ANALGESIC AND CYTOTOXIC ACTIVITIES OF *MICROCOS PANICULATA* L.

Md. Atiqur Rahman[1]*, Kumar Shuvrojjwal Sampad[1], S. N. Hasan[1], Md. Saifuzzaman[1]

[1]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 leaves of *Microcos paniculata* L. (Family - Tiliacece) was investigated for its possible analgesic and cytotoxic 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 crude ethanolic extract also produced the most prominent cytotoxic activity against brine shrimp *Artemia salina* (LC_{50} = 60 µg/ml and LC_{90} = 120 µg/ml). The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Key Words: analgesic activity, cytotoxic activity, *Microcos paniculata* L.

Introduction

Microcos paniculata L. (English Name: Microcos; Family: Tiliacece; Synonym: *Microcos nervosa* (Lour.) S. Y. Hu, *Grewia nervosa* (Lour.) Panigrahi) locally known as 'Kathgua or Fattashi' in Bangladesh. It is also known as Bu zha ye, Po bu ye (Transcribed Chinese); Kaphla (Transcribed Thai). It is a herbaceous plant which looks like a shrub or small tree that widely distributed and naturally grown throughout Bangladesh. It is also native and distributed more or less throughout India, Andaman and Nicobar (Andaman Islands), Sri Lanka, China, Cambodia, Myanmar, Thailand, Vietnam, Indonesia and Malaysia[1-2].

Microcos paniculata L. is added in Chinese herb tea. It's claimed to have medicinal values as well. The taste of it is mildly sour. Traditional beliefs claim it services the digestive system to work better and it is additionally employed for other health conditions inclusive of colds, diarrhea, hepatitis, heat stroke and dyspepsia. This plant traditionally used in wound healing, fever and as an insecticide in Bangladesh[1, 3].

Literature study reveals that the stem bark of *Microcos paniculata* contained a new alkaloid, N-Methyl-6 beta-(deca-1',3',5'-trienyl)-3 beta-methoxy-2 beta-methylpiperidine, which showed good insecticidal activity against *Aedes aegypti* second instar larvae[4]. Another study claims that two new piperidine alkaloids, microcosamines A (1) and B (2), were isolated from the leaves of *Microcos paniculata*. Their structures were elucidated by spectroscopic analysis.

Both new compounds showed significant larvicidal activity against *Culex quinquefasciatus*[5]. On the other hand, the free-radical-scavenging assay of various solvent extracts of stem of *Microcos paniculata* yielded five compounds: a new triterpene named methyl 3beta-O-p-hydroxy-E-cinnamoyloxy-2alpha,23-dihydroxyolean-12-en-28-oate (1), epicatechin (2), 3-trans-feruloyl maslinic acid (3), maslinic acid (4) and sucrose (5). Among them, compound 2 displayed significant free-radical-scavenging activity which is similar to that of standard antioxidant ascorbic acid (V(C)) and therefore may be a promising natural antioxidant[6].

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 analgesic and cytotoxic activities of the ethanol extract of dried leaves of *Microcos paniculata* L.

Materials and Methods

Plant Material

Leaves of *Microcos paniculata* L. were collected from road side area of Bhola Sadar Thana, Bhola, Bangladesh in September 2008 and were authenticated by the experts at National Herbarium (Accession Number: 33881). After collection, leaves were sun dried for several days to remove moisture. After drying, the dried leaves were ground into course powder by 'Hammer' mill. About 400 gm of powdered leaves 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 analgesic 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).

Preliminary Phytochemical Analysis

The ethanol extract of leaves of *Microcos paniculata* L. 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[1, 7].

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
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

Analgesic Activity

Analgesic activity of the ethanolic extract of leaves of *Microcos paniculata* L. was tested using the model of acetic acid induced writhing in mice[8-9]. 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 leaves of *Microcos paniculata* L. 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.

Cytotoxicity Test

The brine shrimps used for cytotoxicity test were obtained by hatching 5 mg of eggs of *Artemia salina* in natural seawater after incubation at about 29°C for 48h. The larvae (nauplii) were allowed another 48 h in seawater to ensure survival and maturity before use. Six doses of plant extract (20, 40, 60, 80, 120 and 140 µg/ml) in 5% DMSO and/or seawater were tested. Each extract preparation was dispensed into clean test tubes in 10 ml volumes and tested in duplicates. The concentration of DMSO in the vials was kept below 10 µl/ml. For control, same procedure was followed except test samples. After marking the test tubes properly, 10 living shrimps were added to each of the 20 vials with the help of a Pasteur pipette[10] .The test tube containing the sample and control were then incubated at 29°C for 24 h in a water bath, after which each tube was examined and the surviving nauplii counted. From this, the percentage of mortality was calculated at each concentration.

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 group tests on the ethanolic extract of leaves of *Microcos paniculata* L. showed the presence of Reducing Sugar, Steroids, Alkaloids, Tannins, Gums and Glycosides (Table 1).

Table 1: Results of different chemical group tests of the extract of leaves of *Microcos paniculata* L.

Extract	Reducing Sugar	Steroids	Alkaloids	Tannins	Gums	Flavonoids	Glycosides	Saponins
Ethanolic extract of leaves of *Microcos paniculata* L.	+	+	+	+	+	-	+	-

Key: + = Presence, - = Absence

Antinociceptive Activity

Table 2 showed the effect of leaves of *Microcos paniculata* L. on acetic acid-induced writhing model in mice. The extract produced about 44.10% and 70.77% writhing inhibition at the dose of 250 and 500 mg/kg of body weight respectively, which were comparable to the standard drug diclofenac sodium where the inhibition was about 83.59% at the dose of 25 mg/kg of body weight (Table 2).

Table 2: Effect of ethanolic extract of leaves of *Microcos paniculata* L. on acetic acid induced writhing in mice

Animal Group / Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% tween-80 in water, p.o.	19.5±1.45 (100)	---
Positive control Diclofenac sodium 25 mg/kg, p.o.	3.2±1.12* (16.41)	83.59
Test group-I Ethanolic extract 250 mg/kg, p.o.	10.9±1.52* (55.90)	44.10
Test group-II Ethanolic extract 500 mg/kg, p.o.	5.7±1.74* (29.23)	70.77

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

Cytotoxic Activity

In brine shrimp lethality bioassay, the extract showed lethality against the brine shrimp nauplii. It showed different mortality rate at different concentrations. From the plot of percent mortality versus log concentration on the graph paper LC_{50} and LC_{90} were deduced (LC_{50} = 60 µg/ml; LC_{90} = 120 µg/ml) (Table 3).

Table 3. Brine shrimp lethality bioassay of the ethanolic extract of dried leaves of *Microcos paniculata* L.

Test sample	Concentration (µg/ml)	Log (concentration)	Number of alive shrimp	Mortality (%)	LC_{50} (µg/ml)	LC_{90} (µg/ml)
Ethanolic Extract	20	1.30	08	20	60	120
	40	1.60	07	30		
	60	1.77	05	50		
	80	1.90	03	70		
	120	2.07	1	90		
	140	2.14	0	100		

Discussion

Analgesic activity of the ethanolic extract of dried leaves of *Microcos paniculata* L. 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[11]. The extract produced significant writhing inhibition comparable to standard drug diclofenac sodium. Based on this, it could be concluded that it might possess analgesic activity.

The cytotoxic activity of the ethanol extract of leaves of *Microcos paniculata* L. was tested by using brine shrimp lethality bioassay. It is a recent development in the bioassay for the bioactive compounds. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor etc.[12]. The extract was found to show potent activity against the brine shrimp nauplii. Therefore the positive response obtained in this assay suggests that the extract may contain antitumor, antibacterial or pesticidal compounds.

In conclusion, it could be suggested that the crude ethanolic extract of leaves of *Microcos paniculata* L. might possess analgesic and cytotoxic 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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