Journal of Traditional and Complementary Medicine 7 (2017) 133–140

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Original article

The possible mode of antitussive and expectorant activity of the ethanol seed extracts of *Picralima nitida* ((Stapf) Th. & H. Durand)

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ARTICLE INFO

Article history:
Received 24 August 2015
Received in revised form
24 March 2016
Accepted 9 May 2016
Available online 13 July 2016

Keywords:
Muco-suppressant
Mast cell stabilizer
Antibacterial
Anxiolytics
Antioxidant

ABSTRACT

It has been established that *Picralima nitida* has antitussive effect. This study therefore aimed at determining the possible mode of antitussive and expectorant activity of an ethanolic seed extract of *P. nitida* (PNE). The muco-suppressant, mast cell stabilization, and the anxiolytic effects of PNE were ascertained using ammonium chloride-induced phenol red secretion in BALB/c mice; compound 48/80-induced mesenteric mast cell degranulation assay; and the open field and the elevated plus maze models respectively. Antibacterial potential was ascertained by the agar plate diffusion method and its antioxidant potential by the 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) free radical scavenging, linoleic acid lipid peroxidation, reducing power, and total antioxidant assays. Data obtained was analyzed using One-way analysis of variance (ANOVA) with Dunnett's Multiple Comparison *post hoc* test. PNE (100–500 mg/kg) reduced ($P \leq 0.05$–0.001) tracheal phenol red secretion. The extract (100–500 μg/ml) also dose-dependently ($P \leq 0.05$–0.0001) stabilized mast cells. PNE (100–500 mg/kg) increased open arm activities in the elevated plus maze ($P \leq 0.05$) as well as central zone exploration ($P \leq 0.05$) in the open field test. PNE (10–50 mg/ml) showed activity against *Staphylococcus aureus*, *Streptococcus pneumonia*, *Escherichia coli*, *Klebsiella pneumonia*, and *Salmonella typhi*. By the assays, PNE showed significant antioxidant effect. The ethanolic seed extract of *P. nitida* has demonstrated very significant mast cell stabilizing, mucus suppressant, and antioxidant activity as well as substantial antibacterial and anxiolytic properties; all of which could contribute to its antitussive and expectorant property.

1. Introduction

Antitussives are pharmacological agents that suppress the cough reflex.[1] They act via mechanisms classified as central or peripheral; though some act through both pathways.[1] The antitussive effect of an ethanolic seed extract of *Picralima nitida* (PNE) has previously been established by the authors.[2] However, the mode of activity of cough medicines could be attributed to at least five reasons: pharmacological, physiological, true placebo, psychological and non-specific action. Plant/plant products exhibiting antitussive activity may not have one mode of expressing this pharmacological effect.[3,4] The purport of this study was to establish the possible mode of antitussive and expectorant activity of an ethanolic seed extract of *P. nitida*. This will be ascertained by; evaluating muco-suppression effect, assessing mast cell stabilization effect, and establishing antibacterial, anxiolytic, antioxidant activity in various rodent models.

2. Materials and methods

2.1. Plant collection and extraction

The pods of *P. nitida* were collected from the KNUST botanical garden in Kumasi, Ghana, in February, 2013. Authentication was done at the Department of Pharmacognosy, KNUST. The pods were

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Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2016.05.003

opened, the seeds removed, air-dried, and milled into powder. The powder weighing four (4) kg was extracted by cold maceration with 70% ethanol over a period of 72 h. The resulting extract was then concentrated at a temperature of 40 °C and under low pressure to a syrupy mass in a rotary evaporator (Rotavapor R-210, Buchi, Switzerland). The syrupy mass obtained was then dried in a hot air oven (Gallenkamp, UK) maintained at 40 °C to obtain 0.532 kg (% yield: 13.3%) of a solid mass of *P. nitida* extract (PNE).

2.2. Drugs and chemicals

Sodium cromoglycate (Ashford Laboratory Ltd., Macau); ammonium chloride (Philip Harris, Hyde-Cheshire; UK); Phenol red and sodium chloride (BDH Chemicals Ltd, Poole, England); Ketotifen fumarate (Novartis Pharma AG, Basle, Switzerland); Compound 48/80 and toluidine blue (Sigma Chemical Co., St. Louis, MO, USA); Sodium hydroxide (Avondale, England); Acetic acid, Diazepam (Sigma–Aldrich Inc., St. Louis, MO, USA), Caffeine (Sigma–Aldrich Inc., St. Louis, MO, USA) were used in this study.

2.3. Animals

BALB/c mice (20–30 g) and a Sprague–Dawley rat (130 g), obtained from the animal house of the Department of Pharmacology, KNUST, Kumasi, Ghana were used in this study. They were fed on standard rodent pellet diet (Agricare Ltd, Tanoso, Kumasi, Ghana) and water *ad libitum*. The animals were kept in the experimental area of the Departmental animal house at ambient conditions of light, temperature and humidity for seven (7) days prior to experimentation and during experimentation.

2.4. Muco-suppressant effect of PNE

The muco-suppressant effect of PNE was determined using the ammonium chloride-induced tracheal phenol red secretion model; described previously.[5] BALB/c mice were put into five groups, I–V, ($n = 5$) and pre-treated, for 30 min, as follows: Group I, two (2) ml/kg normal saline (*p.o*); Group II, 100 mg/kg Sodium cromoglycate (i.p); Groups III–V, 100, 300, and 500 mg/kg of PNE (*p.o*) respectively. Tracheal mucus secretion was then induced with five (5) mg/kg ammonium chloride *per os*. Animals were then injected with 500 mg/kg phenol red, intraperitoneally, 30 min later. The trachea was excised from each mouse and cleared of adhering tissues, after sacrificing it by cervical dislocation (30 min after the phenol red injection). Each excised trachea was washed in three (3) ml physiological saline; sodium hydroxide (0.3 ml NaOH, one (1) M) was then added to the washing to stabilize the pH of the lavage fluid. The absorbance of the mixture was then taken at a wavelength of 460 nm using a spectrophotometer (T90 + UV/VIS Spectrometer – PG Instruments Ltd). A calibration curve for phenol red was made; from which concentrations of phenol red secreted by mice tracheae were extrapolated.

2.5. Compound 48/80-induced mesenteric mast cell degranulation

Mast cell stabilizing effect was ascertained using compound 48/80-induced mesenteric mast cell degranulation as described previously.[6] Sprague–Dawley rat intestinal mesenteries was excised into several pieces and put into five groups, G1–G5 ($n = 5$), in petri dishes containing tyrode solution. The mesenteries were then subjected to the following treatment: G1, normal saline; G2, 20 μg/ml ketotifen fumarate; G3–G5, 100, 300, and 500 μg/ml PNE respectively. The petri dishes were then incubated at 37 °C for 15 min after which one (1) ml of compound 48/80 solution (10 μg/ml) was added and incubated at 37 °C for ten (10) min. The mesenteric pieces were then fixed in 10 % buffered formalin and processed in xylene and acetone. They were then stained with 0.1 % toluidine blue and observed under a Leica DM 750 microscope (Leica Microsystems CM5 GmbH, Wetzlar – Germany) for both intact and degranulated cells. The percentage mast cell degranulation for each treatment was estimated.

2.6. Antibacterial property of PNE

The antibacterial property of PNE was investigated using the agar well diffusion method.[7] Ten (10) test tubes (labeled T1–T10) each containing 20 ml nutrient agar were stabilized. The molten agars were inoculated with 0.2 ml each of the following organisms: T1 and T2, *Staphylococcus aureus*; T3 and T4, *Streptococcus pneumonia*; T5 and T6, *Salmonella typhi*; T7 and T8, E*scherichia coli*; T9 and T10, *Klebsiella pneumonia*. The seeded agars were poured into labeled sterile petri dishes (P1–P10) and allowed to set. Using a sterile cork borer number seven (7), five wells were created in each of the ten petri dishes. Various concentrations of PNE were prepared and poured in the wells to three-quarter (3/4) full as follows: P1, P3, P5, P7 and P9 each received 0.05, 0.5, 5, 25 mg/ml PNE, while P2, P4, P6, P8 and P10 received 0.1, 1, 10, 50 mg/ml PNE, in four of the five wells created. The petri dishes were covered and left on the bench for 45 min in order to allow effective diffusion of the extract. They were then incubated at 37 °C for 24 h, after which they were examined for zones of growth inhibition around the wells. Amoxicillin (1 %) was used as a positive control in the test against *S. aureus*, *S. pneumonia*, *E. coli* and *K. pneumonia*. Ciprofloxacin (0.1 %) was the control in the test against *S. typhi*. The test was carried out in triplicate.

2.7. Anxiolytic effect of PNE

2.7.1. Elevated plus maze

This test has widely been used to measure anxiety in rodents especially mice.[8] The apparatus was made of plexiglas and consisted of two open arms and two closed arms of dimensions 30 cm × 5 cm × 15 cm. These arms extend from a central square platform (5 cm × 5 cm). The maze was elevated to a height of 60 cm above the floor and placed in a lit room. Mice were divided into ten groups ($n = 6$) and received the following treatment: vehicle-control, PNE (100, 300, 500 mg/kg), Diazepam (0.1, 0.3, 1.0 mg/kg), and Caffeine (3, 10, 30 mg/kg). Diazepam and caffeine served as reference anxiolytic and anxiogenic drugs respectively. The vehicle, PNE, caffeine were orally administered to their respective animals' an hour before the experiment, whiles diazepam was given intraperitoneally 30 min before the experiment. At the start of the experiment, animals were individually placed at the center of the maze, facing one of the enclosed arms and their behavior videotaped for 5 min with a digital camera placed 100 cm above the maze. After each test, the maze was carefully cleaned up with 10% ethanol solution. Behavioral parameters were scored from the videotapes as follows:

1) number of closed and open arm entries—(absolute value and percentage of the total number);
2) time spent in exploring the open and closed arms of the maze—absolute time and percentage of the total time of testing
3) number of head-dips (absolute value and percentage of the total number)—protruding the head over the ledge of either an open (unprotected) or closed (protected) arm and down toward the floor;
4) number of stretch-attend postures (absolute value and percentage of the total number)—the mouse stretches forward and