Neuropharmacological potential of methanolic extract and a triterpene isolated from *Madhuca longifolia* L leaves in mice

Triveni S Inganakal¹, Md. Liyakhat Ahmed² & Paramjyothi Swamy¹*  
¹Department of Biochemistry, Gulbarga University, Gulbarga 585106, India  
²Department of Pharmacology, Luqman College of Pharmacy, Gulbarga 585106, India

Received 6 June 2012; revised 10 September 2012

The methanolic extract of *M. longifolia* (MLME) and a compound, a triterpene, derivative of madhucic acid (dMA) isolated from the leaves of *M. longifolia*, were investigated for their possible neuropharmacological activities in mice using phenobarbitone induced sleeping time, spontaneous motor activity, marble burying test and Eddy’s hot plate method. LD₅₀ for MLME and dMA were 100 and 10 mg/kg of body weight, respectively. Both MLME and dMA (10 mg/kg and 2 mg/kg oral route respectively) exhibited significant increase in phenobarbitone induced sleeping time, greater reduction in spontaneous motor activity and marble burying activity, confirming their sedative nature. Both MLME and dMA also exhibited considerable antinociceptive activity in experimental animals. The results suggest that both MLME and dMA have CNS depressant activity in mice.

**Keywords:** Chlorpromazine, Diazepam, *Madhuca longifolia*, Madhucic acid, Neuropharmacological effect, Phenobarbitone

*Madhuca longifolia* L (Sapotaceae; Mahua in Hindi, Mahwa in Bengali, Butter tree in English, Hippemara, Kadippe in Kannada, Madhukamu in Telgu) is an economic plant growing throughout the subtropical region of the indo-pak subcontinent¹. The medicinal properties attributed to this plant are stimulant, demulcent, emollient, heating and astringent². The bark is a good remedy for itch, swellings, fractures, and snake-bite poisoning, internally employed as a lotion in chronic ulcer, in acute and chronic tonsillitis and pharyngitis. Bark, leaves, flowers, fruits and seeds of *M. longifolia* have various uses in the Indian indigenous system of medicine. The tree is valued for its oil bearing seeds and flowers, which are used for alcoholic beverages production. Mahua seeds have edible fats³. Its seeds kernel is rich in saponins⁴. The distilled juice of the flower is considered a tonic, both nutritional and cooling and also used in treatment of helminthes, acute and chronic, tonsillitis. Preliminary phytochemical studies showed the presence of flavonoids, triterpenoids, alkaloids, saponins, phenols and glycosides. The objective of the present study is to isolate the bioactive from the leaves of *M. longifolia* and investigate its neuropharmacological activities in mice.

Anxiety and depression are the most frequent psychiatric condition commonly found. A large number of persons suffer from these conditions at some time during their life. To date, the efficacy of the drugs for these conditions are very limited, so the need for newer, better tolerated and more efficacious treatment is high. Therefore, herbal therapies should be considered as alternative/complementary medicine. Recently the search for novel pharmacotherapy from medicine plants for psychiatric illness has been progressed significantly. This is reflected in large number of herbal medicine whose psychotherapeutics potential has been assured in a variety of animal models. Hence, in present study, an attempt has been made to screen the neuropharmacological activities of methanolic extract of *M. longifolia* leaves and an isolated compound (dMA) in a systematic way using laboratory animals.

**Materials and Methods**

*Plant material*—The leaves of *M. longifolia* were collected during November 2009 from Konchavaram forest, Gulbarga, Karnataka and authentication was done by Prof Y.N. Seetharam, Department of Botany, Gulbarga University, Gulbarga where a voucher specimen has been deposited in the herbarium (HGUG no: 723).

*Extraction and isolation*—Air dried leaves (500 g) of *M. longifolia* were reduced to a fine powder, which

*Correspondent author*  
Telephone: 9341806110  
E-mail: paramjyothiswamy@gmail.com

---

¹Department of Biochemistry, Gulbarga University, Gulbarga 585106, India  
²Department of Pharmacology, Luqman College of Pharmacy, Gulbarga 585106, India

Received 6 June 2012; revised 10 September 2012

The methanolic extract of *M. longifolia* (MLME) and a compound, a triterpene, derivative of madhucic acid (dMA) isolated from the leaves of *M. longifolia*, were investigated for their possible neuropharmacological activities in mice using phenobarbitone induced sleeping time, spontaneous motor activity, marble burying test and Eddy’s hot plate method. LD₅₀ for MLME and dMA were 100 and 10 mg/kg of body weight, respectively. Both MLME and dMA (10 mg/kg and 2 mg/kg oral route respectively) exhibited significant increase in phenobarbitone induced sleeping time, greater reduction in spontaneous motor activity and marble burying activity, confirming their sedative nature. Both MLME and dMA also exhibited considerable antinociceptive activity in experimental animals. The results suggest that both MLME and dMA have CNS depressant activity in mice.

**Keywords:** Chlorpromazine, Diazepam, *Madhuca longifolia*, Madhucic acid, Neuropharmacological effect, Phenobarbitone

*Madhuca longifolia* L (Sapotaceae; Mahua in Hindi, Mahwa in Bengali, Butter tree in English, Hippemara, Kadippe in Kannada, Madhukamu in Telgu) is an economic plant growing throughout the subtropical region of the indo-pak subcontinent¹. The medicinal properties attributed to this plant are stimulant, demulcent, emollient, heating and astringent². The bark is a good remedy for itch, swellings, fractures, and snake-bite poisoning, internally employed as a lotion in chronic ulcer, in acute and chronic tonsillitis and pharyngitis. Bark, leaves, flowers, fruits and seeds of *M. longifolia* have various uses in the Indian indigenous system of medicine. The tree is valued for its oil bearing seeds and flowers, which are used for alcoholic beverages production. Mahua seeds have edible fats³. Its seeds kernel is rich in saponins⁴. The distilled juice of the flower is considered a tonic, both nutritional and cooling and also used in treatment of helminthes, acute and chronic, tonsillitis. Preliminary phytochemical studies showed the presence of flavonoids, triterpenoids, alkaloids, saponins, phenols and glycosides. The objective of the present study is to isolate the bioactive from the leaves of *M. longifolia* and investigate its neuropharmacological activities in mice.

Anxiety and depression are the most frequent psychiatric condition commonly found. A large number of persons suffer from these conditions at some time during their life. To date, the efficacy of the drugs for these conditions are very limited, so the need for newer, better tolerated and more efficacious treatment is high. Therefore, herbal therapies should be considered as alternative/complementary medicine. Recently the search for novel pharmacotherapy from medicine plants for psychiatric illness has been progressed significantly. This is reflected in large number of herbal medicine whose psychotherapeutics potential has been assured in a variety of animal models. Hence, in present study, an attempt has been made to screen the neuropharmacological activities of methanolic extract of *M. longifolia* leaves and an isolated compound (dMA) in a systematic way using laboratory animals.

**Materials and Methods**

*Plant material*—The leaves of *M. longifolia* were collected during November 2009 from Konchavaram forest, Gulbarga, Karnataka and authentication was done by Prof Y.N. Seetharam, Department of Botany, Gulbarga University, Gulbarga where a voucher specimen has been deposited in the herbarium (HGUG no: 723).

*Extraction and isolation*—Air dried leaves (500 g) of *M. longifolia* were reduced to a fine powder, which
was subjected to hot continuous extraction in a soxhlet extractor, successively with petroleum ether (40-60 °C), chloroform, methanol and water. Each time before extracting with the next solvent, the powder material was dried in hot air oven below 50 °C. Each extract was concentrated by distilling off the solvent followed by evaporation to dryness on a water bath. All extracts were kept in a desiccator and stored in a refrigerator for phytochemical and pharmacological studies. The methanolic extract was subjected for column chromatography on silica gel (60-120 mesh) and eluted with following solvent systems: chloroform:methanol (8:2) and (1:9). The chloroform:methanol (1:9) fraction was repeatedly chromatographed on column and the collected fraction was checked on TLC until it gave a single spot of bright red color (100 mg) (Rf value : 0.89). The isolated compound was subject for spectral analysis and the compound was identified as 10-(carboxoxy)-1,2,2,6a,9,9, hexamethyldocasahydro-

picene-4a-carboxylic acid which showed m.p at 310 °C, \( \lambda_{max} \) 254 nm, the IR (KBr) \( \nu_{max} \) cm\(^{-1} \) 3468.32 (OH) stretching, 2922-2808 (C-H) stretching, 1709 (C=O), 1612 (COOH), 1213 (C-O-C); \( ^1 \)H-NMR. (DMSO) suggesting the structural similarities with Madhucic acid which was identified and confirmed by LCMS,IR, \( ^1 \)H-NMR.

Animals—Swiss albino male mice weighing 25-30 g were maintained under standard environmental conditions and had access to standard diet and water ad libitum. Animal were housed in polythene cages with 12:12 h light and dark cycle. All experiments were performed after an overnight fast and confirmed to acceptable protocols for use of animals in experiment and it was approved by Institutional Animal Ethical Committee, Luqman College of Pharmacy, Gulbarga, Karnataka, India.

Drugs and chemicals—Tramadol hydrochloride (Piramal Health Care Ltd Mumbai India), phenobarbitone sodium (Dhar, Madhya Pradesh), diazepam (Runbaxy, Himachal Pradesh), chlorpromazine (Sun Pharmaceuticals Ltd., India) silica gel C (Glaxosmithkline pharmaceuticals Ltd., Mumbai). Other chemicals used were of analytical grade.

Acute toxicity studies—In vivo toxicity was carried out according to the method of Trease and Evans\(^8\). Albino male mice were divided into groups consisting of six animals each. Graded doses of \( M. \) longifolia methanolic extract (MLME) and derivative of madhucic acid (dMA) (dissolved in water) were administered orally. One group serving as control was treated with normal saline. The animals were monitored for 24 h after drug administration for gross behavioural changes and mortality. Dose at 50% mortality in a group was observed and considered as lethal dose (LD\(_{50}\)). Based on the results of preliminary toxicity studies, the doses for the further studies were fixed at 10 mg/kg for \( M. \) longifolia methanolic extract and 2 mg/kg for derivative of madhucic acid.

Phenobarbitone induced sleeping time—Sleeping time is the time interval between losing and regaining of righting reflex\(^7\).

The animals were divided into following 4 groups: Gr. I animals served as control, Gr. II animals were administered with phenobarbitone, Gr. III and Gr. IV animals received \( M. \) longifolia methanolic extract (MLME; 10 mg/kg) and derivative of madhucic acid (dMA; 2 mg/kg) respectively. All the treatments were done 30 min prior to ip administration of phenobarbitone. The onset of sleep and duration of sleeping time for each animal was determined.

Spontaneous motor activity—Spontaneous motor activity was performed using actophotometer (Inco, Ambala, India). The animals were divided into following 4 groups: Gr. I animals served as control, Gr. II animals were administered with chlorpromazine (13 mg/kg, ip) — a CNS depressant standard drug, Gr. III and Gr. IV animals received \( M. \) longifolia methanolic extract (MLME; 10 mg/kg) and derivative of madhucic acid (dMA; 2 mg/kg) respectively. Each animal was kept for 10 min in the cage and the readings were taken at 0, 1, 2 and 4 h of administration of the dose\(^8\).

Marble burying test—The animals were divided into following 4 groups. Gr. I animals served as control, Gr. II animals were administered with diazepam (10 mg/kg), Gr. III and Gr. IV animals received \( M. \) longifolia methanolic extract (MLME; 10 mg/kg) and derivative of madhucic acid (dMA; 2 mg/kg), respectively. Twenty glass marbles (20 mm diameter) were used for each individual test; opaque cages (23 ×14 × 7 cm) of smooth plastic with vinyl ceiling and 5 cm layer of saw dust were constructed. Animals were placed individually in these cages for 15 min (habituation cage) and then returned to their home cage. Twenty glass marbles were evenly spaced 5 cm apart on a 5 cm layer of saw dust in the habituation cages. Animals were then reintroduced. After 15 min, the test was terminated by removing the
animals and counting the number of marbles that were more than 2/3\textsuperscript{rd} covered with saw dust. After each trait sawdust was replaced, and test apparatus and glass marbles were washed with water and cleaned with 70% ethanol\textsuperscript{9}.

Antinociceptive activity—Antinociceptive activity was evaluated using Eddy’s hot plate instrument (Inco-Medcraft Analgesiometer). The animals were individually placed on Eddy’s hot plate maintained at constant temperature (55±0.5 °C) and reaction of animals such as paw licking or jump response to the pain stimulus was considered as the end point. Tramadol (10 mg/kg, ip) was used as the standard drug. Reaction times were determined at 0, 1, 2 and 4 h of administration of the dose; 15 seconds was considered as cut off time for the reaction\textsuperscript{10}.

Statistical analysis—The data are presented as mean ± SE. The level of statistical significance was determined by analysis of variance (ANOVA) followed by Dunnet’s t-test and the results were regarded as significant at $P < 0.05$.

Results

Acute toxicity studies—Preliminary phytochemical screening of the MLME showed the presence of saponin, triterpene, flavonoids, phenols and alkaloids. Toxicity symptoms such as changes in gross animal behaviour were visible with dose level of 50 mg/kg of \textit{M. longifolia} methanolic extract (MLME) and 5 mg/kg of derivative of madhucic acid (dMA) within 8 h of dose administration. The LD\textsubscript{50} for MLME and dMA were found to be 100 and 10 mg/kg body weight for respectively.

Phenobarbitone induced sleeping time—Effect of MLME and dMA on phenobarbitone induced sleeping time in mice are presented in Table 1. Both the test samples exhibited greater increase in phenobarbitone induced sleeping time. These results can be compared with the animals administered with phenobarbitone.

Spontaneous motor activity—Both MLME and dMA exhibited significant reduction in spontaneous motor activity in the experimental animals (Table 2). The effect was significant from 30 min which persisted up to 2 h and gradually decreased up to 4th h of drug administration.

Marble burying test—The CNS depressant effect of MLME and dMA was further studied by marble burying test in which the experimental animals showed a reduction in the marble burying reflex due to sedation and hypnosis (Table 3).

Antinociceptive activity—The effect of MLME and dMA on response of animals to the thermal pain stimulus was investigated using Eddy’s hot plate method. Both MLME and dMA exhibited considerable antinociceptive activity in experimental animals (Table 4).

Discussion

The present study reports CNS depressant effect of MLME and dMA in mice\textsuperscript{11}. The diverse array of

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (dose, mg/kg)</th>
<th>Onset of time (min)</th>
<th>Duration of time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>228.3±1.4</td>
<td>14.3±2.6</td>
</tr>
<tr>
<td>II</td>
<td>Phenobarbitone (20)</td>
<td>14.0±0.5</td>
<td>113.0±2.6</td>
</tr>
<tr>
<td>III</td>
<td>MLME (10)</td>
<td>28±2.3**</td>
<td>273±2.6**</td>
</tr>
<tr>
<td>IV</td>
<td>dMA (2)</td>
<td>19.6±1.2**</td>
<td>340.6±3.8**</td>
</tr>
</tbody>
</table>

MLME: \textit{M. longifolia} methanolic extract (10 mg/kg); dMA (2 mg/kg). $P$ values: *<0.05; **<0.01
terpenoid structures and functions provoked increased interest in their commercial use. Terpenoids have been found to be useful in the prevention and therapy of several disease including cancer and also antimicrobial, antifungal, antiparasitic, antiallergenic, antiviral, antihyperglycemic, anti-inflammatory and antimicrobial, antifungal, antiparasitic, antiallergenic, of several disease including cancer and also determined whether triterpene alone were responsible for the activity 

Earlier studies have related prolongation of barbital hypnosis to phenobarbitone metabolic inhibition or action on CNS involved in regulation of sleep. The MLME and dMA significantly reduced spontaneous motor activity. The activity is a measure of level of excitability of the CNS and this decrease may be closely related to sedation resulting from depression of central nervous system. Marble-burying behaviour of mice is considered as the marker index of compulsive behaviour, which is characteristically evident in Obsessive-compulsive disorder (OCD). Marble-burying behaviour is an unconditioned species specific defensive reaction in rodents, which is not associated with physical danger, and does not habituate upon repeated testing. The burying behaviour in male mice models shows a compulsive behaviour rather than anxiety. The effect of MLME and dMA on response to the thermal pain stimulus was investigated. Both MLME and dMA exhibited considerable antinociceptive activity in experimental animals. The effect of both the test samples persisted up to 1 h of the drug administration. However, the results were not significant when compared with tramadol. The ability of the test samples to prolong the reaction latency to the pain thermally induced in mice by the hot plate, further suggest central analgesic activity.

It’s generally accepted that the sedative effect of drugs can be evaluated by measurement of spontaneous motor activity; marble burying and phenobarbitone induce sleeping time in laboratory animal models. These results corroborate that the enhancement of barbital hypnosis is a good index of CNS depressant activity.

**Conclusion**

The present result demonstrates the potential effectiveness of methanolic extract and dMA from M. longifolia leaves has potential depressant effect that is associated with drowsiness and sedation.

**Acknowledgement**

Thanks are due to Dr Syed Sanaullah, Luqman College of Pharmacy, Gulbarga for permission to do the work in the College.

**References**

3. Dilip Kumar, Santanu S & Majumdar Upal Kanti, Analgesic and anticonvulsant effect of saponin isolated from the leaves


