ANTINOCICEPTIVE AND NEUROPHARMACOLOGICAL ACTIVITIES OF *SWIETENIA MAHAGONI (L.) JACQ*

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Summary

The crude ethanolic extract of seeds of *Swietenia mahagoni (L.) Jacq* was evaluated for its antinociceptive and neuropharmacological activities. When given orally to mice at dose of 300 and 600 mg/kg, the extract showed a significant (P<0.001) writhing inhibition in acetic acid induced writhing in mice, which was comparable to the standard drug diclofenac sodium. Moreover, it potentiated the pentobarbital induced sleeping time in mice and decreased the open field score in open field test, decreased the number of hole crossed from one chamber in the hole cross test and decreased the head dip responses in hole board test. The overall results tend to suggest the antinociceptive and central nervous system depressant activities of the crude ethanolic extract of seeds of *Swietenia mahagoni (L.) Jacq.*

Key words: antinociceptive activity, neuropharmacological activity, *Swietenia mahagoni (L.) Jacq*.

Introduction

Swietenia mahagoni (L.) Jacq (Family: Meliaceae, Synonym(s): *Cedrus mahogani L. , Swietenia fabrilis Salisbury , Swietenia mahogoni (L.) Lam.*) locally known as 'Mahogany' in Bangladesh. It is found almost all parts of Bangladesh. It is native to Bahamas, Cuba, Haiti, Jamaica, Netherlands Antilles, United States of America and exotic to Bangladesh, Benin, Burkina Faso, Cameroon, Chad, Cote d'Ivoire, Fiji, Gambia, Ghana, Guinea, Guinea-Bissau, India, Indonesia, Liberia, Malaysia, Mali, Mauritania, Niger, Nigeria, Philippines, Puerto Rico, Senegal, Sierra Leone, Sri Lanka, Togo.

The whole plant is used for various therapeutics purposes. Previous studies have shown that its bark contains significant hypoglycemic and antioxidant activity[1]. *Swietenia mahagoni (L.) Jacq* seeds extract has high free radical scavenging and xanthine oxidase inhibitory activity[2]. Its seeds extract also has inhibitory effects on the growth of *Candida albicans, Staphylococcus aureus, Pseudomonas aeroginosa, Streptococcus faecalis* and *Proteus mirabillase*[3]. Its seeds extract has also been reported to have medicinal value for the treatment of hypertension, diabetes, and malaria[4], and it has also been reported to have medicinal value for treatment of cancer, amoebiasis, coughs, chest pains and intestinal parasitism[5]. The biologically active ingredients, tetranortriterpenoids and fatty acids are considered to be responsible for these therapeutic effects[6].

Swietenia mahagoni (L.) Jacq seeds extract is high in lipids, particularly neutral lipids, glycolipids and phospholipids, the most abundant of which is phosphatidylcholine. Fatty acid composition of *Swietenia mahagoni (L.) Jacq* seed oil : myristic acid, palmitic acid, stearic acid, oleic acid, arachidic acid etc.[7]

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 neuropharmacological activities of the ethanolic extract of seeds of *Swietenia mahagoni (L.) Jacq*

Materials and methods

Plant material

Seeds of *Swietenia mahagoni (L.) Jacq* were collected from Bogra, Bangladesh in January 2008 and were authenticated by the experts at National Herbarium (Accession Number: 32096). After drying, seed pulps were separated from seeds and powdered by 'Hammer' mill. The powder of seed pulps was extracted by hot extraction process using ethanol as solvent. Each time 50 gm powdered material was extracted with 200 ml of solvent in a soxhlet extraction apparatus. The extraction was carried out until the process was completed. After the extraction, the extract was poured in a 1000 ml beaker and stayed overnight with the mouth of beaker closed with foil paper. Three (3) layers in the extract were found afterwards. The upper layer was yellowish oily liquid layer, the middle layer was dark coffee colored semi solid layer and the bottom layer was off-white colored solid layer. All the 3 layers were separated by a separating funnel. Then evaporated the solvent of each of the layers by using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the crude extract. As the middle layer contains the highest chemical groups among all the three layers, this middle layer was used for all pharmacological screening.

Animals

For antinociceptive and neuropharmacological activity study, Swiss-albino mice of either sex, weighing 20-25 g, bred in the animal house of the Department of Pharmacy, Jahangirnagar University, were used. The animals were kept 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), Pentobarbital (Sigma Chemicals, U.S.A).

Preliminary phytochemical analysis

The ethanol extract of seeds of *Swietenia mahagoni (L.) Jacq* 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.[8-9]

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.

Combined Reducing Sugar test:
1 ml of the extract was boiled with 2 ml of diluted hydrochloric acid for 5 min. After cooling, the mixture was neutralized with sodium hydroxide solution and then Fehling's test was performed as described above.

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
Libermann-Burchard test: 1 ml of the extract was placed in a test tube and then 2 ml Libermann-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 seeds of *Swietenia mahagoni (L.) Jacq* was tested using the model of acetic acid induced writhing in mice.[10-11] 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 seeds of *Swietenia mahagoni (L.) Jacq* at dose of 300 and 600 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.

Neuropharmacological activity

i) Pentobarbital induced hypnosis
Pentobarbital induced hypnosis test was carried out by the method of Williamson *et al*., 1996[12]. The test animals were divided into three groups consisting of seven mice in each group. Group I was the control group and group II and III were the experimental groups. The experimental groups were administered with the ethanolic extract of seeds of *Swietenia mahagoni (L.) Jacq* at dose of 300 and 600 mg/kg body weight intra-peritoneally (i.p.), while the animals of group I (control) were supplied with distilled water containing 0.1% (v/v) tween-80 (i.p.) at the dose of 10 ml/kg of body weight. The total sleeping time were recorded for both control as well as for treated groups. Total sleeping time represents the time between the loss and regain of righting reflex.

ii) Exploratory behavior
This experiment was performed by (i) Open field test[13] (ii) Hole cross test[14] and (iii) Hole board test[15]. The test animals were divided into three groups consisting of seven mice in each group. Group I was the control group and group II and III were the experimental groups. The experimental groups were administered with the ethanolic extract of seeds of *Swietenia mahagoni (L.) Jacq* (prepared by distilled water and tween-80) at dose of 300 and 600 mg/kg of body weight intra-peritoneally (i.p.), while the animals of group I (control) were supplied with

0.1% (v/v) tween-80 (i.p.) at the dose of 10 ml/kg of body weight. The observations were made on 0 min before injection and 30, 60, 120 and 240 min after injections of the test samples and control.

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 seeds of *Swietenia mahagoni (L.) Jacq* (middle layer) showed the presence of alkaloids, reducing sugars, glycosides, tannins, gums and saponins (Table 1).

Table 1: Results of different chemical group tests of the extract of seeds of *Swietenia mahagoni (L.) Jacq* (Middle layer)

Extract	Reducing Sugar	Steroids	Alkaloids	Combined Reducing Sugars	Tannins	Gums	Flavonoids	Glycosides	Saponins
Ethanolic extract of seeds of *Swietenia mahagoni (L.) Jacq* (Middle layer)	+	-	+	+	+	+	-	+	+

Key: + = Presence; - = Absence

Antinociceptive activity

Table 2 showed the effect of seeds of *Swietenia mahagoni (L.) Jacq* on acetic acid-induced writhing model in mice. The extract produced about 41.66% and 72.22% writhing inhibition at the dose of 300 and 600 mg/kg body weight respectively, which were comparable to the standard drug diclofenac sodium where the inhibition was about 88.89% at the dose of 25 mg/kg body weight (Table 2).

Table 2: Effect of ethanolic extract of seeds of *Swietenia mahagoni (L.) Jacq* on acetic acid induced writhing in mice

Animal Group / Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% tween-80 in water, p.o.	9.0±0.65 (100)	---
Positive control Diclofenac sodium 25 mg/kg, p.o.	1.0±0.35* (11.11)	88.89
Test group-I Ethanolic extract 300 mg/kg, p.o.	5.25±0.67* (58.33)	41.66
Test group-II Ethanolic extract 600 mg/kg, p.o.	2.5±0.66* (27.78)	72.22

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

Neuropharmacological activity

i) Pentobarbital induced hypnosis Test

Table 3 showed the effect of *Swietenia mahagoni (L.) Jacq* on pentobarbital induced hypnosis in mice. The total sleeping time were about 39 and 72 min at dose of 300 and 600 mg/kg of body weight respectively where as in control group it was about 20 min.

Table 3: Effect of seeds of *Swietenia mahagoni (L.) Jacq* on pentobarbital induced hypnosis

Animal group	Treatment	Total sleeping time (min)
I (Control)	0.1% Tween 80 solution	20.50±1.10
II (Test group-I)	Eth. Extract 300 mg/kg.	39.70±3.0*
III (Test group-II)	Eth. Extract 600 mg/kg	72.40±5.66*

Values are expressed as Mean±S.E.M (n=7); **P*<0.001; Eth. = Ethanolic

ii) Exploratory behavior Test

Test for exploratory behavior in mice was performed by (i) Open field test (ii) Hole cross test and (iii) Hole board test. It was observed that the extract decreased the number of open field score (Table 4), caused decrease in the number of hole crossed from one chamber to another chamber (Table 4), and also decreased head dip responses (Table 4) in mice at dose of 300 and 600 mg/kg of body weight from 30 min to 240 min.

Table 4: Effect of seeds of *Swietenia mahagoni (L.) Jacq* on exploratory behavior in mice

Group	Response at				
	0 min	30 min	60 min	120 min	240 min
Effect on Open Field Test					
I (Control)	101.28±1.98	99.00±1.85	97.85±2.05	97.00±1.68	96.57±1.72
II (Eth. Ext.) 300 mg/kg	99.85±1.77*	85.42±1.30*	70.71±1.57*	62.57±2.47*	54.28±2.76*
III (Eth. Ext.) 600 mg/kg	100.28±2.01*	71.42±2.90*	54.71±2.95*	42.85±3.33*	36.42±3.62*
Effect on Hole Cross Test					
I (Control)	10.57±0.55	10.42±0.37	9.57±0.46	10.14±0.65	9.71±0.53
II (Eth. Ext.) 300 mg/kg	10.28±0.72*	8.28±0.38*	6.85±0.39*	4.85±0.27*	3.14±0.65*
III (Eth. Ext.) 600 mg/kg	9.85±0.67*	7.0±0.57*	4.71±0.48*	3.28±0.73*	1.57±0.63*
Effect on Hole Board Test (Head dipping)					
I (Control)	16.28±0.84	17.0±0.51	16.57±0.77	17.14±0.69	16.85±0.97
II (Eth. Ext.) 300 mg/kg	16.14±0.67*	14.57±0.33*	11.28±0.57*	8.85±0.56*	6.28±0.30*
III (Eth. Ext.) 600 mg/kg	16.00±0.78*	12.42±0.76*	8.57±0.44*	5.85±0.47*	3.42±0.37*

Values are expressed as Mean±S.E.M (n=7); Eth. = Ethanolic; Ext. = Extract; *P<0.001

Discussion

Antinociceptive activity of the extract 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[16]. 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.

Experimental findings from the pentobarbital induced hypnosis test in mice showed that the extract potentiated the pentobarbital induced sleeping time in mice which suggests its CNS depressant activity[17], thus suggesting the probable tranquilizing action[18]. Moreover, the test for exploratory behavior in mice showed that the extract suppressed the open field score, hole crossing ability and head dip responses in mice, which further support the CNS depressant properties of the extract.

In conclusion, it could be suggested that the crude ethanolic extract of seeds of *Swietenia mahagoni (L.) Jacq* might possess antinociceptive and central nervous system depressant 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.

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