Available online at SciVarse ScienceDirect
Chinese Herbal Medicines (CHM)
ISSN 1674-6384
Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Original article

Evaluation of Antinociceptive Activity of Methanol Extract from *Cleome rutidosperma* in Mice

Prawej Ansari[1, 2*], Mitali Debnath[1], Md. Foyez Ahmad[1], Shofiul Azam[1,2], Shafia Akther[1], Gazi Md. Mustakim[1], Md. Hamza Naquib[1], Shammy Sarwar[3]

1. Department of Pharmaceutical Sciences, North South University, Dhaka-1229, Bangladesh
2. Department of Pharmacy, International Islamic University Chittagong, Chittagong-4203, Bangladesh
3. Department of Pharmacy, Stamford University Bangladesh, Dhaka-1217,Bangladesh

ARTICLE INFO

Article history

Received: January 25, 2016

Revised: March 3, 2016

Accepted: April 13, 2016

Available online:

July 8, 2016

DOI:

10.1016/S1674-6384(16)60050-3

ABSTRACT

Objective *Cleome rutidosperma* (Capparidaceae), commonly known as "Fringed Spider Flower", is a medicinal plant found in Southeast Asia. *C. rutidosperma* is used in folk medicine for diuretic, laxative, analgesic, anti-inflammatory, antipyretic, antimicrobial, anti-oxidant, hypoglycemic, and anthelmintic activities. We have evaluated the anti-nociceptive properties of methanol extract from *C. rutidosperma* (MECR) *in vivo*. **Methods** Thermal method (hot plate test and tail flick test) was induced to judge the anti-inflammatory effect and couple of chemical method also used (formalin induced licking test; writhing test carried by acetic acid) to evaluate analgesic effect. Both of these tests were made over animal models, like mice and rats. Two different doses (100 and 200 mg/kg) were used for each case of test, while morphine sulphate (5mg/kg, ip) was used as reference drug. **Results** MECR demonstrated the significantly anti-nociceptive activity in the analgesic and anti-inflammatory tests by reducing nociception in mice models ($P < 0.001$). In the hot-plate and tail-flick tests, MECR significantly elongated the time to response to the thermal stimuli (100 and 200 mg/kg with $P < 0.05$, 0.001). The remarkable increase in the latency was observed at 90 and 120 min. In acetic acid-induced writhing test and formalin induced licking test for anti-inflammatory activity, MECR at 100 and 200 mg/kg doses exhibited significant ($P < 0.001$) reduction of writhing and licking response. **Conclusion** The anti-inflammatory and analgesic effects of *C. rutidosperma* propose that this effect may be a result of both peripheral and central mechanisms. Further study is required to ensure the proper mechanism of action as well as the active ingredient.

Key words

anti-nociceptive; *Cleome rutidosperma*; hot-plate method; tail-flick tests

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1. Introduction

Pain is a sensation appearing as symptoms of many diseases. It is a protective mechanism of body (Almeida et al, 2001). This unpleasant response of body can be defined as triggering of nociceptive stimuli. The activation of specific sensory neuron stimulates nociceptive sensation that transmit the inflammatory information to the spinal cord from where it

* **Corresponding author: Ansari P** E-mail: chemist89ansari@gmail.com

is related to supraspinal levels (Julius and Basbaum, 2001; Verri et al, 2006).

Cleome rutidosperma DC. (Family: Capparidaceae) is a herb of very limited growth of height up to 70 cm with trifoliate roots and small violet-blue flowers, which gradually turn pink along the time, originated in waste grounds and grassy places. The plant is native to West Africa and is going naturally in various parts of tropical America including Southeast Asia (Waterhouse and Mitchell, 1998; Widespread, 1972). Traditionally, the roots, leaves, and seeds of the plants in *Cleome* L. have used like stimulant, with antiscorbutic, anthelmintic, rubifacient, vesicant, and carminative activities (Kiritikar and Basu, 1991). Scientifically, aerial part of *C. rutidosperma* has wide range of medicinal importance. *C. rutidosperma* has been reported including anti-inflammatory, antipyretic, analgesic (Bose and Mandal, 2007), anti-plasmodial (Bose et al, 2010), anti-arthritic (Chakraborty and Roy, 2010), anti-microbial (Bose et al, 2007), anti-oxidant (Chakraborty et al, 2010), wound healing (Mondal and Suresh , 2012), and CNS depressant effects (Bose et al, 2012), and the roots are noted to have hypoglycemic effect and anthelmintic activity (Mondal et al, 2009). In Malaysia, planting of *C. rutidosperma* encircling a field edges may be pondered as part of a pest control program (Burkill, 2004).

Various chemical compounds like alkaloids, glycosides, carbohydrates, steroids, terpenoids, flavonoids, tannins, saponins, lipids, and sugars, proteins and many other compounds have been extracted from the plant (Mondal and Suresh, 2012; Bose et al, 2012). The use of *C. rutidosperma* has been reported in different painful conditions in folk medicine but do not have any study yet. We are the first to study anti-nociceptive activity in both thermal and chemical induced model using *C. rutidosperma* extract.

2. Materials and methods

2.1 Plant materials and extract preparation

Cleome rutidosperma DC. was collected from Mirpur area, Dhaka, and Bangladesh in October, 2013. The plant was recognized by the taxonomists of National Herbarium, Dhaka, Bangladesh (Accession No. 38625) where a voucher sample has been deposited. The whole plant was washed, dried, and grounded. About 500 g of dried plant powder was dissolved in 1500 mL of methanol and stirred periodically for the next three days. Then it was filtered using a sterilized cotton filter and dried using a rotary evaporator. Finally, 37.67 g (yield 7.53%) of dried plant extract obtained and stored at freezer for future use.

2.2 Chemicals

Morphine sulphate (Gonoshasthaya Pharmaceuticals Ltd. Bangladesh), 0.9 % sodium chloride solution (normal saline) (Orion Infusion Ltd., Bangladesh), formalin (Merck, Germany), acetic acid (Merck, Germany), and other reagents of analytical grade were used.

2.3 Animals

Swiss Albino mice (20−25 g) were obtained from the International Center for Diarrheal Disease Research, Bangladesh (ICDDR, b). Then, under the standard environmental conditions, [temperature of (24.0 ± 1.0) °C; relative humidity of 55%−65%, 12 h light and dark cycle], these animals were housed. Mice were provided with the food and fresh water, *ad libitum*. According to the *Ethical Principles and Guidelines for Scientific Experiments on Animals (1995)* formulated by The Swiss Academy of Medical Sciences and the Swiss Academy of Sciences, treatment for all the experimental animals was designed. The protocol was examined, and then accepted by the Ethics Committee of School of Health and Life Sciences, North South University, Dhaka−1229, Bangladesh.

2.4 Acute toxicity test

Mice were grouped into the control and three test groups (n = 5). The test groups received the methanolic extract of *C. rutidosperma* (MECR) orally at the doses of 1500, 2000, and 3000 mg/kg body weight. Then they were separately caged and provided free excess to food and water. They were observed for the next three days (Walker CIB et al, 2008; Imam and Sumi, 2014) for possible changes in the behavior, allergic reactions (like skin rash, itching), and mortality.

2.5 Hot plate test

With only a slight modification, the hot-plate test was conducted in compliance with the method narrated by Eddy and Leimbach (1953). The metal surface's temperature was set at (52 ± 2) °C. The removal of the paw(s) or leaping response on the hot plate was recorded. The selected mice for this study were fasted all-night with only water, *ad libitum*. The mice were then treated with DMSO as control (10 ml/kg, ig), MECR (100 and 200 mg/kg), and morphine as positive control (5 mg/kg, ip). MECR was administered (100 and 200 mg/kg, ig) 30 min prior to the experiment while morphine sulphate was dispensed (5 mg/kg, ip) 15 min prior to the experiment. The response was noted at 30, 60, 90, and 120 min succeeding the treatment, which was in the form of hind paw licking, removal of the paw(s) or leaping. In order to prevent the paw tissues from being damaged, a discontinuation period of 20 s was provided (Eddy and Leimbach, 1953). The results of the hot plate test are presented as a percentage of the maximal possible effect (MPE), which was worked out by using this given formula:

MPE = (post drug latency − pre drug latency) / (cut off period − pre drug latency)

2.6 Tail flick test

Tail flick test monitors whether the drugs possess morphine like effect in mice or not (Toma et al, 2003; Lapa et al, 2009). This test was conducted according to the description of

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