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In Vivo Antitussive Activity of *Chrysophyllum welwitschii* against Ammonia Liquor-Induced Cough in Rats

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Abstract: ***The study was carried out to evaluate antitussive activity of the aqueous and ethanolic extracts from leaves of Chrysophyllum welwitschii (CHRYSOW) in laboratory animals. The present study was designed to observe pharmacological activities of the leaves extract of the plant Chrysophyllum welwitschii. Antitussive activity was performed by ammonia liquor-induced cough reflex rats. Analgesic and antipyretic activities were evaluated by the dose of 250 and 500 mg/kg body weight. The aqueous and ethanolic extracts of CHRYSOW (500 mg/kg) exhibited significant antitussive activity i.e. 77.82% and 65.21% respectively, but lower dose (250 mg/kg) of these showed less activity i.e. 31.70% and 22.80% respectively, inhibition in ammonia liquor induced cough (P < 0.05). The standard antitussive drug dextromethorphan (20 mg/kg) showed maximum inhibition of cough by 94.11%. It was found that both extracts of Chrysophyllum welwitschii showed antitussive activity and obtained percentage inhibition of cough reflex is approximately comparable as standard drug.***

Keywords: cough, antitussive activity, *Chrysophyllum welwitschii*, dextromethorphan

1. Introduction

The cough is a protective reflex mechanism that removes foreign material and secretions from the bronchi and bronchioles of the airways (foreign objects, catarrhs of the respiratory system, etc.). It can be in various situations inappropriately stimulated; for example, by inflammation in the respiratory tract or neoplasia. In these cases, the cough has a pathological character and it is necessary sometimes to use cough-suppressant drugs (Rang *et al.*, 1999). The most frequently used antitussive drugs in clinical conditions are from a group of narcotic analgesics. Their antitussive action is very effective at doses below those required for pain relief. Their greatest disadvantage is a high rate of unwanted effects, like depression of the respiratory center, decreased secretion in the bronchioles, and inhibition of ciliary activity (Rang *et al.*, 1999). The present study was focused on the antitussive activity of *Chrysophyllum welwitschii* (Sapotaceaes) is the lianescente specie known in West African forests (Aubreville, 1959). *C. welwitschii* has been used in traditional medicine for treatment of naso-pharyngeal affections, diarrhea, dysentery, cough-medicine, arthritis and rheumatism. *Chrysophyllum welwitschii* leaves are used to decrease the cough. But the pharmacological basis for its medicinal use especially in cough remains unknown. Therefore, this study was to evaluate antitussive activity of aqueous and ethanolic extracts of *Chrysophyllum welwitschii*.

2. Materials and Methods

2.1. Plant material

Chrysophyllum welwitschii Engl leaves, were collected from the southern region of Côte d'Ivoire. The specimen was identified and authentified in the National Floristic Center.

2.2. Preparation of extracts

The leaves of CHRYSOW were dried for three weeks and then ground to powder with an electric grinder (IKA Labotechnik). The extraction of secondary metabolites from the powder CHRYSOW was performed using solvents of increasing polarity. 200 g of powder of *C. welwitschii* leaves were macerated under vibrator for 24 hours in 2 liters of ethanol 70%. The macerated obtained was then filtered twice on white cotton and once on Whatman filter paper N°3. Then the vegetable residue was dried at 37°C in an oven for 2 hours and re-extracted under the same conditions with water. The filtrate obtained in ethanol 70% was evaporated to dryness at reduced pressure at a temperature of 40°C using a rotary evaporator type Buchi 161 Water Bath. The aqueous filtrate was concentrated by evaporation at reduced pressure at a temperature of 50°C using a type Med Center Venticell.

2.3 Experimental animals

The apparently healthy Wistar rats and albinos mice were used. The animals were purchased from Department of Toxicology, Unit of research and formation to Biologic and Pharmaceutical Sciences, University of Felix Houphouët Boigny, Côte d'Ivoire. The animals were contained in a cage and maintained under standard laboratory conditions. They were giving rodent pellets (Vital feeds) and water *ad libitum*. They were acclimatized for 2 weeks and were fasted over night with free access to water prior the experiments. The animals were conducted in compliance with NIH Guide for Care and Use of Laboratory Animals.

2.4 Antitussive activity

Antitussive activity of ethanolic and aqueous extracts of *Chrysophyllum welwitschii* leaves against ammonia liquor induced cough in rats.

2.5 Induction of cough in rats with ammonia

The experiment was conducted according to the method described by (Xu *et al.*, 1991) and (Yeo *et al.*, 2008) with modifications. In a tightly cage containing a grid, 3 mL of ammonia (25% NH_4OH) was placed in a bowl under the grid. This grid served as a platform for the movement of the animal. Ammonia was removed using a 5 mL syringe. 30 seconds later, the animal was put on the platform where it was exposed to ammonia for 2 minutes. Cough was detected by observation of the animal. The number of coughs of each animal noted for 5 minutes in the observation cage. The observation time was measured using a chronometer. Animals whose cough frequency was between 15 and 25 were retained for the rest of the experiment.

2.6 Antitussive activity

The selected animals were randomized into 6 groups (5 males and 5 females). Group 1 received distilled water *per os*. Group 2 was treated with dextromethorphan (20 mg/kg bw). Group 3 and 4 was treated with aqueous extract of CHRYSOW at the respective doses of 500 and 250 mg / kg bw. Groups 5 and 6 were treated with different doses 500 and 250 mg/kg bw of ethanolic extract respectively. The drugs were administered orally. 1 hour after drug administration, each animal was returned to cage containing the ammonia liquor (25% NH_4OH). Each animal was exposed to irritant agent, ammonia, for 2 minutes. The number of coughs produced during the last 5 min of exposure was counted.
The frequency of the cough was calculated by the following formula:

Percentage frequency of cough = (1-T / C) × 100

T: the number of coughs after treatment; **C:** the number of coughs of control (Jain *et al.*, 2013).

3. Statistical Analysis

The results were expressed as Mean ± SEM and statistically analysed by one-way analysis of variance (ANOVA) followed by Turkey's test.

4. Results

4.1 Behavior of animals

Animals showed redness in the nose, often with runny nose and red eyes. The cough differentiated from sneezing by changes in posture (stretching of the front feet and stretching towards the front of the neck) and the characteristic opening of the mouth associated with the emission of a precise sound.

4.2 Pharmacological antitussive activity of CHRYSOW

The activity of the various leaves extracts of *Chrysophyllum welwitschii* on ammonia-induced cough in rats are shown in **Figure 1**. The decrease in cough frequency was statistically significant ($P < 0.05$) compared to the control group and the extracts at 250 mg/kg. The aqueous and ethanolic extracts at doses 500 mg/kg of *Chrysophyllum welwitschii* produced a significant reduction of cough reflex. The reduction of the frequency with aqueous and ethanolic extracts of 500 mg/kg bw were 22.18 ± 6.06 and 35.57 ± 7.57 respectively. The cough frequency with dextromethorphan at a dose of 20 mg/kg (8.30 ± 3.00) was no significant compared the aqueous extract at dose 500 mg/kg ($P > 0.05$).

The dextromethorphan (20 mg / kg bw) administered to animals produced 94.11 ± 2.07% inhibition of cough bout induced by ammonia liquor. The extracts of CHRYSOW (500 mg/kg, p.o.) exhibited significant activity i.e. 77.82 ± 6.063% and 65.21 ± 6.05% respectively, but lower dose (250 mg/kg, p.o.) of these showed less activity i.e. 31.70 ± 6.20% and 22.80 ± 3.80% respectively, inhibition in ammonia liquor induced cough. The percentage of inhibition of cough was statistically no significant ($P > 0.05$) compared to the aqueous extract (500 mg/kg), but statistically significant ($P < 0.05$) compared to the ethanolic extract at the same dose. The percentage of inhibition of cough showed by figure 2.

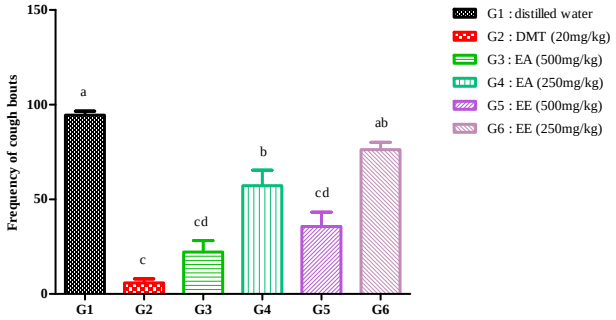

Figure 1: Frequency of induced cough of different doses of extracts of *C. welwitschii*

G1: Group 1 treated with distilled water; G2: Group 2 received DMT (20 mg/kg); G3: Group 3 received the EA (500 mg/kg); G4: Group 4 treated with EA (250 mg/kg); G5: Group 5 received the EE (500 mg/kg); G6: Group 6 treated with EE (500 mg/kg). DMT: dextromethorphan; EA: aqueous extract; EE: ethanolic extract; Ammo: ammonia. The values of the parameters are expressed as mean ± SEM for 10 rats (n = 10). Values with the same letters are not significant ($P > 0.05$).

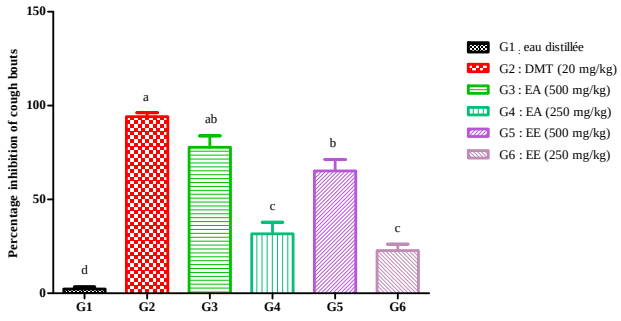

Figure 2: Percentage inhibition of induced cough of different doses of CHRYSOW leaves

The values of the parameters are expressed as mean ± SEM for 10 rats (n = 10). Values with the same letters are not significant ($P > 0.05$).

5. Discussion

This study is the first to report the antitussive activity of *C. Welwitschii* using animal models. The results of the antitussive experiment indicated that the aqueous and ethanolic extracts had antitussive activities and these activities was dependent dose. These results would be comparable to those obtained in the study conducted by Boskabary *et al.* (2006) on *Astragalus gummifer* in guinea pigs. These authors have shown that the extracts of this plant have antitussive activity. The antitussive activity of leaf extracts of *C. welwitschii* could be attributed to the bioactive compounds highlighted in this study. In addition, studies by Shang *et al*. (2010) revealed that only the alkaloid fraction has significant antitussive and anti-asthmatic properties. These results suggest that the activity of the aqueous and ethanolic extracts of *C. welwitschii* is the work of the alkaloids they contain. Cantekin *et al.* (1983) and Gavliakova *et al.* (2013) reported in their respective studies that dextromethorphan has a central action in the central nervous system in the brainstem. It stimulates opioid mu and kappa receptors by depolarization of the vagus nerve. This depolarization results in an increase in the cough threshold, hence the decrease frequency of coughing. Leaves extracts *C. Welwitschii* could have an action on the central nervous system including the brainstem. In addition, Advenier *et al.* (1987) have shown that tachykinin receptor antagonists also have antitussive activity. This suggests that the antitussive activity of *C. welwitschii* is thought to be due to the presence in these extracts of antitussive tachykinin inhibitory substances.

6. Conclusion

The present study indicated interesting results based on the antitussive activity of the CHRYSOW. Antitussive activity obtained varied with solvents used for extraction. It can be concluded that the aqueous and ethanolic extracts of CHRYSOW exerted significant antitussive activity in experimentally induced cough in rats. CHRYSOW plant has a promising medicinal value and treatment for cough of infections. Finally, the biological activity observed in *C. Welwitschii* provided a scientific basis for the use of the plant in traditional medicines. Further studies should be performed to investigate the mechanism of action for antitussive activity *C. welwitschii*.

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