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ANALGESIC AND ANTIEMETIC ACTIVITY OF *CLEOME VISCOSA* L.

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Abstract

The seeds of *Cleome viscosa* are used in traditional systems of medicine for the treatment of many diseases in Asia. This study evaluated fixed oil from the seeds of *Cleome viscosa* for analgesic and antiemetic activity by using the acetic acid induced writhing test in mice (intraperitoneally) and chick emetic model (oral treatment) respectively. The results showed significant analgesic and antiemetic activities of *Cleome viscosa* fixed oil.

Introduction

Cleome viscosa L., commonly known as wild or dog mustard, belongs to the caper family (Capparaceae). It is an annual, sticky herb found as a common weed all over the plains of India, Pakistan and throughout the tropics of the world. The leaves, seeds and roots of the plant are widely used in traditional and folkloric systems of medicine as an anthelmintic, antiscorbutic, antiseptic, cardiac stimulant, carminative, febrifuge and sudorific (Mali, 2010), anticonvulsant (Shah *et al.*, 1983), antidiarrheal (Malhotra & Moorthy, 1973; Sharma *et al.*, 1979), and also for treating skin diseases (Purohit *et al.*, 1985). Pharmacological studies have shown that *Cleome viscosa* possesses analgesic, antidiarrheal, anti-inflammatory, antimicrobial, antipyretic, anthelmintic, hepatoprotective and immunomodulatory activities (Mali, 2010). The seeds contain 18.3% oil, a mixture of amino acids, fatty acids and sucrose (Rukmini & Doesthale, 1979). Linoleic, palmitic, stearic, oleic and linolenic acids are present in the oil (Rukmini, 1978; Afaq *et al.*, 1984; Deora *et al.*, 2003). Aqueous and alcoholic extracts of seeds showed analgesic (Singh & West, 1991), anthelmintic (Mali *et al.*, 2007) and hepatoprotective activity (Sengottuvelu *et al.*, 2007). Seed oil possesses mutagenic properties (Polasa & Rukmini, 1987). The crude methanol extract of *C. viscosa* showed significant analgesic activity using acetic acid induced writhing, tail flick, tail clip and tail immersion methods in mice (Parimaladevi *et al.*, 2003).

Materials and Methods

Plant material: Seeds of *Cleome viscosa* were collected from University of Karachi campus in July 2010. A voucher specimen (CV-02-2010) has been submitted to the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi.

Animals: Swiss albino mice (weighing 20-30 g) of both sexes were purchased from Aga Khan University and Hospital animal house for analgesic activity. Young male chicks, 4 days old and weighing from 32-52 g were obtained from Big-bird Poultry Breeders (Pvt) Ltd., Karachi, Pakistan for antiemetic activity. All animals were kept under laboratory conditions of room temperature with 12 h light and dark cycles and were allowed free access to food and water.

Soxhlet extraction: Seeds of *C. viscosa* (50 g) were separated from capsules, crushed and subjected to solvent extraction using Soxhlet apparatus. The solvent used for extraction was hexane. The extracted *Cleome viscosa* seeds produced a dark brown oil. Several cycles of syphon were run at 70°C until all oil contents were extracted. Finally, the hexane was evaporated from the oil with a rotary evaporator. The required amounts of fixed oil were weighed and solubilized in DMSO for the experiment.

Preliminary screening of fixed oils: The qualitative chemical tests for fixed oils (stain and saponification tests) were carried out following the protocols of Khandelwal, (1995) and Evans & Trease, (2002).

Analgesic activity

Mouse writhing test: The mice were randomly divided into five groups of eight animals each. Doses of 75, 100 and 125 mg/kg of *C. viscosa* fixed oil were administered to three groups while the remaining two groups received distilled water 10 ml/kg and aspirin 150 mg/kg respectively, following protocols established by Koster *et al.*, (1959) and Salawu *et al.*, (2008). All treatments were administered intraperitoneally, and after 30 min 10 ml/kg of 0.6% acetic acid solution in normal saline was injected intraperitoneally. The numbers of writhes were counted for 15 min after acetic acid injection. The percentage inhibition was calculated using formula [Sulaiman *et al.*, 2004]:

$$\frac{(N - N_t)}{N} \times 100$$

where

N = Average number of writhes in control group

N_t = Average number of writhes in test group

Antiemetic activity

Chick emetic model: Chicks were randomly divided into five groups of eight animals each. The antiemetic activity of *C. viscosa* fixed oil was determined by calculating the mean decrease in number of retches compared to the control following the protocol of Yang *et al.*, (1999). Each chick was placed in a large beaker and left to settle for 10 minutes. The fixed oil of *C. viscosa* was prepared as a dose of 150 mg/kg body weight in a volume of 10 ml/kg of 0.9% saline containing 5% DMSO and 1% Tween 80. The dose was administered abdominally. The control group received only saline 0.9%. After 10 minutes, copper

sulfate was administered orally at 50 mg/kg, and the number of retches (an emetic action without emitting gastric material) was observed during the next 10 minutes. Chlorpromazine was used as a standard antiemetic drug (150 mg/kg body weight).

The percent inhibition was calculated by the following formula:

$$\text{Inhibition (\%)} = (A-B/A) \times 100$$

Table 1. Effect of *Cleome viscosa* seed oil on acetic acid induced writhing in mice.

Treatments	Dose (mg/kg) i.p.	Mean number of writhes \pm S.E.M. (15 min)	Inhibition (%) of writhes
Control	---	62.6 \pm 7.25	---
Aspirin	150	11.0 \pm 2.1	82.42*
	75	5.2 \pm 0.4	91.69**
CVFO	100	4.8 \pm 0.4	92.33**
	125	2.5 \pm 0.2	96.00**

CVFO = *Cleome viscosa* fixed oil, N = 8 for each group, i.p.= intraperitoneally, S.E.M = Standard Error of Mean, * $p < 0.05$ and ** $p \leq 0.001$ vs. control

Table 2. Antiemetic effect of *Cleome viscosa* seeds oil.

Treatments	Dose (mg/kg) p.o.	Mean number of retches \pm S.E.M	Inhibition (%) of emesis
Control	---	68.12 \pm 3.88	----
CPZ	150	47.3 \pm 0.28	30.56*
	75	10.6 \pm 1.007	84.43**
CVFO	100	9.83 \pm 0.65	85.56**
	125	5.60 \pm 1.50	91.77**

CPZ= Chlorpromazine, CVFO = *Cleome viscosa* fixed oil, N = 8 for each group, p.o.= per oral, S.E.M.= Standard Error of Mean, * $p < 0.05$ and ** $p \leq 0.001$ vs. control

Results and Discussion

Analgesic activity: Fixed oil of *C. viscosa* seeds at all doses significantly decreased the number of writhes when compared to the aspirin treatment and the control (Table 1 & Fig. 1). Fixed oil in doses of 75, 100 and 125 mg/kg body weight reduced the numbers of writhes by 91.69%, 92.33% and 96.0%, respectively. The group of mice treated with aspirin at a dose of 150 mg/kg body weight had 11 writhes compared to the 62 writhes of the control group, thus aspirin reduced the writhes by 82.42%. The acetic acid induced writhing method is an effective method to evaluate peripherally active analgesics. The abdominal constriction response induced by acetic acid is a sensitive method to test peripherally acting analgesics (Gene *et al.*, 1998). Hyperalgesia, induced by the injection of acetic acid, is characterized by contraction of the abdominal muscle accompanied by body elongation and an extension of the forelimbs. Various peripherally acting analgesic drugs such as ibuprofen, aspirin and indomethacin have been reported to inhibit acetic acid induced writhing in mice (Gene *et al.*, 1998; Okpo *et al.*, 2001). Fixed oil is reportedly effective against acetic acid induced writhing in mice and has been suggested to be a peripherally acting analgesic (Singh & Majumdar, 1995), perhaps via the inhibition of synthesis and release of prostaglandins and other endogenous substances (Salawu *et al.*, 2008). The mechanism of fixed oil activity may be linked to the inhibition of cyclooxygenases.

where

A = Frequency of retching in control group

B = Frequency of retching in test group

Statistical analysis: Analgesic and antiemetic activities were expressed as mean \pm standard error of mean (Tables 1 & 2). The statistical significance of the difference was determined by an unpaired Student's *t*-test.

Antiemetic activity: The antiemetic activity of *C. viscosa* fixed oil on young chicks demonstrated that it has an antiemetic effect (Table 2 & Fig. 2). Fixed oil in doses of 75, 100 and 125 mg/kg body weight reduced the numbers of retches by 84.43%, 85.56% and 91.77%, respectively. The group of chicks treated with chlorpromazine at a dose of 150 mg/kg body weight had 47 retches compared to the 68 retches of the control group, thus chlorpromazine reduced the retches by 30.56%. *C. viscosa* seed oil inhibited emesis to a greater extent than chlorpromazine. On the basis of these results, it may be said that the fixed oil of *C. viscosa* has antiemetic potential and is comparable with chlorpromazine, which can relieve nausea. *C. viscosa* fixed oil reduces copper sulfate induced retching in young chicks, possibly by peripheral action as the oral copper sulfate induces emesis by peripheral action through excitation of visceral afferent nerve fibers of the gastrointestinal tract (Bowman & Rand, 1980). It has also been established that the peripheral 5-HT₄ receptors play an important role in copper sulfate induced emesis (Bhandari & Andrews, 1991; Fukui *et al.*, 1994).

Conclusions

Conclusively, it may be said that *C. viscosa* seed oil has peripheral analgesic and antiemetic activities. Further studies on *C. viscosa* seed oil are required to investigate the molecular mechanism(s) of analgesic and antiemetic activities.

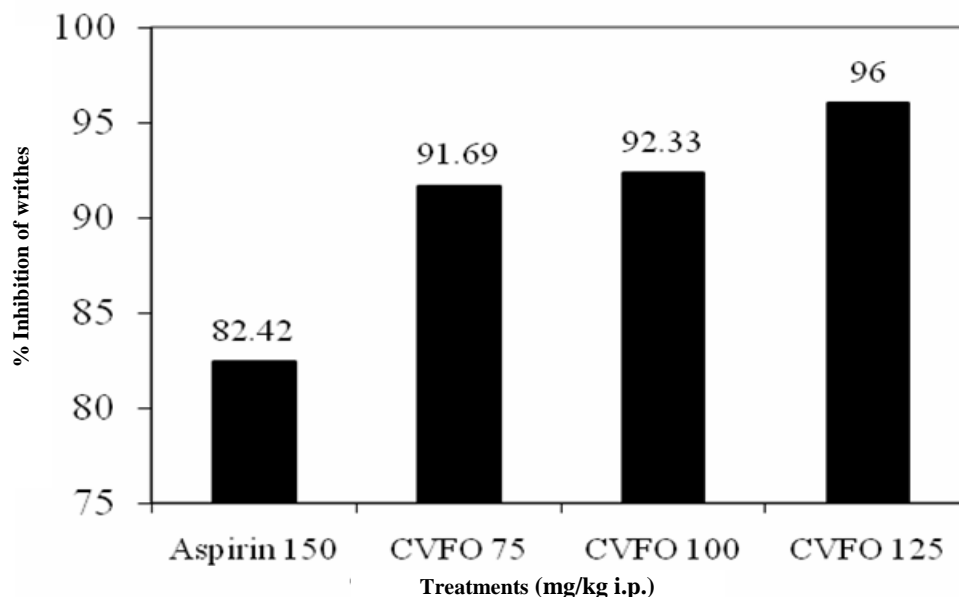


Fig. 1. Percent inhibition of writhes from fixed oil of *Cleome viscosa*.

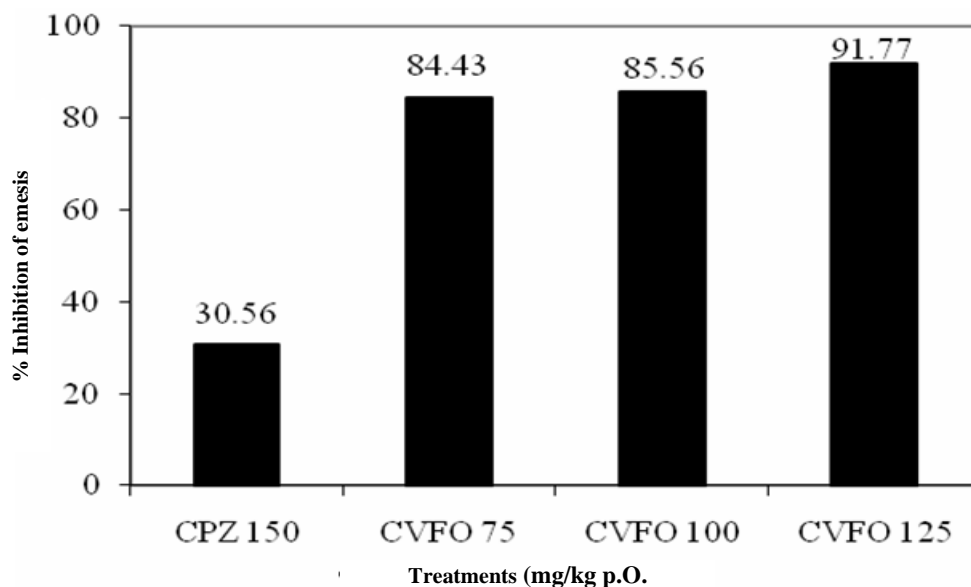


Fig. 2. Percent inhibition of emesis from fixed oil of *Cleome viscosa*.

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