]. In this test rats were placed in a pool of water facing the pool-side to avoid bias and required to escape from water onto a hidden platform whose location can normally be identified only using spatial memory. The time taken by rats to reach the platform was noted [28].

RESULT

The effect of oral administration of Centella asiatica for 10days on different CNS parameters is shown in the following tables.

Table No 1: Effect of drug on stationary rod activity.

Time in seconds required to reach the platform						
Groups	Day 1	t-test	Day 5	t-test	Day 10	t-test
Control	107 ± 0.82	**p< 0.001	6.30 ±2.63	IS	3 ± 1.63	IS
Treated	97 ± 0.876		4 ± 1.63		2 ± 0.816	

Values are mean ± SD
n=10 = total number of animals
*p<0.01= Significant difference by t-test
**p<.001= highly significant difference from the control following t-test
IS= insignificant difference by t-test

Table No 2: Effect of drug on open field activity.

Table No 2.1: Effect of Drug on Central Square Crossing:

Number of central square crosses						
Groups	Day 1	t-test	Day 5	t-test	Day 10	t-test
Control	28.7± 5.48	**p< 0.001	22.2± 5.81	**p< 0.001	13.2± 2.66	IS
Treated	9.4± 4.78		4.2± 1.75		12.4± 7.63	

Values are mean ± SD
n= 10= total number of animals
*p<0.01= Significant difference by t-test
**p<.001= highly significant difference from the control following t-test
IS= insignificant difference by t-test

Table No 2.2: Effect of Drug on Peripheral Square Crossing.

Number of peripheral square crosses						
Groups	Day 1	t-test	Day 5	t-test	Day 10	t-test
Control	103.7 ±35.4	IS	134.4 ±17.9	**p< 0.001	83.8± 14.7	**p< 0.001
Treated	94.1± 9.28		38.8± 16		12.4± 7.63	

Values are mean ± SD
n= 10= total number of animals
*p<0.01= Significant difference by t-test
**p<.001= highly significant difference from the control following t-test
IS= insignificant difference by t-test

Table No 3: Effect of drug on light and dark box activity.

Time in seconds spent in illuminated box						
Groups	Day 1	t-test	Day 5	t-test	Day 10	t-test
Control	78.2± 43.2	IS	60.6± 57.1	IS	10.6± 4.84	**p< 0.001
Treated	41.7± 13.2		43.3± 20.9		33.7 ±12	

Values are mean ± SD
n= 10= total number of animals
*p<0.01= Significant difference by t-test
**p<.001= highly significant difference from the control following t-test
IS= insignificant difference by t-test

Table No 4: Effect of drug on water maze activity.

Time in seconds to reach the platform						
Groups	Day 1	t-test	Day 5	t-test	Day 10	t-test
Control	22±0. 816	**p< 0.001	2.2± 0.789	IS	1.5± 0.471	IS
Treated	18±1. 63		1.2± 0.789		2± 0.816	

Values are mean ± SD
n= 10= total number of animals
*p<0.01= Significant difference by t-test
**p<.001= highly significant difference from the control following t-test
IS= insignificant difference by t-test

Table No 5: Effect of drug on FST activity.

Struggling time in sec						
Groups	Day 1	t-test	Day 10	t-test	Day 13	t-test
Control	227.9 ±34.9	IS	211.5 ±22.8	IS	258± 6.04	**p< 0.001
Treated	234.5 ±11.1		211± 38		290± 8.54	

Values are mean ± SD
n= 10= total number of animals
*p<0.01= Significant difference by t-test
**p<.001= highly significant difference from the control following t-test
IS= insignificant difference by t-test

DISCUSSION

In the present study the plant Centella asiatica was evaluated for its neuro pharmacological effects. It was observed that after the administration of Centella asiatica, the animal crossed the stationary rod in lesser time as compare to control. This effect suggests that Centella asiatica enhances learning and memory. The possibility of this outcome may be because of the fact that Centella asiatica stimulates cholinergic system [29]. The effect of this drug on water maze activity also support the above finding, as the time taken by treated animal to reach the platform was decreased considerably as compare to control. Previous clinical studies on Centella asiatica extract also revealed the presence of memory enhancing effect of this plant [12,5,6,7].

The open field activity especially the peripheral square crosses was decreased significantly literature survey denote that Centella asiatica does not induce locomotor activity, thus does not improve open field exploratory behavior [29]. This finding may be based on the fact that Centella asiatica possess anxiolytic profile which made the animal comfortable enough to decrease open field exploration. In open field model, the central square crossing is the most coherent behavior which demonstrates anxiolytic activity [30] and our study shows that on acute dosing the number of central square crosses are decreased but relatively increase on day 10, comparable to control, give rise to the premise that Centella asiatica shows anxiolytic effect on chronic administration.

Light and dark box test is based on instinctive characteristic of rodents to dislike brightly illuminated areas and on the extemporaneous exploratory behavior of rodents in response to mild stressors, i.e. novel environment and light [31]. According to our study the time spent in light box is decreased on day 1 and day 5 but increased on day 10. This result also witness the anxiolytic effect of plant on chronic use.

Forced swimming test is the behavioral paradigm used to measure the effect of antidepressant drugs [32]. In our research the struggling time was increased significantly which revealed that the plant has antidepressant property. After the acute administration of Centella asiatica showed prolonged struggling time as compare to control leading to the assumption that the plant might possess antidepressant profile on acute administration.

CONCLUSION

On the basis of above finding it can be concluded that Centella asiatica enhances memory, decreases exploratory behavior and show antidepressant activity on acute administration while anxiolytic behavior on chronic use.

REFERENCES

1. WHO Legal Status of Traditional Medicine and Complementary and Alternative Medicine. A World Review. Geneva: WHO; 2001.
2. Sharma PV. Dravyaguna Vignana, 13th ed. New Dehli, India: Chaukhamba Vishwa Bharati Academy; 1992.p.3-5.

3. Premila MS. Ayurvedic Herbs: A Clinical Guide to the Healing Plants of Traditional Indian Medicine: Haworth Press; 2006.p.280-297.
4. Khan UG, Saeed A, Alam MT. Indusyunic Medicine: Traditional Medicine of Herbal, Animal and Mineralorigin in Pakistan. University of Karachi: Bcc & T Press;1997.p.149.
5. Dash PK, Mistry IU, Rao AR. Role of Medhya Rasayana in school children. Ayu 1996;12-15.
6. Shah LP. An open clinical trial of Mentat in hyperkinetic children. Probe 1992;31:125-9.
7. Rao AR, Srinivasan K, Rao KT. The effect of Mandookaparni (Centella asiatica) on the general mental ability (Medhya) of mentally retarded children. J Res Ind Med 1973;8:9-12.
8. Joshi H, Pasle M. Brahmi rasayana improves learning and memory in mice. Evid Based compliment. Alternat Med 2006;3:79-85.
9. Subathra M, Samvel S, Marimuthu S, Muthuswany AD, Chinnakkannu P. Emerging role of Centella asiatica in improving age related neurological antioxidant status. Exp Gerontology 2005;40:707-715.
10. Haleagrahara N, Ponnusamy K. Neuroprotective effect of Centella asiatica extract (CAE) on experimentally induced Parkinsonism in aged Sprague-Dawley rats. J Toxicol Sci 2010;35(1): 41-7.
11. Dhanasekaran M, Holcomb LA, Hitt AR, Tharakan B, Porter JW, Young KA, et al. Centella asiatica extract selectively decreases amyloid beta levels in hippocampus of Alzheimer's disease animal model. Phytother. Res 2009; 23(1):14-9.
12. Sivarajan VV, Indira Balachandran. Ayurvedic drugs and their plant sources. New Delhi. India: Oxford and IBH publishing Co Pvt Ltd. 1994;97:289-90
13. KG Mohandas RAO, S Muddanna RAO, S Gurumadhva RAO. Centella asiatica (linn) induced behavioral changes during growth spurt period in neonatal rats. Neuroanatomy 2005; 4: 18–23.
14. Silviya Rajakumari Jared. Enhancement of memory in rats with Centella asiatica. Biomed Res 2010: 21(4):429-432.
15. Chatterjee TK, Chakraborthy A, Pathak M, Sengupta GC. Effects of plant extract Centella asiatica (Linn.) on cold restraint stress ulcer in rats. Indian J Exp Biol 1992; 30: 889–891.
16. Ji WQ, Zhang CC, Zhang GH. Effect of somatostatin and GABA on long term potentiation in hippocampal CA1 area in rats. Zhongguo Yao Li Xue Bao 1995;16:380–382.
17. Hatfield T, McGaugh JL. Norepinephrine infused into the basolateral amygdala posttraining enhances retention in a spatial water maze task. Neurobiol. Learn Mem 1999; 71: 232–239.
18. Farr SA, Banks WA, Morley JE. Estradiol potentiates acetylcholine and glutamate-mediated post-trial memory processing in the hippocampus. Brain Res 2000;864: 263–269.
19. Wattanathorn J, Mator L, Muchimapura S, Tongun T, Pasuriwong O, Piyawatkul N, et al. Positive modulation of cognition and mood in the healthy elderly volunteer following the administration of Centella asiatica. J of Ethnopharmacol 2008;116:325–332.
20. Rahila Najam. Pharmacological Screening of some bioactive products from marine resources dissertation. University of Karachi; 2003.
21. Ito N, Nagai T, Yabe T, Nunome S, Hanawa T, Yamada H. antidepressant like activity of Kampo (Japanese Herbal) medicine, Kaso-san and its mode of action via the hypothalamic- pituitary- adrenal axis. Phytomed 2006;13:658-667.
22. Deussing JM. Animal model of depression. Drug discovery Today. Disease models 2006; 3(4): 375-383.
23. Haleem DJ, Kennett GA, Curzon G. Adaptation of female rats to stress shift to male pattern by inhibition of corticosterone synthesis. Brain Res 1988; 485, 339-347.
24. Najam R, Ahmed SP, Azhar I. Pharmacological Activities of Hypnea musciformis. Afr J Biomed Res 2010;13(1):69–74.
25. Yadav YC, Jain A, Deb L. A Review: Neuropharmacological Screening Techniques for Pharmaceuticals. Int J of Pharmacy and Pharmaceutical Sciences 2010; 2(2): 10-14.
26. Morris RGM. Spatial localisation does not depend on the presence of local cues. Learning and Motivation. 1981; 12:239-260.
27. Morris RG, Garrud P, Rawlins JN, O'Keefe J. Place navigation impaired in rats with hippocampal lesions. Nature 982; 297(5868):681-683.
28. Richard G, Morris M. Morris water maze. Scholarpedia 2008; 3(8):6315.
29. Sulochana B Rao, Chetana M, Devi PU. Centella asiatica treatment during postnatal period enhances learning and memory in mice. Physiology & Behavior 2005; 86(4): 449-457.
30. Wijeweera A, Arnason JT, Kosyzycki D, Merali Z. Evaluation of anxiolytic properties of gotukola (Centella asiatica) axtract and asiaticoside in rat behavioral models. Phytomed 2006;13(9-10):668-676.
31. Bourin M, Hascoet M. The mouse light/dark box test. Eur. J Pharmacol 2003; 28(2): 1-3.
32. Petit-Demouliere B, Chenu F, Bourin M. Forced swimming test in mice: a review of antidepressant activity. Psychopharmacology (Berl) 2005; 177(3): 245-55.