

PRELIMINARY SCREENING OF *Marsilea quadrifolia* EXTRACTS FOR THEIR ANTIANXIETY POTENTIAL

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Received: 22 Nov 2012, Revised and Accepted: 31 Mar 2013

ABSTRACT

Marsilea quadrifolia a commonly available aquatic plant found to be used in different traditional system and by local people for anxiety and related disorders.

Objective: The study was intended to evaluate the antianxiety potential of *Marsilea quadrifolia* and give a scientific basis for this.

Method: The CNS effect was screened by taking Swiss albino mice and Wistar rats. The extract was found to cause reduction in spontaneous activity, decrease in exploratory behavioural pattern by swimming and pole climbing test at a dose of 500mg/kg. In general behavioral screening the extracts found to show tranquilizing property.

Result and conclusion: These Preliminary tests indicate that the ethanolic extract of *Marsilea quadrifolia* in doses of 500 mg/kg has significant antianxiety activity. The current study thus supports the traditional utilization of this plant against the inflammatory disorders.

Keywords: *Marsilea quadrifolia*; Ethanolic extract; Exploratory behaviour.

INTRODUCTION

The sudden holocaust of anxiety disorders in the present era taken the attention of the researchers for development and search of new drug. Recognition of severe side effects and addiction liabilities associated with long term administration of widely prescribed anxiolytic drugs have aroused the attention of researchers towards natural resources. Inclusion of some plants like *Valeriana officinalis*¹, *Nardostachys jatamansi*², *Withania somnifera*³ and *Panax ginseng*⁴ in the arsenal of modern therapeutics has revived the faith of researchers in the plants. A survey of literature on plant revealed several reports on the traditional uses of *Marsilea quadrifolia* in anxiety and for sedation.

Marsilea quadrifolia Linn, a member of Marsileaceae is a creeping herbaceous perennial plant. It is known as caupatiya, sunsuniya in Hindi and ciklindakura 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 [5]. Charaka gave sprouts cooked as vegetable in cough and spastic conditions of leg muscles. Sushruta prescribed it was also given in cases of poisoning and in folk medicine; the herb is used as a vegetable for inducing sleep [6]. As per the traditional claims the plant has been used for astringent, hypnotic, diuretic, expectorant, aphrodisiac, anodyne, ophthalmic, constipating, psychopathy, leprosy, haemorrhoids, skin diseases, fever, insomnia and febrifuge [6]. 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-glucosylflavones and C-glucosylxanthones have been isolated from the plant [7]. The plant is traditionally used to reduce mental tension and to induce sleep, reducing anxiety and stress in emotional conditions. Therefore, with the reference to traditional and reported uses, the present study was undertaken to investigate the CNS activity of the psychopharmacological activity of the plant and give a scientific rationale for its use.

MATERIALS AND METHODS

Plant material

The entire plant of *Marsilea quadrifolia* was collected from the rural belt of Tadepaligudem, west Godavari district, during month of November. and washed with tap water to remove the dirt particles. The collected plant material was dried under shade and pulverised in a mechanical grinder. The powder was passed through sieve no. 20 and coarse powders were extracted by using Soxhlet apparatus with Ethanol (95%) for 72 hour [8,9]. The dark brown coloured

sticky residue (yield- 9.42% w/w) was collected after complete removal of the solvent under reduced pressure and by keeping in a desiccator.

Animals

Swiss Albino mice of the either sex weighing 20-25gm were used. The animals were housed in standard cages with free access to food (standard laboratory rodent's chow) and water. The animal house temperature was maintained at $23 \pm 5.00^\circ\text{C}$ with a 12-h light/dark cycle (lights ON from 0600 to 1800hrs). Permission from Institutional Animal Ethics Committee constituted for the purpose of CPCSEA Government of India was taken. The guidelines for the investigation of experiments in conscious animals were followed in all tests.

Acute Toxicity Tests:

Acute toxicity studies were conducted by using albino mice of either sex weighing 23-35 gm. The animals were fasted overnight prior to the experimental procedure. The method of Up and Down or staircase was used to determine the dose [10, 11]. During this the animals were observed for signs of behavioral, Neurological toxicity and mortality. The procedure was followed as per OECD 423 guidelines.

Pharmacological screenings:

General behavioural methods [12,13,14]

Evaluation of general behavioural profile was performed by the method of Irwin S. twenty adult albino mice were divided into two groups. one group of animal receive 500mg/kg of extracts by oral intubation and the other group receives the 2 ml of acacia suspension as control. The animals were under observation for behavioral changes if any, at 30 minutes interval in the first hour and at one hour intervals for next 6 h for the following parameters. The awareness and alertness were recorded by visual measure of the animal's response when placed in different positions and its ability to orient itself without bumps or falls. The normal behavior at resting position was scored as 0. Similarly little activity, moderate flexibility, strong response and abnormal restlessness were scored as 1, 2, 3 and 4 respectively. The stereotype movements in mice involve searching movements of the head, circling, self-biting, walking backwards, licking the lips, and tail lashing, which were scored 0 and 1.

The spontaneous activity of mice was recorded by placing the animal in a bell jar. It usually shows a moderate degree of inquisitive behavior. Less or moderate activity was scored as 2, 3 and strong

activity as 4. If three is slight or little motion, the score was 1 while the animal sleeps the score was 0. A similar test was performed with the same scoring, when the animal are removed from the jar and placed on a table. The touch response was recorded by touching the mice with a pencil or forceps at a various parts of the body (i.e. on the side of the neck, abdomen and groin). The pain response was graded when a small artery clamp was attached to the base of the tail, and response was noted. Albino mice normally utter no sound, so that vocalization may indicate noxious stimulus. Pinna reflex is tested by touching the center of the pinna with a hair or other fine instrument. The unaffected mouse withdraws from the irritating hair. In the corneal reflex, a stiff hair, touched to the cornea, causes the animal to withdraw. In the ipsilateral flexor reflex, a toe-pad is compressed with a forceps, which causes the animal to flex its leg in a retiring movement.

Rearing and sniffing [15]

The onset and intensity of rearing (repetitive standing), sniffing (touching the nose to the container) and licking behavior for about 5 min were observed by keeping the mice in a small glass jar. The numbers of times the animal show rearing and sniffing response were tabulated.

Head dip test [16]

The animals were placed on the top of a wooden box with 16 evenly spaced holes, 1 hour after the oral administration of control and extracts. The number of times that each animal dipped the head into the hole was counted for a period of 3 min.

Forced swim test [17]

The mice were individually placed into a glass cylinder (25 cm height, 10 cm diameter) containing 8 cm of water maintained at 22–24°C. Every 30 s for a total of 6 min the mice were rated for immobility which defined as the absence of active, escape-oriented behaviors such as swimming, jumping, rearing, sniffing, or diving. At each time point the mice were observed for 10 s and immobility was recorded as being present or absent. Any mouse appearing to have difficulty keeping its head above water was removed from the cylinder and deleted from the study. The rater was blind to the experimental treatment.

Motor coordination [18]

Animals remain on Rota-Rod (25 rpm) 5 min or more after low successive trials are included in the study. After the administration of control and the test material the fall off time from the rotating rod was noted after 2 hrs. The difference in the fall off time from the rotating rod between the control and the treated mice was taken as an index of muscle relaxation.

Locomotor activity [19,20]

The spontaneous locomotor activity was assessed with the help of actophotometer (V. Suresh et al). Each animal was observed for a period of 5 min in a square closed Field arena (30 x 30 x 30 cm) equipped with 6 photocells in the outer wall. Interruptions of Photocell beams (locomotor activity) were recorded by means of a 6 digits counter. To see the locomotor activity, the Actophotometer was turned on and each mouse was placed individually in the activity cage for 5 minutes. The basal activity score for all the animals was noted. Control and extract was given orally and after 1 hour retesting, activity score for 5 minutes observed. The difference in the activity, before and after drug administration was noted. Percentage decrease in motor activity was calculated.

RESULTS AND DISCUSSION

The results obtained from the experiments are presented in Table 1. *Marsilea quadrifolia* ethanolic extracts at a dose of 500mg/kg significantly affected spontaneous activity, irritability gait and touch responses. They also produce moderate or slight depression relating to awareness and alertness animals. However, the righting reflex corneal reflex and vocalization remain normal throughout the study process. Above studies indicates that there is a mild sedation action of the extract on the animals. The loss of irritability, reactivity and

grooming [12] indicates anxiolytic effect of the extracts on the animal. Irritability is an extension of restlessness and in higher degree it is demonstrated by aggressiveness. The test extract suppress the normal irritability when expose to stressful external stimuli. The gait of the animal indicates the control disturbance or neuromuscular blocker upon administration of the test drug some animals show bit staggering gait. Above studies indicates that there is a mild sedation action of the extract on the animals. The loss of irritability, reactivity and grooming indicates a anxiolytic effect of the extracts on the animal

Table 1: Effect of *Marsilea quadrifolia* ethanolic extracts on general behavioural profiles in mice (n=10).

Evaluation Parameters	Scores (average of 10 animals)	
	Control (2% gum acacia)	Test (500mg/kg extract)
Alertness	0	0.3
Grooming	0.1	0.4
Vocalization	0	0
Restlessness	0.1	0.2
Irritability	0	0.8
reactivity	0.1	0.8
Spontaneous action	4	2.6
Touch response	0	0.2
Pinna response	0.1	0.2
Corneal reflex	0	0
gait	4	3.2
Righting reflex	0	0

Table 2: Effect of *Marsilea quadrifolia* ethanolic extracts on forced swimming test.

Group No.	Treatment Group	Immobility(sec)
1	Vehicle (2% gum acacia)	188.33±8.6
2	<i>Marsilea quadrifolia</i> hydroalcoholic (500 mg/kg)	160.66±10.2 ^a

The observations are mean ± SEM of 6 animals. p<0.05 as compared to that of vehicle treated group (One way ANOVA followed by Dunnett's test).

The ethanolic extract of *Marsilea quadrifolia* exhibited significant decrease in immobility induced by Forced swimming. There are reports establishing that the Forced swimming decreases serotonin, noradrenaline (NA) and increases corticosteroids level. As the extract is having significant effect by decreasing immobility in forced swimming test, the action of extract may be mediated through increasing the effect or, level of serotonin and NA in brain [21].

Many medications originally approved for the treatment of depression have been found to relieve symptoms of anxiety. These include certain selective serotonin reuptake inhibitors (SSRIs)[10], tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and the newer atypical antidepressants. Antidepressants are often preferred over the traditional anti-anxiety drugs because the risk for dependency and abuse is smaller.

Table 3: Effect of *Marsilea quadrifolia* ethanolic extracts on exploratory behaviour (head dip test) in mice.

Group No.	Treatment Group	Immobility(sec)
1	Vehicle (2% gum acacia)	88.4±2.16
2	<i>Marsilea quadrifolia</i> hydroalcoholic (500 mg/kg)	71.6±3.76 ^a

The observations are mean ± SEM of 10 animals. p<0.05 as compared to that of vehicle treated group (One way ANOVA followed by Dunnett's test).

In head dip test, there was a significant reduction in the head tip responses occurred in mice treated with the extract, compared with the control (Table 3). These indicate the decrease in the spontaneous curiosity in the mice which can be considered as the decrease in anxiety and depression.

Table 4: Effect of *Marsilea quadrifolia* ethanolic extracts on locomotor activity (Actophotometer test) in mice

Group No.	Treatment Group	locomotor activity (no.)
1	Vehicle (2% gum acacia)	281.5 ± 12.8
2	<i>Marsilea quadrifolia</i> hydro alcoholic (500 mg/kg)	216 ± 18.6

The observations are mean ± SEM of 10 animals. $p < 0.05$ as compared to that of vehicle treated group (One way ANOVA followed by Dunnett's test).

Locomotor activity is considered as an index of alertness and a decrease in that indicates a sedative effect. One hypothesis regarding the significance of motor activation in relation to anxiety-like state is the increase in locomotor stimulation may be indicative of a stress-like state [22]. The decrease in behavioral arousal by the suppression in locomotor activity may be indicative of an anxiolytic-like state. The ethanolic extract of *Marsilea quadrifolia* exhibited significant decrease in the locomotor activity when compared to control which is depicted in table 4. This result has indicated the probable antianxiety effect of the extracts.

Table 4: Effect of *Marsilea quadrifolia* ethanolic extracts on Rearing and Sniffing Response in Mice

Group No.	Treatment Group	Rearing	sniffing
1	Vehicle (2% gum acacia)	23.6 ± 1.6	26.4 ± 0.9
2	<i>Marsilea quadrifolia</i> hydro alcoholic (500 mg/kg)	14.25 ± 0.5	17.75 ± 0.6

The observations are mean ± SEM of 10 animals. $p < 0.05$ as compared to that of vehicle treated group (One way ANOVA followed by Dunnett's test).

Anxiety many a time leads to increase in the stereotype behavior like repetitive standing (rearing) and continuous sniffing (touching the nose to wall of the container) and licking the wall of the container. These purposeless behaviors are decreased by the neuroleptic significance. However the best compound decreases the rearing and sniffing rate than the normal animal indicating the decrease in the spontaneous activity which is common manifestation of anxiolytic drugs. Therefore it can be assumed that the decrease in the rearing and sniffing response by the test extract is due to the anxiolytic property.

CONCLUSION

From all these above studies it is found that the test extract of *Marsilea quadrifolia* has got CNS depressant or anxiolytic property. Further studies are to be carried out to find out the exact mechanism for these actions and establish the proper use of the drug. The Behavioural tests for anxiety are useful for the better understanding of potential activity in human and mechanism of action of drug. From the above observation we found that *Marsilea quadrifolia* ethanolic extract possess anxiolytic activity which is supported by decrease in the spontaneous curiosity locomotory behavior and differentiate behavioural parameters in the animal our findings

substantiates and give a scientific rational for the use of *Marsilea quadrifolia* in different anxiety conditions

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