

Antinociceptive, cytotoxic and antibacterial activities of *Cleome viscosa* leaves

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Article

Received 7 Jul 2010

Accepted 26 Aug 2010

Available online 25 Feb 2011

Keywords:

antinociceptive
cytotoxic
antibacterial
Cleome viscosa
Capparidaceae

ISSN 0102-695X

doi: 10.1590/S0102-695X2011005000023

Abstract: The methanol extract of the dried leaves of *Cleome viscosa* L., Cleomaceae, was investigated for its possible antinociceptive, cytotoxic and antibacterial activities in animal models. The extract produced significant writhing inhibition in acetic acid-induced writhing in mice at the oral doses of 250 and 500 mg/kg body weight ($p < 0.001$) comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight ($p < 0.001$). The crude extract produced the most prominent cytotoxic activity against brine shrimp *Artemia salina* (LC50 28.18 $\mu\text{g/mL}$ and LC90 112.20 $\mu\text{g/mL}$). The extract of *C. viscosa* L. exhibited significant in vitro antibacterial activity against *Staphylococcus saprophyticus*, *Shigella sonnie*, *Salmonella typhi*, *Vibrio cholera*, *Streptococcus epidermidis*, *Shigella flexneri* and *Staphylococcus aureus* with the zones of inhibition ranging from 10.76 to 16.34 mm. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Introduction

Cleome viscosa L., Cleomaceae, is a widely distributed sticky herb with yellow flowers and long slender pods containing seeds, which similar those of mustard (Hindi), Hurhuria (Bengali), Nayikkadugu (Tamil) in South Asian folk medicine, found throughout the larger part of Indian Subcontinent, often in waste places (Tandon et al., 2010). The plant finds its use in the traditional system of local medicine as a laxative and diuretic (Mali, 2010). It is reported to be useful in the treatment of malarial fevers, fever due to indigestion, skin diseases, leprosy, blood diseases, and uterine complaints (Kirtikar & Basu, 1984). In the Unani system of medicine, the seeds of the plant are documented as anthelmintic and detergent, and are given to treat fever and diarrhoea (Chopra et al., 1956). The seeds are used for anthelmintic while the leaves are useful for healing wounds (Parimaladevi et al., 2003). A poultice made from the plant is efficacious as a counterirritant in chronic painful joints. Juice is used to remove pus from ear (Nadkarni, 1976). Fatty constituents of the seed oil of *C. viscosa* have been reported to contain oleic (18.6%), linoleic (32.8%), palmitic (2%), stearic (20%) and linolenic acid (26.6%) and the oil has been reported as edible (Rukmini, 1978). Different types of chemical compounds are reported *i.e.* coumarino-lignan, glucosinolates, cleomeolide, stigmasta-5, kaempeferide-3-glucuronide (Sudhakar et al., 2006). Subsequent to a large number

of claims about the wide range of traditional medicinal properties of the plant, considerable efforts have been made to identify its efficacy as a curative agent through pharmacological investigations. The plant reported to possess antipyretic (Devi et al., 2003), antidiarrhoeal (Devi et al., 2002), anti-inflammatory (Parimaladevi et al., 2003), immunomodulatory (Tiwari et al., 2004), local anesthetic activities (Singh & West, 1991), antimalarial activity (Saxena et al., 2000). Traditional medicines hold a great opportunities as sources of easily available, effective healing agents to the people, particularly in tropical developing countries, including Bangladesh. It is in this context that the people consume several plants or plant-derived preparations to cure different diseases. In the present study we evaluated antinociceptive, cytotoxic and antibacterial potential of methanol extracts of leaves of *C. viscosa*.

Materials and Methods

Plant material collection and extraction

The leaves of *Cleome viscosa* L., Cleomaceae, were collected from the Khulna, Bangladesh in July 2007, and were taxonomically identified by experts at the Bangladesh National Herbarium (accession number: 34556). About 400 g of powdered leaves were taken in a clean, flat-bottomed glass container and soaked in 1,300 mL of 80% methanol. The container with its contents was

sealed and kept for a period of seven days accompanying occasional shaking and stirring. The whole mixture 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.

Drugs

Diclofenac sodium (Opsonin Chemical Industries Ltd, Bangladesh), Loperamide (Square Pharmaceuticals Ltd., Bangladesh), Gentamycin (Square Pharmaceuticals Ltd., Bangladesh), DMSO (Sigma Aldrich, Dhaka), Acetic acid (Azad Chemicals Ltd., Dhaka).

Animals

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 for the test. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase under standard laboratory conditions (relative humidity 55-65%, room temperature 25±2 °C and 12 h light-dark cycle) and fed with standard diets (ICDDR, B formulated) 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.

Phytochemical tests

Desirable amount of *C. viscosa* extract was solubilized in water for phytochemical tests. The extract solution was tested for alkaloids, glycosides, steroids, flavonoids, saponins and tannins according to the protocol described by Evans (1989).

Pharmacological studies

Antinociceptive activity

Antinociceptive activity of the crude extract was tested using the model of acetic acid-induced writhing in mice (Ahmed et al., 2007; Roome et al., 2008) The experimental animals were randomly divided into four groups, each consisting of ten animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 in water at the dose of 10 mL/kg of body weight; 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 the extracts at dose of

250 and 500 mg/kg of body weight respectively. Control vehicle, standard drug and extracts were administered orally, 30 min prior to acetic acid (0.7%) injection in peritoneum. Then after an interval of 10 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 48 h. The larvae (nauplii) were allowed another 48 h in seawater to ensure survival and maturity before use. Five doses of plant extract (1, 2, 4, 6, 8 and 10 µg/mL) in 5% DMSO (dimethylsulfoxide) 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, ten living shrimps were added to each of the 20 vials with the help of a Pasteur pipette (Meyer et al., 1982). 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.

Antibacterial activity

The antibacterial activity of *C. viscosa* extract was studied against *Staphylococcus saprophyticus*, *Shigella sonnie*, *Salmonella typhi*, *Vibrio cholera*, *Streptococcus epidermidis*, *Shigella flexneri* and *Staphylococcus aureus* clinical isolates. All bacterial strains were kindly provided by IMTECH, Chandigarh (India). Cultures of these bacteria were grown in a nutrient broth at 37 °C and maintained on nutrient agar (Himedia, India) slants at 40 °C. The antibacterial property was studied by the disc diffusion method (Chattopadhyay et al., 2002) using extract 200 mg/disc. Control disks contained solvents only (50% aqueous ethanol). Gentamycin was used as positive controls. Minimum inhibitory concentration (MIC) was evaluated by the micro dilution method using 5 mL of liquid broth with different concentrations of extract (Ahmad & Beg, 2007; Bayoud et al., 2007).

Statistical analysis

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

Results

Preliminary phytochemical analysis

Results of different chemical tests on the methanol crude leaves extract of *C. viscosa* showed the presence of alkaloids, glycosides, steroids, flavonoids, saponins and tannins.

Antinociceptive activity

Table 1 showed the effect of the methanol extract of *C. viscosa* on acetic acid-induced writhing in mice. At dose of 250 and 500 mg/kg of body weight, the extract produced about 35.80% and 47.94% writhing inhibition in test animals, respectively. The results were statistically significant ($p < 0.001$) and were comparable to the standard drug diclofenac sodium, which showed about 64.17% writhing inhibition at the dose of 25 mg/kg ($p < 0.001$).

Cytotoxic activity

In brine shrimp lethality bioassay (Table 2), 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 LC50 and LC90 were deduced (LC50: 28.18 $\mu\text{g/mL}$; LC90: 112.20 $\mu\text{g/mL}$).

In vitro antibacterial activities

The extract of *C. viscosa* exhibited significant in vitro antibacterial activity (Table 3) against *Staphylococcus saprophyticus*, *Shigella sonnie*, *Salmonella typhi*, *Vibrio cholera*, *Streptococcus epidermidis*, *Shigella flexneri* and *Staphylococcus aureus* with the zones of inhibition ranging from 10.76 to 16.34 mm.

Table 1. Effects of *Cleome viscosa* L., Cleomaceae, crude leaves extract on writhing effect on acetic acid induced mice.

Treatment	Dose (mg/kg)	Mean writhing	% Inhibition	SD	p value (One way Anova)
Experimental control (1% Tween80)	10	33.50±1.33	-	2.97	$p < 0.001$
Positive control (diclofenac sodium)	25	12.00±1.24	64.17	2.76	$p < 0.001$
Test sample	250	20.50±2.01	35.80	2.87	$p < 0.001$
Test sample	500	17.7±1.93	47.94	3.85	$p < 0.001$

Test sample: *C. viscosa* crude extract: 30 min after treatment, 0.7% acetic acid was injected *i.p.* 10 min after injection writhing responses was recorded for 5 min. n=5.

Table 2. Brine shrimp lethality bioassay of *Cleome viscosa* L., Cleomaceae, crude leaves extract.

Test sample	Conc. ($\mu\text{g/mL}$)	No. of alive shrimp			% mortality	LC50 ($\mu\text{g/mL}$)	LC90 ($\mu\text{g/mL}$)
		Test 1	Test 2	Average			
Methanolic extract	1	8	7	7.5	25	28.18	112.20
	2	7	7	7	30		
	4	5	6	5.5	45		
	6	4	5	4.5	55		
	8	2	2	2	80		
	10	0	0	0	100		

Table 3. *In vitro* antibacterial activity of the methanolic crude leaves extract of *Cleome viscosa* L., Cleomaceae.

Bacterial stains	<i>C. viscosa</i> (mm)	Control (mm)
<i>Staphylococcus saprophyticus</i>	12.22	30.00
<i>Shigella sonnie</i>	16.34	44.88
<i>Salmonella typhi</i>	14.65	36.88
<i>Vibrio cholera</i>	14.42	32.44
<i>Streptococcus epidermidis</i>	12.45	34.28
<i>Shigella flexneri</i>	10.76	28.90
<i>Staphylococcus aureus</i>	10.80	30.50

Control (50% aqueous ethanol); Diameter of inhibition zones (mm).

Discussion

Plants are employed as important source of medication in many traditional medications (Grover et al., 2002; Keung & Vallee, 1998; Neves et al., 2009). *C. viscosa* commonly found tree plant was taken to explore the phytochemical and pharmacological properties as there was very limited work done on the plant. The leaves, which are mostly used as a source of medication in traditional medicines was considered to examine the properties of the plant.

Antinociceptive activity of the methanol extract of *C. viscosa* was tested by acetic acid-induced writhing model in mice. Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algnesia by liberation of endogenous substances, which in turn excite the pain nerve endings (Taesotikul et al., 2003). Increased levels of PGE2 and PGF2 α in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid (Derardt et al., 1980). The extract produced significant writhing inhibition comparable to the standard drug diclofenac sodium (Table 1). The polar compounds present in the plant extract may be responsible for the obtained antinociceptive activity. Based on this result it can be concluded that the methanol extract of *C. viscosa* might possess antinociceptive activity.

The cytotoxic activity of the methanol extract of *C. viscosa* was tested by using brine shrimp lethality bioassay. It is a recent well known developed bioassay for the bioactive compounds (Ara et al., 1999; Hartl & Humph, 2000). The plant is reported to contain saponins (Ghani 2003). There is growing interest in natural saponins caused as much by the scientific aspects extraction and structural analysis of these compounds, as by the fact of their wide spectrum of pharmacological activities; for instance, bactericidal, antiviral, cytotoxic, analgesic, anti-inflammatory, anti-cancer and antiallergic (Ahmed et al., 2007; Attele et al., 1999). Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, and antitumor (Anderson et al., 1988). 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 this experiment, methanolic extract of *C. viscosa* showed moderate sensitivity to the five of the test organisms both gram positive and gram negative type of bacteria. The highest zone on inhibition (16.34 mm) was recorded against *Shigella sonnie*. Moreover, the experiment was only conducted with five species of

bacteria as test samples. Therefore further research is essential to evaluate the sensitivity of the plant extract against other species of bacteria, fungi, virus of other microorganisms.

Conclusion

Finally, it could be suggested that the methanol extract of *C. viscosa* leaves possesses antinociceptive, cytotoxic and antibacterial activities. These facts indicate the scientific basis of *C. viscosa* being used as a traditional medicine. However, further experiments may help to determine the pharmaceutical potentialities of the plant as a medicine.

Acknowledgements

The authors are thankful to Prof. Dr. Samir Kumar Sadhu, Head, Pharmacy Discipline, Khulna University; Dr. Asish Kumar Das, 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.

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