

ANTINOCICEPTIVE AND ANTIDIARRHOEAL ACTIVITIES OF  
*MADHUCA INDICA* J. F. GMEL.

Md. Atiqur Rahman<sup>1\*</sup>, Md. Ekramul Haque<sup>1</sup>, Md. Solaiman<sup>1</sup>, Md. Saifuzzaman<sup>1</sup>

<sup>1</sup>Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh.

\* **Corresponding Author:** Md. Atiqur Rahman

Present Address: Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh.

Phone: +8801717584231. E-mail: satikrahman@gmail.com

### Summary

The ethanol extract of the dried bark of *Madhuca indica* J. F. Gmel. (Family - Sapotaceae) was investigated for its possible antinociceptive and antidiarrhoeal activities in animal models. The extract produced significant ( $P<0.001$ ) writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 250 and 500 mg/kg of body weight comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight. The extract also showed antidiarrhoeal activity on castor oil induced diarrhoea in mice, it increased mean latent period and decreased the frequency of defecation significantly ( $P<0.001$ ,  $P<0.01$ ) at the oral dose of 500 mg/kg body weight comparable to the standard drug Loperamide at the dose of 50 mg/kg of body weight. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

**Key Words:** antinociceptive activity, antidiarrhoeal activity, *Madhuca indica* J. F. Gmel.

### Introduction

*Madhuca indica* J. F. Gmel. (English Name: Indian Butter Tree, Family: Sapotaceae, Synonym: *Madhuca longifolia* (J.Konig) J.F.Macbr.) locally known as 'Mahua or Maul' in Bangladesh. It is also known as Mahua (Hindi), Madhuka (Sanskrit), Mahwa (Marathi), Illuppai(Tamil), Yappa (Telugu). It is a large, shady deciduous tree both wild and cultivated, found in different parts of Bangladesh. It is also distributed more or less throughout India especially in the states of Jharkhand, Uttar Pradesh, Bihar, Madhya Pradesh, Kerala, Gujarat and Orissa<sup>1-2</sup>.

*Madhuca indica* J. F. Gmel. is mainly valued for its seeds oil and flowers which are utilized for alcoholic beverage production. Mahua seeds are a good source of edible oil<sup>3</sup>. Distilled juice of its flower is considered a tonic, both nutritional and cooling and also in treatment of helminthes, acute and chronic tonsillitis, pharyngitis<sup>4</sup> as well as bronchitis<sup>5</sup>. Its leaves are applied as a poultice to relieve eczema. The medicinal properties attributed to this plant are stimulant, demulcent, emollient, heating and astringent. The bark is good remedy for itch, swelling, fracture and snake bite poisoning, internally employed in diabetes mellitus. Its bark is used to cure leprosy and wounds. Its flowers are prepared to relieve coughs, biliousness and heart-trouble while its fruits are given in cases of consumption and blood diseases<sup>2</sup>.

From the existing information it is evident that the plant may possess some important biological activities. The main objective of this study was to evaluate the antinociceptive and antidiarrhoeal activities of the ethanol extract of dried bark of *Madhuca indica* J. F. Gmel.

### Materials and Methods

#### Plant Material

Barks of *Madhuca indica* J. F. Gmel. were collected from Jessore, Bangladesh in February 2008 and were authenticated by the experts at National Herbarium (Accession Number: 32529). After collection, barks were pieced into small size by hand with the help of a sharp knife. The small pieces of bark were then sun dried for several days to remove moisture. After drying, the dried pieces of bark were ground into coarse powder by 'Hammer' mill. About 400 gm of powdered bark was taken in a clean, flat-bottomed glass container and soaked in 1,300 ml of 80% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture was then underwent a coarse filtration by a piece of cotton followed by a filtration through Whatmann filter paper and the filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the crude extract. And this crude ethanolic extract was used for all phytochemical and pharmacological screening.

#### Animals

For antinociceptive and antidiarrhoeal activity study, young Swiss-albino mice of either sex, weighing 20 - 25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B), were used. After purchase, the animals were kept at animal house of Pharmacy Discipline, Khulna University, for adaptation under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0±2.0°C and 12h light-dark cycle) and fed with standard diets and had free access to tap water. The experimental met the national guidelines on the proper care and use of animals. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol. All the experiments were conducted on an isolated and noiseless condition.

#### Drugs

Diclofenac sodium (Opsonin Chemical Industries Ltd, Bangladesh), Loperamide (Square Pharmaceuticals Ltd., Bangladesh).

#### Preliminary Phytochemical Analysis

The ethanol extract of bark of *Madhuca indica* J. F. Gmel. was subjected to a preliminary phytochemical screening for major chemical groups. In each test, 10% (w/v) solution of the extract in ethanol was used unless otherwise specified in individual test<sup>1,6</sup>.

#### Tests for Reducing Sugar

**Benedict's Test:** 0.5 ml of the extract was placed in a test tube and then 5 ml Benedict's solution was added to it, boiled for 5 min and allowed to cool spontaneously.

**Fehling's Test (Standard Test):** 2 ml of the extract was added in 1 ml of a mixture of equal volumes of Fehling's solutions A and B, and was boiled for few min.

**Tests for Tannins**

Ferric Chloride Test: 5 ml of the extract was placed in a test tube and then 1 ml of 5% Ferric chloride solution was added to it.

Potassium dichromate test: 5 ml of the extract was placed in a test tube and then 1 ml of 10% potassium dichromate solution was added.

**Test for Flavonoids**

A few drops of concentrated hydrochloric acid were added to 5 ml of the extract.

**Test for Saponins**

1 ml of the extract was placed in a graduated cylinder and was diluted to 20 ml with distilled water and shaken gently for 15 min.

**Test for Gums**

5 ml of the extract was placed in a test tube and then Molish's reagent and sulphuric acid were added to it.

**Tests for Steroids**

Liebermann-Burchard test: 1 ml of the extract was placed in a test tube and then 2 ml Liebermann-Burchard reagent was added to it.

Sulphuric acid test: 1 ml of the extract was placed in a test tube and 1 ml sulphuric acid was added to it.

**Tests for Alkaloids**

Mayer's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube and 1ml of Mayer's reagent was added to it.

Dragendroff's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube and then 1 ml Dragendroff's reagent was added.

Wagner's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of iodine solution (Wagner's reagent) was added.

Hager's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of picric acid solution (Hager's reagent) was added.

**Tests for Glycosides**

A small amount of extract was taken in 1 ml water. Then few drops of aqueous sodium hydroxide were added. Yellow precipitate is considered as an indication for the presence of glycosides.

In another test, a small amount of extract was taken in 1 ml water and boiled with 5 ml Fehling's solution in a boiling water bath. Brick-red precipitate is considered as an indication for the presence of glycosides.

In another test, a small amount of extract was boiled with few drops of dilute sulfuric acid, neutralized with sodium hydroxide solution and boiled with 5 ml Fehling's solution in a boiling water bath. Brick red precipitate is considered as an indication for the presence of glycosides.

### Pharmacological Studies

#### Antinociceptive Activity

Antinociceptive activity of the ethanolic extract of bark of *Madhuca indica* J. F. Gmel. was tested using the model of acetic acid induced writhing in mice<sup>7-8</sup>. The experimental animals were randomly divided into four groups, each consisting of ten animals. Group I was treated as 'control' which received 1% (v/v) Tween-80 solution in water; group II was treated as 'positive control' and was given the standard drug diclofenac sodium at dose of 25 mg/kg of body weight; group III and group IV were test groups and were treated with ethanolic extracts of bark of *Madhuca indica* J. F. Gmel. at dose of 250 and 500 mg/kg of body weight respectively. Control vehicle, standard drug and the ethanolic extracts were administered orally 30 min prior to the intra-peritoneal injection of 0.7 % acetic acid, then after an interval of 15 min, the number of writhes (squirms) was counted for 5 min.

#### Antidiarrhoeal Activity

Antidiarrhoeal activity of the ethanolic extract of bark of *Madhuca indica* J. F. Gmel. was tested using the model of castor oil-induced diarrhoea in mice<sup>9</sup>. The mice were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the final experiment. The test animals were randomly chosen and divided into three groups having five mice in each. Group-I was kept as control and received 1% Tween-80 at the dose of 10 ml/kg of body weight; group II was treated as 'positive control' and was given the standard drug loperamide at a dose of 50 mg/kg of body weight; group III was test group and was treated with the extract at a dose of 500 mg/kg of body weight. Control vehicle, standard drug and the extract were administered orally, 1 h prior to the oral administration of castor oil at a dose of 0.5 ml per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhoea every hour in five hours study after the castor oil administration. Number of stools or any fluid material that stained the adsorbent paper was counted at each successive hour during the experiment. The latent period of each mouse was also counted. At the beginning of each hour old papers were replaced by the new ones.

#### Statistical Analysis:

Student's *t*-test was used to determine a significant difference between the control group and experimental groups.

## Results

#### Chemical Group Test

Results of different chemical tests on the ethanolic extract of bark of *Madhuca indica* J. F. Gmel. showed the presence of alkaloids, tannins, gums, saponins, reducing sugars and glycosides (Table 1).

**Table 1:** Results of different chemical group tests of the extract of bark of *Madhuca indica* J. F. Gmel.

Extract	Reducing Sugar	Steroids	Alkaloids	Tannins	Gums	Flavonoids	Glycosides	Saponins
Ethanollic extract of bark of <i>Madhuca indica</i> J. F. Gmel.	+	-	+	+	+	-	+	+

Key: + = Presence, - = Absence

**Antinociceptive Activity**

Table 2 showed the effect of bark of *Madhuca indica* J. F. Gmel. on acetic acid-induced writhing model in mice. The extract produced about 42.86% and 72.45% writhing inhibition at the dose of 250 and 500 mg/kg body weight respectively, which were comparable to the standard drug diclofenac sodium where the inhibition was about 79.08% at the dose of 25 mg/kg body weight (Table 2).

**Table 2:** Effect of ethanolic extract of bark of *Madhuca indica* J. F. Gmel. on acetic acid induced writhing in mice

Animal Group / Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% tween-80 in water, p.o.	19.6±1.26 (100)	---
Positive control Diclofenac sodium 25 mg/kg, p.o.	4.1±1.01* (20.92)	79.08
Test group-I Ethanolic extract 250 mg/kg, p.o.	11.2±1.37* (57.14)	42.86
Test group-II Ethanolic extract 500 mg/kg, p.o.	5.4±1.62* (27.55)	72.45

Values are expressed as Mean±S.E.M (n=10), \*P&lt;0.001, % = Percentage, p.o. = per oral.

**Antidiarrhoeal Activity**

Antidiarrhoeal activity of the ethanol extract of bark of *Madhuca indica* J. F. Gmel. was tested by castor oil induced diarrhoea in mice. The extract caused an increase in latent period (1.79 h) i.e. delayed the onset of diarrhoeal episode at the dose of 500 mg/kg body weight significantly ( $P<0.001$ ) which was comparable to the standard drug loperamide at the dose of 50 mg/kg of body weight in which the value was 1.98 h ( $P<0.001$ ) (Table 3a). The extract also decreased the frequency of defecation at the same dose where the mean numbers of stool at the 1st, 2nd, 3rd, 4th and 5th h of study were 1.4, 1.2, 1.0, 1.4 and 1.6 respectively and in standard drug the values were 1.2, 1.0, 1.0, 1.2 and 1.4 respectively (Table 3b).

**Table 3a.** Effect of the extract of bark of *Madhuca indica* J. F. Gmel. on castor oil induced diarrhoea in mice (latent period)

Animal Group / Treatment	Dose(/kg, p.o)	Latent Period (h)
Group-I (control) 1% tween-80	10 ml	0.86 ± 0.140
Group-II (positive control) Loperamide	50 mg	1.98 ± 0.114*
Group - III Ethanollic extract	500 mg	1.79 ± 0.106*

Values are expressed as Mean±S.E.M (n=5), \* $P<0.001$ , p.o. = per oral.

**Table 3b.** Effect of the ethanolic extract of bark of *Madhuca indica* J. F. Gmel. on castor oil induced diarrhoea in mice (Number of stools)

Animal Group/Treatment	Dose (/kg, p.o.)	Period of study (h)	Total number of stool
Group-I (control) 1% tween-80 solution in water	10 ml	1	3.0 ± 0.304
		2	3.2 ± 0.357
		3	3.0 ± 0.295
		4	3.4 ± 0.364
		5	3.2 ± 0.390
Group-II (positive control) Loperamide	50 mg	1	1.2 ± 0.219*
		2	1.0 ± 0.320*
		3	1.0 ± 0.332*
		4	1.2 ± 0.287*
		5	1.4 ± 0.224*
Group-III Ethanolic extract	500 mg	1	1.4 ± 0.357*
		2	1.2 ± 0.444*
		3	1.0 ± 0.469*
		4	1.4 ± 0.455*
		5	1.6 ± 0.265*

Values are expressed as Mean±S.E.M (n=5), \* $P<0.01$ , p.o. = per oral.

### Discussion

Antinociceptive activity of the extract of bark of *Madhuca indica* J. F. Gmel. tested by acetic acid induced writhing model in mice. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which then excite the pain nerve endings<sup>10</sup>. The extract produced significant writhing inhibition comparable to standard drug diclofenac sodium. Based on this, it could be concluded that it might possess antinociceptive activity.

Antidiarrhoeal activity of the ethanol extract of bark of *Madhuca indica* J. F. Gmel. was tested using the model of castor oil induced diarrhoea in mice<sup>9</sup>. Castor oil, which is used to induce diarrhoea in mice, mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinoleic acid remains in the intestine and produces its anti absorptive or secretory effect. The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of the intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Most agreed view is that ricinoleate salts stimulates the intestinal epithelial cell's adenylyl cyclase<sup>11</sup> or release prostaglandin<sup>12</sup>. The extract caused an increase in latent period i.e. delayed the onset of diarrhoeal episode and decreased the frequency of defecation as well as the number of stool. On the basis of the result of castor oil induced diarrhoea, it can be concluded that the ethanol extract of bark of *Madhuca indica* J. F. Gmel. might possess antidiarrhoeal activity.

In conclusion, it could be suggested that the crude ethanolic extract of bark of *Madhuca indica* J. F. Gmel. might possess antinociceptive and antidiarrhoeal activities. However, further studies comprising of thorough phytochemical investigations of the used plant to find out the active principles and evaluation for these activities using other models are essential to confirm its pharmacological properties.

### Acknowledgements

The authors are thankful to Prof. Dr. Samir Kumar Sadhu, Head, Pharmacy Discipline, Khulna University; Dr. Asish Kumar Das, Associate professor, Pharmacy Discipline, Khulna University; Md. Saifuzzaman, Assistant professor, Pharmacy Discipline, Khulna University; Dr. Mahiuddin Alamgir, Research Scientist, National Measurement institute (NMI), Australia, for their encouragement during the research time. All the informants of the study area are cordially acknowledged for their valuable cooperation.

### References

1. Ghani A. Medicinal Plants of Bangladesh, 1<sup>st</sup> edition, Dhaka, Bangladesh, The Asiatic Society of Bangladesh, 1998: 134-135, 202-204.
2. Kirtikar, K. R. and Basu, B. D. In: Indian medicinal plants, 2<sup>nd</sup> edition, Dehradun, India, International Book Distributors and Book sellers, 1987: 350-353.

3. Ramadan, M.F., G. Sharanabasappa, S. Parmjyothi, M. Seshgiri and J.T. Moersel. Profile and levels of fatty acids and bioactive constituents in mahua butter from fruit seeds of butter cup tree (*Madhuca longifolia* (Koenig)). Eur. Food Res. Technol. 2006; 222: 710-718.
4. Nadkarni, K.M. Indian Materia Medica, 3<sup>rd</sup> edition, Bombay, India, Popular Books, 1954: 253-56.
5. Varier, P.S.V. Indian Medicinal Plants, 1<sup>st</sup> edition, India, Orient Longman, Arya Vaidyasala Kottakkal, 1995: 362.
6. Evans WC. Trease and Evan's Textbook of Pharmacognosy, 13th edition, London, Cambridge University Press, 1989: 546.
7. Whittle BA. The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. Br. J. Pharmacol. Chemother. 1964; 22: 246-253.
8. Ahmed F, Selim MST, Das AK, Choudhuri MSK. Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn. Pharmazie 2004; 59: 329-330.
9. Chatterjee TK. Handbook of laboratory Mice and Rats, 1st edition, India, Jadavpur University, 1993: 133-139.
10. Taesotikul T, Panthong A, Kanjanapothi D, Verpoorte R, Scheffer JJC. Anti-inflammatory, antipyretic and antinociceptive activities of *Tabernaemontana pandacaqui* Poir. J. Ethnopharmacol. 2003; 84: 31-33.
11. Racusen LC, Binder HJ. Ricinoleic acid stimulation of active anion secretion in colonic mucosa of the rat. J. Clin. Invest. 1979; 63: 743-749.
12. Beubler E, Juan H. Effect of Ricinoleic acid and other Laxatives in Net Water Flux and Prostaglandin E release by the Rat Colon. J. Pharm. Pharmacol. 1979; 31: 681-685.