



Evaluation of the anti-asthmatic and antitussive potentials of methanol leaf extract of *Napoleona vogelii* in rodents

Adesola A. Adejayan^a, Raymond I. Ozolua^{a,*}, Dickson O. Uwaya^b, Gerald I. Eze^c, Adaobi C. Ezike^d

^a Department of Pharmacology & Toxicology, Faculty of Pharmacy, University of Benin, Benin City 300001, Nigeria

^b Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City 300001, Nigeria

^c Department of Anatomy, Faculty of Basic Medical Sciences, University of Benin, Benin City 300001, Nigeria

^d Department of Pharmacology & Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria

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ABSTRACT

Napoleona vogelii is used in ethnomedicine for the treatment of asthma and cough. This study evaluated anti-asthmatic and antitussive properties of its methanol leaf extract (NVE) in rodents. Phytochemical screening was conducted using established methods. Acute oral toxicity test was done in mice and guinea pigs. Ovalbumin (OA)-sensitized guinea pigs were orally pretreated with 100, 200 or 400 mg/kg/day of NVE or 0.5 mg/kg/day of salbutamol for 14 days before exposure to 0.2% histamine aerosol. Latency to preconvulsive dyspnea (PCD), tracheal fluid volume (TFV), flow rate (FR), and tracheal morphometry (TM) were evaluated. Tracheal rings from sensitized guinea pigs were tested in organ baths for antispasmodic and spasmolytic effects. Citric acid and ammonium hydroxide cough models were used to evaluate antitussive effects in guinea pigs and mice respectively. Tannins, alkaloids, flavonoids, and phenolic substances were found in NVE. LD₅₀ values in mice and guinea pigs were greater than 5000 mg/kg. NVE caused a significant ($P < 0.05$) increase in the latency to PCD and a decrease in TFV in the group treated with 200 mg/kg. TM indicated a reduction in airway narrowing in NVE-treated groups. The presence of NVE significantly attenuated responses of tracheal rings to carbachol. Its addition to carbachol precontracted rings resulted in significant relaxation. E_{max} for calcium concentration-response was significantly ($P < 0.01$) decreased in the presence of NVE. Cough bouts dose-dependently decreased significantly ($P < 0.05$) in guinea pigs and mice. We conclude that NVE seems safe and possesses anti-asthmatic effect that involves inhibition of calcium influx. It also has antitussive properties that may be peripherally mediated.

1. Introduction

Asthma is a disease with significant morbidity, mortality and huge socioeconomic burden [1]. It is estimated that lifetime prevalence of the disease ranges from 1% to as high as 18% of the general population [2]. Although there are regional differences in its prevalence, it is about 13% in Africa [3]. Essentially, the disease is marked by airway inflammation and hyperresponsiveness [4]. In many settings, treatment of acute attacks and management of chronic forms of the disease have been by use of orthodox drugs. Generally, these drugs minimize or eliminate symptoms, reduce the need for emergency department visits and hospitalization, normalize pulmonary function, establish a normal life style without limitations on activities including exercise [5]. Cough is also a respiratory disease with varied etiology that causes significant reduction in the quality of life [6,7] and may co-exist with asthma [8,9]. About 40% of the population reports cough [10]. Oftentimes,

drugs with antitussive effects are useful in asthma [11,12]. Recently, complementary and alternative medicine of which herbal medicine is a part, has gained popularity in the treatment of cough and asthma [13,14]. Factors responsible for the increased use include alignment with cultural beliefs and practices [15], cost of orthodox drugs, the supposed fewer incidence of adverse effects, and perhaps most importantly, aggressive marketing by manufacturers [16–18].

Napoleona vogelii Hook (Lecythidaceae) commonly called Wallia is found mostly in rain forests and along seashores [19]. In West Africa, its distribution extends from Sierra Leone to Nigeria; grows up to 15 m high with few high branches and it is used in making wooden poles, wraps, chewing sticks and mats [19,20]. The leaves are widely used in South-Eastern Nigeria for the treatment of stomach aches, diarrhoea and treatment of cough and asthma [21]. Traditional healers often dry the leaves under shade and prepare the extracts in alcohol. The ethnomedicinal uses of *N. imperialis* which is distinguishable from *N. vogelii*

* Corresponding author.

E-mail address: ozolua@uniben.edu (R.I. Ozolua).

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by its smaller leaves include the treatment of cough and asthma, and as a tonic and as an analgesic [22]. The methanol leaf extract of *N. vogelii* has been shown to possess anti-ulcer and hypoglycemic [21], wound healing [23,24], antidiabetic, and hypolipidemic [25] properties. Reported secondary plant metabolites in the leaves of *N. vogelii* include alkaloids, tannins, saponins, phenols and hydrogen cyanide [26,27]. Pure substances have not been characterized in the plants. There is paucity of scientific information on the anti-asthmatic and antitussive potentials of the plant in spite of the acclaimed ethnomedicinal usefulness in these conditions. We therefore used rodent models to test these properties and the underlying mechanisms with the hope that it will provide the scientific basis for the use of the plant in ethnomedicine.

2. Materials and methods

2.1. Plant material and extraction

Fresh leaves of *N. vogelii* were obtained from Nsukka in Enugu State, Nigeria. The plant was duly authenticated by staff of International Centre for Ethnomedicine and Drug Development, University of Nigeria, Nsukka where a herbarium voucher with number (Inter CEDD/507) has been deposited. The leaves were dried under shade and then powdered using a mill. The powdered leaf material (2 kg) was immersed in 8 l of methanol for 72 h and then filtered. The filtrate was concentrated to dryness using hot water bath. The extract (NVE) was weighed (yield = 6.10% w/w), and then stored in amber-colored bottles. The extract was reconstituted in distilled water for oral administration to animals.

2.2. Animals

Guinea pigs of either sex weighing 350–500 g and Swiss albino mice of either sex weighing between 20–30 g were used for the experiments. The guinea pigs were purchased from the animal facility of Department of Physiology, Ambrose Alli University, Ekpoma, Nigeria and were used for anti-asthmatic and antitussive evaluations. The mice were purchased from a private animal farm in Benin City, Nigeria and were used only for antitussive experiments. All animals were kept in plastic cages; males separate from females and allowed two weeks acclimatization. The animals had free access to dry rodent pellets and tap water and were exposed to natural light/dark cycle and room temperature. They were handled according to standard protocols for the use of laboratory animals [28]. The experimental protocol was approved by Ethics Committee of the Faculty of Pharmacy, University of Benin, Benin City with reference number EC/FP/018/12.

2.3. Drugs and chemicals

The drugs include ovalbumin (Sigma-Aldrich), histamine dihydrochloride (Sigma-Aldrich), salbutamol (GlaxoSmithKline), dihydrocodeine (Bristol Laboratories), carbachol (Sigma-Aldrich), isoprenaline (BDH), theophylline (BDH), and ethylene glycol-bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA; Sigma-Aldrich). All chemicals and reagents were purchased from reputable manufacturers such as Sigma-Aldrich and British Drug House (BDH). Solutions of drugs and chemicals were freshly prepared before use.

2.4. Qualitative and quantitative analysis of phytoconstituents

The methanol leaf extract of *N. vogelii* was subjected to qualitative chemical screening for secondary plant metabolites using conventional methods [29,30]. Alkaloids and tannins were quantified using the method described by Lawal et al. [31]. Flavonoid content was estimated using the method described by Ordonez et al. [32]. Total phenol content was determined using methods described in Otang et al. [33].

2.5. Acute toxicity test

The median lethal dose (LD₅₀) was estimated in mice and guinea pigs using the Lorke [34] method. In the first phase, the animals were randomly allotted to four groups of three animals each. Group I was orally administered 2 ml/kg of distilled water and groups II–IV were orally administered 10, 100, and 1000 mg/kg of NVE respectively. In the absence of death within 24 h, the second phase was conducted. In this phase, the animals were randomly allotted to four groups of one animal each. Group I was given 2 ml/kg of distilled water and groups II to IV were given doses of 1600, 2900, and 5000 mg/kg of the extract. For each phase of the model, the animals were observed continuously for 2 h immediately after dosing and for 30 min each day for a maximum of 14 days to note any gross toxicological manifestations.

2.6. Anti-asthmatic evaluation

2.6.1. Histamine-induced preconvulsive dyspnoea

Guinea pigs were randomly allotted to six groups (n = 5 per group): group 1, non-sensitized control given 2 ml/kg/day of distilled water; group 2, sensitized control given 2 ml/kg/day distilled water; group 3, sensitized and treated with 100 mg/kg/day of NVE; group 4, sensitized and treated with 200 mg/kg/day of NVE; group 5, sensitized and treated with 400 mg/kg/day of NVE; and group 6, sensitized and treated with 0.5 mg/kg/day of salbutamol. Treatment was for 14 consecutive days. The guinea pigs were sensitized by modifying the method described by Bramley et al. [35]. They were given intraperitoneal (i.p.) injections of 100 mg/kg ovalbumin (OA), a booster dose of 50 mg/kg OA (i.m.) after 24 h, and a final dose of 50 mg/kg (i.p.) 24 h before exposure to histamine aerosol. On the 14th day, one hour after the various treatments, the animals were exposed to 0.2% histamine dihydrochloride (dissolved in distilled water) aerosol in a glass chamber (60 × 56 × 60 cm) using Omron® (Omron Healthcare Ltd Japan) compressor nebulizer (rate of 0.4 ml/min and particle size of 5 μ m) until preconvulsive dyspnea (PCD) was observed. The animals were removed from the chamber and placed in fresh air to recover. The latency to PCD was noted for each animal [36,37].

2.6.2. Evaluation of tracheal fluid volume and flow rate

The guinea pigs used in the experiment above were euthanized with chloroform and 2 cm length of trachea up to the point of thoracic bifurcation was isolated from each animal. The isolated trachea was held in a vertical position by clamps and then flushed with 2 ml of deionized water (37 °C). The effective tracheal fluid volume was taken as the difference between the flushing volume and final volume collected [36,37]. The trachea fluid flow rate was determined by an indirect method [38]. Briefly, the fluid was withdrawn into a 1 ml syringe fitted with a 21 G needle and allowed to drop under the influence of gravity after gently removing the plunger. The flow rate calculated as volume (ml) divided by time (s) was used as an index of viscosity.

2.6.3. Isolated guinea pig tracheal ring experiments

NVE was tested on isolated tracheal rings obtained from sensitized guinea pig [39]. On the 14th day of sensitization, the guinea pigs were mercifully sacrificed; the tracheas were dissected out quickly and placed in a Petri dish containing physiological salt solution (PSS). The tracheas were cleaned of adherent connective tissues as much as possible and cut into rings of 2.5 mm length. The rings were suspended in L-shaped wire loops in 10 ml organ baths containing the PSS. The composition of the PSS was (g/l): NaCl 7.52, KCl 0.44, MgSO₄ 0.29, KH₂PO₄ 0.16, NaHCO₃ 2.10, glucose 2.18, CaCl₂ 0.37. The PSS was bubbled throughout the experiment with 95% O₂ and 5% CO₂ gas mixture (BOC Gases, Nigeria Plc) and temperature was maintained at 37 °C. Responses were measured with a force displacement transducer (FT 302) which was connected to a Grass 7D polygraph (Grass instrument Co, Quincy, MA, USA). The rings were given a resting force of 1 g

and allowed an equilibration period of 45 min during which the PSS was changed four times. After equilibration, the rings were depolarized with 80 mM KCl before experimental protocols.

Antispasmodic effect was evaluated by first applying cumulative concentrations of carbachol (1×10^{-10} to 4×10^{-4} M) to the organ baths. Then in the presence of different concentrations (1 mg/ml, 2 mg/ml and 4 mg/ml) of the extract, cumulative concentrations of carbachol were applied. The concentrations of carbachol producing 50% of maximum response (EC_{50}) and maximum response (E_{max}) by the tracheal rings in each experiment were calculated.

Spasmolytic effect was evaluated by precontracting tracheal rings with the EC_{70} of carbachol (2×10^{-4} M). At maximum contractile response, cumulative concentrations of NVE (1–4 mg/ml) were injected into the organ bath. Similar concentration-response relationships were obtained to isoprenaline and theophylline (1×10^{-10} to 4×10^{-4} M) in rings precontracted with the EC_{70} of carbachol. The E_{max} was compared across the three concentrations of the extract.

The normal PSS was replaced by Ca^{2+} -free 2 mM EGTA-containing PSS for 20 min. The rings were then stimulated with 2×10^{-4} M (EC_{50}) of carbachol and then washed four times with Ca^{2+} -free PSS. This was repeated a second time in which 2×10^{-4} M carbachol gave no response. Subsequently in Ca^{2+} -free PSS (without EGTA), with 2×10^{-4} M carbachol, cumulative concentrations of $CaCl_2$ were added to the organ bath. Using the same protocol of Ca^{2+} -depletion, the IC_{70} of NVE (3.3 mg/ml), was injected into the organ bath. After 10 min, the rings were contracted with 2×10^{-4} M carbachol before cumulative addition of $CaCl_2$. The effect of NVE on calcium loading was calculated by comparing the EC_{50} and E_{max} of Ca^{2+} in the absence and presence of NVE [40].

2.6.4. Tracheal morphometry

The morphometry of the trachea was done using the modified Reid index [41]. The index is defined as the ratio between the thickness of the submucosal secreting glands and the thickness between the epithelium and cartilage that covers the trachea. Left overs of the dissected tracheas from the evaluation of PCD described earlier were first fixed in 10% formo-saline and then processed histologically [42]. Stained slides were viewed using an optical photomicroscope (Leica MC170 HD, Leica Biosystems, Germany) at $\times 100$ magnification.

2.7. Antitussive models

2.7.1. Citric acid induced cough

In this model [43], guinea pigs were screened a day before experiment by placing them individually for 5 min in the chamber described for histamine-induced PCD. Cough was induced by exposure to citric acid aerosol (7.5%w/v) using the Omron® compressor nebulizer described for histamine-induced PCD, for 10 min. Guinea pigs exhibiting 10–20 bouts of cough were selected for the experiment and were fasted overnight but with access to water. They were randomly allotted to 5 groups (n = 5) as follows: control group given 2 ml/kg of distilled water; groups that received 100, 200, 400 mg/kg of NVE respectively; and positive control group given 25 mg/kg of dihydrocodeine. Treatment was by the oral route. An hour after treatment, the animals were re-exposed to citric acid aerosol and the numbers of cough bouts were recorded.

2.7.2. Ammonia-induced cough model

In this model [44], mice were randomly allotted into five groups (n = 5) as follows: control group given 2 ml/kg of distilled water; groups given 100, 200, 400 mg/kg of NVE respectively; and positive control group given 25 mg/kg of dihydrocodeine. An hour after the oral administration of distilled water, different doses of NVE and dihydrocodeine, each mouse was placed in a $10 \times 10 \times 10$ cm glass chamber and exposed to 0.3 ml of 25% NH_4OH for 45 s. Thereafter, the mouse was removed from the chamber and placed in a beaker in which

Table 1

Phytochemical constituents of methanol leaf extract of *N. vogelii*.

Constituent	Presence	Quantity (mg/g)
Tannins	+	61.88
Saponins	+	NQ
Alkaloids	–	8.49
Anthraquinone	+	NQ
Flavonoids	+	5.06
Cardiac glycosides	+	NQ
Other phenolics	+	19.59
Terpenoids	+	NQ

NQ, not quantified; + = present; – = absent.

the number of cough within 5 min was counted.

2.8. Statistical analysis

The results obtained are expressed as mean $\pm$ S.E.M and analysed by one-way analysis of variance and Student-Newman Keul's post hoc (GraphPad Prism Software version 7, USA). $P < 0.05$ was considered significant.

3. Results

3.1. Phytochemical constituents of NVE

The methanol extract of *N. vogelii* leaves contained tannins, alkaloids, anthraquinone, flavonoids, saponins, terpenoids, cardiac glycosides and phenolic compounds (Table 1). The Table also shows that tannins (61.88 mg/g) and other phenolic compounds (19.59 mg/g) were highest in relative abundance.

3.2. Oral acute toxicity of NVE

NVE did not cause death in both phases of the LD_{50} determination in mice and guinea pigs. The oral LD_{50} value for the extract was therefore greater than 5000 mg/kg in both rodent species. No notable gross toxicological symptom was seen within the period of observation.

3.3. Anti-asthmatic effects of NVE

3.3.1. Effect on histamine-induced preconvulsive dyspnea

Fig. 1 shows the latency to preconvulsive dyspnea (PCD) following the exposure of the various groups of guinea pigs to histamine aerosol. The oral doses of 100 mg/kg/day and 400 mg/kg/day of NVE for 14 consecutive days did not significantly inhibit bronchospasm induced by

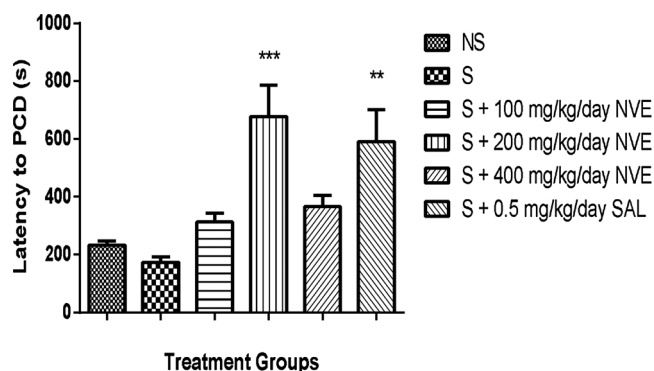


Fig. 1. Effect of 14-day oral administration of methanol extract of *N. vogelii* (NVE) on latency to preconvulsive dyspnea (PCD) in ovalbumin-sensitized (S) guinea pigs. ** $P < 0.001$, *** $P < 0.0001$ compared with sensitized untreated group. NS, non-sensitized, SAL, salbutamol. n = 5 per group.

Table 2

Effect of methanol leaf extract of *N. vogelii* on guinea pig tracheal fluid volume and flow rate.

Group	Doses (mg/kg)	Volume (ml)	Flow Rate (ml/s)
Non-sensitized	–	1.60 ± 0.68	29.00 ± 0.77
Sensitized	–	0.60 ± 0.24	26.60 ± 0.68
NVE	100	0.80 ± 0.14	26.60 ± 0.81
NVE	200	0.14 ± 0.09*	25.60 ± 0.40
NVE	400	0.78 ± 0.37	26.00 ± 0.32
Salbutamol	0.5	0.45 ± 0.26	27.25 ± 1.75

* $P < 0.01$ versus non-sensitized group. NVE, *N. vogelii* methanol extract. n = 5 per group.

0.2% histamine aerosol. The extract at 200 mg/kg ($P < 0.0001$) and salbutamol ($P < 0.001$) significantly increased the latency when compared to the sensitized groups.

3.3.2. Effect on tracheal fluid volume and flow rate

The doses of 100 mg/kg, 400 mg/kg of NEV and salbutamol did not reduce tracheal fluid volume (Table 2). The dose of 200 mg/kg of NEV significantly ($P < 0.01$) reduced the tracheal fluid volume (0.14 ± 0.09 ml) when compared with non-sensitized group control (1.60 ± 0.67 ml). Neither the extract nor salbutamol significantly altered the flow rate of tracheal fluid in the guinea pigs (Table 2).

3.3.3. Effect on isolated guinea pig tracheal ring contractility

The concentration versus response curves for carbachol alone, and carbachol in the presence of 1, 2, and 4 mg/ml of NVE are shown in Fig. 2. The responses in the presence of 1 and 2 mg/ml of the extract are superimposable but the response in the presence of 4 mg/ml was more attenuating. Table 3 shows that the EC_{50} of carbachol in the presence of 4 mg/ml of NVE was significantly ($P < 0.001$) increased compared to carbachol alone.

The comparative E_{max} for isoprenaline, theophylline and the concentrations of the extract are shown in Fig. 3. Whereas isoprenaline and theophylline completely relaxed the rings, the concentrations of the extract did not. The relaxant effect of 4 mg/ml was significantly superior to those of 1 and 2 mg/ml.

The concentration versus response curves of calcium-depleted but carbachol-stimulated isolated guinea pig tracheal rings exposed to calcium alone or in the presence of IC_{70} of the extract (3.3 mg/ml) are

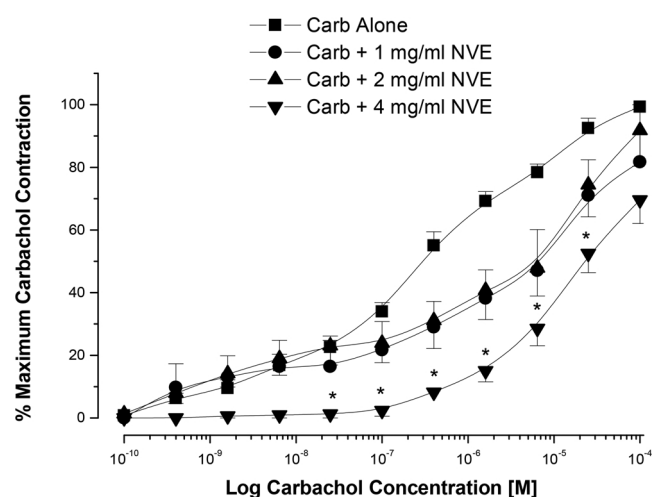


Fig. 2. Concentration versus response curves for isolated guinea pig tracheal rings exposed to carbachol in the absence or presence of 1 mg/ml, 2 mg/ml and 4 mg/ml methanol leaf extract of *N. vogelii* (NVE). Carb, Carbachol. NVE, * $P < 0.001$ versus carbachol alone. n = 5 guinea pigs per group.

Table 3

Maximum contractile responses (E_{max}) and concentrations producing 50% contractile effect (EC_{50}) of isolated guinea pig tracheal rings in the absence or presence of methanol leaf extract of *N. vogelii* (NVE).

	EC_{50} (M)	E_{MAX} (%)
Carbachol alone	$26.40 \pm 7.30 \times 10^{-8}$	100 ± 0
1 mg/ml NVE + carbachol	$1.08 \pm 61.20 \times 10^{-6}$	84.28 ± 17.88
2 mg/ml NVE + carbachol	$1.14 \pm 73.20 \times 10^{-5}$	97.74 ± 13.24
4 mg/ml NVE + carbachol	$7.36 \pm 1.69 \times 10^{-5***}$	80.04 ± 10.42

*** $P < 0.0001$ versus carbachol alone, n = 5 per group.

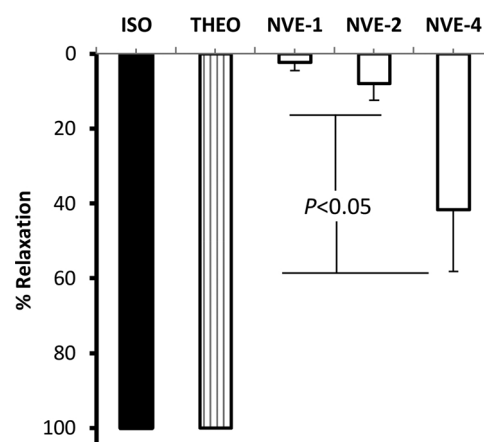


Fig. 3. Comparative maximum relaxant effect (E_{max}) of isoprenaline (ISO), theophylline (THEO) and 1, 2, and 4 mg/ml methanol leaf extract of *N. vogelii* (NVE) on isolated guinea pig tracheal rings. n = 5 per group.

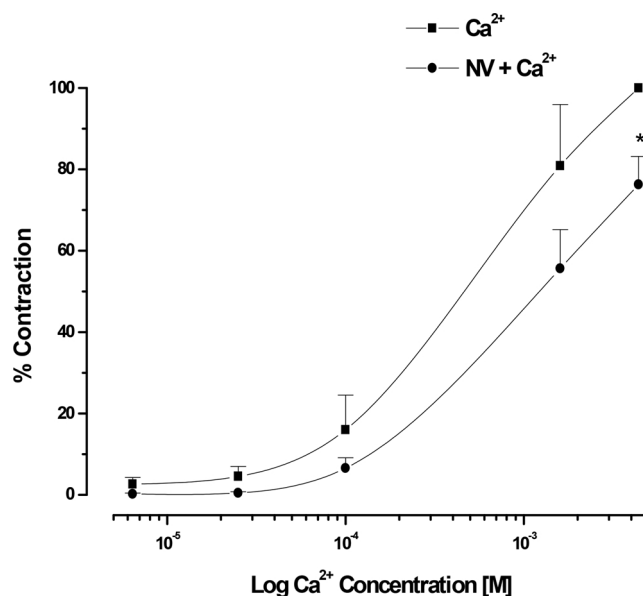


Fig. 4. Calcium concentration versus response for isolated guinea pig tracheal rings in the presence or absence of IC_{70} of methanol leaf extract of *N. vogelii* (NV). * $P < 0.01$ versus Ca^{2+} . n = 5 per group.

shown in Fig. 4. The EC_{50} of calcium-induced tracheal ring contractility was not significantly altered in the presence of IC_{70} of the extract whereas there was significant attenuation in the E_{max} ($P < 0.01$) of calcium induced tracheal ring contractility.

Table 4 shows the morphometric analysis of NEV on the guinea pig bronchus using the modified Reid index. Doses of NEV and salbutamol reduced the thickness of the bronchial wall when compared to the

Table 4

Morphometric analysis of tracheal segments of guinea pigs given doses of methanol leaf extract of *N. vogelii* (NVE).

Groups	Guinea pigs	MT (cm)	BWT (cm)	MRI	AMRI
Non-sensitized Control	1	2.5	3.0	0.83	0.79
	2	2.5	3.3	0.75	
Sensitized	1	4.5	2.0	2.25	3.15
	2	6.1	1.5	4.06	
Sensitized + 100 mg/kg NVE	1	0.8	0.5	1.60	1.15
	2	1.4	1.7	0.70	
Sensitized + 200 mg/kg NVE	1	1.8	1.7	1.05	1.15
	2	1.5	1.2	1.25	
Sensitized + 400 mg/kg NVE	1	1.5	1.0	1.50	1.27
	2	1.3	0.9	1.04	
Sensitized + 0.5 mg/kg SAL	1	1.0	0.8	1.25	1.12
	2	1.3	1.3	1.00	

MRI = MT ÷ BWT; MRI, Modified Reid index; MT, Mucosal Thickness; BWT, Bronchial Wall Thickness; AMRI, Average of Modified Reid Index; SAL, salbutamol.

sensitized untreated group. The Reid index in the sensitized untreated group was 3.15 which shows marked thickening of the bronchial wall (with resultant luminal narrowing) when compared to the treatment groups.

3.4. Antitussive effect of NVE

3.4.1. Effect on citric acid induced cough

Fig. 5a shows the mean number of cough bouts of the treatment groups compared with the control group. The dose of 100 mg/kg of NVE gave cough bouts of 16.40 ± 3.27 which was not significantly lower than the cough bouts of the distilled water group (18.20 ± 2.35). The doses of 200 mg/kg and 400 mg/kg significantly reduced the number of cough bouts to 8.80 ± 2.34 ($P < 0.01$) and 6.40 ± 1.24 ($P < 0.001$) respectively when compared to the control (18.20 ± 2.35). The effect of NVE was therefore dose-dependent. The standard drug, dihydrocodeine, significantly ($P < 0.001$) reduced the number of cough bouts to 9.60 ± 0.51 when compared to the control.

3.4.2. Effect on ammonium induced cough

The number of cough bouts of NVE treated groups compared with the control is shown in Fig. 5b. The doses of 100 mg/kg and 200 mg/kg of the extract did not significantly reduce the number of cough bouts when compared to the untreated group. Dihydrocodeine and 400 mg/

kg significantly reduced the number of cough bouts to 10.20 ± 0.96 ($P < 0.01$) and 14.20 ± 2.05 ($P < 0.001$) respectively when compared to untreated group. The effect of NVE in this model of cough was dose-dependent.

4. Discussion

The study has shown that methanol extract of *N. vogelii* (NVE) appears safe, inhibits histamine-induced bronchospasms in guinea pigs and prevents chemically-induced cough in guinea pigs and mice. With an oral LD₅₀ > 5000 mg/kg and neither death nor other toxicological symptoms observed within fourteen days, NVE can be said to have a high safety margin [45,46]. Safety of the leaf extract has been reported by others [23,25].

Sensitization of guinea pigs with ovalbumin (OA) is a well-established animal model of asthma that has been used extensively [35,37]. Increased latency to PCD by 200 mg/kg of NVE indicates an inhibitory action on bronchospasm induced by histamine [35–37]. Spasmogens such as histamine and carbachol activate calcium release and utilization processes that accentuate airway smooth muscle contraction [47,48]. NVE has shown both antispasmodic and spasmolytic effects by attenuating responses to carbachol and relaxing carbachol-precontracted tracheal rings. These effects seem accounted for at least partly by its ability to block the inflow of calcium into airway smooth muscles. NVE contains flavonoids which are known to have bronchodilatory effects [49,50]. Flavonoids inhibit antigen-induced release of histamine from mast cells [51] and also inhibit contractions induced by bronchoconstrictors such as histamine [52,53], among many other anti-asthmatic properties [54]. Many plants with similar phytoconstituents as NVE have also exhibited anti-asthmatic properties [36,37,55]. Substances that reduce trachea fluid volume and decrease the viscosity may be useful in the management of asthma since mucus plugging of the airways occurs in the disease [56]. In this study, there was a reduction in the tracheal fluid volume without any significant decrease in viscosity. NVE has high concentration of tannins (61.9 mg/g) which are known to have astringent and antisecretory effects [57,58]. This may account for the decrease in tracheal fluid volume at the dose of 200 mg/kg. The Reid index has been used to assess the degree of hyperresponsiveness of the bronchi [41,59]. In this study, the index showed marked bronchoconstriction in the ovalbumin sensitized untreated group when compared to the non-sensitized group. Reduction in the index in NVE and salbutamol treated groups indicates bronchodilatation. Reactive oxygen species (free radicals) are produced in the cells infiltrating the bronchial mucosa in asthmatic patients [60,61]. Natural antioxidants such as

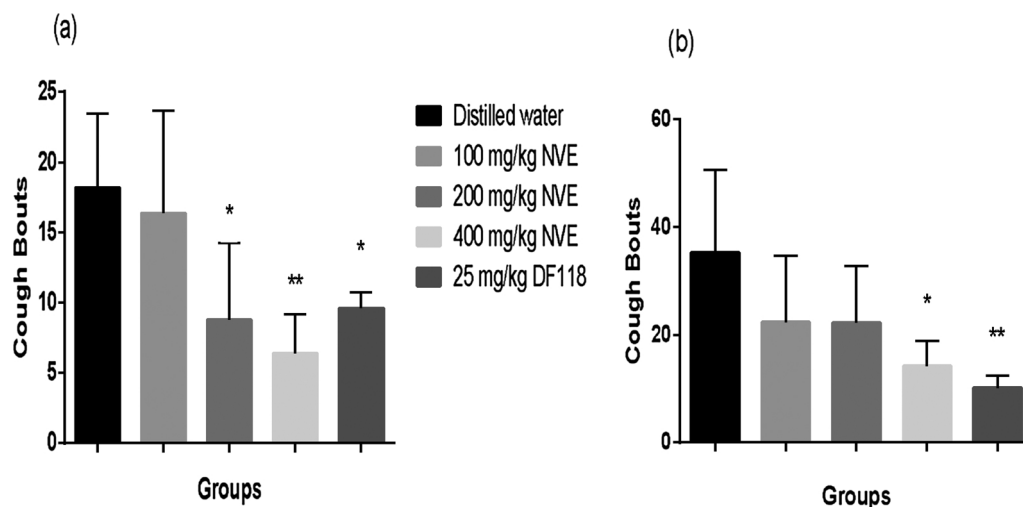


Fig. 5. Antitussive effect of methanol leaf extract of *N. vogelii* (NVE) on citric acid-induced cough in guinea pigs (a); and ammonium hydroxide-induced cough in mice (b) * $P < 0.01$, ** $P < 0.001$ compared with control. DF 118, dihydrocodeine. $n = 5$ per group.

flavonoid and other phenolic constituents of NVE could also contribute to the anti-asthmatic effects of NVE [62,63].

In this study citric acid and ammonium were used to induce cough that was significantly and dose-dependently inhibited by NVE. Chemical models of cough are believed to be similar to the physiological event in man [64]. Acidification of the airway of guinea pigs by citric acid activates A δ fibres and vagal C-fibres, partly through activation of transient receptor potential vanilloid-1 (TRPV-1) [64]. TRPV-1 expression has been found in epithelial nerves in several species [65–67] and antagonism of this receptor inhibits tussive response [68,69]. Ammonium hydroxide also causes airway irritation that may stimulate these receptors. These suggest that NVE may be acting peripherally unlike dihydrocodeine that acts centrally by inhibiting the cough centre through mu and kappa receptors [70].

5. Conclusion

The extract (NVE) contains some phytochemical constituents (e.g. tannins, flavonoids, terpenoids, and steroids) which may be responsible for its anti-asthmatic and antitussive effects. The extract appears safe owing to the very high LD₅₀ value (> 5000 mg/kg). NVE relaxes airway smooth muscles by inhibiting Ca²⁺ influx into the cells. The antitussive effect is possibly peripheral by blunting chemical irritant induced impulses in the airways. The study lends credence to the ethnomedicinal use of the leaves in asthma and cough.

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Declaration of interest

The authors declare that there is no conflict of interest.

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