Pharmacognosy Communications
Volume 1 | Issue 2 | Oct-Dec 2011
www.phcogcommn.org

Research Article

Investigation into the Mechanism of Action of *Madhuca longifolia* for its Anti-epileptic Activity

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ABSTRACT: **Background:** Heart wood of *Madhuca longifolia* J.F. Macbr. (Sapotaceae) is used in traditional medicine of India to treat seizure. **Objectives:** The heart wood extract of *Madhuca longifolia* was investigated for anticonvulsant activity and the possible mechanism of action involved in this activity. **Materials and methods:** The anticonvulsant activity of the methanol extract of heart wood of *Madhuca longifolia* was assessed in pentylenetetrazole (PTZ) - induced convulsion in mice with benzodiazepine as standard drug. Mechanistic studies were conducted using both flumazenil, a $GABA_A$-benzodiazepine receptor complex site antagonist, and naloxone a non-specific opioid receptor antagonist. **Results:** *Madhuca longifolia* at the dose of 400 mg/kg prolonged the onset time of seizure and decreased the duration of seizures compared to saline group ($p < 0.001$). Flumazenil and naloxone suppressed anticonvulsant effects of *Madhuca longifolia*. **Discussion and conclusion:** It appears that *Madhuca longifolia* may be useful for the treatment of absence seizures and that these effects may be related to its effect on GABAergic and opioid systems. This result suggests that *Madhuca longifolia* possesses biological active constituents which may contribute to the anti-convulsant activity of *Madhuca longifolia*. This supports the ethnomedical claims of the use of plant in the management of epilepsy.

KEYWORDS: Pentylenetetrazole, Flumazenil, Naloxone

INTRODUCTION

Epilepsy is a chronic disorder characterized by paroxysmal brain dysfunction, and usually associated with some alteration of consciousness. It is one of the most common chronic diseases with approximately 0.5% of all human beings suffering from epilepsy.[1] Each year, about 125,000 new epilepsy cases are diagnosed; of these, 30% are in people younger than the age of 18 at the time of diagnosis. Seizures occur because small numbers of neurons discharge abnormally.[2] The causes of seizures in the elderly may be multifactorial and include cerebrovascular disease (both ischemic and hemorrhagic stroke), neurodegenerative disorders, tumor, head trauma, metabolic disorders, and CNS infections. The agitated neuronal activity that occurs during a seizure is caused by a sudden imbalance between the inhibitory and excitatory neurotransmitters in the brain with γ-aminobutyric acid (GABA) and glutamate, respectively and opioid receptor function being the most important factors involved.[3]

Many patients with epilepsy fail to experience adequate control of their seizures, despite the concurrent use of two or more antiepileptic drugs and because of increasing side effects of available synthetic drugs for epilepsy, there is need to focus on the scientific exploration of herbal drugs having fewer side effects. *Madhuca longifolia* J.F. Macbr. (Sapotaceae), commonly known as mahwa or mahua, is an Indian tropical tree found largely in the central and north Indian plains and forests. It is a fast growing tree that reaches to approximately 20 m in height, possesses evergreen or semi-evergreen foliage. The plant parts are used in ethnomedicine for wide variety of illnesses, such as epilepsy,[4] inflammation, diabetes mellitus, analgesic, anthelmintic, pneumonia, piles and skin diseases.[5]

Anti-inflammatory activity,[6] antiulcer activity,[7] and analgesic activity[8] of *Madhuca longifolia* have also been reported. The

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DOI: 10.5530/pc.2011.2.4

fruit pulp yields a number of triterpenoids (including α- and β-amyrin acetate); also *n*-hexacosanol, β-D-glucoside of β-sitosterol and free sitosterol. The nut shell contains β-sitosterol glucoside, quercetin and dihydroquercetin. The carollas are rich source of sugars, vitamins, minerals. The seeds yielded saponins 2,3-*di*-*O*-glucopyranoside of bassic acid (saponin A and saponin B). Trunk bark contained lupeolacetate, α-amyrin acetate, α-spinasterol, erythrodiol monocaprylate, betulinic acid and oleanolic acid caprylates.[5]

In this study, we examined anticonvulsant effects of a methanol extract of *Madhuca longifolia* using pentylenetetrazole (PTZ) induced seizure as *petit mal* epilepsy model in mice. Furthermore, we partially elucidated the possible mechanisms underlying the actions of *Madhuca longifolia* on the CNS and assessed the probable involvement of GABAergic and opioid system.

MATERIALS AND METHODS

Chemicals

Drugs used as follows: PTZ (National Chemicals Limited, India), flumazenil ampoule (Roche), diazepam ampoule (Ind-Swift Limited, India), naloxone ampoule (Troikaa Pharmaceuticals Limited, India). PTZ was dissolved in normal saline.

Preparation of extract

The heartwood of *Madhuca longifolia* was collected from Anand Agriculture University (Anand, Gujarat, India) in the month of September and authenticated by Dr A. S. Reddy, Department of Biosciences, Sardar Patel University, and Gujarat. Shade dried heart wood was powdered and extracted by Soxhlet-apparatus with methanol. The heart wood extract was filtered and dried at room temperature (yield 5.7% w/w) and the residue was stored at 4°C.

Phytochemical screening

Phytochemical analysis revealed the presence of flavonoids, tannins, carbohydrates and saponins in the methanol extract of heart wood of *Madhuca longifolia*.[9] The total concentration of polyphenolics and flavonoids was determined spectrophotometrically by using standard methods.[10,11]

Animals

Female ICR Mice (25-30 g) were obtained from the Zydus Research Center (Ahmedabad) and were housed in polypropylene cages and maintained under ambient room temperature. They were fed with a standard pellet diet (Pranav Agro Ltd. Pune, India) and water *ad libitum*. The Institution Animal Ethics Committee (CPCSEA/IAEC/ARCP/2009-10/05) approved the study.

PTZ -induced seizure

The mice were divided into groups of ten animals each. In the two groups, the mice were given methanol extract of *Madhuca longifolia* at the different doses (200, 400 mg/kg p.o.) 30 min before the administration of PTZ (80 mg/kg i.p). One group of mice were injected with diazepam (1 mg/kg i.p.) whilst the other group were injected normal saline 30 min before the administration of PTZ (80 mg/kg i.p.). Each animal was placed into an individual plastic cage for observation lasting 1 h. The onset of a general clonus was used as the endpoint. The general clonus was characterized by forelimb clonus followed by full clonus of the body. The time taken before the onset of clonic convulsions, the duration of clonic convulsions, and the percentage of seizure and mortality protection were recorded.

Effect of flumazenil on the anticonvulsant activity of Madhuca longifolia

In order to investigate the probable involvement of benzodiazepine receptor, we studied the effects of a selective benzodiazepine receptor antagonist, flumazenil, on the anticonvulsant activity of *Madhuca longifolia*. Six groups of ten mice each were selected randomly for the study. The first group of mice were initially given flumazenil (2 mg/kg, i.p.) follow by *Madhuca longifolia* (400 mg/kg p.o.) extract with a 5 min interval between treatments. After a further 30 min the injection of PTZ were given. In the second group of animals flumazenil (2 mg/kg, i.p) was given 5 min before the administration of diazepam (1 mg/kg, i.p.). Mice in the 3rd, 4th, 5th and 6th groups received diazepam (1 mg/kg i.p.), flumazenil (2 mg/kg i.p.), normal saline and *Madhuca longifolia* (400 mg/kg p.o.) extract 30 min before the administration of PTZ (80 mg/kg i.p.) respectively. The anticonvulsant activity of *Madhuca longifolia* and diazepam in mice pretreated with flumazenil were assessed and compared with normal saline (10 ml/kg p.o.), flumazenil (2 mg/kg i.p.), diazepam (1 mg/kg i.p.) and *Madhuca longifolia* (400 mg/kg p.o.) treated animals.

Effect of Naloxone on the anticonvulsant activity of Madhuca longifolia

Four groups of ten mice each were selected for further investigation of the probable modulatory activities of opioid receptors on the anticonvulsant activity of *Madhuca longifolia*. Naloxone (an opioid receptor antagonist) was administered at a dose of 5 mg/kg i.p., followed by Madhuca longifolia (400 mg/kg p.o.) extract with 5 min interval. After a 30 min interval, the injection of PTZ (80 mg/kg i.p.) was given. The anticonvulsant activity of *Madhuca longifolia* in groups pretreated with Naloxone were assessed and compared with animals pretreated only with *Madhuca longifolia* (400 mg/kg p.o.) , Naloxone (5 mg/kg i.p.) and normal saline (10 ml/kg i.p.) groups.

Statistical analysis

The data were expressed as mean values ± S.E.M. and tested with one-way ANOVA followed by the multiple comparison test of Tukey-Kramer.

RESULTS

Estimation of total polyphenolics and flavonoids

The level of total polyphenolic compounds was found to be 52.08 ± 2.3 mg of gallic acid equivalents per gram of ethanolic extract of *Madhuca longofolia* heart wood. The total flavonoids content was found to be 27.5 ± 1.2 mg of quercetin equivalents per gram of extract.

PTZ-induced seizure

Madhuca longifolia extract at a dose of 400 mg/kg prolonged the onset time of seizure and decreased the duration of seizures compared to saline group ($p < 0.001$) (Table 1). *Madhuca longifolia* at the dose of 200 mg/kg also prolonged the onset time of seizure and decreased the duration of seizures, compared to saline group ($p < 0.001$), but was less effective than the 400 mg/kg dose. As it is shown in Table 1, *Madhuca longifolia* exhibited its protection against seizure in a dose-dependent manner. Furthermore, diazepam also was shown to prolong the latency and shortened the duration of seizures compared to saline group (Table 1).

The effect of flumazenil on the anticonvulsant activity of Madhuca longifolia

In the PTZ-induced seizure model, the administration of flumazenil (2 mg/kg) 5 min before *Madhuca longifolia* (400 mg/kg) reversed the effect of *Madhuca longifolia* in prolonging seizure latency and reducing the duration of clonic seizures. There was significant difference between the latency and duration of seizure in mice which received *Madhuca longifolia* (400 mg/kg, p.o.) pretreated with Flumazenil and the saline group. Furthermore, flumazenil could also reverse the anticonvulsant activity of diazepam (Table 2).

The effects of Naloxone on the Anticonvulsant activity of Madhuca longifolia

In the PTZ-induced seizure model, the administration of Naloxone (5 mg/kg) 5 min before *Madhuca longifolia* (400 mg/kg) reversed the effect of *Madhuca longifolia* in prolonging seizure latency and reducing the duration of clonic seizures. There was significant difference between the latency and duration of seizure in mice which received *Madhuca longifolia* (400 mg/kg, p.o.) pretreated with Naloxone and the control group (Table 3).

Table 1: Effect of *Madhuca longifolia* extract on pentylenetetrazole induced convulsion in mice

Treatment (Dose)	Onset of Convulsion (second)	Duration for Convulsion (second)	Seizure Protection (%)	Mortality (%)
Normal Saline(10 ml/kg)	30.00 ± 2.082	300.00 ± 14.271	0	66.66
Diazepam (0.5 mg/kg)	127.25 ± 8.662***	102.33 ± 9.969***	33.33	0
Diazepam (1 mg/kg)	251.50 ± 11.026***	80.66 ± 8.417*	33.33	0
Madhuca longifolia Extract (200 mg/kg)	74.66 ± 3.252***	130.00 ± 0.899***	0	33.33
Madhuca longifolia Extract (400 mg/kg)	92.00 ± 6.126***	111.83 ± 5.437***	0	33.33

Normal Saline (i.p), Diazepam (i.p) and *Madhuca longifolia* (p.o.) were administered 30 min before the injection of pentylenetetrazole.
Values are the mean of ± S.E.M for 10 mice.
*$p < 0.05$ compared to saline group
***$p < 0.001$ compared to saline group

Table: 2 Effect of Flumazenil on the Anti-Convulsant activity of Madhuca longifolia extract in Pentylenetetrazole induce Convulsion in Mice

Treatment (Dose)	Onset of Convulsion (second)	Duration for Convulsion (second)	Mortality (%)
Normal Saline (10 ml/kg)	30.00 ± 2.082	300.00 ± 14.271	66.66
Flumazenil(2 mg/kg)	41.67 ± 1.922	195.16 ± 3.103***	100
Diazepam (1 mg/kg)	251.50 ± 11.026***	80.66 ± 8.417*	0
Diazepam + Flumazenil	151.83 ± 5.102	115.33 ± 3.363	0
Madhuca longifolia Extract (400 mg/kg)	92.00 ± 6.126***	111.83 ± 5.437***	33.33
Madhuca longifolia Extract + Flumazenil	74.16 ± 3.544#	136.33 ± 4.052#	33.33

Normal Saline (i.p), Diazepam (i.p), Flumazenil (i.p) and *Madhuca longifolia* (p.o.) were administered 30 min before the injection of Pentylenetetrazole.
Values are the mean of ± S.E.M for 10 mice.
*$p < 0.05$ compared to saline group
***$p < 0.001$ compared to saline group
#$p < 0.05$ compared with *Madhuca longifolia* extract (400 mg/kg)

Table 3: Effect of Naloxone on the Anti-convulsant activity of *Madhuca longifolia* extract in Pentylenetetrazole induced convulsion in Mice

Treatment (Dose)	Onset of Convulsion (second)	Duration for Convulsion (second)	Mortality (%)
Normal Saline (10 ml/kg)	30.00 ± 2.082	300.00 ± 14.271	66.66
Naloxone (5 mg/kg)	47.75 ± 1.922***	246.50 ± 5.096*	100
Madhuca longifolia Extract(400 mg/kg)	92.00 ± 6.126***	111.83 ± 5.437***	33.33
Madhuca longifolia Extract+ Naloxone	72.50 ± 2.456#	166.50 ± 4.916#	33.33

Normal Saline (i.p), Naloxone (i.p) and *Madhuca longifolia* (p.o.) were administered 30 min before the injection of Pentylenetetrazole.
Values are the mean of ± S.E.M for 10 mice.
*p < 0.05 compared to saline group
***p < 0.001 compared to saline group
#p < 0.05 compared with *Madhuca longifolia* extract (400 mg/kg)

DISCUSSION

Within the context of present study, anticonvulsant activity of the methanol extract of *Madhuca longifolia* was investigated by using PTZ induced convulsion in order to assess the validity of the use of this plant. We further investigated the probable mechanism(s) of action for its anticonvulsant effects. The present study investigated the anticonvulsant effect of *Madhuca longifolia* extract using the PTZ-model. Single dose, intraperitoneally administration of PTZ (80 mg/kg) caused clonic convulsions as well as lethality in mice. Pretreatment of the mice with the methanol extract of *Madhuca longifolia* caused a dose-dependent protection against PTZ-induced convulsions. *Madhuca longifolia* extract could suppress onset and duration of clonic seizure in PTZ model and it seems that this effect increased dose dependently. Also, the seizure and mortality protection percentage increased dose dependently. We observed that at the 400 mg/kg dose, all animals displayed significantly suppressed onset and duration of clonic seizure. Diazepam, a standard antiepileptic drug, has been shown to exert their antiepileptic effects by enhancing GABA-mediated inhibition in the brain.[12] It is possible that both phenobarbitone and diazepam antagonize PTZ convulsion by enhancing GABA neurotransmission. It is also possible that Phenytoin, a standard antiepileptic drug, did not alter PTZ convulsion because it is thought to exert its antiepileptic effect by blocking sodium ion channels and preventing the influx of sodium ions into brain cells, thus inhibiting the generation of a repetitive action potential. Since the methanol extract of *Madhuca longifolia* delayed the occurrence of PTZ convulsion, it is probable that it may interfer with GABAnergic mechanism(s) to exert its anticonvulsant effect.

The majority of currently available antiepileptic drugs (AEDs) fall into one of two main pharmacological classes, those that modulate neuronal voltage-gated sodium channels (e.g. Carbamazepine, Phenytoin, Lamotrigine, and Topiramate) and those that modulate inhibitory GABAnergic neurotransmission (e.g. benzodiazepine, Vigabatrin and Tiagabine). A small number of AEDs such as Ethosuximide, Gabapentin and possibly Levetiracetam, may exert their effects via an interaction with voltage-operated calcium channels.[13] Regarding the possible contribution of the GABAnergic system in the anticonvulsant activity of *Madhuca longifolia*, flumazenil (a benzodiazepine receptor antagonist) was used. In our study, flumazenil decreased the prolongation of seizure latency induced by *Madhuca longifolia* and it also antagonized the effect of *Madhuca longifolia* extract on decreasing the duration of clonic seizures in the PTZ model. It is noticeable that the anticonvulsant effect of *Madhuca longifolia* extract is blocked by an antagonist of benzodiazepine receptor. So this effect of *Madhuca longifolia* extract seems to be related to benzodiazepine receptor activation. Many flavonoids were found to be ligands for the γ aminobutyric acid type A ($GABA_A$) receptors in the central nervous system (CNS); which led to the hypothesis that they act as benzodiazepine-like molecules.[12] This is supported by their behavioral effects in animal models of anxiety, sedation and convulsion. The quantitative estimation of total polyphenolics and flavonoids confirmed that the methanol extract contains substantial quantity of polyphenolics and flavonoids which could be contributed to the anticonvulsant activity. However, further investigations are needed to make clear which of these flavonoids or other compounds have anticonvulsant effects.

Evidence supports the view that endogenous opioid peptides are activated as a result of seizure activity and they are involved in ictal, postictal and interictal activity.[14-,17] At present, the effects of opioid receptors on seizure activity are controversial. Several reports indicate that mu receptors, which interact with Go, Gi[18] and Gs proteins,[19] induce anticonvulsant effects.[20] Anticonvulsant activity of kappa opioid receptor (KOPr) agonists has been established in wide range of previous animal studies. KOPr agonists are effective against bicuculline-, maximal electroshock- and excitatory amino acid-induced convulsions. Furthermore, they attenuate the kindling of seizures produced by repeated

administration of PTZ.[21] In contrast, other experimental evidence suggests that activation of mu opioid receptors produces proconvulsant effects.[22] Further studies support dual effects of mu receptors (i.e., they facilitate the epileptogenesis process, but increase refractoriness to subsequent seizures during the postictal period).

We also observed other mechanistic details about the anticonvulsant effects of *Madhuca longifolia* extract. It was revealed from our study that Naloxone antagonized the effect of *Madhuca longifolia* extract on decreasing the duration of clonic seizures in the PTZ model compared to saline group. Naloxone decreased the prolongation of seizure latency induced by *Madhuca longifolia* extract. Thereby, it showed significant reversal of anticonvulsant effect of *Madhuca longifolia*. It seems that some part of anticonvulsant effects of *Madhuca longifolia* extract related to activation of opioid system which was attenuated by Naloxone.

From the above explanation, we may assume that *Madhuca longifolia* extract could activate KOPr and produce protective effects against PTZ- induced seizure. However, the mechanism of anticonvulsant effects with KOPr agonist, have not been globally accepted. Protective effects of KOPr agonist on seizure induced by $GABA_A$ receptor antagonists have been reported.[23] Furthermore, its agonist could inhibit glutamate release.[24] Thus, there are two possibilities which could explain the anticonvulsant activity of the *Madhuca longifolia* extract via the KOPr activation: 1) Enhancement GABAnergic activity and/or 2) Supression of glutamatergic activity.

The results of the study have demonstrated that the *Madhuca longifolia* extract possessed anticonvulsant activity on the animal models investigated and this provides a rationale for its use in traditional medicine for the management of epilepsy. Further work to establish the active chemical constituent(s) of the extract and the exact mechanism of action is required.

ACKNOWLEDGEMENT

The authors are grateful to SICART, Vallabh vidyanagar, Anand and Principal A.R. College of Pharmacy, New Vallabh Vidyanagar, Anand for providing necessary facilities.

CONFLICT OF INTEREST

The Author(s) declare(s) that they have no conflicts of interest to disclose.

REFERENCES

1. Kasper DL, Fauci AS, Longo DL, Braunwald E, Hauser SL, Jameson JL. Harrison's Principle of Internal Medicine. New York: McGraw-Hill; 2005.
2. Dipiro JT, Talbert RT, Yee GC, Matzke GR, Wells BG, Posey LM. Pharmacotherapy- A pathophysiologic Approach. New York: McGraw-Hill; 2002.
3. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. New Delhi: Reed Elsevier India Pvt Ltd; 2006.
4. Warrier PK, Nambiar VPK, Ramankutty C. Indian Medicinal Plants: A Compendium Of 500 Species. Chennai: Orient longman Ltd; 1995.
5. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. New York: Springer Science; 2007.
6. Gaikwad RD, Ahmed LM, Khalid MS, Swamy P. Anti-inflammatory activity of *Madhuca longifolia* seed saponin mixture. Pharm Biol 2009; 47(7):592-97.
7. Roy SP, Shirode D, Patel T, Prabhu K, Shetty SR, Rajendra SV. Antiulcer activity of 70% ethanolic extract of Bark of *Madhuca longifolia*. Ind J Nat Prod 2008; 24(4):8.
8. Chandra D. Analgesic activity of aqueous and alcoholic extract of Madhuca longifolia. Ind J Pharmacol, 2001; 33:108-11.
9. Trease EG, Evans WC. Textbook of pharmacognosy. Singapore: Alden Press; 1983.
10. Singleton VL, Orthofer, RM, Ramuela-Raventos RM. Analysis of total phenols and antioxidants and other substrates by means of Folin–Ciocalteu reagent. Methods Enzymol 1999; 299:152-78.
11. Hsu C. Antioxidant activity of extract from *Polygonum aviculare* L. Biol Res 2006; 39:281-8.
12. Przewłockaa B, Lasońa W, Turchana J, Bruinb N, Luijtelaarb GV, Przewłockia R, Coenenb A. Anatomical and functional aspects of *μ* opioid receptors in epileptic WAG/Rij rats. Epilepsy Res, 1999; 29(2):167-3.
13. Porter RJ, Meldrum BS. Basic and Clinical Pharmacology. New York: Lange Medical Books/McGraw-Hill; 2001.
14. Joshi D, Naidu PS, Singh A, Kulkarni SK. Protective effect of quercetin on alcohol abstinence-induced anxiety and convulsions. J Med Food 2005; 8:392-96.
15. Caldecott-Hazard S, Engel J. Limbic postictal events: Anatomical substrates and opioid receptor involvement. Neuropsychopharmacology 1987; 11:389-418.
16. Rocha L, Engel J, Ackermann RF. Effects of chronic Naloxone pretreatment on amygdaloidal kindling in rats. Epilepsy Res 1991; 10:103-10.
17. Rocha L, Maidment NT, Evans CJ, Ackermann RF, Engel J. Microdialysis reveals changes in extracellular opioid peptide levels in the amygdala induced by amygdaloidal kindling stimulation. Exp Neurol 1994; 126:277-83.
18. Wickenden AD. Potassium channels as antiepileptic drug targets. Neuropharmacology 2002; 43:1055-0.
19. Vindrol O, Briones R, Asai M, Fernández-Guardiola A. (1981). Amygdaloid kindling enhances the enkephalins content in the rat brain. Neuroscience 1981; 21:39-3.
20. Chan JS, Chiu TT, Wong YH. Activation of type II adenylylcyclase by the cloned mu-opioid receptor: Coupling to multiple G proteins. J Neurochem 1995; 65:2682-9.
21. Chakrabarti S, Regec A, Gintzler AR. Biochemical demonstration of mu-opioid receptor association with Gs alpha: Enhancement following morphine exposure. Mol Brain Res 2005; 135, 217-4.
22. Yajima Y, Narita M, Takahashi-Nakano Y, Misawa M, Nagase H, Mizoguchi H, Tseng LF, Suzuki T. Effects of differential modulation of μ-, δ- and κ-opioid systems on bicuculline-induced convulsions in the mouse. Mol Brain Res 2000; 862:120-6.
23. Beckert A, Braun H, Schroder H, Grecksch G, Hollt V. Effects of enadoline on the development of pentylenetetrazole kindling, learning performance, and hippocampal morphology. Mol Brain Res, 1999; 823:191-7.
24. Bohme GA, Stutzmann JM, Roques BP, Blanchard JC. Effects of selective mu and delta-opioid peptides on kindled amygdaloid seizures in rats. Neuroscience 1987; 74:227-1.