

Original Article**Anti-aggressive activity of a standardized extract of *Marsilea minuta* Linn. in rodent models of aggression****Om P. Tiwari¹, Subhrata K. Bhattamisra², Pushpendra K. Tripathi³, Paras N. Singh^{4,*}**¹ Pharmacology Laboratory, Institute of Pharmacy, Harish Chandra PG College, Bawan Beegha, Varanasi, India;² Pharmacology Division, Torrent Research Centre, Village Bhat, Gandhinagar, India;³ Rajarshi Ranajay Singh College of Pharmacy, Amethi, Sultanpur, India;⁴ Neuropharmacology Research Laboratory, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi, India.**Summary**

The present study was undertaken to evaluate *in vivo* anti-aggressive potential of a standardized extract of *Marsilea minuta* Linn. (Marsileaceae). The standardized extract of *Marsilea minuta* was evaluated for its potential effects against defensive and offensive aggressive behavior models of rodents. *Marsilea minuta* extract was orally administered at three dose levels (100, 200, and 400 mg/kg BW) once daily for 14 consecutive days as a suspension in polyethylene glycol (PEG), diazepam (2.5 mg/kg, *p.o.*) was used as a standard anti-aggressive agent. Control group animals were given an equal volume of vehicle (10%, v/v, PEG suspension). Anti-aggressive activity was evaluated using the following validated models of aggression, viz.: foot shock-induced aggression, isolation-induced aggression and resident-intruder aggression, in rodents. As a result, *Marsilea minuta* extract showed dose dependant anti-aggressive activity in the aforementioned, validated models of aggression. This suggests that the extract from *Marsilea minuta* has a promising anti-aggressive activity qualitatively comparable to that of diazepam.

Keywords: *Marsilea minuta*, aggression, stress, foot shock, isolation

1. Introduction

Marsilea minuta Linn. (Marsileaceae), a common species of water fern, is widely found in wet and humid places (1). In Ayurveda, the plant is recommended for treatment of psychopathy, diarrhea, cough, bronchitis, and skin diseases (2). A standardized extract of *Marsilea minuta* has been reported to possess anti-amnesic (3), anxiolytic (4), and antidepressant activities (5). Marceline, an ester of 1-triacontanol and hexacosanoic acid, isolated from *Marsilea minuta* is known to have sedative and anticonvulsant activity (6). Gupta *et al.* (7) reported hypocholesterolemic activity of the methanolic extract of the plant in gerbils. Other reported activities include antifertility activity (8), tranquilizing activity

(9), antibacterial (10), and antifungal activity (11). In our earlier study (12), we reported adaptogenic and anti-stress activity of the standardized extract of *Marsilea minuta*. Aggression is now a significant public health problem and association between mental illness and aggression is well established (13,14). Besides this, stress is another major factor promoting aggression and violence in humans (15,16). Keeping in view the beneficial effect of *Marsilea minuta* Linn. in neurological disorders such as amnesia, depression, anxiety and antistress activity we decided to investigate the anti-aggressive activity of *Marsilea minuta* Linn.

2. Materials and Methods**2.1. Materials**

Whole plants of *Marsilea minuta* were collected during the month of July 2004 from Berhampur, Orissa, India. *Marsilea minuta* Linn. (Marsileaceae) was authenticated by Prof. N. K. Dubey, Incharge herbarium, Department of Botany, Banaras Hindu University, Varanasi, India.

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A specimen copy of the same (Sept-2004-1) was deposited in the herbarium, Department of Botany, Banaras Hindu University. All other reagents used were of analytical grade.

2.2. Preparation of extract

The whole plant of *Marsilea minuta* was dried under shade in a drying room with a relative humidity of 40%. The room temperature was maintained between 37 and 40°C. The drying process was carried out for 5-7 days. The shade-dried plant was reduced to coarse powder in a roller grinder and was finely powdered further. The fine powder was then passed through a No. 40 sieve. About 500 g of plant powder was thoroughly extracted with 2.5 liters of 90% ethanol in a soxhlet apparatus for 48 h. The extract was concentrated under vacuum at 50°C and then lyophilized (yield 16.3%, w/w), and was stored at -20°C until required. The presence of steroids, flavonoids, alkaloids, and saponins was confirmed in a preliminary phytochemical investigation of the ethanolic extract of *Marsilea minuta* (17). Marsiline was isolated as described previously (6) and characterized. The extract was standardized for marsiline (purity, 94.32%) using a Perkin Elmer HPLC with a diode array detector. The method was standardized and validated with an initial sample of 5 µg/mL. Eight replicates of this concentration (5 µg/mL) were prepared and analyzed. The limit of detection and limit of quantification obtained was 1.53 and 5.11 µg/mL, respectively. The average percent recovery and coefficient of variation was found to be 91.75 and 1.11%, respectively. A standard curve was prepared using five standards at 10, 20, 50, 100, and 200 µg/mL. The curve showed good linearity with an r^2 value of 0.942. The standardized ethanolic extract of *Marsilea minuta* (Mm) (1.15%, w/w of marsiline) was used for the pharmacological evaluations.

2.3. Animals

Swiss albino mice (20 ± 2 g) and Wistar rats (200-250 g) of either sex were obtained from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University (Regd. No. 542/02/ab/CPCSEA). Animals were randomly housed in groups of six in polypropylene cages at an ambient temperature of 25 ± 1°C and 45-55% relative humidity, with a 12 h light/dark cycle (lights on at 7 am). The animals had free access to standard pellet (Hindustan Lever, India) and water *ad libitum*. Experiments were conducted between 8:00 and 14:00. The experiments were conducted according to the norms of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Prior permission was obtained from the Institutional Animal Ethics Committee (IAEC) to carry out the experiments.

2.4. Drug treatments

Based on our earlier studies, the standardized ethanolic extract (1.15%, w/w of marsiline, HPLC) of Mm was administered orally, as a polyethylene glycol (PEG) suspension in doses of 100, 200, and 400 mg/kg of body weight, once daily for 14 consecutive days. Experiments were conducted on day 14, 1 h after the last oral treatment. Diazepam (2.5 mg/kg, *p.o.*) was used as the standard anti-aggressive agent for comparison. Control animals were treated with an equal volume of vehicle (10%, v/v, PEG suspension).

2.5. Experimental methods

The three most widely used rodent models, often used to detect potential effects of a therapeutically used anxiolytic drug on aggression were chosen to evaluate the effect of Mm on aggressive behavior, viz.: foot shock-induced aggression, isolation-induced aggression, and resident-intruder aggression.

2.5.1. Foot shock-induced aggression

Weight matched Swiss mice were divided into five groups (each containing 6 pairs), treated with vehicle, Mm (100, 200, and 400 mg/kg BW) or diazepam respectively, once daily for 14 consecutive days. On the 14th day, 1 h after the last oral treatment, all pairs of mice were subjected to foot shock by placing them in an aggressometer (Techno) for 3 min. During a 3 min observation period, every 5 sec a 60-Hz current was delivered for 5 sec. Each pair of mice was dosed and tested without previous exposure. The total number of fights were recorded for each pair (18,19).

2.5.2. Isolation-induced aggression

Male Swiss mice (body weight of 25 ± 5 g) were kept isolated in small cages for two months. Prior to the drug treatment, the aggressive behavior of the isolated mouse was assessed against a male mouse (similar in weight to that of the isolated mouse, and accustomed to living in a group and put into the cage of an isolated mouse for 5 min). Immediately, the isolated mouse started to attack the "intruder". The aggressive behavior of the isolated mouse was characterized by hitting the tail on the bottom of the cage, screaming and biting. Isolated mice not exhibiting aggressive behavior were excluded from the test. One day after the initial trial, isolated animals were distributed into five groups ($n = 6$) and were treated with vehicle, Mm (100, 200, and 400 mg/kg BW) or diazepam for 14 consecutive days. One hour after the last dose, aggressive behavior of the isolated mouse against a male mouse was evaluated for 5 min (19-21). Aggressive behavior related parameters assessed during this test were latency to first attack, screaming, pursuit frequency,

tail rattle, aggressive posture, and total number of fights.

2.5.3. Resident-intruder aggression

Male rats (400 ± 20 g) were tested in their home cages for aggression against a smaller (200 ± 20 g) male intruder. Before the start of the experiments, each resident male rat was kept in a pair with one female rat in a polypropylene cage for 15 days, and they were randomly divided into 5 groups ($n = 6$). Drug treatment was started from the 16th day onward, and only male rats of each pair were administered with vehicle, Mm (100, 200, and 400 mg/kg BW) or diazepam for 14 consecutive days. The resident female was removed from the cage 30 min prior to the start of the test. One hour after the last oral treatment, a male intruder (~ 200 g) was placed in the territorial cage of the resident male, and behavior of the resident male was observed for the next 15 min. During this period, the time until the first attack (in seconds), number of attacks, and duration of each attack (in seconds) were recorded by a blind observer (19).

3. Results

3.1. Foot shock-induced aggression

All three doses of Mm (100, 200, and 400 mg/kg) significantly reduced the total number of fights as compared to controls. Diazepam treatment also significantly reduced foot shock-induced fighting behavior in mice (Figure 1).

3.2. Isolation-induced aggression

All three doses of Mm (100, 200, and 400 mg/kg) significantly increased latency time to first attack (Figure 2) while the number of aggressive postures,

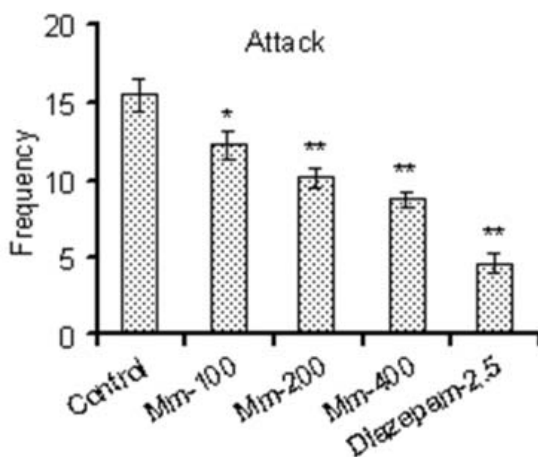


Figure 1. Effect of *Marsilea minuta* on foot shock-induced aggressive behavior. Values are given as mean \pm S.E.M. ($n = 6$). * $p < 0.05$, ** $p < 0.01$ compared to control.

aggressive pursuit, tail rattle frequency and attacks were significantly reduced by all three doses of Mm. These effects of Mm (100, 200, and 400 mg/kg) were identical to that of diazepam (2.5 mg/kg) (Figure 3).

3.3. Resident-intruder aggression

All three doses of Mm (100, 200, and 400 mg/kg) significantly prolonged the latency period of first attack (Figure 4) and significantly reduced the frequency of aggressive posture, aggressive grooming and total number of attacks (Figure 5). The total duration of fighting was also reduced significantly by all three doses (100, 200, and 400 mg/kg) of Mm (Figure 6). The observed effects of diazepam in this model were qualitatively similar to those of Mm.

4. Discussion

The present anti-aggressive study was carried out to explore knowledge about the beneficial effect of Mm in



Figure 2. Effect of *Marsilea minuta* on latency time to isolation-induced first attack. Data are given as mean \pm S.E.M. ($n = 6$). * $p < 0.01$ compared to control.

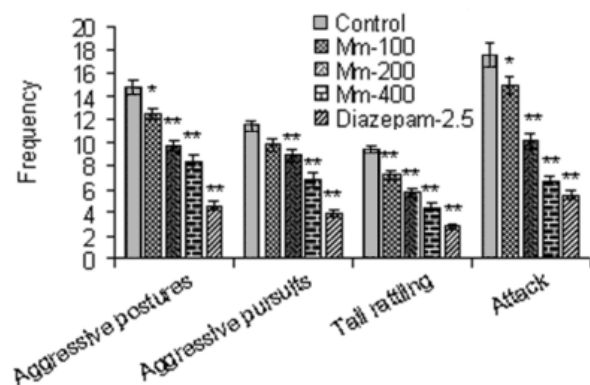


Figure 3. Effect of *Marsilea minuta* on frequency of various isolation-induced aggressive behavior. Data are given as mean \pm S.E.M. ($n = 6$). * $p < 0.05$, ** $p < 0.01$ compared to control.

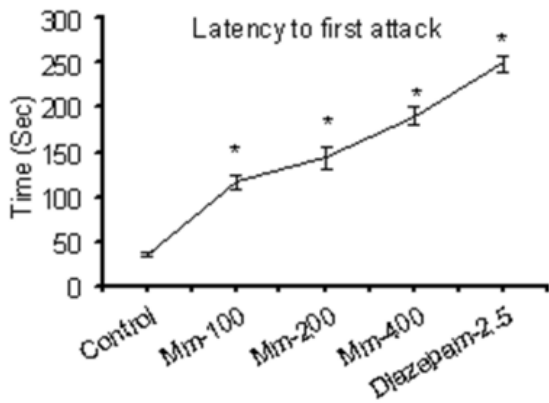


Figure 4. Effect of *Marsilea minuta* on latency period of first attack against resident intruder. Data are given as mean \pm S.E.M. ($n = 6$). * $p < 0.01$ compared to control.

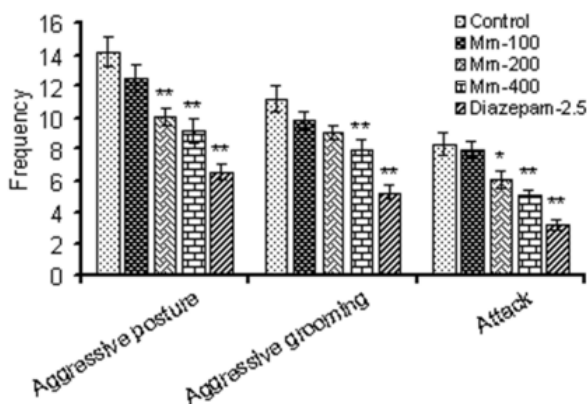


Figure 5. Effect of *Marsilea minuta* on frequency of various resident intruder aggressive behaviors. Data are given as mean \pm S.E.M. ($n = 6$). * $p < 0.05$, ** $p < 0.01$ compared to control.

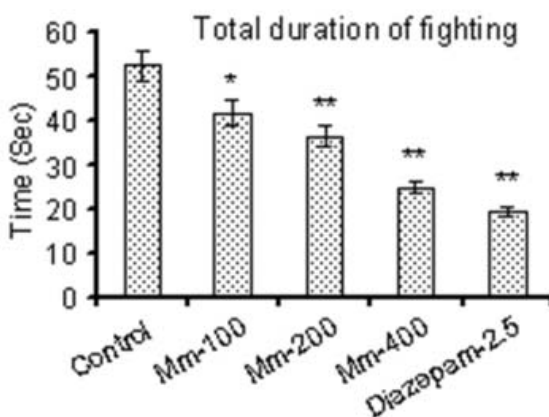


Figure 6. Effect of *Marsilea minuta* on total duration of fighting against resident intruder. Data are given as mean \pm S.E.M. ($n = 6$). * $p < 0.05$, ** $p < 0.01$ compared to control.

neurological disorders as already established in anxiety (4), depression (5), amnesia (3), and convulsions (6). The result of the study indicates that Mm has a dose dependent significant anti-aggressive activity which is comparable to diazepam. All three doses of Mm (100, 200, and 400 mg/kg, *p.o.*) significantly reversed

the parameters of aggression in all three models of aggression used, *viz.*: foot shock-induced aggression, isolation-induced aggression, and resident-intruder aggression.

The term aggression is widely employed to indicate various patterns of psychological or sociological behavior resulting from pathological, biochemical or physiological alteration of central nervous system constituents. There are many psychiatric disorders such as schizophrenia and Alzheimer's disease which show close association with aggression (14).

Like any other behavior, aggression is also controlled and modulated by neurotransmitters. The agonist of 5-HT_{1A}/5-HT_{1B} and antagonist of 5-HT_{2A}/5-HT_{2C} receptors have been reported to possess anti-aggressive properties (22,23). Bernard *et al.* (24) showed that dopamine levels and measurement of dopamine synthesis and turnover in the whole brain have increased in aggressive strains of mice and in mice that have just engaged in aggressive behavior. In the isolation-induced aggressive behavior model the level of dopamine increases in the striatum (25). In a postmortem study, Clement *et al.* (26) showed that the levels of GABA and glutamic acid decarboxylase, are low in brain areas such as the striatum and olfactory lobes of mice and rats which exhibited aggressive behavior. Initial studies targeting the α subunit of the GABA_A receptor point to their significant role in the aggression-heightening effect of alcohol, benzodiazepines, and neurosteroids (27). Tsuda *et al.* (28) and Tanaka *et al.* (29) have reported that expression of aggression is an alternative mechanism to decrease the stress related increase of noradrenaline.

Antidepressants, anxiolytics, cognitive function modulators, anticonvulsants, and other psychoactive agents are now identified as potential anti-aggressive therapeutics because of their neurotransmitter modulator properties. Mm has been investigated in various experimental models of depression, anxiety and memory and learning to reveal its modulator action on a variety of neurotransmitters. In this regard the effect of Mm on serotonin levels is of particular interest. Bhattamisra *et al.* (3) showed that Mm significantly decreases the serotonin level in the whole brain region of mice. That activation of 5-HT_{1A} receptors which results in decreased release of serotonin is accompanied by anti-aggressive behavior after administration of a 5-HT_{1A} agonist is a well established fact (30,31). Based on this premise, it can be concluded that the observed anti-aggressive property of Mm is due to its serotonin inhibitory action in whole brain. Besides the possible role of neurotransmitters in mediating aggression, stress has also been implicated to promote aggression and violence in humans (15,16). An earlier study (12) in the author's laboratory reported anti-stress activity of the standardized extract of Mm. Thus, it is concluded that the anti-aggressive activity of Mm may be

supplemented by its anti-stress property along with its neuromodulatory action.

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