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Evaluating Muco-suppressant, Anti-tussive and Safety Profile of *Polyscias fruticosa* (L.) Harms (Araliaceae) in Asthma Management

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GAK and AB designed, wrote the protocols, supervised, and performed all statistical analysis for the study. Authors JOA and SK conducted the toxicological studies for this research and wrote the first draft of the manuscript. Authors CKN and APD managed data collection, the literature searches, and laboratory work. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Polyscias fruticosa* is been used in Ghanaian folkloric medicine for the management of asthma and its related complications.
Aim: This study evaluated the muco-suppressant, anti-tussive, and safety profile of an ethanolic leaf extract of *Polyscias fruticosa* in its use as an anti-asthmatic.
Place and Duration of Study: Department of Pharmacology, Faculty of Pharmacy and

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Pharmaceutical Sciences, KNUST, Kumasi, Ghana and the School of Physical Sciences, University of Cape-Coast, Cape-Coast, Ghana; between December, 2013 and May, 2014.
Methodology: Preliminary phytochemical screening was carried out on the extract. Ammonium chloride-induced tracheal mucus phenol red secretion in ICR mice and the suppression of citric acid-induced cough in Dunkin-Hartley guinea pigs were determined after treatment of experimental animals with 100 mg/kg sodium cromoglycate, or 20 mg/kg dihydrocodeine respectively, as well as with 100, 250, or 500 mg/kg of the extract. A 100, 250, and 500 mg/kg dose of the extract was administered daily for 28 days to groups of guinea pigs to establish a safety profile in a sub-chronic toxicity study.
Results: Phytochemical screening revealed the presence of saponins and cyanogenetic glycosides, alkaloids, and sterols. The extract significantly inhibited ($P \leq .01$ - 0.001) tracheal mucus phenol red secretion, and suppression of citric acid-induced cough. There were no significant changes in body weight, haematological profile, as well as liver and kidney functions in the sub-chronic toxicity study.
Conclusion: The findings indicate that the ethanolic leaf extract of *Polyscias fruticosa* has muco-suppressant and anti-tussive properties, and is safe to use; hence a suitable adjunct/remedy for the management of asthma.

Keywords: Asthma; tracheal mucus phenol red secretion; citric acid-induced cough; muco-suppressant; liver and kidney function tests; sub-chronic toxicity study.

1. INTRODUCTION

Asthma is one common disorder encountered in clinical medicine in both children and adults. It is characterized by inflammation of the airways which causes airway dysfunction [1]. About 300 million people around the globe suffer from it, of which about 250 thousand deaths have been recorded annually [2].

Airway mucus hyper-secretion, indicative of poor asthma control, is a feature of many patients with asthma and this contributes to morbidity and mortality due to airway obstruction, air flow limitation, ventilation perfusion mismatch, and impairment of gas exchange [3]. Excess mucus not only obstructs airways but also contributes to airway hyper-responsiveness and turns cough, a protective reflex which helps expel the mucus from the airways [4]. A longer duration of cough however causes chest and thorax pain which is further discomfort for the asthmatic patient.

Although conventional mucolytic and anti-tussives, which have been used for decades, have variable efficacy in inhibiting airway mucus hyper-secretion, these are limited by their many side-effects [5]. Currently, the most commonly used anti-tussives are the centrally acting opiates such as codeine, dihydrocodeine or pholcodeine

Several medicinal plants which have anti-inflammatory properties have proved effective in the treatment of asthma. *Polyscias fruticosa* (L.) Harms, a shrub-to-small-tree which possesses anti-inflammatory, anti-pyretic and analgesic properties [6,7] is one plant used in Ghana in the traditional management of asthma. Thus, this study aims at determining the muco-suppressant and anti-tussive property of an ethanolic leaf extract of *Polyscias fruticosa* using mice and guinea-pig models, and to ascertain its safety for use in a sub-chronic toxicity study.

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

The leaves of *Polyscias fructicosa* were collected from Esiama, a community in the Ellembelle constituency in the Western Region of Ghana. The plant was identified and authenticated at the Department of Herbal Medicine, of the Faculty of Pharmacy and Pharmaceutical Sciences, Kumasi, Ghana, where a voucher specimen with number (KNUST/HM/13/W010) has been deposited.

2.2 Preparation of the Ethanolic Extract of *Polyscias fruticosa* (PFE)

Polyscias fruticosa leaves were washed, shade dried, powdered using a mechanical blender, and sieved through a mesh of 40 µm. A 700 g quantity of the powder was soaked in 1 liter of absolute ethanol in a volumetric flask for 72 h on a rotary mixer. The filtrate obtained after extraction was concentrated in a rotary evaporator (Rotavapor R-210, Buchi, Switzerland). The residue (13.6 g; Percentage

yield: 1.94%) obtained was dried in a desiccator, labelled PFE and stored at 4°C in a refrigerator. PFE was reconstituted in a suitable vehicle for use in this study.

2.3 Phytochemical Screening of PFE

Preliminary phytochemical screenings were carried out on PFE by methods described by Trease and Evans [8] and Sofowora [9].

2.4 Chemicals and Drugs Used

Sodium cromoglycate (Ashford Laboratory Ltd., Macau), Ammonium chloride (Philip Harris, Hyde-Cheshire), Citric acid (Fisons Scientific Equipment, Loughborough), dihydrocodeine (Bristol Laboratories Ltd., UK), Phenol red and Sodium chloride (BDH Chemicals Ltd, Poole, England), and Sodium hydroxide (Avondale, England), were the main chemicals used in this study.

2.5 Experimental Animals

Healthy male ICR mice (20-30 g) and male Dunkin-Hartley guinea pigs (400-500 g) were used for the muco-suppressant, and anti-tussive studies respectively. All experimental animals were maintained under ambient conditions of temperature, relative humidity and dark-light cycle, and housed in sanitized aluminum cages (70 × 42 × 28 cm) with a base dressing of sawdust as bedding. They were given access to standard pellet diet (Agricare Ltd, Kumasi, Ghana) and water *ad libitum*.

2.6 Dosing of PFE to Experimental Animals

Dosing of PFE, selected based on its known traditional usage, was by oral gavage. Individual dose volumes were calculated based on the animal's most recent body weight.

2.7 Ammonium Chloride-Induced Tracheal Mucus Phenol Red Secretion

This assay used to evaluate tracheal mucus secretion was based on the method of Engler and Szelenyi [10]. Twenty five mice were allotted to five different treatment groups. Mice in Groups 1-5 were pretreated with 2 ml/kg normal saline (p.o), 100 mg/kg sodium cromoglycate (SCG) intraperitoneally, and 100, 250, or 500 mg/kg PFE (p.o), respectively, for 30 min; after which mice were administered with 5 mg/kg ammonium chloride (p.o). After 30 min, the mice were then injected with 500 mg/kg phenol red intraperitoneally. The mice were then sacrificed by cervical dislocation 30 min after phenol red injection. The trachea of each mouse was excised, cleared of adhering tissues and washed in 3 ml physiological saline. A 0.3 ml quantity of 1 ml sodium hydroxide was added to stabilize the pH of the lavage fluid. The absorbance of phenol red was read at 460 nm using a spectrophotometer (T-70 UV/visible). The concentration of phenol secreted was estimated from the absorbance measured. A graph of absorbance against concentration was plotted, from which concentrations of phenol red were extrapolated.

2.8 Citric Acid-Induced Cough

A modified anti-tussive profile was evaluated in conscious guinea pigs against citric acid-induced cough using methods by McLeod et al. [11]. The guinea-pigs were put into a perspex box (20 × 12 × 14 cm) and exposed to 7.5% citric acid aerosol (delivered by an ultrasonic nebulizer) for 5 min. During this period, the animals were watched for cough reflexes (procedure was filmed). The numbers of coughs (basal values) were counted. After overnight fasting (with water *ad libitum*), the guinea pigs were then put into five groups (n = 4). Group 1 (normal control) was treated with 2 ml/kg Normal saline. Groups 2 (positive control) was treated with 20 mg/kg dihydrocodeine (DHC) *per os*, whereas Groups 3 - 5 were treated with 100, 250, or 500 mg/kg of PFE orally, 1 h before re-exposure to the citric acid. The percentage cough suppression was calculated for each animal as follows:

$$\text{Percentage Cough Suppression } [1-(C1-C2)/C1] \times 100$$

(where, C1 = Basal value, and C2 = number of coughs after drugs administration).

Anti-tussive activity was then evaluated in each guinea pig as the reduction in the number of coughs in comparison with the previously established basal value. The animals were then treated for seven [7] continuous days and the anti-tussive activity again evaluated.

2.9 Sub-chronic Toxicity Studies

This study was based on methods described by Koffuor et al. [12] with modification. Twenty male

guinea pigs were put into five groups (n=4). Group 1 served as normal control. Group 2 (negative control) was given 1 ml normal saline while Groups 3, 4, and 5 were given 100, 250 and 500 mg/kg of PFE by gavage daily for 28 days. The animals were observed daily for physical and clinical symptoms of toxicity. The weight of each animal was recorded on day 0, 14, and 28. Blood samples for biochemical analyses were collected into tubes containing gel and clot activator (Channel MED, China) and centrifuged at 3,000 g for 5 min, to obtain plasma for liver and kidney function tests. Blood samples were also collected into EDTA tubes to estimate the full blood count. The liver, kidneys, heart, and lungs were then harvested and the relative organ weight (ROW) of each organ calculated as follows:

ROW =

$$\frac{\text{Absolute organ weight (g)}}{\text{Animal body weight (g) on day of sacrifice}} \times 100$$

2.10 Analysis of Data

Graph- Pad Prism Version 5.0 for Windows (Graph-Pad Software, San Diego, CA, USA) was used for all statistical analyses. Data were presented as mean ± SEM and also analyzed by one - way ANOVA followed by Dunnett's multiple Comparison test (*post hoc* test). P ≤ .05 was considered statistically significant.

3. RESULTS

3.1 Phytochemical Screening

The ethanolic leaf extract of *Polyscias fruticosa* (L.) Harms, revealed the presence of glycosides (cyanogenetic), saponins, alkaloids, and sterols. (See Table 1).

Table 1. Results obtained for phytoscreening of PFE

Phytochemical	Result (-): absent; (+): present
Alkaloids	+
Flavonoids	–
Cyanogenetic glycosides	+
Phytosterols	+
Saponins	+
Tannins	–
Triterpenoids	-

3.2 Ammonium Chloride-induced Tracheal Mucus Phenol Red Secretion

Ammonium chloride-induced mucus phenol red secretion from the mice tracheae for the PFE and SCG-treated mice were significantly lower (P ≤ .05) than that of the normal saline-treated (control) group, except the 100 mg/kg PFE treatment (P > .05) (Fig. 1). This is an indication of the suppression of tracheal mucus secretion.

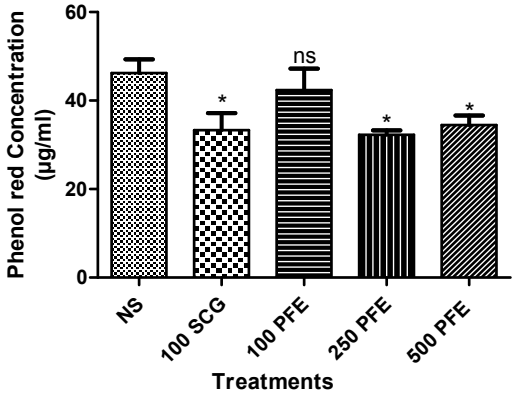

Fig. 1. The effect of 2 ml/kg Normal saline (NS), 100 mg/kg Sodium cromoglycate (SCG), as well as 100, 250, and 500 mg/kg PFE on ammonium chloride–induced mucus secretion measured by tracheal phenol red secretion

*ns implies P > .05; * implies P ≤ .01. Values plotted are means ± SEM (n = 5)*

3.3 Citric Acid-Induced Cough

PFE and Dihydrocodeine suppressed the cough response induced by 7.5% citric acid significantly (P ≤ .05-0.01) after a day's treatment (Fig. 2). After a 7-day treatment, the inhibitory effects of PFE and standard comparator were very significant (P ≤ .01 - 0.001).

3.4 Sub-Chronic Studies

3.4.1 Effects of PFE on liver and kidney functions

There were no test drug-induced liver and kidney toxicity (Tables 2 and 3) estimated in the blood samples of the various treated groups.

3.4.2 Effects of PFE on haematological profile

Full Blood Count (FBC, Table 4) estimated for all the treated groups did not show any abnormality in relation to the control group. Statistically, the

extract had no significant effect on these parameters.

3.4.3 Effects of extract on body and organ weights

The various organs did not undergo any abnormal weight changes (Table 5). Thus, there were no statistically significant differences in the relative organ weights (ROW) between treated and control groups. Overall body weights for 100 mg/kg PFE treated group had slightly reduced and that of 500 mg/kg PFE treated group slightly increased. However, these findings were not statistically significant.

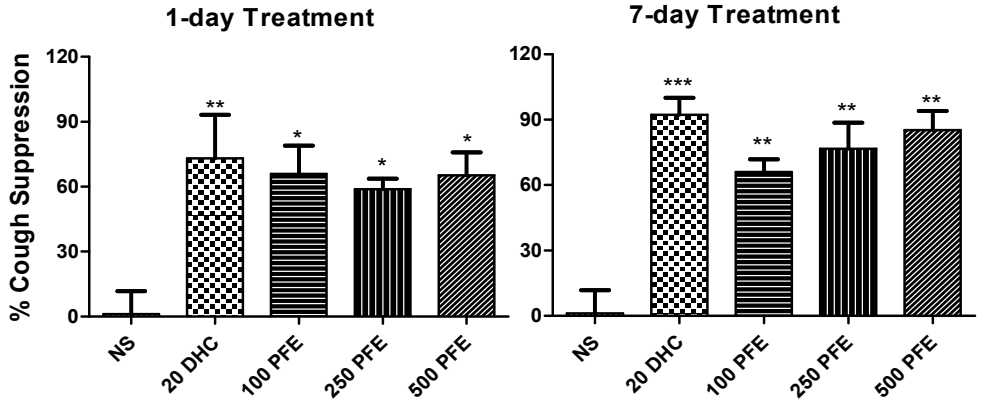

Fig. 2. Percentage suppression of citric acid-induced cough caused by 2 ml/kg Normal saline (NS), 20 mg/kg Dihydrocodeine (DHC) and 100, 250, and 500 mg/kg PFE in guinea pigs after 1 and 7 days of treatment

** implies P ≤ .05, **implies P ≤ .01, ***implies P ≤ .001 Values plotted are means ± SEM (n = 4)*

Table 2. The effect of PFE on the measured parameters in a liver function test performed on Dunkin-Hartley guinea-pigs

Parameter	Control	2 ml/kg NS	100 mg/kg PFE	250 mg/kg PFE	500 mg/kg PFE
Albumin (g/l)	37.07±0.73	37.40±0.64 ns	37.93±0.94 ns	38.43±0.49 ns	37.37±0.50 ns
Globulins (g/l)	29.80±1.26	31.50±0.81 ns	29.00±2.50 ns	31.40±0.70 ns	30.43±2.09 ns
TP (g/l)	66.87±0.84	65.83±0.71 ns	63.83±2.12 ns	71.17±0.62 ns	67.77±1.68 ns
ALT (U/l)	67.47±0.73	67.37±1.17 ns	66.60±0.40 ns	67.47±0.96 ns	66.90±0.12 ns
AST (U/l)	199.0±7.02	209.9±4.25 ns	214.6±4.52 ns	194.5±0.41 ns	212.9±7.15 ns
ALP (U/l)	148.6±5.42	153.8±4.63 ns	156±8.23 ns	149. 5±6.12 ns	151.8±5.42 ns
Bil. Direct (µmol/l)	0.87±0.03	0.73±0.09 ns	1.10±0.06 ns	0.80±0.06 ns	1.0±0.06 ns
Bil. Indirect (µmol/l)	6.167±0.38	6.33±1.02 ns	6.23±0.09 ns	4.73±0.09 ns	5.80±0.21 ns
TBil (µmol/l)	7.10±0.35	7.17±1.14 ns	7.33±0.13 ns	5.50±0.06 ns	6.87±0.29 ns
GGT (µmol/l)	0.3±0.0	0.27±0.03 ns	0.233±0.03 ns	0.30±0.0 ns	0.30±0.0 ns

Values are Means ± S.E.M. (N=3). ns implies P > .05 for insignificant increments or decrements. ALT/GPT = Alanine Transaminase/Glutamic Pyruvate Transaminase; AST/GOT = Aspartate transaminase/Glutamic Oxaloacetic transaminase; ALP = Alkaline Phosphatase; TP =Total protein; Bil = Bilrubin; TBil = Total bilirubun; GGT = Gamma GlutamylTransferase

Table 3. The effect of PFE on the measured parameters in a kidney function test performed on Dunkin-Hartley guinea-pigs

Parameter	Control	2 ml/kg NS	100 mg/kg PFE	250 mg/kg PFE	500 mg/kg PFE
Creatinine (µmol/l)	666.7±10.92	620.1±2.95 ns	678.0±50.14 ns	643.7±0.27 ns	610.9±1.25 ns
Urea (mmol/l)	15.50±0.12	12.40± 2.25 ns	12.83±0.19 ns	14.30±0.06 ns	15.10±0.40 ns

Values are Means ± SEM. (N=3). ns implies P > 0.05 for insignificant increments or decrements

Table 4. The effects of 100 mg/kg, 250 mg/kg and 500 mg/kg of PFE on the haematological profile of guinea-pigs

Parameter	Control	2 ml/kg NS	100 mg/kg PFE	250 mg/kg PFE	500 mg/kg PFE
WBC ($x10^9$/L)	4.833±0.38	5.0±0.40 ns	4.68±0.47 ns	5.03±0.233 ns	4.63±0.26 ns
HGB (g/dl)	8.23±0.23	7.80±0.23 ns	7.80±0.35 ns	7.43±0.20 ns	7.80±0.25 ns
RBC ($x10^{12}$/L)	3.53±0.19	3.60±0.23 ns	3.87±0.28 ns	3.80±0.40 ns	3.70±0.26 ns
HCT (%)	26.57±0.52	26.93±1.23 ns	27.37±0.74 ns	27.30±0.42 ns	28.30±0.66 ns
MCV (fL)	77.30±3.37	79.57±3.68 ns	77.67±0.94 ns	83.70±2.38 ns	77.37±2.17 ns
MCH (pg)	24.20±0.40	25.63±0.88 ns	25.70±1.17 ns	25.10±0.85 ns	24.40±1.23 ns
MCHC (g/dl)	29.03±0.69	28.23±1.59 ns	29.43±1.33 ns	27.77±1.13 ns	26.87±1.44 ns
RDW-CV (%)	41.07±0.44	40.13±0.55 ns	39.17±0.76 ns	40.77±0.74 ns	39.87±0.62 ns
RDW-SD (fL)	12.90±0.35	13.57±0.20 ns	13.07±0.32 ns	12.87±0.20 ns	12.87±0.09 ns
PLT ($x10^9$/L)	212.0±38.02	195.7±24.50 ns	206.7±19.53 ns	226.3±16.02 ns	185.0±8.89 ns
MPV (fL)	7.37±0.07	6.90±0.32 ns	7.13±0.12 ns	6.933±0.09 ns	7.37±0.28 ns
PDW	8.27±0.32	8.40±0.15 ns	8.70±0.32 ns	8.67±0.24 ns	9.07±0.15 ns
PCT (%)	13.63±2.71	12.87±2.21 ns	12.90±2.49 ns	14.63±1.20 ns	14.43±0.45 ns

*Values are Means ± S.E.M. (n=3). ns implies P > .05 for insignificant increments or decrements. For significant increments: * implies P ≤ .05; ** implies P ≤ .01, *** implies P ≤ .001*

Table 5. Organ weight to body weight ratio of PFE treated guinea pigs in a toxicity study

	% Organ weight to body weight ratio			
Group	**Liver**	**Kidney**	**Heart**	**Lungs**
Normal Control	3.98±0.09	0.99±0.07	0.33±0.01	1.15±0.11
2 ml/kg NS	4.89±0.25 ns	1.04±0.01 ns	0.41±0.03 ns	1.14±0.10 ns
100 mg/kg PFE	3.90±0.39 ns	1.14±0.02 ns	0.43±0.02 ns	1.04 ±0.02 ns
250 mg/kg PFE	3.58±0.02 ns	1.04±0.02 ns	0.4±0.00 ns	1.09±0.03 ns
500 mg/kg PFE	3.89±0.57 ns	1.05±0.02 ns	0.47±0.07 ns	1.24±0.13 ns

Values are Means ± SEM. (n=3). ns implies P > .05 for insignificant increments or decrements

4. DISCUSSIONS

Bronchial asthma is an inflammatory disorder of the airways characterized by airway obstruction, airway inflammation and bronchial hyper-responsiveness [13]. The inflammatory mediators in asthma are responsible for broncho-constriction, mucus secretion and increase in airflow obstruction [14]. Since mucus hyper-secretion and cough are clinical features of asthma [5], it was justifiable in this study to evaluate muco-suppressant and antitussive properties of *Polyscias fruticosa*, a plant used to manage asthma in Ghana, and to ascertain its safety for you. The ethanolic leaf extract of *Polyscias fruticosa* (PFE) administered resulted in a significant decreased the concentration phenol red secreted in tracheal mucus in mice; an effect similar to sodium cromoglycate. As the concentration of tracheal phenol red secretion is directly proportional to mucus secretion, PFE and sodium cromoglycate caused muco-suppression. Koyama et al. suggested that sodium cromoglycate is effective in reducing excessive airway mucus [15]. Sodium cromoglycate prevents the release of inflammatory mediators, such as histamine from mast cells; and the inhibition of calcium influx and chloride channels, and thus helps prevent the release of preformed cytokines from inflammatory cells [16]. Mice have been identified to possess Muc5ac- (the predominant gel-forming mucin gene expressed) and Muc5b (which localizes almost exclusively to the sub-mucosal glands at baseline, becomes detectable within the surface epithelium after antigen challenge) [17]. Congruently, PFE might prevent the activity of Muc5, Muc5b and Clara cells by inhibiting the inflammation mediators that enhances their activities [17,18].These findings are consistent with humans during mucus productions. Hence, PFE would be useful in the management of asthma. Rodgers and Barns [5] indicated that conventional therapies, including anti-cholinergics, adrenoceptor agonists, corti-costeroids, mucolytics and macrolide antibiotics, have variable efficacy in inhibiting airway mucus hypersecretion. Moreover, current information indicates that the most effective use of mucolytic drugs is long-term therapy for reduction of exacerbations of severe asthma [19].

In anti-tussive evaluation of PFE, Citric acid was used to induce cough [20]. Inhaled Citric acid stimulates the C-fibers throughout the respiratory

tract. The activated sensory nerves interact with airway neurons in higher neuronal centres to elicit the cough [21]. Since most guinea-pigs breathe nasally, the whole of the respiratory tract, from the nasal cavity to the bronchi, will be stimulated after inhalation. However, the sneeze reflex is likely to be induced upon nasal cavity stimulation [22] and the cough and sneeze reflexes are generally indistinguishable in inhalation experiments. An active expiratory effort with an explosive sound therefore, was conventionally counted as a cough in this study.

PFE produced significant cough suppression, similar to dihydrocodeine after a one-day and seven-day treatment. Dihydrocodeine has a high affinity for μ-opioid receptors [23], which are located in the periphery and central nervous system. It is therefore possible that dihydrocodeine reduces the afferent fibre nerve inputs or inhibits the activation of airway sensory receptors (C-fibers) [24] and hence abolish cough. Binding studies concerning guinea-pig and human opioid receptors demonstrated that codeine and dihydrocodeine, gold standard narcotic anti-tussives, are more selective to the μ-opioid receptor than κ- or δ-opioid receptors. *Polyscias fruticosa* therefore has a good potency to inhibit chemically induced cough; as it may contain phytochemicals that act in a similar manner as dihydrocodiene.

After one-day treatment with PFE, the percentage cough suppression increased to 65%, 58.89% and 65.23% for the three doses administered. In acute phase models, it is generally accepted that the allergen induce an eosinophilic mediated inflammation in an attempt to clear the airway [25]. It is the allergen that stimulates cytokines release to sensitize the nerves to elicit cough. PFE mimics the pathway of DHC in inhibiting cough and probably might have desensitized the afferent neurons lined in the tracheal area. The C-fibers are lined in the broncho-tracheal area and their excitation might have been decreased or inhibited by PFE. There is quite an appreciable role of the extract in minimizing this mechanism, but it is well exemplified in the seven-day treatment. Moreover, this phase of treatment was rapid; as such the immunologic mediated pathway might have played a minimal role in alleviating the stimulant.

In the chronic phase model (seven-day treatment), a greater inhibitory effect of the plant was established (Fig. 2). The plant might have established this property due to the presence of saponins, sterols and alkaloids in the extract. Secondly, this property could have been due to its ability of PFE to mimic the DHC pathway. It is observed that the extract presented a dose dependent effect on inhibiting the anti-tussive property. Continuous treatments with PFE for seven days could have induced an immunity state to the organisms. In asthma, an eosinophilic or neutrophilic infiltrate is a common feature of allergic airway inflammation and this is clinically correlated to airway hyper-responsiveness [25,26]. The inflammatory sequence in asthma is essential; hence inhibition of the sequence without adverse side effect may lead to a breakthrough in asthmatics medicine hence the safety assessment of PFE in this study. The anti-inflammatory property of PFE has been established by Koffuor et al. [7].

Preliminary phytochemical screening of PFE revealed the presence of saponins, and sterols. Saponins are well known for their anti-inflammatory and mast cell stabilizing effect. They inhibit the formation of cyclo-oxygenase metabolites, activities of histamine, bradykinin and serotonin which in turn inhibit the formation of reactive oxygen species. Sterols (steroid alcohols) too possess a very strong anti-inflammatory activity by inhibiting cytokines release (IL-1, 2 and 6), migration of leukocytes into the airways [27,28]. Cumulatively, these properties exerts an anti-inflammatory reaction, hence minimize the cough pattern. These anti-inflammatory mediators inhibit the release of cytokines and other mediators that trigger the activation of the sensory nerves [29]; hence inhibiting the fibers decreases the cough response.

It is established that substance P peripherally heightens the cough reflex mechanism by the release of prostaglandins and bradykinin. Hence, the presence of saponins in the extract will decrease or inhibit the released mediators that potentiate the activity of substance P [30,31]. This antagonistic mechanism is likely to occur at the periphery. The probable inhibition of substance P by PFE could also result in the cough suppression observed after seven days of treatment (Fig. 2). Comparing the one-day treatment to the seven-day treatment reveals a very potent anti-tussive extract when administration of the extract is prolonged. This effect confirms the assumption that long term therapy for PFE will yield a better effect. This is indicative that PFE will play a significant

therapeutic role in both acute and chronic phases of cough.

Acute and delayed toxicity studies conducted on PFE by Koffuor et al. [7], established its safety at lower doses (NOAEL: < 1000 mg/kg), and on short term use. The usefulness of data/information obtained in acute toxicity studies could however be limited clinically as drug-related toxicities could be cumulative even when drugs are taken in low doses over a period of time [32]. Multiple dose studies are therefore necessary in trying to establishing the safety profile of drugs with favorable therapeutic effects. In such a study, daily clinical observations as well as that made at the end of the study period, which are of prime importance in repeated dose studies [33], were made using lower doses (100, 250 and 500 mg/kg) of PFE. Observations did not reveal significant changes in behavioral and clinical manifestations. Feeding and water intake were normal.

There were no significant changes in the serum levels of *Aspartate transaminase* (AST), *Alanine transaminase* (ALT), and *Alkaline phosphatase* (ALP), suggesting no hepatocyte damage in animals exposed to PFE over the 28 days period. AST and ALT are enzyme present in hepatocytes/associated with liver parenchymal cells. When a cell is damaged, these enzymes leak into the blood, where it is measured. ALT rises dramatically in acute liver damage. AST is also raised in acute liver damage, but because it is also present in red blood cells, as well as in cardiac and skeletal muscle, AST is not a specific indicate of liver damage [34]. Since ALT is localized primarily in the cytosol of hepatocytes, this enzyme is considered a more sensitive marker of hepatocellular damage. A mutual rise in serum AST and ALT would be a sure indication of hepatocellular damage [35,36]. Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. ALP levels in plasma will rise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver [37].

Serum total proteins (albumin and globulin) is usually within normal ranges in liver disease; as globulin levels tend to increase, albumin levels fall. Elevation of total protein is often realized in chronic active hepatitis (which could be drug-induced), and in liver cirrhosis. Total proteins are also elevated in conditions that cause immune system over activity (production of globulins), and chronic inflammatory disorders [38]. Cirrhosis and other liver diseases could also cause significant reduction in total protein [38]. A significant reduction in serum albumin could be as a results of protein loss through renal disease, failure of protein synthesis through extensive loss of functioning liver tissue, and some inflammatory conditions where the liver switches to making other proteins [39]. As findings from the study indicated no significant change in total plasma proteins, after 28 days of PFE treatment, the extract could be said to have no destructive effect or may not have induced inflammatory disorders in the liver, as well as in other parts of the body.

After treatment with PFE, 'conjugated' (direct), 'unconjugated' (indirect) and total bilirubin were not elevated. Serum bilirubin is considered in a liver function test, since it reflects the liver's ability to take up, process, and secrete bilirubin into the bile. A raised blood level of 'conjugated' bilirubin occurs in various liver and bile duct conditions. It could therefore be said that the extract did not have any deleterious effects on hepatic metabolism or biliary excretion.

The kidneys eliminate many drugs and their metabolites. It is therefore necessary in preclinical toxicity studies, to consider renal function as this is liable to occur particularly because of the high doses of drugs given [40,41]. In this study, blood urea nitrogen (plasma urea) and creatinine were determined as markers of kidney function [42]. High urea levels can indicate kidney dysfunction, but because blood urea nitrogen is also affected by protein intake and liver function, the test is usually done in conjunction with blood creatinine, a more specific indicator of kidney function. Per the findings, there were no significant differences in serum levels of creatinine and urea in the PFE-treated groups compared to controls.

The hematological profile is very necessary in safety assessment as it has a higher predictive value (91%) for toxicity in humans [43]. Drugs administered by any route finds its way into blood. Blood is a very important tissue in the body. Its three prominent functions; transport, protection and regulation, are achieved due to its components i.e. red blood cells (RBCs), white blood cells (WBCs), platelets (PLT) and plasma. Damage and destruction of blood cells are therefore inimical to the normal function of the body. The hematological profile which estimates RBCs and its indices (Mean corpuscular hemoglobin (MCH), Mean corpuscular

hemoglobin concentration (MCHC), Mean corpuscular volume (MCV), Red blood cell distribution width (RDW), Hemoglobin concentration (HB), hematocrit (HCT), WBC (Total and Differential), as well as PLT and platelet indices, assesses the health status of an individual. Some disorders associated with blood cells include several forms of anaemia, Leucopenia, and thrombocytopenia. These disorder which could affect energy production, protection of the body against foreign substances and pathogen, and blood coagulation could be the toxic effects of xenobiotics. Since PFE did not have any significant effect on haematological profile, It suggests a high level of safety (low toxicity) with its use.

A comparison of body weights (Table 4) showed that animals treated with 100, 250, 500, and 1000 mg/kg PFE had no significant differences in comparison with the control group. Decreased activity, weight loss, reduced feed and water consumption were typical of the 2000 mg/kg PFE treated group [7]. This then becomes obvious that the extract may have exhibited some level of toxic effect at the 2000 mg/kg dosage. Significant body weight loss may be one of the most sensitive indicators of toxicity in animals; its condition is deteriorating [44]. Body weight loss is usually accompanied by a change in food and water consumption. Changes in organ weight that are observed following exposure to test drugs could be interpreted as signs of toxicity on that organ (or another) or a generalized systemic toxicity [45,46]. Important organs in the body that could be affected include the liver, kidney, heart, lungs, spleen, testis and ovaries. The absence of any significant differences in the body weight and weights of the liver, kidney, heart and lungs, and in feeding and drinking after PFE dosing provides support for the safety (within limits) of PFE.

Within the limit of experimental errors, it is worth nothing that PFE can be a new novel for long term therapy and in severe forms of respiratory distress secondary to mucous activity. Further investigations are requested on isolation, purification and characterization of chemical constituents from PFE, which are responsible for its anti-tussive and mucolytic activity.

5. CONCLUSION

The ethanolic leaf extract of *Polyscias fruticosa* has muco-suppressant and anti-tussive properties. PFE showed no significant toxic effect after 28 days of administration at doses of 100, 250, and 500 mg/kg.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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