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The antitussive effects of Korean red ginseng on citric acid-induced cough in Guinea pigs

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Abstract
Cough which is also called "tussis" entails the rapid expulsion of air from the lungs against a closed glottis and has a characteristic sound. This study was done to investigate the cough suppression potential of Korean Red Ginseng extract in Guinea Pigs. The animals exhibiting 10-20 bouts of cough were selected for the study. The selected animals were randomly allotted to 5 groups (n=5 per group). The animals were treated orally: group 1, being the control, received 2ml of normal saline, group 2 received 25 mg/kg of dihydrocodeine, group 3 received 150 mg/kg of Korean Red Ginseng extract, group 4 received 300 mg/kg of the extract and group 5 received 600 mg/kg of the extract. An hour after administration, they were re-exposed to citric acid aerosol, and the latency of cough and cough count were recorded. The procedure was repeated after 2 hours and after 3 hours of treatment. The antitussive activity was then evaluated in each guinea pig as the percentage reduction in the number of coughs also known as percentage suppression of cough and percentage increase in latency of cough. The results revealed that Korean Red Ginseng extract exhibited a dose-dependent percentage increase in cough latency period as well as a percentage increase in suppression of cough which were inferior to dihydrocodeine, but significantly greater than normal saline and basal levels. It is probable that the Korean Red Ginseng extract possesses cough suppression and central nervous system effects similar to that of dihydrocodeine.

Keywords: Citric acid, cough, cough suppression, dihydrocodeine, Korean red ginseng, guinea pigs

1. Introduction
Cough, also called "tussis", entails the rapid expulsion of air from the lungs against a closed glottis and has a characteristic sound [1]. Cough could be a voluntary or involuntary action aimed at clearing particles, excess mucus, irritants, microbes, or other substances from the respiratory tract [2]. As a reflex action, cough is considered a protective mechanism against unwanted substances entering the respiratory tract [3]. Viruses such as the common cold virus as well as bacteria such as *Mycobacterium tuberculosis* which infects the respiratory system could result in coughing [3]. Also, respiratory diseases such as asthma, chronic bronchitis, and emphysema could result in coughing [1]. Cough could be a side-effect of some drugs such as angiotensin-converting enzyme (ACE) inhibitors (e.g., lisinopril and captopril) and beta-blockers [4]. The mechanism is believed to be through the accumulation of bradykinin. Dust, food particles, particulate matter, smoke, and other irritants may induce the cough reflex in order to expel them from the respiratory tract [4]. Apart from infections that directly affect the respiratory system, some other illnesses have cough as a symptom. Such illnesses include malaria and gastroesophageal reflux disease. Cough is prevalent in many societies, especially, due to its association with several diseases. The prevalence of chronic cough (CC) reported in studies in Europe and the USA varies between 9% and 30% [5], while Song *et al*. [6] reported a global prevalence of 9.6% with the Oceania region having a prevalence of 18.1% and Africa a prevalence of 2.3%. In Nigeria, the prevalence of acute, sub-acute, and chronic cough is 3.8%, 1.7%, and 1.1% [7]. This is an indication that cough impacts the quality of life of many individuals. Despite the prevalence of cough in many societies, few drugs currently exist for its effective management [4], and could even be toxic with chronic use. Some cough drugs such as codeine have addictive potential and if abused, there is a risk of respiratory depression associated with these drugs. Others may produce sedative effects post-administration and this may result in accidents when the patient drives a vehicle or operates machines. There is also an emerging concern about resistance to conventional cough remedies [8, 9]. For instance, a meta-analysis of five studies with dextromethorphan and codeine in adults concluded that these central antitussives drugs have demonstrated marginally superior to placebo [3].

All these highlight the need for newer, more effective, and less toxic alternatives to conventional antitussives. A number of naturally-occurring compounds have been reported to possess activity against cough. Such agents include ginseng, honey, licorice, apricot kernel, ginger, red date, cinnamon bark, tangerine peel, etc. [4]. Thus, the essence of this study is to scientifically evaluate the cough suppression potential of ginseng extract. Ginseng has a medical history for thousands of years and become one of the most widely used traditional herbal medicines [10]. It belonged to the *Panax* genus of the Araliaceae family. The word *Panax* means "all heal" in Greek, which is based on the view that ginseng is powerful to heal any kind of disease. Ginseng originated from the Chinese words "Jen Sheng," meaning "man-herb" because the shape in the root of the plant resembles a humanoid form. The most extensively investigated ginsengs are *Panax* ginseng (Korean ginseng), *Panax quinquefolius* L. (American ginseng), and *Panax notoginseng* (Chinese ginseng) [11]. It has been documented that ginseng and its constituents exhibit a wide variety of beneficial pharmacological effects. Constituents of the ginseng plant have been shown to produce adaptogenic, restorative, vasodilatory, immunomodulatory, anti-inflammatory, antioxidant, antiaging, anticancer, antifatigue, anti-diabetic, anti-stress, and anti-depressive effects in animals and humans [11]. Ginseng is also known to affect the nervous system, due to various effects that are beneficial to the brain. Ginsenosides and other active constituents from ginseng are known to show neuroprotective properties and work as cognitive performance and memory enhancers [12].

2. Materials and Methods

2.1 Procurement of animals

Twenty-five adult Guinea Pigs of either sex weighing 460-600g were obtained from an animal facility in Ogoni, Rivers State, and brought to the animal house of the Department of Pharmacology, Faculty of Basic Clinical Sciences, College of Health Sciences, and University of Port Harcourt, Nigeria. All animals were allowed two weeks of acclimatization in the animal facility of the Department of Pharmacology, University of Port Harcourt. They were all allowed free access to food and tap water and were exposed to the natural light-dark cycle and room temperature.

2.2 Ethics consideration

All animals were managed in compliance with established laboratory animal protocols. Adherence to the National Research Council's 'Guide for the Care and Use of Laboratory Animals' [13] ensured strict adherence to all animal procedures and experimental protocols. Ethical approval for the research involving animals was obtained from the University of Port Harcourt's Research Ethics Committee.

2.3 Maceration extraction methods

One gram (1g) of dried Korean Red Ginseng root obtained from a fruit garden in Port Harcourt, Rivers State, Nigeria was ground to powder form and was mixed with 1 liter of 70% ethanol for 48 hours with agitation at room temperature in a 2-liter conical flask. After that, the extracts were taken and filtered by using a 0.45 millipore filter paper. Then, the extracts were concentrated using a rotary evaporator at 40 °C under reduced pressure. Finally, the extract was weighed and stored in a fridge at 3.3 °C till their usage for the study.

2.4 Drugs and chemicals

Chemicals and reagents were of analytical grade. They were obtained from internationally known suppliers such as Sigma (UK) and BDH (UK). Dihydrocodeine phosphate was purchased from the University of Port Harcourt Teaching Hospital, Choba, Port Harcourt. Drug and extract solutions were freshly prepared before administration.

2.5 Experimental procedure

2.5.1 Cough induction with citric acid

This was based on the guinea pig cough model of Pistia-Brueggeman and Hollingsworth [14] and Nadig and Laximi [15] with minor alterations. A day before the test, Guinea Pigs were placed individually in a transparent chamber (60 × 36 × 60 cm) for 5 minutes before cough was induced by exposure to 15% citric acid, delivered using an Omron (Omron Health Care Ltd, Japan) compressor nebulizer (rate of 0.4 ml/minute and particle size 5µm) for 10 minutes. The animals were then monitored visually within this exposure time for cough; the latency and counts, of which, were taken as the basal values. The animals exhibiting 10 - 20 bouts of cough were selected for the study and fasted overnight but with access to water.

2.6 Experimental grouping

The selected animals were randomly allotted to 5 groups (n=5 per group). The animals were treated orally thus: Group 1 was the control group and received 2 mg/kg normal saline; Group 2 received 25 mg/kg of dihydrocodeine, while group 3 received 1.439 mg/kg of extract and groups 4 and 5 received 2.878mg/kg and 5.756mg/kg of the extract respectively. An hour after administration, they were re-exposed to citric acid aerosol (as earlier described) and the latency of cough and cough count were recorded [16, 17]. The procedure was repeated at hours 2 and 3 after treatment. The antitussive activity was then evaluated in each guinea pig as the percentage reduction in the number of coughs, and percentage increase in latency of cough and was compared with the previously established control basal value, using the formulas shown below:

$$Percentage\ reduction\ in\ cough\ count = \left[1 - \left(\frac{C2}{C1}\right)\right] x\ 100 \text{ - Equation 1}$$

Where;
C1 is basal values and
C2 is the total number of coughs after treatment.

$$Percentage\ increase\ in\ latency\ of\ cough = \left[1 - \left(\frac{L2}{L1}\right)\right] x\ 100 \text{ - Equation 2}$$

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

2.7 Statistical analysis

All results are expressed as mean ± Standard Error of the Mean (SEM). Between the means of the treated and untreated groups, the significance of differences was evaluated using analysis of variance (ANOVA) followed by a Post-hoc analysis between the different groups performed with a Dunnett's t-test. A value of $p \leq 0.05$ was considered significant. Data were analyzed using Statistical Package for Social Sciences (IBM SPSS, Version 26).

3. Results

Tables 1 to 4 present the effects of Korean Red Ginseng extract on coughing behavior and latency periods in Guinea Pigs subjected to a tussive protocol induced by citric acid. The results showed the number of cough bouts, latency

periods, and percentage reduction in cough counts, and percentage increase in cough latency for different treatment groups compared to the control (Normal Saline) and reference (Dihydrocodeine) groups. Korean Red Ginseng extract significantly (p<0.05) suppressed citric acid-induced coughing in guinea pigs, demonstrating a dose-dependent response. Its anti-tussive effect was comparable to Dihydrocodeine, a known cough suppressant. The extract significantly reduced cough bouts and increased the latency period, indicating delayed cough onset.

Table 1: Effect of Korean Red Ginseng extract on the Number of cough bouts in Guinea Pigs treated with citric acid in a tussive protocol

Group	PCB	OCB	TCB	THCB
Normal Saline (NS)	17.60±0.24	14.60±0.25^{b}	13.80±0.37^{b}	13.40±0.25^{b}
Dihydrocodeine (DH)	17.60±0.24	4.00±0.32^{a}	3.20±0.20^{a}	3.00±0.02^{a}
Low Dose (Ginseng) (LDG)	17.60±0.24	8.00±0.31^{a,b}	8.20±0.38^{a,b}	7.80±0.37^{a,b}
Medium Dose (Ginseng) (MDG)	17.60±0.24	6.80±0.37^{a,b}	6.40±0.24^{a,b}	5.80±0.37^{a,b}
High Dose (Ginseng) (HDG)	17.60±0.24	5.60±0.40^{a}	5.40±0.26^{a,b}	4.60±0.41^{a}

Values are expressed as Mean ± Standard error of mean (SEM), n=5. ^{a}value is significant at p<0.05 when compared to control group 1 (Normal Saline); ^{b}value is significant at p<0.05 when compared to group 2 (Dihydrocodeine).

Hint: PCB = Pre-treatment cough bouts; OCB= Cough bouts after one hour of drug administration; TCB = Cough bouts after 2 hours of drug administration; THCB = Cough bouts after 3 hours of drug administration

Table 2: Effect of Korean Red Ginseng extract on latency period (in seconds) in Guinea Pigs treated with citric acid in a tussive protocol

Group	BLP (seconds)	OLP (seconds)	TLP (seconds)	THLP (seconds)
Normal Saline (NS)	43.60±0.24	44.60±0.21^{b}	44.80±0.37^{b}	45.40±0.40^{b}
Dihydrocodeine (DH)	43.60±0.24	59.80±0.20^{a}	60.00±0.01^{a}	60.60±0.22^{a}
Low Dose (Ginseng) (LDG)	43.60±0.24	48.20±0.20^{b}	48.40±0.25^{b}	48.60±0.24^{b}
Medium Dose (Ginseng) (MDG)	43.60±0.24	51.80±0.21^{a,b}	52.40±0.25^{a,b}	52.80±0.37^{a,b}
High Dose (Ginseng) (HDG)	43.60±0.24	55.40±0.25^{a}	56.40±0.21^{a,b}	56.80±0.20^{a,b}

Values are expressed as Mean ± Standard error of mean (SEM), n=5. ^{a}value is significant at p<0.05 when compared to control group 1 (Normal Saline); ^{b}value is significant at p<0.05 when compared to group 2 (Dihydrocodeine).

Hint: BLP = Basal Latency Period; OLP = One Hour Latency Period; TLP = Two Hours Latency Period; THLP = Three Hour Latency Period

Table 3: Effect of Korean Red Ginseng extract on percentage reduction in cough counts in Guinea Pigs treated with citric acid in a tussive protocol

Group	OPRCC	TPRCC	THPRCC
Normal Saline (NS)	17.06±0.24	21.63±1.28^{b}	23.86±1.02^{b}
Dihydrocodeine (DH)	77.19±2.03^{a}	81.83±1.04^{a}	82.94±0.24^{a}
Low Dose (Ginseng) (LDG)	52.35±2.45^{a,b}	55.03±1.83^{a,b}	56.14±2.21^{a,b}
Medium Dose (Ginseng) (MDG)	60.19±1.85^{a,b}	63.66±1.10^{a,b}	67.13±1.74^{a,b}
High Dose (Ginseng) (HDG)	68.10±2.53^{a,b}	69.28±1.83^{a,b}	73.92±2.04^{a,b}

Values are expressed as Mean ± Standard error of mean (SEM), n=5. ^{a}value is significant at p<0.05 when compared to control group 1 (Normal Saline); ^{b}value is significant at p<0.05 when compared to group 2 (Dihydrocodeine).

Hint: OPRCC = Percentage reduction in cough count after one hour of drug administration; TPRCC = Percentage reduction in cough count after two hours of drug administration; THPRCC = Percentage reduction in cough count after three hours of drug administration

Table 4: Effect of Korean Red Ginseng extract on percentage increase in cough latency in Guinea Pigs treated with citric acid in a tussive protocol

Group	OPICL	TPICL	THPICL
Normal Saline (NS)	2.29±0.15	2.75±0.45^{b}	4.13±0.43^{b}
Dihydrocodeine (DH)	37.13±0.58^{a}	37.56±0.73^{a}	38.92±0.18^{a}
Low Dose (Ginseng) (LDG)	11.06±0.50^{a,b}	11.51±0.07^{a,b}	11.92±0.43^{a,b}
Medium Dose (Ginseng) (MDG)	19.28±0.67^{a,b}	20.20±0.95^{a,b}	21.11±0.90^{a,b}
High Dose (Ginseng) (HDG)	27.07±0.54^{a,b}	29.38±0.99^{a,b}	30.30±1.01^{a,b}

Values are expressed as Mean ± Standard error of mean (SEM), n=5. ^{a}value is significant at p<0.05 when compared to control group 1 (Normal Saline); ^{b}value is significant at p<0.05 when compared to group 2 (Dihydrocodeine).

Hint: OPICL = Percentage increase in cough latency after one hour of drug administration; TPICL = Percentage increase in cough latency after two hours of drug administration; THPICL = Percentage increase in cough latency after three hours of drug administration

4. Discussion

The study investigated the potential antitussive effects of Korean Red Ginseng extract on citric acid-induced cough in Guinea Pigs. Citric acid aerosol was used to induce a tussive protocol in Guinea Pigs, which have observable cough reflexes [18]. The research aimed to assess the impact of the Ginseng extract on cough behavior and latency periods in comparison to a control group and the standard drug, Dihydrocodeine. The study measured the number of cough bouts and latency periods at different time frames to evaluate the extract's effectiveness in suppressing coughing and its potential as an antitussive agent [19]. The results of the study indicated that there were no significant differences between the basal or pre-treatment cough bouts and those of the normal saline group for various time frames. However, there were significant differences observed between the basal

cough bouts and those of the Dihydrocodeine group and the three doses (1.439mg/kg, 2.878 mg/kg, and 5.756 mg/kg) of the Ginseng extract. Statistical analysis using Dunnett t-tests revealed significant differences in the number of cough bouts between normal saline and Dihydrocodeine for various time frames, as well as between normal saline and the different doses of Ginseng extract. These findings suggest that normal saline did not have any antitussive effect, while both Dihydrocodeine and the Ginseng extract significantly reduced the number of cough bouts [19].

Furthermore, the study showed that the Ginseng extract's effect on cough behavior was dose-dependent, with the extract having inhibitory effects on the intrapulmonary rapidly adapting receptor (RAR). Dihydrocodeine, as the standard drug, demonstrated stronger antitussive effects than the Ginseng extract. Dihydrocodeine's known mechanism of action involves the stimulation of mu (μ) opioid receptors in the central nervous system (CNS), resulting in the suppression of the cough reflex [20]. It is hypothesized that the Ginseng extract's antitussive effects may also be related to CNS stimulation of μ opioid receptors, similar to Dihydrocodeine [19-22]. Regarding the latency periods, the study revealed that the basal latency period was significantly lower than that produced by Dihydrocodeine and the Ginseng extract for various time frames. Normal saline's latency periods were also significantly lower than those of Dihydrocodeine and the different doses of Ginseng extract. These findings suggest that normal saline did not suppress the cough reflex, whereas Dihydrocodeine and the Ginseng extract significantly increased cough latency. The percentage reduction of coughs produced by normal saline was significantly lower than that produced by Dihydrocodeine and the Ginseng extract, indicating that both Dihydrocodeine and the extract reduced the cough-stimulating mechanism. The increase in cough latency brought about by the Ginseng extract was dose-dependent, similar to Dihydrocodeine. It can be inferred that the Ginseng extract's impact on the CNS is comparable to Dihydrocodeine's, resulting in increased cough latency [19]. Overall, the study supports the potential of Korean Red Ginseng extract as an antitussive agent. It demonstrated significant antitussive effects comparable to the standard drug Dihydrocodeine. The Ginseng extract's dose-dependent response and similarities in efficacy with Dihydrocodeine suggest that it may modulate the cough reflex through CNS mechanisms, possibly involving μ opioid receptors. However, further research is needed to fully elucidate the underlying mechanisms and to explore the clinical applications of Korean Red Ginseng extract as a potential antitussive therapy.

5. Conclusions

Both dihydrocodeine and an extract of Korean Red Ginseng were discovered to be potent antitussives. Additionally, it was discovered that the Korean Red Ginseng extract had a dose-dependent antitussive effect that reduced cough duration and increased cough counts (latency) in a manner comparable to dihydrocodeine. It is possible that the extract has a comparable effect to dihydrocodeine as the latter also has a central nervous system action that reduces coughing.

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8. Ethical Declaration: Declared in the study

9. Declaration of interest: No conflict of interest is declared by the authors

10. Data sharing Statement: Data supporting this study are available upon request from the corresponding authors

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