

Antitussive, expectorant and analgesic effects of the ethanol seed extract of *Picralima nitida* (Stapf) Th. & H. Durand

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Abstract

Picralima nitida is used traditionally for management of cough. This study, therefore, investigated the antitussive, expectorant, and analgesic properties of the ethanolic seed extract of *Picralima nitida* (PNE), and ascertained its safety for use. Presence of secondary metabolites, and safety of PNE (10-2000 mg/kg) were evaluated by preliminary phytochemical screening, and by Irwin's test respectively. Percentage reduction in cough count, percentage increase in latency of cough, and percentage protection offered by PNE were established by the citric acid-induced cough, acetylcholine- and Histamine-induced bronchoconstriction models. Dunkin-Hartley guinea pigs were treated with 100-500 mg/kg PNE or reference drugs, dihydrocodiene, atropine, mepyramine. Expectorant property of PNE (100-1000 mg/kg) was determined using the tracheal phenol red secretion; with ammonium chloride as a reference medication. Percentage maximal possible analgesic effect in the tail immersion test and the total nociceptive score in acetic acid-induced abdominal writhes, after treatment of BALB/c mice with PNE (100-500 mg/kg), diclofenac, and morphine were also estimated. Phytochemical screening revealed the presence of tannins, alkaloids, glycosides, saponins, steroids, terpenoids and anthraquinones. PNE did not cause any extract-related physical, pharmacological and CNS toxicities or mortality; sedation was observed at doses 1000-2000 mg/kg. It showed significant dose-dependent reduction in cough count, and increased cough latency. PNE (1000 mg/kg) enhanced tracheal phenol red secretion. PNE (100-500 mg/kg) significantly and dose dependently increased tail withdrawal latencies, and nociceptive score. PNE has antitussive, expectorant, and analgesic properties, with an LD₅₀>2000 mg/kg.

Keywords: Tracheal phenol red secretion; Citric acid-induced cough; Total nociceptive score; Tail withdrawal latency; *Picralima nitida*

INTRODUCTION

Cough is defined as a forced expulsive maneuver, usually against a closed glottis which is associated with a characteristic sound (1). Cough can be the result of several respiratory tract disorders which may require drug treatment for its relief. Chronic cough is disturbing to the patient as it monumentally affects the patient's quality of life (2).

Bronchoconstriction is significant in cough induction since the process stimulates intrapulmonary rapidly adapting receptor (RAR), a type of cough receptor to cause or

enhance the sensitivity of the cough (3). RAR activation initiates bronchospasm and mucus secretion via parasympathetic reflexes.

Cough can be described as non-productive (dry) or productive (chesty). Antitussives are effective in managing non-productive cough but not as effective with productive cough except when the antitussive has expectorant property (4). Recently, many pharmacological agents have been shown to have both antitussive and expectorant effect making them useful for both chesty and dry cough (4). The essence of such dual effect is affirmed by the fact that many pharmaceutical

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formulations have such combination. Some herbal preparations have also shown both antitussive and expectorant effects (5-7). Cough just like other medical conditions lead to complications (8). Notable, worrisome and widely reported among them is pain (9,10). Cough and pain have many things in common; they originate in afferent nervous systems that detect and signal real or impending threats to the organism. There is therefore the need to find drugs with bronchodilator, expectorant, and analgesic properties.

A lot of medicines have been used over the past centuries as cough suppressants or antitussives but due to various reasons, especially adverse effects, very few are still in use. The management of symptomatic cough has usually been with the opioid antitussives like codeine. Codeine and dextromethorphan were previously questioned due to their adverse effects, and recently the efficacy of codeine as a “gold standard” for cough has been found to be questionable (11). This has called for the need to find new effective antitussives with relatively less adverse effect.

Picralima nitida is a plant that promises to be an effective antitussive. Apart from the evidence obtained from the indigenous population with regard to its efficacy in cough, other pharmacological properties confirmed on the plant by retrospective researchers give good reasons why the antitussive effect should be confirmed experimentally: the alkaloids in the plant have demonstrated significant activity on opioid receptors (12). Anti-inflammatory (13,14) antibacterial (15-17) as well as stimulatory effect on the β_2 -adrenoreceptors in the trachea may all contribute to it being a potential therapeutically effective antitussive against several forms and etiologies of cough.

This study, therefore, sought to establish the antitussive, expectorant, as well as analgesic properties of *P. nitida* after preliminary phyto-chemical screening, and Irwin’s test which ascertain its safety for the use.

MATERIALS AND METHODS

Plant collection

The pods of *P. nitida* were collected from the botanical garden, KNUST, Kumasi,

Ghana, in December, 2012. This was authenticated by Dr. Kofi Annan, of the Department of Pharmacognosy, KNUST.

Preparation of plant extract

The pods of *P. nitida* were opened, and the seeds removed, air-dried, and milled into powder. A 3 kg quantity of the powder was extracted with 70% ethanol by cold maceration over a 72 h period. The extract obtained was concentrated at 40 °C and under low pressure using a rotary evaporator (Rotavapor R-210, Buchi, Switzerland) to a syrupy mass. The syrupy mass obtained was then dried in a hot air oven (Gallenkamp, UK) maintained at 40 °C to obtain 0.389 kg (% yield: 12.9%) of a solid mass of *P. nitida* extract (PNE) for use in this study.

Drugs and chemicals

Phenol red and sodium chloride (BDH Chemicals Ltd, Poole, England), sodium hydroxide (Avondale, England), ammonium chloride (Philip Harris, Hyde-Cheshire), citric acid (Fisons Scientific Equipment, Loughborough) and dihydrocodeine (Bristol Laboratories Ltd., UK) were some chemicals used in the current study.

Animals

Dunkin-Hartley guinea-pigs (270-350 g) and BALB/c mice (15-20 g) were all obtained from the Animal house of the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST; and fed on standard rodent pellet diet obtained from the Agricare Ltd, Tanoso, Kumasi, Ghana, and given water *ad libitum*. The animals were kept in the experimental area of the departmental animal house under ambient conditions of temperature, humidity, and light for 10 days prior to the experimentation. All animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals and experiments were approved by the Faculty Ethics Committee.

Phytochemical screening

Preliminary phytochemical screening was conducted on PNE, to determine the secondary metabolites present, by methods described by Sofowora and Trease and Evans (18,19).

Safety assessment

This test was carried out as described earlier (20) to establish safe doses to use in this study. In this test BALB/c mice were treated *per os* with distilled water or 10, 30, 100, 300, 1000 and 2000 mg/kg PNE and observed critically at times 0, 15, 30, 60, 120, and 180 min and then at 24 h. The animals were observed for acute toxicity including physical appearance, behavioral changes, central and autonomic nervous system effects, clinical symptoms and death.

Antitussive effect of PNE

The antitussive effect of PNE was investigated using the citric acid-induced cough model (21) with modifications, as well as the acetylcholine- and histamine-induced bronchoconstriction model (22,23).

Citric acid-induced cough

Guinea pigs were individually placed in a perspex chamber (24 × 14 × 24 cm) and exposed to 15% citric acid, delivered by an ultrasonic nebulizer, for 5 min. The animals were then monitored visually within this exposure time for cough; the latency and counts, of which, were taken as the basal values. The cough count was also taken for five minutes outside the chamber, post citric acid-exposure, as a way of monitoring its recovery. The guinea pigs were put into five groups, I-V, (n = 5) and treated orally as follows: Group I, 2 ml/kg normal saline, Group II, dihydrocodeine (20 mg/kg), Groups III-V PNE (100, 300 and 500 mg/kg, respectively). An hour later, the animals were again exposed to the citric acid and the latency of cough and cough count were recorded. The procedure was repeated at hours 2 and 3 after treatment. Antitussive activity was then evaluated in each guinea-pig as the percentage reduction in the number of coughs, and percentage increase in latency of cough in comparison with the previously established control basal value, calculated as below:

$$\text{Percentage reduction in cough count} = [1 - (C2/C1)] \times 100 \quad (1)$$

where, C1 is basal values and C2 is the total number of coughs after treatment.

$$\text{Percentage increase in latency of cough} = [(L2/L1)-1] \times 100 \quad (2)$$

where, L1 is basal values, and L2 is the latency of coughs after treatment.

Acetylcholine- and histamine-induced bronchoconstriction

In acetylcholine-induced bronchoconstriction, twenty five Dunkin-Hartley guinea pigs were each placed in a perspex chamber (24 × 14 × 24 cm) and exposed to an atomized mist of 0.5% acetylcholine aerosol using a nebulizer. As exposure to acetylcholine causes respiratory distress and cough due to bronchoconstriction, time to onset of cough (TOC) was recorded as basal values for each animal. After 24 h (full recovery), the guinea pigs were grouped into five (n = 5) and given the following treatments: Group I, 2 ml/kg normal saline (control); Group II, 5 mg/kg atropine; Groups III-V, 100, 300, or 500 mg/kg PNE. After an hour, the animals were exposed to acetylcholine aerosol and TOC were determined. The protection offered by the treatment against bronchoconstriction and cough was calculated as follows:

$$\text{Percentage protection} = [1 - (TOC1/TOC2)] \times 100 \quad (3)$$

where, TOC1 is basal TOC, and TOC2 is TOC after drug treatment.

The same protocol was used in histamine-induced bronchoconstriction, using 0.8% histamine and 8 mg/kg mepyramine.

Expectorant property of PNE

The expectorant effect of PNE was determined using the tracheal phenol red secretion as described previously (24,25). BALB/c mice, acclimatized for a week in the experimental laboratory, were grouped into six (n=5). Group 1, the control, was administered normal saline. Group 2 received 1000 mg/kg ammonium chloride *per os*, while groups 3, 4, 5 and 6 received respectively 100, 300, 500, and 1000 mg/kg PNE respectively. Treatments were continued for four (4) consecutive days. One hour after drug administration on day 4, all animals were injected intraperitoneally with

5% phenol red in normal saline (0.1 ml/10 g). Thirty minutes after phenol red injection, animals were sacrificed by cervical dislocation and their tracheae removed and each placed into 2 ml normal saline immediately. After 15 min of ultra-sonication, 2 ml of 5% sodium bicarbonate was added to the saline and the optical density of each mixture was measured at 558 nm using a UV/Visible spectrophotometer (UV-7501).

Analgesic property of PNE

The analgesic property of PNE was assessed by the tail immersion and acetic acid-induced abdominal writhing models.

Tail immersion test

The extreme end of the tail (3 cm) of BALB/c mice was immersed in a water of temperature 50 ± 0.5 °C. The time taken for the mouse to flick its tail was regarded as the tail withdrawal latency. A cut off latency of 10 s was set in order to prevent tissue damage.

Selected mice were randomly assigned to ten groups, I-X, (n = 5) and treated as follows: Group I, normal saline (control), Groups II-IV, diclofenac treatment (10, 30 and 100 mg/kg, i.p, respectively); Groups V-VII, morphine treatment (1, 3 and 10 mg/kg, i.p, respectively); Group VIII-X, PNE treatment (100, 300 and 500 mg/kg, p.o respectively). Thirty minutes after intraperitoneal administration or an hour after oral administration, their tail was immersed in the water bath at times 30, 60, 90, 120, 150, 180 min and the post-drug latency was determined. The percentage maximal possible effect (% MPE) was calculated from the reaction times using the following formula:

$$\% \text{ MPE} = [(T_2 - T_1) / (T_0 - T_1)] \times 100 \quad (4)$$

where, T_1 and T_2 are the pre- and post- drug treatment latency times, and T_0 is the cut-off time

Acetic acid-induced writhing assay

The test was done with the method as described previously (26) with slight modifications. BALB/c mice were divided into seven groups, I-VII, (n = 5) and received the following treatment: Group I, Normal saline (control); Groups II-IV, diclofenac treatment

(10, 30, or 100 mg/kg, i.p respectively); Groups V-VII, PNE treatment (100, 300, or 500 mg/kg, p.o, respectively). Thirty minutes (for orally administered drugs) or 1 h (for drugs administered intraperitoneally) later, the mice were injected with acetic acid (0.6 %, 10 ml/kg, i.p.) and each placed individually in a perspex chamber (testing chamber: $15 \times 15 \times 15$ cm). A mirror inclined at 45° below the floor of the chamber allowed a complete view of the mice. Ten minutes after acetic acid administration, responses were captured (for 20 min) by a camcorder (Everio™, model GZ-MG1300, JVC, Tokyo) placed directly opposite the mirror and attached to a computer for analysis. Acetic acid-induced writhing is characterized by extension of the abdomen combined with the outstretching of the hind limbs. The behavior was tracked by JWatcher TM Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sidney, Australia available at <http://www.jwatcher.ucla.edu/>). The antinociceptive effect was ascertained by the extent to which the various treatments reduced the number of writhes in the animals.

Data analysis

Data obtained were expressed as mean \pm SEM. Statistical analyses were done by analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test (*post-hoc*test) using Graph-Pad Prism for Windows Version 6.0.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening of PNE indicated the presence of alkaloids, tannins, steroids, glycosides, anthraquinones, and terpenoids.

Safety assessment

Cage side examination of mice (even up to two weeks) revealed no physical signs of toxicity (such as unkemptness, hair loss or ulcers on the skin), neurotoxicity, stimulation of autonomic function, or CNS excitation or depression with PNE treatment at doses of 10, 30, 100 and 300 mg/kg relative to control mice. However, mild sedation was observed at doses of 1000 and 2000 mg/kg. No mortality was recorded at all doses.

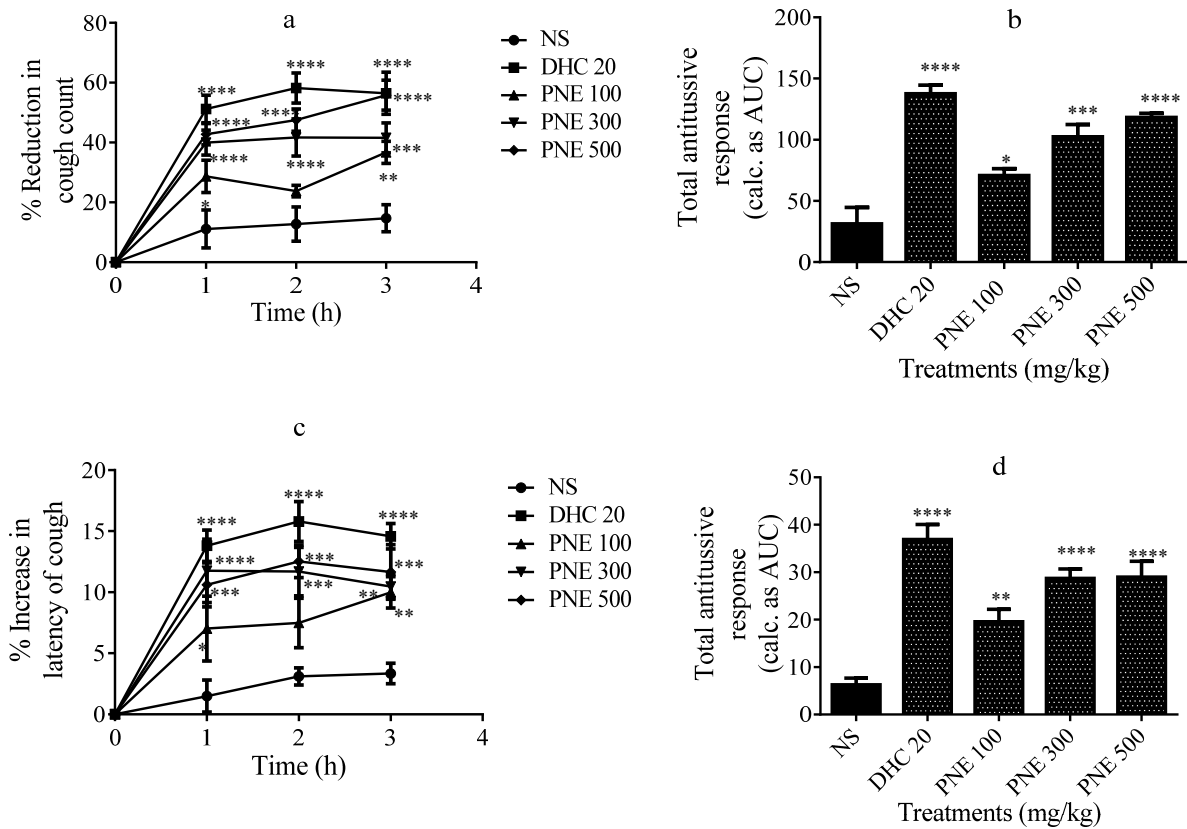


Fig. 1. Effects of PNE, dihydrocodeine (DHC), and normal saline (NS) on the time course curve of a; percent of reduction in cough count and c; percent of increase in latency to cough and b and d; their AUC's respectively in the citric acid-induced cough test. Data plotted are means \pm SEM; (n = 5). **** $P \leq 0.0001$, *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, compared to vehicle-treated group (ANOVA followed by Dunnett's post-hoc test).

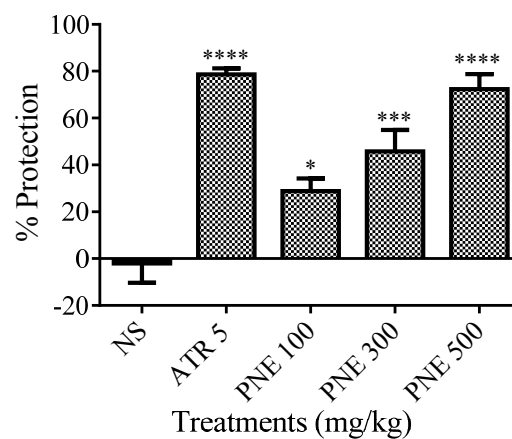


Fig. 2. Effect of PNE, atropine (ATR) and normal saline (NS) on acetylcholine-induced bronchoconstriction. Values plotted are means \pm SEM; (n = 5). **** $P \leq 0.0001$, *** $P \leq 0.001$, * $P \leq 0.05$, compared to vehicle-treated group (ANOVA followed by Dunnett's post-hoc test).

Antitussive effects

Our results showed that both dihydrocodeine and PNE (100, 300, and 500mg/kg) dose-dependently reduced ($P \leq 0.05$) cough count, and increased significantly ($P \leq 0.01$) the latency of cough (Fig. 1). Atropine and PNE (100, 300, 500 mg/kg) significantly ($P \leq 0.05-0.0001$) protected the animals against bronchoconstriction and cough induced by acetylcholine. The percentage protection offered by PNE was dose-dependent (Fig. 2). Similarly, mepyramine and PNE (300, 500 mg/kg) reduced significantly ($P \leq 0.01-0.0001$) bronchoconstriction and cough induced by histamine (Fig. 3).

Expectorant property of PNE

PNE at doses of 100, 300, 500 mg/kg did not show any significant increase ($P > 0.05$) in tracheal phenol red secretion, compared to the control. However, the 1000 mg/kg dose exhibited a significant effect ($P \leq 0.05$) although lesser in magnitude than that for ammonium chloride ($P \leq 0.01$), the reference expectorant drug (Fig. 4).

Analgesic property of PNE

Tail immersion test

Both PNE and the reference drugs,

compared to the control, caused an increase in the tail withdrawal latency, calculated as a percentage of % MPE. There were significant effects of drug treatment on tail withdrawal latencies (PNE: $P \leq 0.0001$; diclofenac: $P \leq 0.0001$; morphine: $P \leq 0.0001$) (Figs. 5a, 5c, and 5e). PNE (100–500 mg/kg) dose dependently increased ($P \leq 0.0001$) tail withdrawal latencies. Diclofenac (10-100 mg/kg) likewise exhibited increased tail withdrawal latencies ($P = 0.0002$). Morphine (1-10 mg/kg) also showed a significant ($P \leq 0.0001$) and dose dependent increase in tail withdrawal latencies (Figs. 5b, 5d, and 5f).

Acetic acid-induced writhing assay

PNE (100, 300, 500 mg/kg) and diclofenac (10, 30, 100 mg/kg) suppressed this writhing (Fig. 6). There were significant effects of drug treatment on number of writhes (PNE: $P \leq 0.0001$; diclofenac: $P \leq 0.0001$) compared to the control (Figs. 6a, 6c). PNE significantly and dose-dependently reduced abdominal writhes over the 20 min observation ($P \leq 0.0001$; Fig. 6b). Diclofenac also significantly reduced abdominal writhes ($P \leq 0.0001$; Fig. 6d).

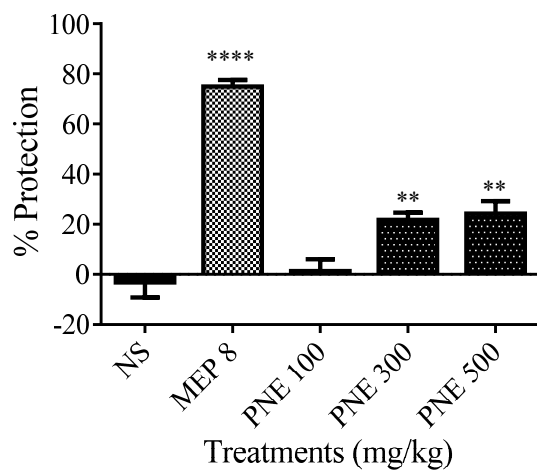


Fig. 3. Effect of PNE, mepyramine (MEP) and normal saline (NS) on bronchospasm induced by histamine. Values plotted are means \pm SEM; (n = 5). **** $P < 0.0001$, ** $P < 0.01$ compared to vehicle-treated group (ANOVA followed by Dunnett's post-hoc test).

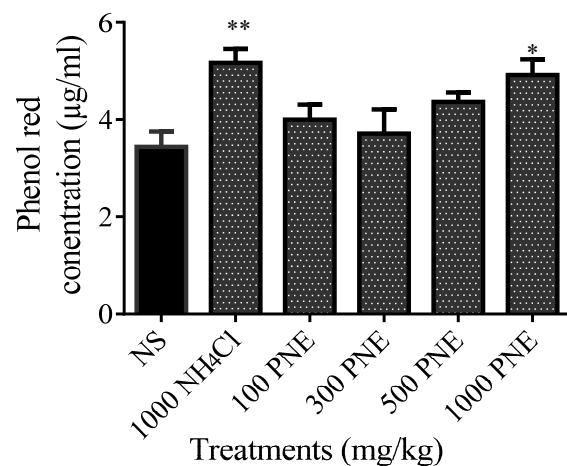


Fig. 4. Effect of PNE, ammonium chloride (NH₄Cl), and normal saline (NS) on tracheal phenol red secretion in mice as a measure of the expectorant effect. Values plotted are means \pm SEM; (n = 5). ** $P < 0.01$, * $P < 0.05$ compared to vehicle-treated group, (ANOVA followed by Dunnett's post-hoc test).

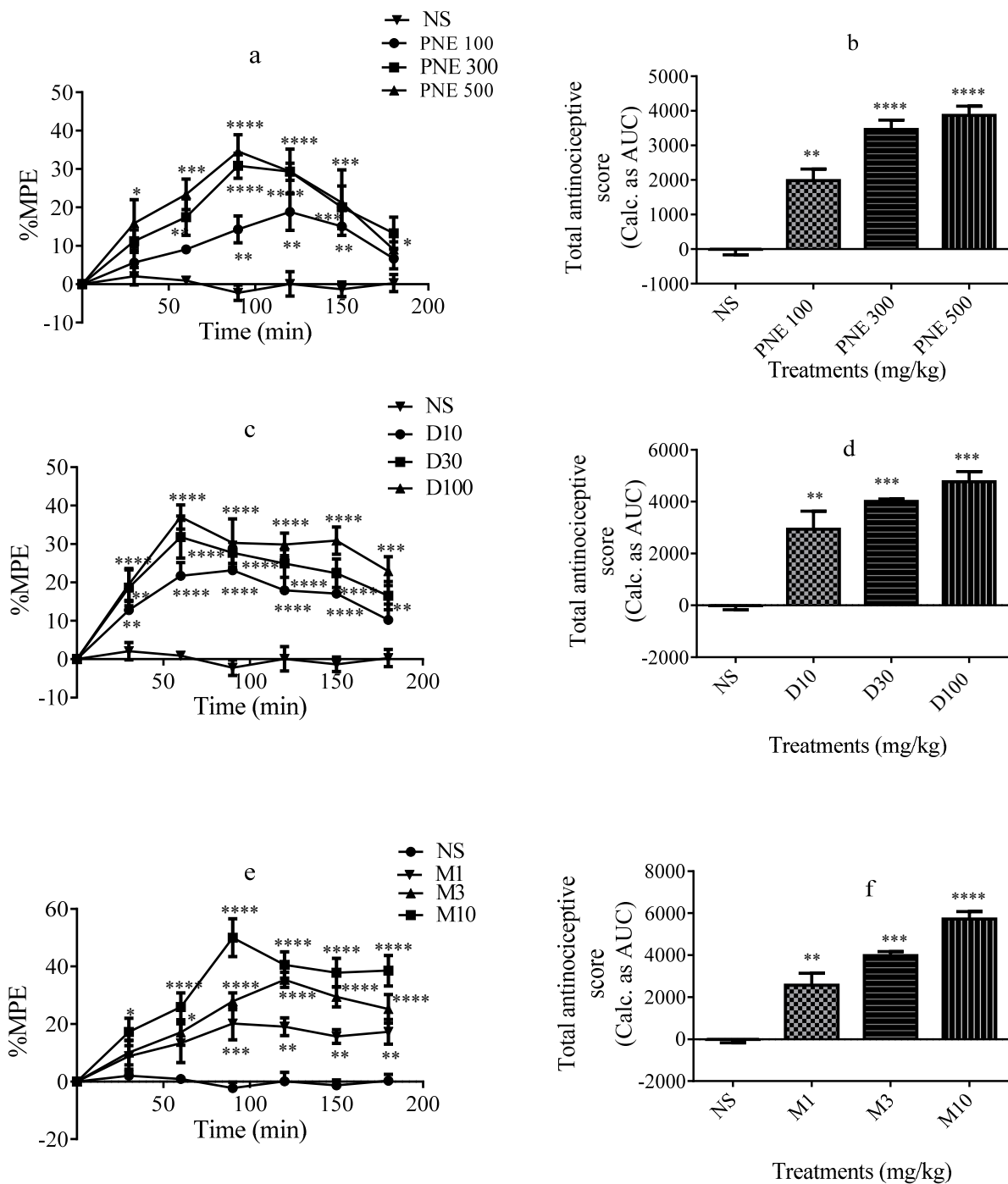


Fig. 5. Effect of PNE, diclofenac (D), morphine (M) and normal saline (NS) on the time course curve a, c and e; of the tail immersion test and b, d and f, the AUC in rats. Values plotted are means \pm SEM; (n=5). **** $P \leq 0.0001$, *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$ compared to vehicle-treated group, (ANOVA followed by Dunnett's post-hoc test).

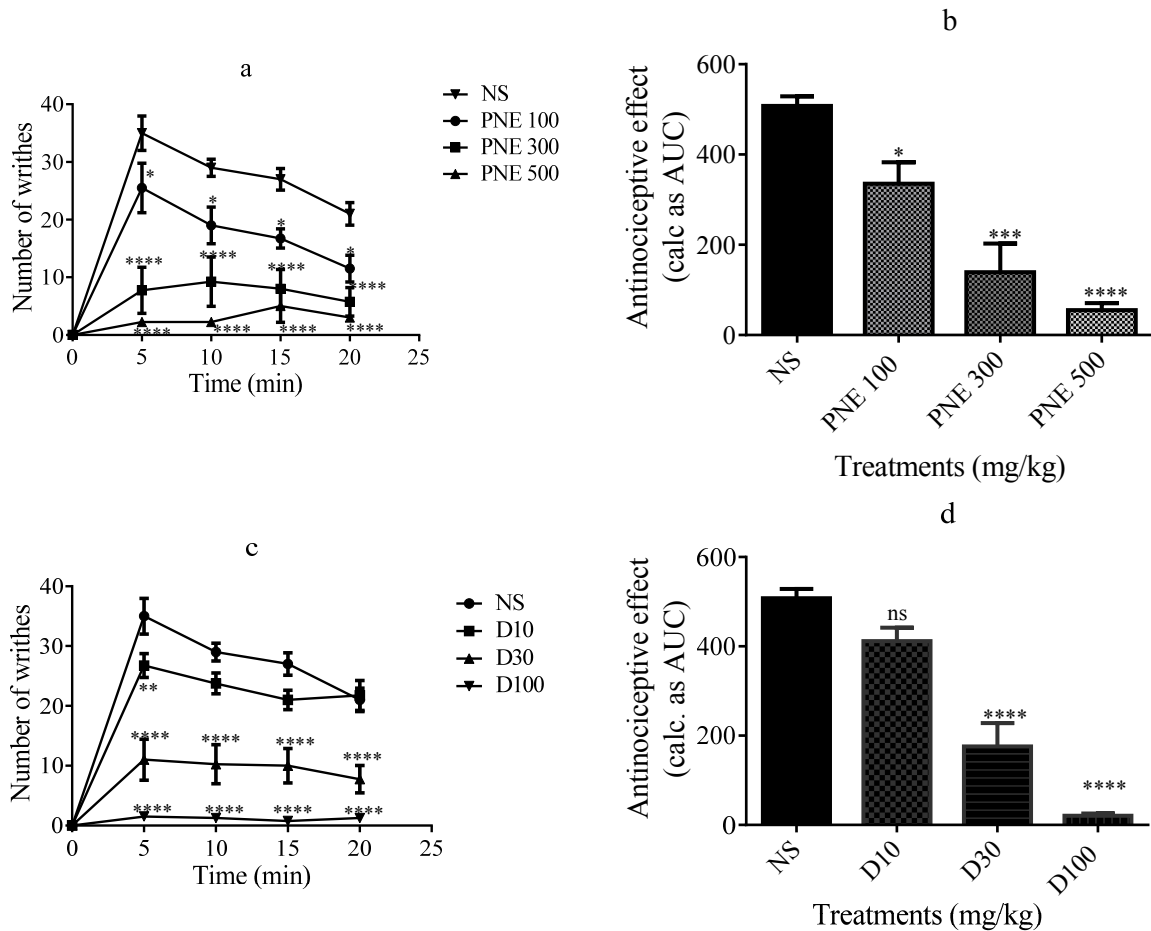


Fig. 6. Effect of PNE, diclofenac (D) and normal saline (NS), a and c; on the time course curve of acetic acid-induced abdominal writhes; and b and d; on the total nociceptive score in the mice. Data are expressed as mean \pm SEM, (n = 5). **** $P \leq 0.0001$, *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$ compared to vehicle-treated group, (ANOVA followed by Dunnett's post-hoc test).

DISCUSSION

This study investigated antitussive, expectorant, and analgesic properties of the PNE, and ascertained its safety for use. To achieve this, preliminary phytochemical screening and an acute exposure safety profile test were conducted. Phytochemical screening revealed the presence of tannins, alkaloids, glycosides, saponins, steroids, terpenoids and anthraquinones which could possibly contribute to the antitussive, expectorant and the analgesic effects being studied. It was determined from the safety studies that PNE has an $LD_{50} > 2000$ mg/kg. This obviously speaks well of the safety of the extract. The reduction in cough count, increase in latency

of cough, and finally the protection offered by PNE against histamine and acetylcholine all give an indication of the extract's antitussive potential. The expectorant property of PNE as confirmed in this work will make it a remedy for productive cough. PNE demonstrated analgesic effect indicating its possible effectiveness in the management of pain associated with cough.

Guinea pigs were used in the antitussive investigation because their airways possess the needed afferent nerves and can produce cough, just like in humans (27). Cough was detected with a characteristic sound and by stretching of limbs accompanied by inspiration and then expiration similar to that described by Morice and coworkers (1). These criteria were adopted

so as to distinguish it from other respiratory reflexes like sneezing and expiratory reflex. As a tussigenic agent when inhaled, citric acid is known to stimulate transient receptor potential vanilloid1 on the C-fibers. This then causes the release of tachykinins to mediate bronchoconstriction and mucus secretion, which in turn stimulates RAR (28,29), a widely studied cough receptor. The impulse is then conveyed through the vagus nerve to the CNS and then back to respiratory muscle through the efferent pathway to cause cough. From the results obtained, dihydrocodeine and PNE were found to be effective antitussive agents. Dihydrocodeine was used as the positive control because it is the second most specific antitussive of the commonly used opioids (30). Dihydrocodeine acts on the μ opioid receptors to suppress the cough reflex (31). PNE also exhibited a dose-dependent antitussive effect reducing both the cough count and latency of cough, similar to dihydrocodeine.

Unlike the C-fiber, it is very difficult to get a chemical which directly stimulates the RAR. Rather, stimulation of RAR relies on indirect effects like bronchoconstriction and presence of mucus (29); hence, the essence of establishing the antitussive effect of PNE using the acetylcholine and histamine-induced bronchoconstriction model.

Acetylcholine causes bronchoconstriction by interacting with the M_2 and M_3 receptors on the airway smooth muscle of guinea pigs. The M_3 muscarinic receptors mediate smooth muscle contraction. Stimulation of these receptors by acetylcholine activates inositol triphosphate (IP_3) - and phosphoinositide-specific phospholipase C to increase intracellular calcium. This results in contraction of airway smooth muscle (32). The M_3 muscarinic receptors also mediate mucus, water and electrolyte secretion (33). Blood vessels of the tracheobronchial circulation dilate in response to acetylcholine released by vagal nerve stimulation (34,35). This acetylcholine-induced vasodilatation is undoubtedly mediated by M_3 receptors (34). Majority of the muscarinic receptors on airway smooth muscle are M_2 (36). They, however, appear to inhibit beta-adrenoceptor-induced

bronchodilation by preventing the activation of adenylate cyclase and calcium -dependent potassium ion channels (37).

Histamine causes bronchoconstriction by stimulation of H_1 receptors on smooth muscles in the airways (38). Mucosal oedema from increased vascular permeability in addition to mucus secretion is also mediated by the H_1 receptor (39). Histamine also induces airway smooth muscle contraction, reflex hyperpnoea and bronchoconstriction indirectly via stimulation of lung irritant receptors through vagal (cholinergic) pathways (29,40).

The abolishing of bronchoconstriction by PNE, seen as significant increase in TOC and hence increased percentage protection to induced bronchoconstriction and cough. Its antitussive effect therefore could be based on its reduced stimulation of RAR, as bronchoconstriction enhances RAR activity. The fact that PNE inhibited bronchoconstriction induced by acetylcholine and histamine (in a manner similar to atropine and mepyramine) could also bring to mind that PNE could have component(s) acting via antimuscarinic or antihistaminic mechanisms to inhibit bronchoconstriction and onset of cough. It could also be due to the activities of phytochemicals present in PNE which is discussed later on in this section. It should also be emphasized that the bronchodilator effect established also contributes to its mucokinetic effect as cough clearance also depends on the properties of tracheal mucus (41). Antimuscarinics decrease tracheal and bronchial mucus secretion (muco-suppressant effect) by inhibiting muscarinic receptor mediated mucus secretions which would stimulate RAR to induce cough. These RAR receptors comprise of fibers that fire in response to tussive stimuli leading to the elicitation of the cough reflex. Their activity is largely increased by mechanical stimuli such as mucus secretion or oedema (42).

Antihistamine activity could also alleviate cough. Older-generation antihistamines have been proposed to possess antitussive effects via a peripheral indirect mechanism involving cholinergic mechanisms (43). Consistent with this premise is the fact that some antihistamines possess anticholinergic actions (44).

This mechanism cannot be conclusive as direct acting bronchodilators like β_2 agonist may have similar effect (45). For instance, the cough due to bronchiectasis may be successfully controlled with inhaled β_2 agonist, which apart from reversing any bronchoconstriction, also improves mucocilliary clearance.

The inhibition of bronchoconstriction shown by PNE in this study could in a way be attributed to the alkaloids because of their stimulatory effect on the opioid receptors. Previously, the various alkaloids in the plants have shown significant activity on the various opioid receptors (12). Phytochemical screening confirmed the presence of alkaloids. The significant antitussive effect observed therefore could be attributed to the activity of the alkaloids of the extract on the opioid receptors. Opioids are known to inhibit non-adrenergic non-cholinergic nerve-mediated bronchoconstrictor responses both *in vitro* in guinea pig bronchi (46) and *in vivo* in guinea pig airways (47). Akuammidine and akuammicine as μ and κ agonists respectively may contribute to the antitussive effect. It can also be added that akuammidine may be acting through the μ_2 activity since it has been established that antitussive action of μ -agonists occurs via μ_2 -receptors (48,49).

To determine the expectorant activity of PNE, the trachea phenol red secretion assay was used. This model is developed on a principle that when phenol red is injected after an expectorant is given for four consecutive days, there will be enhancement of phenol red secretion from the trachea. PNE at doses of 100, 300 and 500 mg/kg did not cause significant phenol red secretion from the trachea. Ammonium chloride and 1000 mg/kg PNE however caused significant tracheal phenol red secretion. Ammonium chloride is known to irritate the bronchial mucosa; leading to the production of excess fluid in the tracheobronchial airways for easier clearance of mucus (50)

The fact that the higher dose of PNE gave similar results as ammonium chloride indicates that PNE has expectorant effect at high doses. The basis for using expectorants as cough relieving agents lays in the possibility that

alteration of the volume of secretions or their composition will lead to suppression of the cough reflex (51). Cough and pain have many things in common; they originate in afferent nervous systems that detect and signal real or impending threats to the organism. As with pain, cough can also be evoked in experimental animals by stimulation of nociceptive C-fibres as well as by faster conducting $A\delta$ -fibres (52). Several stimulants known to selectively stimulate nociceptive C-fibres (e.g. capsaicin, bradykinin) also evoke cough in laboratory animals and humans (53). Central sensitization that has been known to cause allodynia also results in hypertussive states. From the results obtained from the tail immersion test, PNE showed significant antinociceptive effect at all the doses given. This was also observed for both diclofenac and morphine. The thermal model of the tail immersion test is considered to be a spinal reflex, but could also involve higher neural structures and this method identifies mainly central analgesics (54,55). As such it fits as a model to test centrally acting analgesics. Consequently, the extract can be said to be acting through a centrally mediated pathway by elevating pain threshold of animals towards heat.

The writhing test helps in identifying central and peripheral analgesic compounds (55). The extract may act via either of the two pathways. The peripheral mechanisms can be due to reduction of sensitization by such agents like prostaglandins. The probable non-sensitizing role of PNE was demonstrated in the antitussive experiment. The experiment confirms the analgesic effect (56) of *P. nitida* which may be due to the activity of the alkaloid akuammidine which is known to be an agonist at μ receptors.

From the preliminary phytochemical screening conducted, PNE has been shown to contain alkaloids, glycosides, tannins, saponins, terpenoids, steroids and anthraquinones. Consequently, these secondary metabolites will be the ones responsible for the antitussive and other activities that have been established for the plant in this work. Retrospective experiments have given similar secondary metabolites (57,58). Alkaloids in PNE could

have contributed to its antitussive and analgesic activity as these have shown to have activity on μ and k opioid receptors e.g. the alkaloid akuammidine acts as an agonist on μ receptors (12), while akuammicine is a k agonist. Both μ (especially μ_2) and k receptor ligands mediate antitussive activity (49,59). The antitussive effect established therefore could suggest the involvement of akuammidine and akuammicine. Apart from the alkaloids, other secondary metabolites will all have a role in one way or the other in making the plant exhibit the effects that has been established: it has severally been reported that saponins confer expectorant activities on plants (60,61). Various tannins have also exhibited antimicrobial activity against broad spectrum organisms (62,63). Such antibacterial effect will contribute to the cough suppressive effect since bacterial infection has been implicated in cough and antibiotics are usually taken for cough remediation (64).

It was determined from the safety studies that PNE has an $LD_{50} > 2000$ mg/kg. This safety profile has also been established in a study by N'dri in which a phytochemical and toxicological study was conducted on the seed extract of *P. nitida* where the LD_{50} was about 9000 mg/kg (65). This obviously speaks well of the safety of the extract.

CONCLUSION

The ethanolic seed extract of *P. nitida* has antitussive, expectorant (only at high doses), and analgesic properties, as well as a good safety profile; with an $LD_{50} > 2000$ mg/kg.

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