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Study of anti-inflammatory, analgesic and antipyretic activities of seeds of *Hyoscyamus niger* and isolation of a new coumarinolignan

Sajeli Begum [a,*], Bhagawati Saxena [a], Madhur Goyal [a], Rakesh Ranjan [b], Vijaya B. Joshi [a], Ch V. Rao [c], Sairam Krishnamurthy [a], Mahendra Sahai [b]

[a] Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi, India
[b] Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India
[c] National Botanical Research Institute, Lucknow, India

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ABSTRACT

A chemical and biological validation of the traditional use of *Hyoscyamus niger* seeds as anti-inflammatory drug has been established. The methanolic extract of seeds of *H. niger* (MHN) was evaluated for its analgesic, anti-inflammatory and antipyretic activities in experimental animal models at different doses. MHN produced significant increase in hot plate reaction time, while decreasing writhing response in a dose-dependent manner indicating its analgesic activity. It was also effective in both acute and chronic inflammation evaluated through carrageenin-induced paw oedema and cotton pellet granuloma methods. In addition to its analgesic and anti-inflammatory activity, it also exhibited antipyretic activity in yeast-induced pyrexia model. Furthermore, the bioactive MHN under chemical investigation showed the presence of coumarinolignans as major chemical constituent and yielded a new coumarinolignan, cleomiscosin A methyl ether (1) along with four known coumarinolignans, cleomiscosin A (2), cleomiscosin B (3), cleomiscosin A-9′-acetate (4) and cleomiscosin B-9′-acetate (5). The structure elucidation of 1 was done by spectroscopic data interpretation and comparative HPLC analysis. Cleomiscosin A, but not its isomer cleomiscosin B, reduced dry and wet weight of cotton pellet granuloma in mice. This suggests that cleomiscosin A is an important constituent of MHN responsible for anti-inflammatory activity.

1. Introduction

Hyoscyamus niger (L), of Solanaceae family, commonly known as henbane, is widely distributed in Asia and Europe. In India, it is found from Kashmir to Garhwal Himalayas, from 8000 to 11,000 ft high [1]. *H. niger*, a good source of anticholinergic tropane alkaloids also contain nor-tropane alkaloids like calystegins, potent to moderate glycosidase inhibitory activities [2]. Besides alkaloids, the presence of some non-alkaloidal constituents like withanolides [3], tyramine derivatives [3], lignanamides [4] and lignans [5,6] have also been reported.

H. niger also known as Tian-Xian-Zi in China, is well documented in the traditional system of Chinese medicine for its use in stomach cramps, heavy coughs, neuralgia and manic psychosis [1,3]. In Tibetan medicine, the seeds of *H. niger* are used as anthelmintic, antitumor and febrifuge. They are also found to be useful in the treatment of stomach/intestinal pain due to worm infestation, toothache, inflammation of the pulmonary region and tumors [7]. Thus, based on the above facts, we hypothesized that methanolic extract of *H. niger* seeds (MHN) may have potential analgesic and anti-inflammatory activity.

H. niger is traditionally used in Chinese as well as Indian medicine. However, there are no reports on the analgesic, anti-inflammatory and/or antipyretic activities. As the traditional uses of *H. niger* seeds have not been scientifically evaluated, the present study was designed to evaluate the analgesic, anti-

* Corresponding author. Tel.:+91 09455663498 (mobile); fax:+91 542 2368428.
E-mail addresses: sajeli1@rediffmail.com, asbegum.phe@itbhu.ac.in (S. Begum).

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inflammatory and antipyretic effects of MHN in rats and mice using several animal models. Further to decipher the possible basis for such activities, the chemical components of MHN were also investigated.

2. Experimental

2.1. General

IR spectra were recorded on a JASCO-FT/IR-5300 spectrometer. UV spectra were recorded on a Shimadzu UV-1600PC spectrophotometer using spectroscopic grade methanol. ^{1}H and ^{13}C NMR spectra were measured using a Bruker DRX-500 spectrometer with TMS as internal reference. FAB-Mass spectra were recorded on JEOL JMS-700 spectrometer using a direct inlet system. Optical rotations were recorded on JASCO DIP-360 polarimeter (cell length 5 cm). Silica gel used for column chromatography refers to Centron Research Laboratories (India) materials.

2.2. Plant material

The seeds of *H. niger* were purchased from local market in Varanasi, India and was authenticated by Dr. V. K. Joshi, Department of Dravya Guna, IMS, Banaras Hindu University, Varanasi, India. A voucher specimen (AS/HN/02) has been deposited in the Department of Medicinal Chemistry of the same Institution.

2.3. Preparation of extract

Dried and coarsely powdered seeds of *H. niger* (1 kg) were extracted with methanol in a soxhlet extractor for 48 h. The methanolic extract thus obtained was concentrated under reduced pressure and the yield was calculated to be 20% w/w.

2.4. Animals

Experiments were conducted using adult male Wistar rats (150–200 g) and male swiss albino mice (25–30 g) obtained from the central animal house of Institute of Medical Sciences, Banaras Hindu University, Varanasi (Reg. No.542/02/ab/CPCSEA). The animals were housed in groups for a minimum of 7 days prior to pharmacological experiments. The animals had free access to commercial food pellets (Doodh dhara Pashu Ahar, India) and water unless stated otherwise. The minimum number of animals and duration of observation required to obtain consistent data were employed. "Principles of laboratory animal care" (NIH publication number 85–23, revised 1985) guidelines were followed.

2.5. Drugs

Pentazocine was obtained from Ranbaxy Laboratories Ltd. (New Delhi, India). Acetylsalicylic acid (ASA), phenylbutazone, indomethacin and carrageenin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Paracetamol was purchased from Cipla Ltd. (Mumbai, India). Acetic acid used was of analytical grade obtained from Loba Chemicals (Mumbai, India), while pentobarbitone sodium was procured from Loba Chemie Indo Austranal Co. (Mumbai, India).

2.6. Evaluation of analgesic activity

2.6.1. Hot plate method

The method originally developed by Woolfe and McDonalds [8] was followed. The mice ($n=6$) were placed in Eddy's hot plate kept at a temperature of 55 ± 0.5 °C. A cutoff time of 30 s was fixed to avoid damage to the paw. Reaction time of response was recorded using a stopwatch. Control animals were treated with vehicle (0.3% CMC, oral) and test groups were pretreated with 100, 200 and 400 mg/kg of MHN once a day for 5 consecutive days. Pentazocine (10 mg/kg, i. p.) was administered as a positive control. Experiments were performed 1 h after last dose of test or standard drug. Percentage analgesia was calculated using the following formula.

$$\%\,\text{Analgesia} = (\text{LT}/\text{LC} - 1) \times 100$$

LT Latency in treated rats
LC Latency in control group of rats

2.6.2. Writhing test

Mice ($n=6$) of control group were treated orally with vehicle (3 ml/kg) of 0.3% CMC, while those of test groups with MHN (100, 200, and 400 mg/kg) once a day for 5 consecutive days. Acetyl salicylic acid (200 mg/kg, oral) was administrated to mice as a positive control. Writhing was induced in mice by intraperitoneal injection (10 ml/kg) of 0.6% acetic acid after 1 h of the oral administration of tested drugs on the 5th day. The number of writhings was counted over a 15 min period as previously reported [9,10].

2.7. Evaluation of anti-inflammatory activity

2.7.1. Carrageenin-induced rat paw edema

Rats ($n=6$) of control group were treated orally with vehicle (3 ml/kg) of 0.3% CMC and those of the test groups with MHN (50, 100 and 200 mg/kg) once a day for 5 consecutive days. Phenylbutazone (100 mg/kg, oral) was administered as a positive control. On the 5th day, 1 h after drug treatment, 1% carrageenin was injected into the plantar tissue of the right hind paw [11]. The contra-lateral hind paws were injected with 0.1 ml of saline as control. Paw volume was measured plethysmographically at 0 and 3 h after carrageenin injection.

Percentage inhibition of oedema was calculated as follows:

$$\%\,\text{inhibition} = (1 - \text{VT}/\text{VC}) \times 100$$

where VT and VC are the paw volume in treated rats and control group of rats respectively.

2.7.2. Cotton pellet method

Male Wistar rats were anesthetized using 25 mg/kg of pentobarbitone sodium. Under sterilized conditions, cotton pellets of 30 mg were implanted subcutaneously in the interscapular area. Animals ($n=5$) were treated orally with 3 ml/kg of vehicle (0.3% CMC), MHN (100, 200 and 400 mg/kg) and indomethacin (5 mg/kg, oral) as standard, once a day for 5 consecutive days. On the 5th day, after 1 h of drug treatment animals were killed via cervical dislocation and the cotton pellets with the granuloma tissue around them were removed.