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Pharmacological evalution of *Marsilea qudrifolia* plant extracts against Alzheimer's disease

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Abstract

Dementia is one of the age related mental problems and characteristic symptom of various neurodegenerative disorders including Alzheimer's disease which is age related. The whole plant of *Marsilea quadrifolia* isused to enhance the memorypower. The present work was undertaken to justify the traditional claim of the plant *Marsilea quadrifolia* as anti alzheimeric agent in mice. The ethanolic extract of the whole plant was selected for the study. The exteroceptive behavioral models such as elevated plus maze, Morris water maze and Y-maze were used to evaluate the learning and memory, where as scopolamine is the natural ageing inducing amnesia served as interoceptive models. Two doses of (250mg/kg, 500mg/kg) ethanolic extract of *Marsilea qudrifolia were* orally administered for seven successive days in separate groups of animals and the doses were selected according to the animal weight. The both doses of ethanolic extract of plant *marsilea* significantly improved the learning and memory in mice. Furthermore the both doses were significantly reversed the amnesia induced by scopolamine (0.4mg/kg I.P). The anti oxidant property and presence of steroids of *Marsilea quadrifolia* may be contributing favorably to memory enhancement effect. Since scopolamine induced amnesia was reversed by Marsilea, it is possible that the beneficial effect on learning and memory was due to facilitation of cholinergic transmission in mouse brain. However further studies are necessitated to identify the exact mechanism of action. In the present investigation *Marsilea quadrifolia* has shown promise a memory enhancing agent in all the laboratory models employed.

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Key words:
Marsilea quadrifolia, Alzheimer disease, Swiss albino mice, Pharmacological studies

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INTRODCUTION:

Alzheimer's damages and kills brain cells. Alzheimer's is a complex disease likely caused by a combination of factors such as infection or reduced circulation and genetic susceptibility. Although all

the contributing factors may never be known, scientists have identified several common threads. They include Age, Heredity, Genetic history, Lifestyle, Sex, Head injury, Head size, vascular risk factors and Diet [1-4].

Currently, there's no cure for Alzheimer's disease. Doctors sometimes prescribe drugs to improve symptoms that often accompany Alzheimer's, including sleeplessness, wandering, anxiety, agitation and depression. The drugs currently used are tacrine hydrochloride (Cognex) [5-7] and donpezil hydrochloride (Aricept) [8, 9], rivastigmine (Exelon) [10, 11] and galantamine (Reminyl) [12, 13]. They bolster the efficiency of the nerve cells most affected by Alzheimer's disease. However, the effects are short lived and don't cure the disease. Researchers have searched for molecules which inhibit the 'parent' molecule of the beta amyloid protein, to reduce the production of the proteins. Studies using Vitamin E have shown small but significant improvements in function in one group of Alzheimer's disease sufferers [14]. Researchers are testing a range of antioxidants to see if they help protect nerve cells. Researchers believe that beta amyloid proteins may become toxic as they build up. If the accumulated proteins could be broken down, they may be less harmful [15, 16].

Marsilea quadrifolia is the perfect alternative to higher light demand foreground plants. There are a few different varieties of *Marsilea* species, but the true Marsilea quadrifolia is most distinguishable by the four-leaf clover it produces when grown submerged. Present pharmacological study was to prove the activity of plant extracts on Alzheimer's diseases. Results of various studies had shown that positive effect to treat the disease [17-19].

MATERIAL AND METHOD:

Plant description: A creeping perennial herb with slender long dichotomously branching rhizome; rooting at the nodes. Leaves quadrifoliate, circinate, when young, petioles long, slender, flexible, lamina divided into four leaflets, sporocarps are bean like, born on short or long stalks inserted a short distance above the base of the petiole. .

Growth Characteristics: *Marsilea quadrifolia* is an undemanding plant species that is able to adapt to low light and high light conditions. It does not require carbon dioxide or heavy water column fertilization. However a nutrient rich substrate, high light, and carbon dioxide injection will encourage it to grow faster than its natural slow growth propensity.Marsilea quadrifolia spreads through runners across the substrate floor. As it creeps along the surface, new clover like leaves will emerged that are supported by petioles that can grow to a length of 4-6 inches (10-15 cm). When provided with more light the height will be slight shorter. .

Propagation is completed by splitting the runners and replanting them. *Marsilea quadrifolia* is best suited for low to moderate light aquariums, with aquascapers who desire to have slower foreground growth and want limited maintenance efforts.

Medicinal properties: Plant pacifies vitiated pitta, cough, bronchitis, diabetes, psychiatric diseases, eye diseases, diarrhea, skin diseases, antidote, antiphloristic, depurative, diuretic and febrifuge.

Chemical constituent: Thiaminase enzyme is majorly present in this plant. And also present steroids and some carbohydrates.

Toxicity: Although we have found no reports of toxicity for this species, a number of ferns contain carcinogens so some caution is advisable. Many ferns also contain thiaminase, an enzyme that robs the body of its vitamin B complex. In small quantities this enzyme will do no harm to people eating an adequate diet that is rich in vitamin B, though large quantities can cause severe health problems. The enzyme is destroyed by heat or thorough drying, so cooking the plant will remove the thiaminase.

Plant Material and extraction: The plant, *M. quadrifolia* was collected from the village of yellapur. The whole plant of *Marsilea quadrifolia* collected,

shade dried for seven days and ground. The powder was passed through sieve number- 40. The dried powder of *M. quadrifolia* (200gm) was extract with 600ml of ethanol by using soxhlet apparatus. The collected extract is evaporated to remove ethanol using rotary vacuum evaporator. Dried herbal extract is mixed with Carboxy methyl cellulose (CMC) and administered to the animals.

Animals: Swiss albino mice weighing 25gm -35gm were used in this study. Animals were housed in plastic cages in groups. They had free access to food and water, and they were kept in a regulated environment (23_1_C, 40–60% humidity). Experiments were carried out between 9:00 a.m. and 5:00 p.m., in an experimental room with in the animal facility. All animal procedures were conducted in strict under the rules of ethical committee.

Equipments and chemicals: Electronic balance, Morris water maze , Elevated plus Maze, Y-Maze, Syringes and needles, Ethanol, Carboxy methyl cellulose and Scopolamine.

Preliminary Phytochemical Analysis: The extracts were subjected to preliminary phytochemical screening for the presence or absence of various phytoconstituents by suitable methods

Experimental Design:

Grouping of Animals: Animals were divided into four groups each of six animals.

Group I: Control group oral administered by CMC ((Carboxy methyl cellulose).

Group II: Animals oral administered by scopolamine hydro Chloride which is dissolved CMC (Negative control).

Group III: Animals oral administered by extract which is dissolved in CMC (250mg/kg) and Alzheimer's induced with Scopolamine.

Group IV: Animals oral administered by extract which is dissolved in CMC (500mg/kg) and Alzheimer's induced with scopolamine.

PHARMACOLOGICAL STUDIES:

Induction of Alzheimer's disease:

Alzheimer's disease mainly induced by using Scopolamine into the mice.

Scopolamine, also known as levo-duboisine, and hyoscine, is a alkaloid drug with muscarinic antagonist effects. It is among the secondary metabolites of plants from Solanaceae (nightshade) family of plants. Scopolamine exerts its effects by acting as a competitive antagonist at muscarinic acetylcholine receptors, specifically M1 receptors.

Scopolamine is used as a standard/reference drug for inducing amnesia in man and Animals. The effects are generally interpreted as a cholinergic deficit and related to the hypothesis that acetylcholine is involved in memory functions. Scopolamine, besides influencing learning and memory, affects various types of behavior (e.g., loco motor activity, anxiety, attention).

Behavioral studies:

Elevated plus-maze: Elevated plus-maze served as the exteroceptive behavioral model to evaluate memory in mice. The procedure, technique and endpoint for testing memory were followed as per the parameters described by the investigators working in the area of psychopharmacology. The elevated plus-maze for mice consisted of two open arms (16cm×5cm) and two covered arms (16cm×5cm ×12cm) extend from a central platform (5cm×5cm), and the maze was elevated to a height of 25cm from the floor. On the first day (i.e. eighth day of drug treatment), each mouse was placed at the end of open arm, facing away from the central platform. Transfer latency (TL) was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arm with all its four legs. TL was recorded on the first day (training session) for each animal. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned task (memory) was examined 24 hours after the first day trial (i.e. ninth

day, 24 hours after last dose). Significant reduction in the TL value of retention indicated improvement in memory.

Morris water maze task: A spatial test was performed by the method of Morris with minor modification. The water maze is a circular pool (120cm in diameter and 50cm in height) with a featureless inner surface. The pool was filled to a depth of 35cm with water containing 500mL of milk (20±1 ◦C). The pool was divided into four quadrants of equal area. A white platform (6cm in diameter and 29cm in height) was then placed in one of the pool quadrants.

The first experimental day was dedicated to swimming training for 60 s without the submerged platform. During the five subsequent days, the mice were given two daily trials with an inter-trial interval of 30 min in the presence of the platform in place. When a mouse located the platform, it was permitted to remain on it for 10 s. If the mouse did not locate the platform within 120 s, it was placed on the platform for 10 s. The animal was taken to its home cage and was allowed to dry up under an infrared lamp after each trial.

During each trial session, the time taken to find the hidden platform (latency) was recorded. One day after the last training trial sessions, mice were subjected to a probe trial session in which the platform was removed from the pool, allowing the mice to swim for 120 s to search for it. A record was kept of the swimming time in the pool quadrant where the platform had previously been placed. Memory impairment was induced in mice with scopolamine (0.4 mg/kg, I.P.) at 60 min after treatment of test samples. Control group received 1% CMC solution only.

Y-maze task: Y-maze task is used to measure the spatial working through the spontaneous alternation of behavior. The maze is made of black painted wood. Each arm is 40 cm long, 13 cm high, 3 cm wide at the bottom, 10 cm wide at the top, and converges at an equal angle. Each mouse is placed at the end of one arm and allowed to move freely through the maze during an 8-min session. Mice tend to explore the maze systematically, entering each arm in turn. The ability to alternate requires that the mice know which arm they have already visited. The series of arm entries, including possible returns into the same arm, are recorded visually. Alternation is defined as the number of successive entries into the three arms, on overlapping triplet sets. The percentage of alternation is calculated as the ratio of actual alternations, defined as the total number of arm entries minus two, and multiplied by 100.

Statistical Analysis:

All the values were expressed as mean ± SEM. The data was statistically analyzed by one way ANOVA followed by Dunnet's T test. The data of behavioral and biochemical parameters were analyzed using ANOVA followed by Dunnet's T test. P values< 0.01 were considered significant.

RESULTS AND DISCUSSION:

Preliminary Phyto chemical Investigation: The revealed results of the preliminary phyto chemical screening of ethanolic extract of whole plant of *Marselia quadrifolia*. The results were shown below.

Table no 1: Preliminary phyto chemical screening +Ve: indicates the presence of compounds, -Ve: indicates the absence of compounds

SL. No.	Phytochemical Tests	Results
1	Test for Alkaloids	-Ve
2	Test for Carbohydrates	+Ve
3	Test for Proteins	-Ve
4	Test for Steroids	+Ve
5	Test for Sterols	-Ve
6	Test for Phenols	-Ve
7	Test for Flavonoids	-Ve
8	Test for Gums and mucilage	-Ve
9	Test for Glycosides	+Ve
10	Test for Saponins	-Ve
11	Test for Terpenes	-Ve

Elevated plus maze:

Scopolamine significantly decreased the spontaneous aiteration behavior compared with control group. However this decreased spontaneous alteration behaviour induced by scopolamine was significantly inhibited by EEMQ 500 mg/kg.

Table no 2: Effect of EEMQ on Transfer latency of mice using Elevated plus maze

Group	Treatment	TL on 8th day	TL on 9th day
I	Control	69.33±0.76	53±0.73
II	Scopolamine{1mg/kg}	104±2.42**	80.83±0.79**
III	EEMQ(250mg/kg)	60.33±0.80**	34.33±0.76**
IV	EEMQ(500mg/kg)	54±0.93**	24.83±0.60**

Values are expressed as mean± SEM of 6 animals.

Symbol represents the statistical significance done by ANOVA, followed by Dunnet's "t" test. *P<0.05, **P<0.01, #P>0.05 non significant.

Morris water maze:

There is an increase in escape latency in negative control group when compared with the control group (P<0.01) of the two groups of amnesia induced animals, both showed decreased time to escape on to the escape platform. The group treated with 250 & 500 mg/kg EEMQ showed the significance of (P<0.01 and P<0.001) respectively as shown in table

Table no 3: Effect of EEMQ on Escape latency of mice using Morris water maze.

Group	Treatment	Escape latency
I	Control	14.16±0.74
II	Scopolamine{1mg/kg}	23.16±0.60**
III	EEMQ(250mg/kg)	19.16±0.76**
IV	EEMQ(500mg/kg)	17.00±0.73**

Values are expressed as mean± SEM of 6 animals.

Symbol represents the statistical significance done by ANOVA, followed by Dunnet's "t" test. *P<0.05, **P<0.01, #P>0.05 non significant.

Y-maze:

The amnesia induced group (negative control) indicated decrease in the alternation of behavior by the (P<0.01) in comparison with the control group I. The results presented by the treatment groups shows significance by (P<0.01) increase in the alternation of behaviour in respect of 250 mg/kg of EERC and 500 mg/kg of EEMQ when compared with that of the negative control group as shown in Table no:4

Table no 4: Effect of EEMQ on Percentage alteration in mice using Y- maze

Group	Treatment	Percentage alteration
I	Control	90.16±0.60
II	Scopolamine{1mg/kg}	5105±0.42**
III	EEMQ(250mg/kg)	55.16±0.60**
IV	EEMQ(500mg/kg)	67.83±0.70**

Values are expressed as mean± SEM of 6 animals.
Symbol represents the statistical significance done by ANOVA, followed by Dennett's "t" test. *P<0.05, **P<0.01, #P>0.05 non significant.

CONCLUSION:

Alzheimer's disease is a neurodegenerative disorder currently without an effective treatment. Impairment of memory is the initial and most significant symptom of AD. AD is associated with a decline in cognitive abilities. The most common cause of dementia in the elderly is probably AD. Despite the severity and high prevalence of this disease, the allopathic system is yet to provide a satisfactory antidote. The central cholinergic system plays an important role in learning and memory. In the present study, *Marsilea quadrifolia* extract (250mg/kg and 500mg/kg) administered orally improved learning and memory of mice assessed by the behavioral models like Elevated Plus Maze, Morris water maze, Y-maze. In Scopalamine induced amnesia there is loss of memory. The EEMQ extract contains majorly Steroids and antioxidant prosperity which may responsible for the anti-amnesic effect.

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REFERENCE:

1) Alzheimer's disease, information was sourced from www.mayoclinic.com.
2) Kedar N. Prasad, PhD, William C. Cole, PhD, K. Che Prasad, Risk Factors for Alzheimer's Disease: Role of Multiple Antioxidants, Non-Steroidal Anti-inflammatory and Cholinergic Agents Alone or in Combination in Prevention and Treatment, Journal of the American College of Nutrition, Vol. 21, No. 6, 506–522 (2002)
3) Peter Wostyn, Kurt Audenaert and Peter Paul De Deyn, Alzheimer's disease-related changes in diseases characterized by elevation of intracranial or intraocular pressure, Clin Neurol Neurosurgery. 2008 Feb; 110(2):101-9.
4) Patrick L.McGeer and Edith G.Mcgeer, Inflammation and the Degenerative Diseases of Aging, Ann. N.Y. Acad. Sci. 1035: 104–116 (2004).
5) Sally S. Roach, Introductory Gerontological Nursing, Lippincott Williams & Wilkins, 20-Oct-2000 page no.202-205
6) Richard B. Silverman, The organic chemistry of drug design and drug action, Academic Press, 2004,page No .535-550
7) COGNEX (tacrine hydrochloride) capsule [Sciele Pharma, Inc.] source:http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=f31d1e8d-d0f3-4bfd-88b0-b2917365b0ca .
8) Ogura H, Kosasa T, Araki S, Yamanishi Y., Pharmacological properties of donepezil hydrochloride (Aricept), a drug for Alzheimer's disease, Nihon Yakurigaku Zasshi. 2000 Jan;115(1):45-51
9) Aricept (donpezil Hydrochloride tablets) Source http://www.aricept.com/
10) Philip O. Anderson, James E. Knoben, William G. Troutman, Handbook of clinical drug data, McGraw-Hill Professional, 11-Sep-2001, Page No.493-495
11) Kwai Dzy Mak, Clinical toxicology review published by Massachusetts /Rhode island poisons control system, Volume.23 no.9 June 20001
12) Maelicke A. The pharmacological rationale for treating vascular dementia with galantamine (Reminyl), Int J Clin Pract Suppl. 2001 May ;(120):24-8.
13) Antonio Verdoliva,* Vincenzo Rivieccio and Maria Rossi, Simplified β-amyloid peptides for safer Alzheimer vaccines development Human Vaccines 6:11 936-947; November 2010.
14) Michael Grundman, Vitamin E and Alzheimer disease: the basis for additional clinical trials1,2,3, American Journal of Clinical Nutrition, Vol. 71, No. 2, 630S-636s, February 2000.
15) Rutten BP, Steinbusch HW, Korr H, Schmitz C, Antioxidants and Alzheimer's disease: from bench to bedside (and back again), Curr Opin Clin Nutr Metab Care. 2002 Nov; 5(6):645-51.
16) Frank B, Gupta S, a review of antioxidants and Alzheimer's disease, Ann Clin Psychiatry. 2005 Oct-Dec; 17(4):269-86.
17) Bhadra S, Mukherjee PK, Bandyopadhyay A., Cholinesterase inhibition activity of Marsilea quadrifolia Linn. an edible leafy vegetable from West Bengal, India, Nat Prod Res. 2011 Oct 6.
18) Dongare SS, Maske AP, Patil SM, Umbare RP and Mate GS, Antidiabetic Activity of Marsilea quadrifolia linn in Alloxan-Diabetic Rats, RESEARCH JOURNAL OF PHARMACOGNOSY AND PHYTOCHEMISTRY (RJPP), Volume 01, Issue 01, July- August, 2009, page no.15-17
19) Farhana Alam Ripa et al, Antibacterial, Cytotoxic and Antioxidant Activity ofCrude Extract of Marsilea Quadrifolia, European Journal of Scientific Research, Vol.33 No.1 (2009), pp.123-129