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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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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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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

retracts to original position from a closed (protected) or an open (unprotected) arm.

The behavior was tracked by JWatcher™ Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sidney, Australia; available at http://www.jwatcher.ucla.edu/.)

2.7.2. Open field

The open field method has previously been described.[9] The test was conducted in clear plexiglas boxes (40 cm × 40 cm × 30 cm). The floor of this box was divided by red lines into 16 equal squares. For behavioral analysis, the arena of the open field was designated as;

1) corner (one of the four corner squares);
2) periphery (the squares along the walls); or
3) center (the four inner squares).

The animals were divided into ten groups (n = 6), and received either the extracts (100, 300, or 500 mg/kg, *p.o.*), the vehicle or the reference drug diazepam (0.1, 0.3 or 1 mg/kg, i.p.). Thirty minutes after i.p and 1 h after oral administration, the animals were placed at the center of the open field and were allowed to explore for 5 min. This was recorded by a video camera which was suspended 100 cm above the arena. Behavioral parameters for all the tests were scored from videotapes with the aid of the public domain software JWatcher™ Version 1.0. The numbers of entries, as well as the duration of stay in individual zones were assessed.

2.8. Antioxidant property of PNE

The antioxidant property of PNE (0.1–30 mg/ml) was evaluated in 2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) free radical scavenging, linoleic acid lipid peroxidation, reducing power, and total antioxidant assays by standard procedures previously described by Amoateng *et al.*, 2011.[10]

2.9. Data analysis

Data obtained in all experiments were expressed as mean ± SEM. Statistical analyses were done by one-way analysis of variance (ANOVA) with Dunnett's Multiple Comparison test (*post hoc* test) using Graph-Pad Prism for Windows Version 6.0 (Graph-Pad Software, San Diego, CA, USA). Some aspects of the open field and elevated plus maze were done with two-way analysis of variance followed by Bonferroni's *post-hoc* test. Differences between means of treated groups and the control were regarded as statistically significant at $P \leq 0.05$.

3. Results

3.1. Muco-suppressant and mast cell stabilizing effect of PNE

Sodium cromoglycate, and PNE (100–300 mg/kg) significantly ($P \leq 0.05$–0.001) reduced tracheal phenol red secretion compared to the control (Fig. 1a). Treatments with ketotifen fumarate, and PNE (100–500 mg/kg) were also able to reduce significantly ($P \leq 0.05$–0.001) inhibit mast cell degranulation induced by compound 48/80 (Fig. 1b).

3.2. Antibacterial effects

Significant ($P \leq 0.05$–0.0001) antibacterial activity was observed *E. coli*, *S. typhi*, *K. pneumonia*, *S. pneumonia,* and *S. aureus* from 10–50 mg/ml. The lowest effect was against *S. typhi* at 5 mg/ml with a zone of 13.0 ± 0.00 mm whiles the highest response was observed against *S. pneumonia* at 50 mg/ml with a zone of 22.3 ± 0.88 mm (Table 1).

3.3. Anxiolytic effect of PNE

3.3.1. Elevated plus maze

PNE (100, 300, 500 mg/kg) enhanced, dose-independently, activities in the open arm by increasing percentage of entry into open arms ($P \leq 0.0001$; Fig. 2f) and percentage time spent in open arms ($P = 0.1278$; Fig. 2c). There was also reduction in risk assessment by decreasing both the percentage protected head dips ($P = 0.0030$; Fig. 3c) and percentage protected stretch attend postures ($P = 0.0860$; Fig. 3f). Diazepam (0.1–1 mg/kg) also dose dependently and significantly increased percentage of open arm entries ($P = 0.0005$; Fig. 2e) and percentage time spent in open arm ($P = 0.0004$; Fig. 2b). Diazepam also reduced risk assessment by decreasing both the percentage protected head dips ($P = 0.0002$; Fig. 3b) and percentage protected stretch attend postures ($P = 0.0192$; Fig. 3e). These effects confirmed the anxiolytic effects of both PNE and diazepam. With regard to caffeine, it increased open arm avoidance by reducing percentage of open arm entries ($P = 0.8997$; Fig. 2d) and percentage time spent in open arms ($P = 0.0285$; Fig. 2a). Also, caffeine increased both the percentage protected head dips ($P = 0.7205$; Fig. 3a) and percentage protected stretch attend postures ($P = 0.0247$; Fig. 3d), all indicative of anxiogenic effect.

3.3.2. Open field

PNE (100, 300, 500 mg/kg) dose-independently increased the percentage entry into central zone ($P = 0.0201$; Fig. 4d) and percentage time spent in central zones ($P = 0.0427$; Fig. 4b). These observations support the claim that PNE acts as an anxiolytic. The reference anxiolytic, diazepam (0.1–1 mg/kg) dose dependently increased the percentage entry into central zone ($P = 0.0023$; Fig. 4c) and percentage time spent in central zones ($P = 0.0603$; Fig. 4a).

3.4. Antioxidant activity

The reference antioxidant, n-propyl gallate (0.001–0.1 mg/ml), and PNE (0.1–30 mg/ml) exhibited DPPH free radical scavenging effect (Fig. 5a), and showed concentration dependent ability to inhibit the autoxidation of linoleic acid lipid peroxidation (Fig. 5b). PNE and n-propyl gallate also dose dependently reduced Fe^{3+} to Fe^{2+} in the reducing power assay (Fig. 5c). PNE (0.1–30 mg/ml) showed a concentration dependent increase in total antioxidant capacity expressed as ascorbic acid equivalent (AAE) (Fig. 5d). EC_{50} values for the reference antioxidants and PNE are as shown in Table 2.

4. Discusssion

This study carried out on PNE to determine muco-suppressant, antibacterial, mast cell stabilizing, anxiolytic, and antioxidant properties in a bid to establish its mode of activity in the management of cough evolved from previous work on PNE, by the authors, which established antitussive, and expectorant properties of the extract.[2] Tracheal mucus secretion, mast cell degranulation, bacterial infection of the airways, psychogenic factors (e.g. anxiety) and oxidative stress could culminate into cough.

Sodium cromoglycate and PNE significantly reduced tracheal phenol red secretion after ammonium chloride induction, indicating muco-suppressant activity. Sodium cromoglycate is known

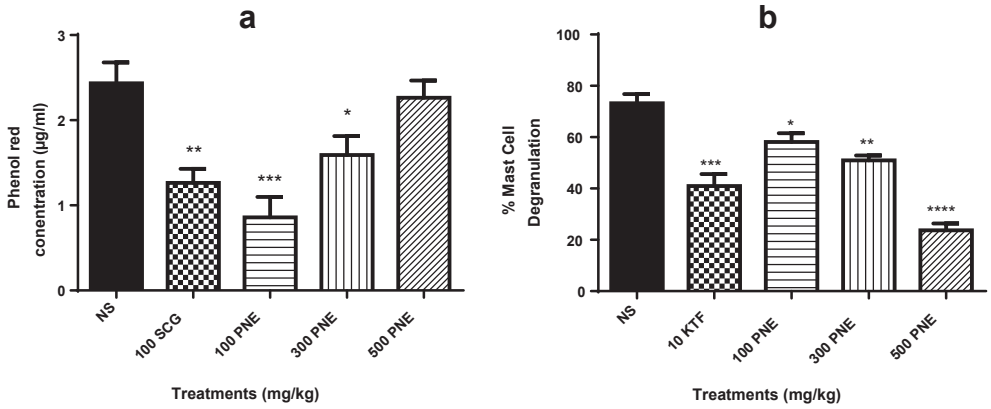

Fig. 1. Effect of PNE on (a) ammonium chloride-induced tracheal phenol red secretion as a measure of muco-suppressant effect and (b) mast cell degranulation induced by Compound 48/80. Values plotted are means ± SEM; (n = 4). ns implies ****P > 0.05; ***P ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05 compared to vehicle-treated group.

Table 1
Effect of 0.05–50 mg/ml PNE on *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Streptococcus pneumonia* and *Staphylococcus aureus*

Conc. (mg/ml)	*S. aureus*	*S. pneumonia*	*S. typhi*	*E. coli*	*K. pneumoniae*
0.05–1	–	–	–	–	–
5	14.67 ± 0.33	14.00 ± 0.00	13.00 ± 0.000	–	–
10	17.00 ± 0.58*	15.33 ± 0.33ns	15.00 ± 0.56ns	13.33 ± 0.33	14.33 ± 0.33
25	18.67 ± 0.88**	17.67 ± 0.67**	17.67 ± 0.88***	14.00 ± 1.16ns	16.00 ± 0.00**
50	20.00 ± 0.00***	22.33 ± 0.88****	19.33 ± 0.33***	16.67 ± 0.33*	17.67 ± 0.33***

Values quoted as zones of inhibitions are means ± SEM; n = 3. (–) indicates "no zones of inhibition were observed". Diameter of borer: 11 mm. ns P > 0.05, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001.

to exhibit muco-suppressant effect by prevent the release of inflammatory mediators[11] resulting from chronic inflammation of the airways. The importance of this result lies in the fact that mucus in the airways mechanically stimulates and increases rapidly the activity of rapidly adapting receptor (RAR); a cough sensor.[12] The presence of excess mucus moves the cough sensor response up the stimulus/response curve and gives the impression of sensitization.[13] By PNE's ability to inhibit mucus secretion from the airway,

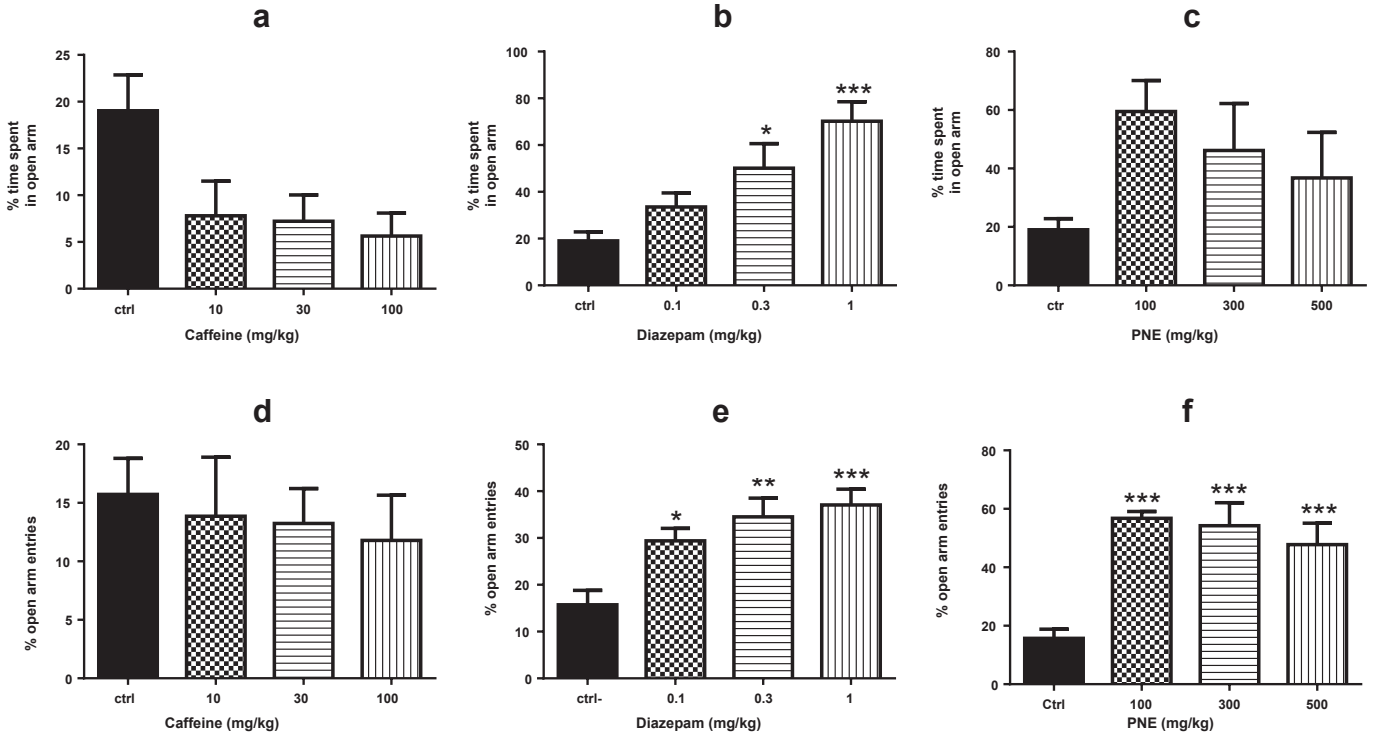

Fig. 2. The % time spent in open arm for Caffeine (a), Diazepam (b), PNE (c) and the % number of arm entries for Caffeine (d), Diazepam (e), PNE (f) in the elevated plus maze. Data are presented as group means ± SEM; (n = 7). Significantly different from control: *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

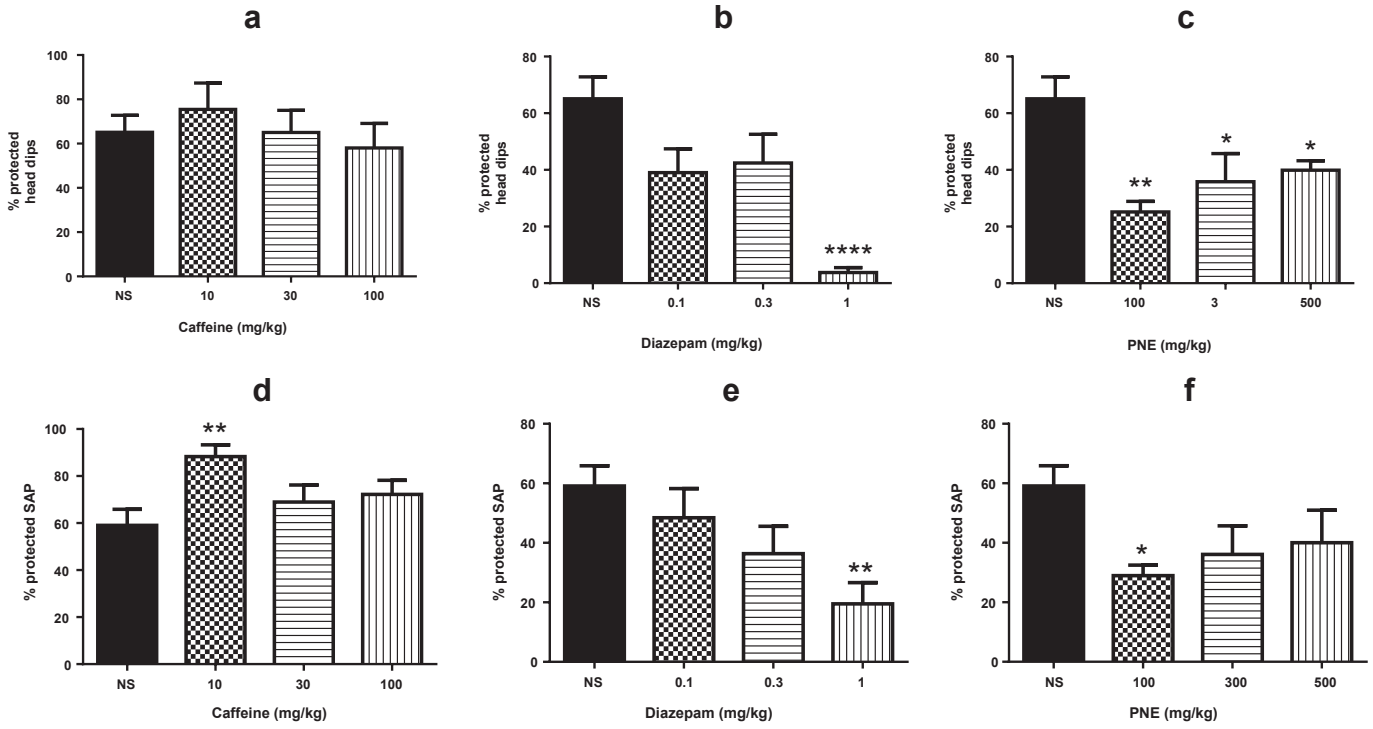

Fig. 3. Effect of Caffeine, Diazepam and PNE on the percentage protected head-dips (a, b, c), and percentage protected stretch-attend postures–SAP (d, e, f), in the EPM. Values plotted are mean ± SEM, (n = 7). *P ≤ 0.05, **P ≤ 0.01 represent significantly different between treatments and control.

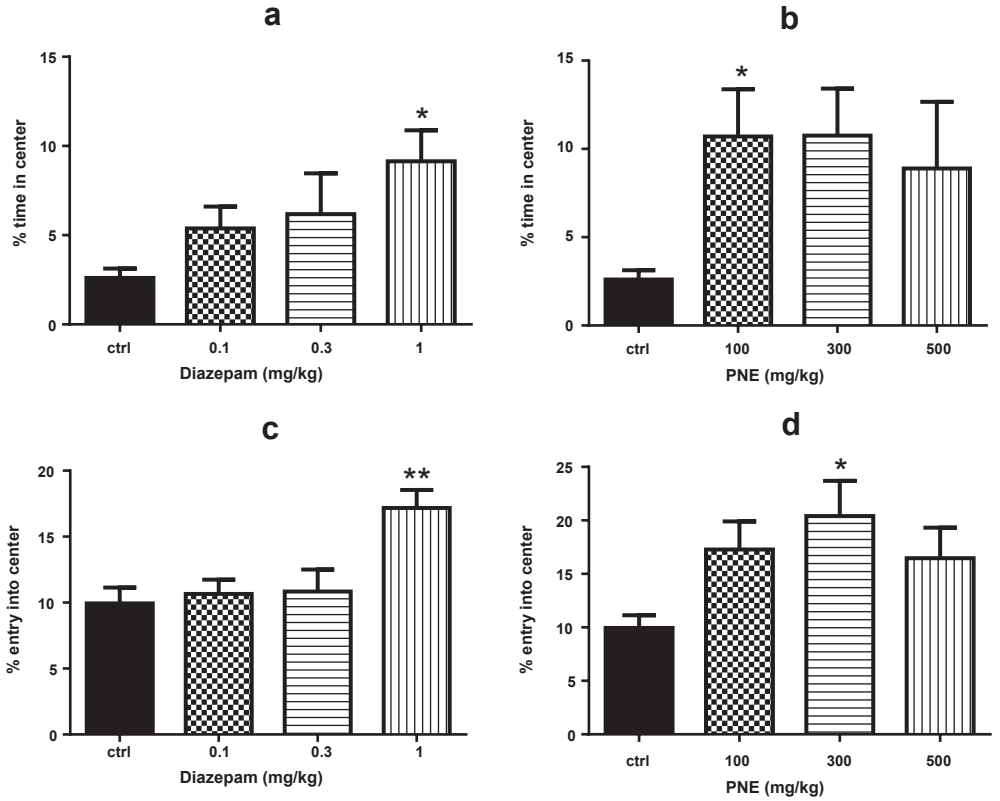

Fig. 4. Effect of Diazepam (0.1, 0.3, 1 mg/kg), and PNE (100, 300, 500 mg/kg) on the percentage time spent in center for (a) Diazepam, (b) PNE; and the percentage entry into central zone for (c) Diazepam, (d) PNE in the open field test. Values plotted are mean ± SEM, (n = 8). *P ≤ 0.05, **P ≤ 0.01, represent significantly different between treatments and control: (n = 8).

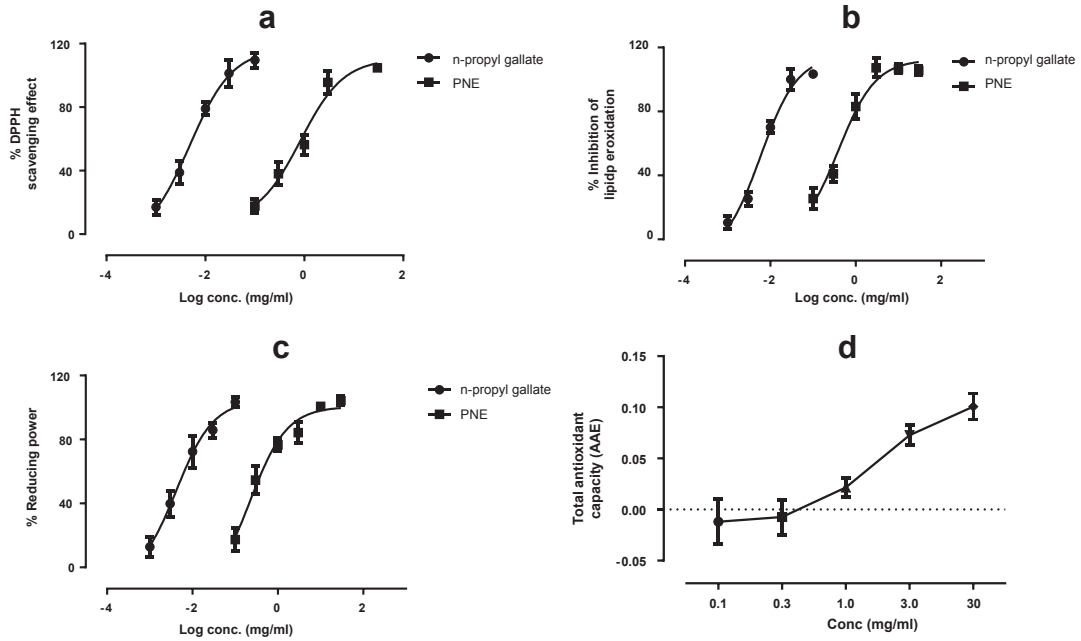

Fig. 5. DPPH free radical scavenging, linoleic acid lipid peroxidation, reducing power, and total antioxidant assays in the determination of antioxidant property of PNE (0.1–30 mg/ml). Values plotted are means ± SEM, n = 3.

Table 2
EC_{50} values for the reference antioxidants and PNE in an antioxidant assay.

	DPPH	LALP	RP
n-propyl gallate	4.821×10^{-3}	5.906×10^{-3}	4.195×10^{-3}
PNE	0.8386	0.3975	0.2216

DPPH = 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) free radical scavenging assay; LALP = Linoleic acid lipid peroxidation assay; RP = Reducing power, TAC = Total antioxidant assay.

stimulation of RAR will be hampered and that could contribute to antitussive effect.

Compound 48/80-induced mast cell degranulation is used to test mast cell stabilizing effects of potential therapeutic agents. Compound 48/80 causes mast cell degranulation by increasing intracellular Ca^{2+}.[14] Reduction in number of degranulated mast cell is a measure of mast cell stabilization. Ketotifen and PNE significantly stabilized mast cells. Ketotifen stabilizes mast cells by inhibiting the release of histamine, serotonin and other inflammatory mediators.[15] Mast cell stabilization could contribute to antitussive effect in that leukotriene and histamine (which can stimulate C-fibers) are not release.[16] Apart from such direct effect, leukotrienes and histamine can induce mucus secretion and bronchoconstriction which can in turn stimulate RAR receptors. Mast cell stabilization also reduces chronic inflammation of the airway which can lead to hypertrophy and hyperplasia of surface goblet (mucous) cells and sub-mucosal glands, which consequently increases mucus production.

PNE demonstrated antibacterial activity against two gram positive and three gram negative bacteria, indicating a possible remedy for cough arising from bacterial infection of the airway. Chronic cough could be a post infectious cough of viral or bacterial origin.[17] Increase in cough sensitivity seems to be the most likely reason why infection contributes to cough.[18] Treatment and/or prevention of airway infection could be an important way of reducing inflammation and hyper-secretion and hence cough sensitivity. Antibiotics are usually considered 'mucoregulatory medications' when used to treat respiratory infection.[19] It is worth noting that there could be a potential reduction in C-fiber stimulation as a result of anti-bacterial effect. Bacterial infection is known to reduce the level of endopeptisase[20] especially substance P which gets released from C-fiber. Considering the fact that substance P can augment C-fiber activity[21] and that of the RAR through mucus secretion and bronchospasm it induces suppressing bacterial proliferation will enhance the level of endopeptidase, which in turn will reduce substance P mediated events.[22]

Anxiety in particular has been found to be an independent risk factor for both productive and non-productive cough. This may reflect a somatic manifestation of a wide range of psychological problems, an increased awareness of physical symptoms in anxious individuals as well as difficulty in maintaining voluntarily cough suppression.[23,24] Therapeutically, therefore, anxiolytics may help to relieve cough. Psychogenic cough is generally regarded as a respiratory tic and may be precipitated by a variety of emotional stimuli.[25] In an attempt to establish the fact that PNE could be useful in managing anxiety-related cough, its anxiolytic effect was established using the elevated plus maze and the open field tests. The open-field test examines anxiety-related behavior characterized by avoidance of an open, brightly lit area by rodents.[26] Animals placed in a novel environment express anxiety and fear, by showing alteration in certain parameters, such as decreases in ambulation and exploration time in the center of the open field with an attendant increased peripheral movement.[27] These behaviors are attenuated by classical anxiolytics, and potentiated by anxiogenic agents. The elevated plus maze utilizes the fear of a novel, brightly lit open space and fear of balancing on a relatively narrow, raised platform. This is considered a natural stimulus because it can induce anxiety even in humans.[28] Generally, anxiolytics increase the number of entries into and the time spent in the open arms of the EPM. This was the case with PNE pointing to potent anxiolytic activity. The fact that PNE reduced the percentage protected head dips and stretch attend posture in the EPM also confirm its ability to reduce fear related behavior.

Oxidative stress contributes to cough in that reactive oxygen species (ROS) have the potential of stimulating C-fibers.[29] In testing the antioxidant effect, DPPH, 2, 2-diphenyl-1-picrylhydrazyl

hydrate a stable radical having a characteristic violet color (and maximum absorption at 517 nm) and which accepts an electron or hydrogen in the presence of a suitable free radical scavenger (reducing agent) was used. From the IC_{50} values obtained, PNE and ascorbic acid exhibited antioxidant activity. Oxidants can cause damage by interacting with anti-proteases or other processes leading to the development of chronic lung damage.[30] Cough and phlegm are symptoms frequently accompanying chronic obstructive pulmonary disease. This may be caused by oxidative stress–mediated inflammation and tissue damage.[31] Intake of fruit and vegetable, which are major sources of antioxidants, have been associated with higher lung function and reduced symptoms of cough with phlegm.[32]

It is to be noted that the mucolytic effect of acetylcysteine have been attributed to their antioxidant activities[33] and that gives an indication of the fact that PNE may also have mucolytic activity. There seems to be notable similarities between mucolytics and expectorants: Mucolytics just like expectorants have been found to be effective in productive cough[34,35] also the model used in testing expectorant effects i.e. the tracheal phenol red secretion has been used in ascertaining mucolytic activity of various extracts.[36] The antioxidant effect established in this work can then be said to contribute to the extracts ability to manage productive cough; a property which is typical of expectorants. However this antioxidant effect could also account for PNE's antitussive activity since reactive oxygen species (ROS) are known activators of C-fibers.

The mast cell stabilization, muco-suppressant, and antioxidant effects were the most prominent effect of PNE even though it shows significant antimicrobial and anxiolytic activity. Preliminary phytochemical screening of PNE indicated the presence of alkaloids, tannins, steroids, glycosides, anthraquinones, and terpenoids[2] some of which would have contributed to its properties.[37–39] A review of the medicinal uses, phytochemistry and pharmacology of *P. nitida* has indicated that its alkaloids (especially from the seed) have significant antimicrobial, analgesic, and anti-inflammatory properties[40] while its coumestan glycosides have antimicrobial activity; these which could make it useful as antitussive.[41] Alkaloids, phenolic compounds (tannins), glycosides, saponins, sterols, and terpenoids has been reported to have mast cell stabilizing effect.[42–44] It is possible that the anxiolytic action could be mediated by synergistic action of these phytochemicals as seen in several studies.[45] The lower dose of HIE (i.e. 100 mg/kg) seemed to be more effective; giving more consistent results than the higher doses (i.e. 300 and 500 mg/kg). This may not be surprising since the extract may have several other components, some of which may have antagonist effects to the agonist effect being elicited; but would only be of significance at higher doses i.e. proportional to the dose administered.

5. Conclusion

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.

Conflict of interest

Authors do not have any conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jtcme.2016.05.003.

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