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Research Article

PHYTO CHEMICAL SCREENING AND ANTI-AMNESIC EFFECT OF *MARSILEA QUADRIFOLIA*

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ABSTRACT

Marsilea quadrifolia 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. It is belongs to family Marsileaceae. Parts are used Whole Plant. Thiaminase enzyme is majorly present in this plant And also present steroids and some carbohydrates. Plant pacifies vitiated pitta, cough, bronchitis, diabetes, psychiatric diseases, eye diseases, diarrhea and skin diseases. *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 property which may responsible for the anti-amnesic effect.

INTRODUCTION

Herbal medicine is still the mainstay of about 75 - 80% of the world population, mainly in the developing countries, for primary health care.[1] This is primarily because of the general belief that herbal drugs are without any side effects besides being cheap and locally available (Gupta and Raina, 1998). [2]According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times (Evans, 1994).[3] *Marsilea quadrifolia* 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. It is belongs to family Marsileaceae. Parts are used Whole Plant. Thiaminase enzyme is majorly present in this plant And also present steroids and some carbohydrates. Plant pacifies vitiated pitta, cough, bronchitis, diabetes, psychiatric diseases, eye diseases, diarrhea and skin diseases.

Experimental

Collection of Plant Materials

The plant, *M. quadrifolia* was collected from the village of Nalgonda in the month of Febravary and identified and authenticated.

Preparation of Plant Extracts

The whole plant of *Marsilea quadrifolia* collected, shade dried for seven days and ground. 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 vaccum evaporator. Dried herbal extract is mixed with Carboxy methyl cellulose (CMC) and administered to the animals.

Experimental Design

Preliminary Phytochemical Screening[51]

The ethanolic extract of *Marsilea quadrifolia* was subjected to preliminary Phyto chemical screening for the presence or absence of active Phytochemical constituents by the following methods.

Test for alkaloids: Treated with dilute hydrochloric acid and filtered. The filtrate was treated with various alkaloidal agents.

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Mayer's- test: Treated with Mayer's reagent. Appearance of cream colour indicates the presence of alkaloid.

Dragendorff's-test: When little amount of the sample was treated with the Dragendorff's reagent, the appearance of reddish brown precipitate indicates the presence of alkaloid.

Hager's-test: Treated with the Hager's reagent, the appearance of yellow colour precipitate indicates the presence of alkaloid.

Quinoline alkaloids test: Little amount of extract is added with glacial acetic acid gives reddish brown fumes and with concentrated sulphuric acid gives blue fluorescence in U.V. light.

Test for carbohydrates: A small quantity (300 mg) of ethanolic extract was dissolved in 4ml of distilled water filter. The filtrate was subjected to (a) Molisch's test, (b) Fehling;s solution A and B, (c) Benedict's reagents and (d) Barfoed's reagents to detect the presence of different sugars.

Test for steroids

Libermann burchard test: When the extract was treated with concentrated sulphuric acid, few drops of glacial acetic acid, followed by the addition of acetic anhydride, appearance of green colour indicates the presence of steroids.

Test for proteins

Biuret's-test: When the extract was treated with copper sulphate solution, followed by the addition of sodium hydroxide solution, appearance of violet colour was observed.

Millon's-test: When the extract was treated with millon's reagent, appearance of pink color indicates the presence of proteins.

Test for tannins: When the extract was treated with 10% lead acetate solution, appearance of white precipitate indicates the presence of tannins. When the extract was treated with aqueous bromine solution, appearance of white precipitate indicates the presence of tannins.

Test for phenols: When the extract was treated with neutral ferric chloride solution, the appearance of violet color indicates the presence of phenols.

When the extract was treated with 10% sodium chloride solution, the appearance of cream color indicates the presence of phenols.

Test for flavanoids: 5 ml of the extract solution was hydrolyzed with 10% v/v sulphuric acid and cool. Then, it was extracted with diethyl ether and divided into three portions in three separate test tubes. 1ml of dilute sodium carbonate, 1ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow color demonstrated the presence of flavonoids.

Shinoda's test: The extract was dissolved in alcohol, to that one piece of magnesium followed by conc. HCI were added dropwise and heated. Appearance of magenta colour shows the presence of flavonoids.

Test for gums and mucilage: The extract was treated with 25 ml of absolute alcohol, and then solution was filtered. The filtrate was examined for its swelling properties.

Test for glycosides: When a pinch of the extract was dissolved in the Glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated sulphuric acid, formation of red ring at the junction of two liquids indicates the presence of glycosides.

Test for saponins

Foam test: 1ml of the extract was diluted to 20 ml with distilled water and shaken well in a test tube. Formation of foam in the upper part of test tube.

Test for Terpenes: When the extract was treated with Tin and thionly Chloride, appearance of pink colour indicates the presence of terpenes.

Vehicle: The plant extract was diluted in carboxy methyl cellulose which is used as solvent. Scopolamine hydrochloride was also dissolved in carboxy methyl cellulose.

Behavioral Studies

Elevated Plus-Maze[37-42]

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[45]

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 (9cm 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[50]

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[47]

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

RESULTS

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

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

Positive: indicates the presence of compounds Negative: indicates the absence of compounds

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	68.33±0.76	52±0.73
II	Scopolamine(1mg/kg)	103±2.42**	81.83±0.79**
III	EEMQ(250mg/kg)	61.33±0.80**	33.33±0.76**
IV	EEMQ(500mg/kg)	52±0.93**	23.83±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.

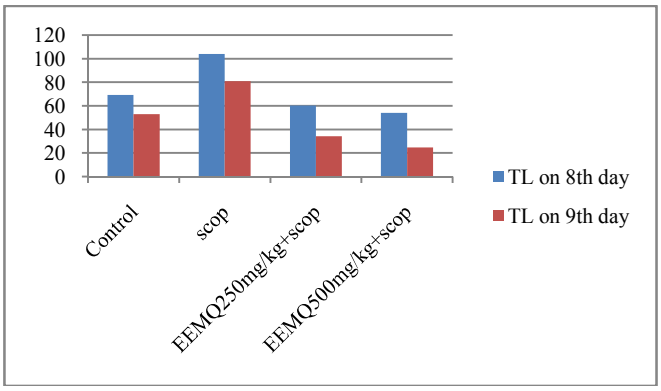

Fig no 3 Effect of EEMQ on Transfer latency of mice using Elevated plus maze

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

Group	Treatment	Escape latency
I	Control	13.16±0.74
II	Scopolamine(mg/kg)	22.16±0.60**
III	EEMQ(250mg/kg)	18.16±0.76**
IV	EEMQ(500mg/kg)	18.00±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

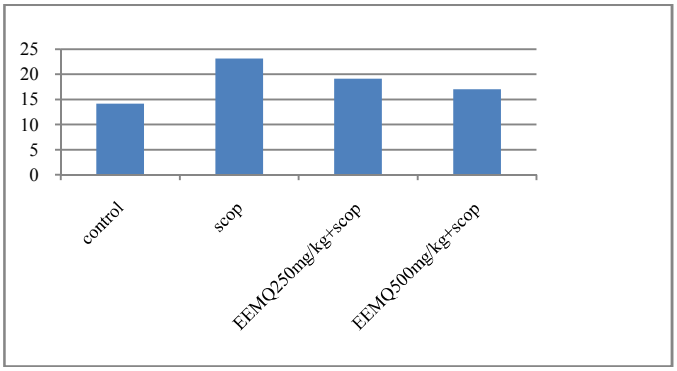

Fig no 4 Effect of EEMQ on Escape latency of mice using Morris water maze

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

Group	Treatment	Percentage alteration
I	Control	91.16±0.60
II	Scopolamine{1mg/kg}	52.05±0.42**
III	EEMQ(250mg/kg)	53.16±0.60**
IV	EEMQ(500mg/kg)	66.83±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.

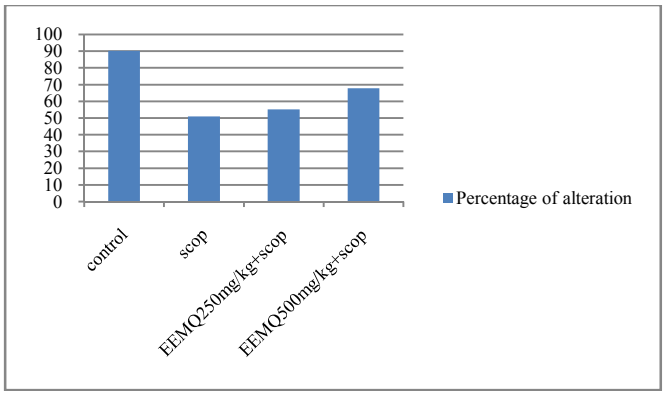

Fig no 4 Effect of EEMQ on Percentage alteration in mice using Y- maze

Discution

Preliminary Phyto chemical Investigation: Ethanolic extract of whole plant of *Marselia quadrifolia contains* Carbohydrates, Steroids, Glycosides, The results were shown in table-1

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

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 [52] EEMQ showed the significance of ($P<0.01$ and $P<0.001$) respectively as shown in

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

CONCLUSION

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 property which may responsible for the anti-amnesic effect.

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