

Short Communication

**Psychopharmacological Studies of Hydro Alcoholic Extract of Whole Plant of *Marsilea quadrifolia***

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**Abstract**

The hydro alcoholic extract of the entire plant *Marsilea quadrifolia* (HEMQ) was evaluated for different psychopharmacological actions such as behaviour, exploratory behaviour, muscle relaxant activity and phenobarbitone induced sleeping time. The extract was found to cause reduction in spontaneous activity, decrease in exploratory behavioural pattern by swimming and pole climbing test., reduction in the muscle relaxant by traction test. In addition, the extract significantly potentiated the phenobarbitone-induced sleeping time. Preliminary tests indicate that the hydro alcoholic extract of *Marsilea quadrifolia* in doses of 200-400 mg/kg has significant psychopharmacological activity.

**Keywords:** *Marsilea quadrifolia*; Hydro alcoholic extract; Muscle relaxant; Exploratory behaviour.

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**1. Introduction**

*Marsilea quadrifolia* Linn, a member of Marsileaceae is a creeping herbaceous perennial plant. It is known as caupatiya, sunsunia in Hindi and ciklintakura in Telugu. It is widely distributed in tropical and temperate regions of world and found throughout India, in marshy places and along the banks of canals and rivers [1]. Charaka gave sprouts cooked as vegetable in cough and spastic conditions of leg muscles. Sushruta prescribed the plant, cooked as a potherb, to harmonize internal body functions and for invigorating eye sight. As a vegetable, it was also given in cases of poisoning and in folk medicine; the herb is used as a vegetable for inducing sleep [2].

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As per the traditional claims the plant has been used for astringent, hypnotic, diuretic, expectorant, aphrodisiac, anodyne, ophthalmic, constipating, strangury and dyspepsia. It is useful in psychopathy, leprosy, haemorrhoids, skin diseases, fever, insomnia and febrifuge [2]. The phytochemicals like marsilin (1-triacontanol-cerotate), 3-hydroxy-triacontan-11-one, hentriacontan-6-ol, methylamine, beta-sitosterol, marsileagenin A, flavonol-O-mono-and-diglycoside, C-glucoylflavones and C-glucosylxanthenes have been isolated from the plant [3]. The crude extract of *Marselia quadrifolia* caused prompt hypotensive response and is also found to be effective against electro convulsions [3].

Since the plant was reported to be used by the tribes to have sound sleep at night, the author had a keen interest to test scientifically the psychopharmacological activity of the plant and support the claim.

## **2. Materials and Methods**

### **2.1. Collection and identification of plant material**

The entire plant of *M. quadrifolia* was collected from the rural belt of Eturunagaram, Warangal district, during early winter and authenticated by Prof. V S Raju, Department of Botany, Kakatiya University, Warangal. The collected plant material was dried under shade and pulverised in a mechanical grinder. The powder was passed through sieve no. 40 and used for further studies.

### **2.2. Preparation of extract**

The powdered plant material (400 g) was extracted with 2 liters of ethanol-water (1:1) by maceration in a closed vessel for 72h. The dark brown coloured sticky residue (yield-14.42% w/w) was collected after complete removal of the solvent under reduced pressure.

### **2.3. Selection and maintenance of animals**

Adult Wistar albino rats weighing between 150-200 g (for swimming test and pole climbing test) and adult Swiss albino mice weighing between 20-25 g (for the gross behavioural studies, traction test and potentiation of phenobarbitone induced sleep test) of either sex were used for the study. The animals were housed in standard polypropylene cages at room temperature and provided with standard diet and water *ad libitum*. The experimental protocol has been approved by the Institutional Animal Ethics Committee and by the Regulatory body of the government (Regd no. 1047/ac/09/CPCSEA).

### **2.4. Gross behavioural studies**

The HEMQ was tested for gross behavioural studies using mice as per the method suggested by Seth *et al.* [4]. The selected mice were divided into eight groups of six in each. The control group received 2 ml/kg of the vehicle intraperitoneally. The other groups received the extract in one of the following doses, 100, 200, 400, 800, 1000, 2000

and 3000 mg/kg in a similar manner. Immediately after dosing, the animals were observed continuously for first four hours for behavioural changes and for mortality if any at the end of 72 h.

### **2.5. Traction test**

The HEMQ was subjected to Traction test as per the method Turner [5]. Selected mice were divided into four groups of six animals each. Each group of mice received one of the following through intraperitoneal route. First group received the vehicle (20% v/v tween-20 in normal saline 0.1 ml/10g), second and third groups received the extract at 200 and 400 mg/kg and the fourth group, diazepam (10 mg/kg). Forty-five minutes after injection of test samples, the mice were suspended separately by means of their forepaws to a metallic wire stretched horizontally. The number of animals that were incapable of touching the wire at least one of the hind paws within five seconds were noted.

### **2.6. Potentiation of phenobarbitone induced sleep**

The HEMQ was subjected to potentiation of phenobarbitone induced sleep test [6, 7]. Selected mice were divided in to four groups of six each. Each group of the animals received one of the following through intraperitoneal route. First group received the vehicle (20% v/v tween-20 in normal saline 0.1 ml/10 g), second and third groups received the extract at 200 and 400 mg/kg and the fourth group, chlorpromazine (4 mg/kg). Thirty minutes after receiving test samples, each animal was injected intraperitoneally, Sodium phenobarbitone (45 mg/kg). The sleeping time was noted by recording the interval between the loss and regaining of righting reflex.

### **2.7. Exploratory behaviour studies**

The HEMQ was subjected to Swimming test and Pole climbing test [8-10] for studying the exploratory behaviour of the animals.

#### **2.7.1. Swimming test**

In this test, the selected group of rats, 30min after injection with the vehicle (1ml/kg), diazepam (10 mg/kg) or the extract (200 and 400 mg/kg) intraperitoneally, were placed singly inside a specially designed apparatus with swimming provisions and with ladder steps. The number of rotations made within 5 min by each rat was recorded as a measure of their behavioural studies to this set of experimental conditions and compared against a blank group (animals treated with vehicle).

#### **2.7.2. Pole climbing test**

In this test, the selected animals were divided in to four groups of six animals in each. The vehicle (1 ml/kg), chlorpromazine (4 mg/kg) and the extract (200 and 400 mg/kg) were

administered to different groups through intraperitoneal route. After 30 Minutes, the animals were treated inside the apparatus as per standard procedure [7]. The time taken by the animals to climb the pole following electric shock was noted.

### 2.8. Statistical analysis

The results were expressed as mean±S.E.M. Significance of difference between control and treated groups was determined by using one way ANOVA followed by Student's *t*-test.

## 3. Results and Discussion

### 3.1. Gross behavioural studies

The results on the gross behavioural studies revealed a marked sedation of HEMQ at all tested dose levels. The extracts affected the spontaneous activity, sound and touch response from 200 mg/kg dose levels. These effects were found to be dose dependent. Awareness and alertness were also found to be depressed. However, no mortality was reported even after 72 h.

### 3.2. Effect of HAMQ whole plant extract on traction test

The experimental observations of traction test indicated decrease number of animals capable of taking a better posture in less than 5 sec when compared with the control group. Results are shown in Table 1.

Table1. Effect of HAMQ whole plant extract on traction test.

Group (treatment)	NA1	NA2	NA3	NA4 (%)
Control (vehicle)	6	6	0	-
200mg/kg (HAMQ)	6	2	4	66.67
400mg/kg (HAMQ)	6	1	5	83.33
10mg/kg (diazepam)	6	0	6	100

HAMQ = Hydro alcoholic extract of whole plant of *Marsilea quadrifolia*; NA1= Number of animals used; NA2= Number of animals capable of touching the wire with in 5 sec; NA3= Number of animals incapable of touching the wire with in 5 sec; NA4= percentage of animals incapable of touching the wire within 5 sec.

### 3.3. Effect of HAMQ whole plant extracts on potentiation of phenobarbitone induced sleep

The results of potentiation of phenobarbitone sleep are shown in Table 2. The extract significantly potentiated ( $p < 0.01$  at 200 mg/kg and  $p < 0.001$  at 400 mg/kg dose levels) the

phenobarbitone induced sleeping time in mice at all tested dose levels when compared to the control group.

Table 2. Effect of HAMQ whole plant extracts on Potentiation of phenobarbitone.

Group (treatment)	Duration of sleep (min) (mean $\pm$ SEM)
Control (vehicle)	29.84 $\pm$ 1.485
200mg/kg (HAMQ)	36.65 $\pm$ 1.513*
400mg/kg (HAMQ)	41.32 $\pm$ 1.934**
4mg/kg (chlorpromazine)	50.16 $\pm$ 2.447**

HAMQ = Hydro alcoholic extract of whole plant of *Marsilea quadrifolia*; n= 6, Student's *t*-test, \*  $p < 0.01$ , \*\* $p < 0.001$  when compared with vehicle control.

### 3.4. Effect of HAMQ whole plant extracts on swimming test

As regards to the tests for exploratory behaviour effects, in the swimming test, it is observed that there is a significant decrease in the number of rotations with the rats treated with the extract at tested dose levels ( $p < 0.01$  at 200 mg/kg and  $p < 0.001$  at 400 mg/kg dose levels) when compared to the control (Table 3).

Table 3. Effect of HAMQ whole plant extracts on swimming test.

Group (treatment)	Number of rotations (mean $\pm$ SEM)
Control (vehicle)	14.6 $\pm$ 0.782
200mg/kg (HAMQ)	10.82 $\pm$ 0.635*
400mg/kg (HAMQ)	9.34 $\pm$ 0.712**
10mg/kg (diazepam)	6.32 $\pm$ 0.853**

HAMQ = Hydro alcoholic extract of whole plant of *Marsilea quadrifolia*; n= 6, Student's *t*-test, \*  $p < 0.01$ , \*\* $p < 0.001$  when compared with vehicle control.

### 3.5. Effect of HAMQ whole plant extract on pole climbing test

In pole climbing test, the time taken to climb the pole after electrical stimulus was also found to be delayed in the extract treated groups ( $p < 0.01$  at 200 mg/kg and  $p < 0.001$  at 400 mg/kg dose levels) than the control. Results are presented in Table 4.

Table 4. Effect of HAMQ whole plant extract on pole climbing test.

Group (treatment)	Duration of time of response (sec)
Control (vehicle)	4.34 ± 0.478
200mg/kg (HAMQ)	8.67 ± 0.985*
400mg/kg (HAMQ)	11.82 ± 0.648**
4 mg/kg (chlorpromazine)	14.60 ± 0.812**

HAMQ = Hydro alcoholic Extract of Whole Plant of *Marsilea quadrifolia*;  $n=6$ , Student's *t*-test, \*  $p < 0.01$ , \*\* $p < 0.001$  when compared with vehicle control.

#### 4. Conclusion

In the present study, the findings revealed that the hydro alcoholic extract of whole plant of *Marsilea quadrifolia* (HAMQ) possess effects in alteration of general behaviour pattern. The extract significantly potentiated the phenobarbitone induced sleeping time suggesting probable tranquilizing action as well as CNS depressant action. The possible effects of the extract were further examined on CNS for some other psychopharmacological effects (exploratory behaviour pattern; swimming test and pole climbing test). The findings of these tests substantiate the effects produced with other CNS depressant drugs. Therefore, it may be concluded that the hydro alcoholic extract of whole plant of *M. quadrifolia* possesses most of the pharmacological characteristics of the psychoactive group of drugs like minor tranquilizers at the tested dose levels. Further studies were needed to isolate and characterize the bio active compounds responsible for the above activity.

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