

Research Article

Validation of Safety and Efficacy of Antitussive Herbal Formulations

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Background: Cough is an important defensive pulmonary reflex that removes irritants, fluids or foreign materials from the airways. Frequently, cough is non-productive and requires suppression and opioid receptor agonists such as codeine are commonly used as antitussive agents. However, opioids produce side effects that include sedation, addiction potential and constipation. Novel cough suppressant therapies should maintain or improve upon the antitussive efficacy profile of opioids but with minimum or no side effects.

Objective: To evaluate antitussive activity of combination of herbal medicines as formulations in sulphur dioxide - induced cough model in rats.

Methodology: Wister rats of either sex, weighing 150 - 200 g, were divided into 7 groups (n = 6). Group 1 served as a control and received normal saline, groups 2 received codeine phosphate, group 3 and 4 received the coded market samples and groups 5, 6 and 7 received the test samples, respectively. Thirty or sixty minutes following administration, the rats were exposed to sulphur dioxide gas for 1 minute and then placed in an open chamber for counting of cough bouts.

Results: The formulations exhibited cough inhibitions of between 15 and 27%, and 14 and 38%, with respect to the control group, 30 and 60 minutes after sample administration respectively.

Conclusion: The herbal formulations demonstrated significant ($p < 0.05$) antitussive activity in sulphur dioxide induced cough model.

Key words: Antitussive activity; herbal formulations; sulphur dioxide; cough

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1. Introduction

Cough is a defense mechanism that prevents the entry of noxious materials into the respiratory system and clears foreign materials and excess secretions from the lungs and respiratory tract (Dicpinigaitis and Gayle, 2003). Cough is also the most common symptom of respiratory tract disorders and the pharmacological

modulation of pathological cough is still unsatisfactory despite the existing large number of cough formulations. Dry coughs are treated with cough suppressants (antitussives), while productive coughs are treated with expectorants (Mason, 2002). Antitussives are widely available in the form of natural and semi-synthetic opiates such as codeine, ethylmorphine, dihydrocodeine and benzylmorphine

among others. However, these opioids alkaloids have inherent side effects like sedation, constipation, and also some addiction liability. Furthermore, their use in severe cough conditions like asthma is contraindicated, as they are known to further compromise the respiratory function (Braga and Allegra, 1989). Therefore, there is need to have effective antitussives which can successfully alleviate chronic cough without side effects.

Many medicinal plants such as *Ocimum sanctum* (Nadig and Laxmi, 2005), *Adhatoda vasica* (Indian Herbal Pharmacopoeia, 2002; Dhule, 1999) *Glycyrrhiza glabra* (Chang and Butt, 1986), *Zingiber officinale* (Suekawa et al, 1984), *Asparagus racemosus* (Mandal et al, 2000), *Passiflora incarnate* (Dhawan and Sharma, 2002) and *Ficus racemosa* (Bhaskara et al, 2003) have been reported to have antitussive activity and it is apparent that the different medicinal plants work by different mechanisms in suppressing cough. However, there are very few studies available on combined activity of the different medicinal plants. This study evaluated safety and efficacy of some formulations with a combination of natural active plant components in sulphur dioxide induced cough in rat model.

2. Materials and Methods

2.1 The herbal cough formulation samples

Honikof syrup, Herbigor Honey and Lemon syrup and Honikof tablets herbal formulations were obtained from the manufacturer, Universal Corporation Limited (UCL), Kenya. The codeine Phosphate reference standard (purity 99.9%) and two market samples (coded sample H and sample V) were also supplied by UCL. The composition of the test formulations and the coded samples are shown in **Table 1**.

2.2 Animals

Six months old male and female adult Sprague Dawley rats weighing 150 - 200 g subjected to natural lighting

conditions (12 hrs of day/ 12 hrs of night) and housed in the animal facility at the Department of Veterinary Physiology, University of Nairobi, Chiromo Campus, were used for the antitussive evaluation experiments. The animals were put in groups of 5 in plastic bottomed cages with wire tops (45 × 28 × 21 cm) bedded with wood chips and fed on chow (rat feed, UNGA feeds Limited) and water ad libitum. The wood chip dressings in the cages were changed every 3 days. Before the start of the experiments the animals were observed for 14 days in an isolated section of the animal house.

Six to eight weeks old healthy Swiss albino male and female mice weighing 20 ± 2 g random-bred and housed within the KEMRI animal facility were used for the *in vivo* safety studies. The mice were moved into the experimental room for acclimatization one week before the onset of experiments. Six Swiss albino mice were put in standard polypropylene 15 × 21 × 29 cm transparent plastic cages bedded with a wood chips and equipped with continuous-flow nipple watering devices. The cages were clearly labelled with experimental details and the mice were fed on pellets (Mice pellets, UNGA feeds Limited) and water ad libitum. The wood chip dressings in the cages were changed every two days. All dying animals in the course of experiments were placed in well-labelled biohazard bags and sent for incineration. After each experiment, surviving animals were euthanized in carbon dioxide (CO₂) chamber and placed in biohazard disposable bags and sent for incineration.

2.3 Sample preparation

The animal doses for both the test and market syrups samples were extrapolated from the human dosages as per the National Institutes of Health (NIH) protocol (Guidance August, 2007). This was done based on maximum adult dose as given in label claim. The average weight of 20 tablets was taken, powdered and some powder weighed out accurately and dissolved in 10% Tween 80 in double distilled water to give a final concentration of 223 mg/ml for safety studies.

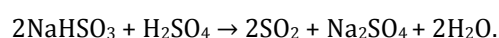
Table 1: Composition of the different herbal formulations

Honikof syrups (in mg/5 mL)	Honikof tablets (in mg/tablet)	Herbigor Honey & Lemon Syrup (per 5 mL)
<i>Ocimum sanctum</i> 30	<i>Ocimum sanctum</i> 15	Lemon juice 0.9 ml
<i>Glycyrrhiza glabra</i> 40	<i>Glycyrrhiza glabra</i> 15	Purified Honey 290 mg
<i>Adhatoda vasica</i> 40	<i>Adhatoda vasica</i> 15	Liquid glucose 4gm
<i>Zingiber officinale</i> 5	<i>Zingiber officinale</i> 5	
<i>Piper longum</i> 10	<i>Piper longum</i> 1.5	
<i>Curcuma longa</i> 2.5	<i>Curcuma longa</i> 1.5	
<i>Piper nigrum</i> 2	<i>Piper nigrum</i> 1.5	
<i>Pudina satva</i> 5	<i>Pudina satva</i> 10	
Honey 1750		

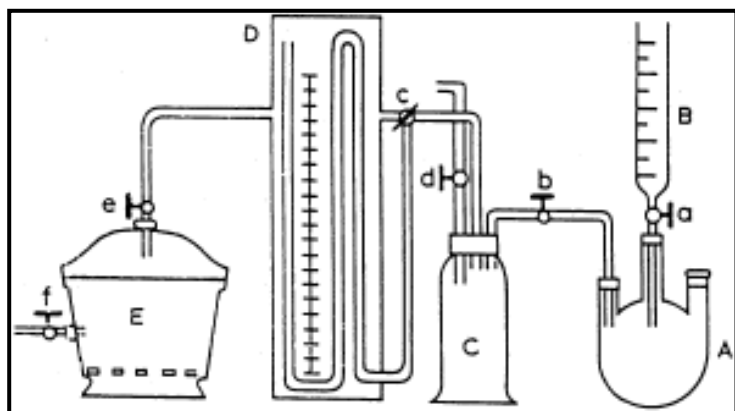
For the antitussive activity, accurately weighed powdered drug was dissolved in normal saline to give a final concentration of 361 mg/ml. For syrup; it was 15 ml (or 20 g based on weight per ml) per 60 kg body weight (bw); that is 330 mg/kg body weight administered in a single dose. Similarly, for tablets, the maximum human adult dose per day is 6 tablets and the average weight per tablet was 1.78 g. Hence, 1.78 g x 6 tablets per day translates to 10.68 g per 60 kg adult body weight (i.e. 178 mg/kg, body weight).

2.4 Sulphur dioxide (SO₂) induced cough

Cough was induced in rats by exposure to sulphur dioxide gas as per the experiment model shown in **Figure 1**. Sulphur dioxide gas was generated by introducing concentrated sulphuric acid in B into a sodium hydrogen sulphite solution in A, according to the following reaction, according to the following reaction:



The generated SO₂ gas was then introduced into the gas reservoir by opening cock b, the pressure in C was recorded using a water manometer D while the stop-cock b and e are closed and the stop-cock c opened slightly till pressure in D reached 75 mm H₂O. The rat was placed on the platform in the dessicator and exposed to SO₂ for 60 sec. The rat was then removed from the dessicator and placed in an observation chamber for counting of bouts of cough for 5 min thereafter. In the same fashion the frequency of cough was measured for all the rats in the treated groups. Cough bouts were counted by 2 independent observers using digital counters (DK-100 Electronic hand counter, Japan) and stopwatches.



A: flask containing aqueous sodium hydrogen sulphite solution
B: burette filled with concentrated sulphuric acid
C: gas storage chamber
D: water manometer
E: desiccator

Figure 1: Experiment model used to generate sulphur dioxide gas for induction of cough in rats (Miyagoshi et al, 1986)

2.5 Antitussive evaluation

Evaluation of the antitussive activity was carried out based on the method of Miyagoshi et al (1986). Both female and male Sprague Dawley rats weighing

between 150 and 200 g were randomly selected and divided into 7 groups of 6 animals each (3 males and 3 females). The animals had free access to a standard animal feed and water. One served as a control group and received only normal saline (2 ml/kg, p.o). Two groups were administered with the market samples (H and V, 2 ml/kg, p.o), another three groups were given the test samples (Honikof and Herbigor Honey & Lemon syrups 2 ml/kg, p.o and Honikof tablets at 722 mg/kg bw, p.o) and the remaining group was given the standard antitussive drug codeine phosphate (250 mg/kg, bw p.o).

The animal was first given either the normal saline, test samples, market samples or the reference drug by gavage at time 0 (zero) min then 30 min after administration, the animal was then put into chamber E for one minute, taken out and the frequency of coughs was observed for 5 minutes in an open chamber. In the same way the frequency of coughs were observed for all the animal groups at 0 min (before drug administration) and at 30 and 60 min interval following drug administration. Fresh animals were used for the 30 and 60 min experiments.

2.6 In vivo safety studies

Toxicity studies of the herbal formulations were performed with Swiss albino mice weighing 20 ± 2 g. Healthy male and female mice were randomly selected and put in groups of 6 animals (3 males and 3 females) in each cage. The animals had free access to a standard pellet diet and tap water *ad libitum*, except for a 12 hr fasting period before oral administration of the drug. Both test and market sample syrups were administered by gavage at 0.4 ml/20 gm bw /mouse (26.6 gm/kg bw) in two equal divided doses at an interval of 2 hr for the test samples (Waako et al, 2005) and a single dose of 2.235 gm/kg bw (44.6 mg in 0.2 ml) for the reference. Tween 80 was administered as the negative control. The general behaviour of mice was observed continuously for 1hr after the treatment and then intermittently for 4 hr, and thereafter over a period of 48 hr for any deaths. The mice were further observed for up to 14 days following treatment for any signs of toxicity and the latency of death (Twaij et al, 1983).

2.7 Data analysis

The pharmacological effect was monitored as the number of coughs over a period of 5 minutes. The experimental results are expressed as the mean ± the standard error of mean (S.E.M) and the percentage inhibition in number of cough bouts calculated. Statistical Package for Social Scientists (SPSS) – computer statistics software version 19.0 was used for analysis. The baseline data was compared with number of coughs obtained in the presence of test formulation and codeine made using ANOVA. The statistical significance was evaluated by using the Student's t-test. P-values less than 0.05 imply significance.

2.8 Ethical issues

The study was approved by the KEMRI Scientific Steering Committee (SSC) and Ethics Review Committee (ERC) (**Proposal SSC No. 1686**). The study animals were handled in conformity with guidelines for the

handling of laboratory animals of World Health Organization (WHO) and of KEMRI animal care and use committee (ACUC).

3. Results

Antitussive evaluation test

The effect of the various test formulations on sulphur dioxide-induced cough in experimental animals is shown in **Figure 2**. Codeine phosphate, a prototype antitussive agent, produced 64.63 and 82.44%

inhibition of cough 30 and 60 min after administration, respectively. The cough inhibition for Herbigor Honey & Lemon was 24.63 and 30.38 % while that of sample V syrup, a similar product in the market, was 24.39 and 14.35 %, 30 and 60 minutes after drug administration, respectively. Thirty and 60 min after treatment, the cough inhibition were 15.28 and 37.40 % respectively, for Honikof tablets. The cough inhibition for Honikof syrup was 17.24 and 30.38 % while that of sample H syrup, a product in the market with similar herbal ingredients, was 26.02 and 23.36%, 30 and 60 min after treatment, respectively.

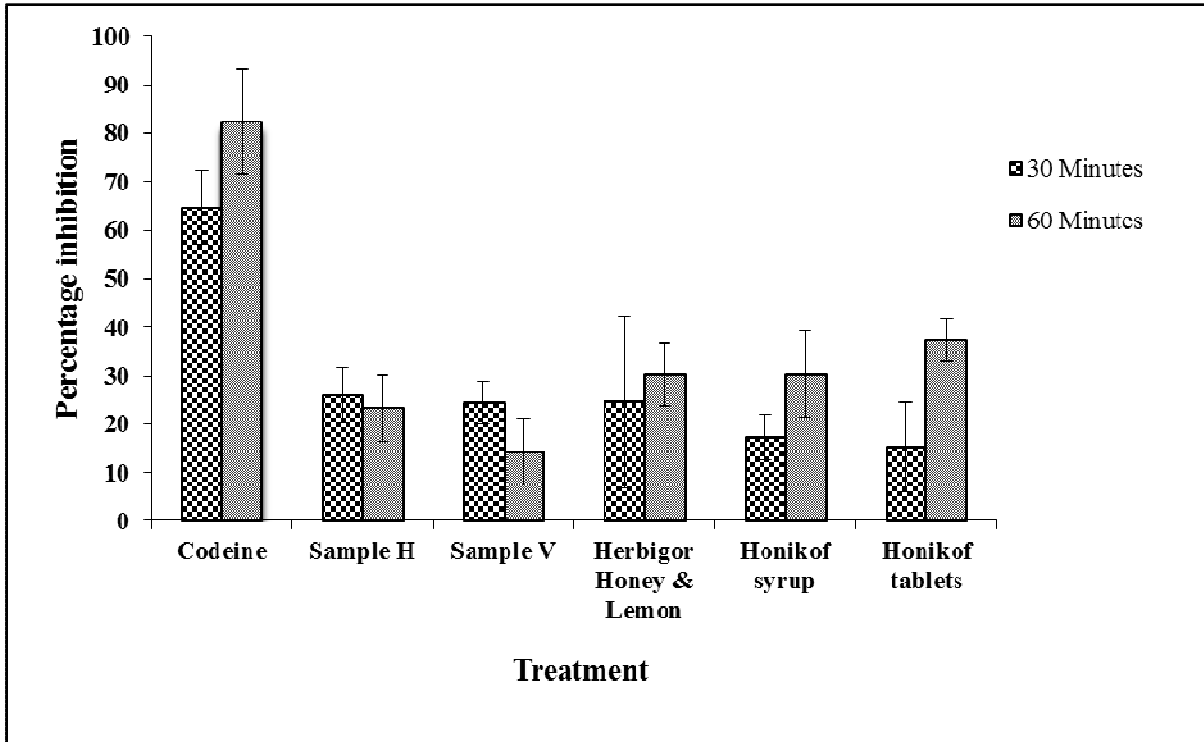


Figure 2: Effects of formulations and codeine phosphate on the cough induced by sulphur dioxide gas in rats (30 and 60 min after sample administration)

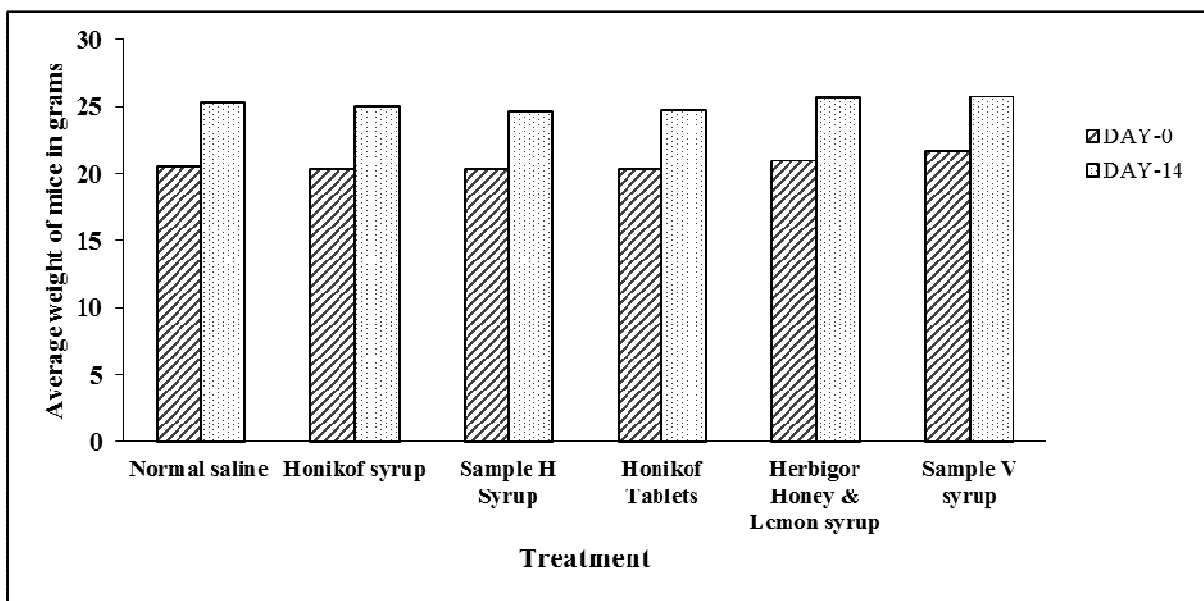


Figure 3: Average weight of mice before treatment and 14-days after treatment

In vivo safety test:

Figure 3 shows the animal weight variations before and 14 days after test samples administration. All the animals registered weight gain however; those that received sample V syrup recorded the lowest weight gain of an average of 4.1 g. On general observation it was noted that the control group behaved normal while the animals were sedated in codeine group but in the test groups (mice treated with formulations) the mice were as active as the control group. There was no apparent sedation. No animal mortalities were observed in all the groups.

4. Discussion

The successful health-care system in most developing countries has been due to the support provided by traditional medicine and the World Health Organization estimates that up to 80 per cent of the world's population relies mainly on herbal medicine for primary health care (WHO, 2002). Furthermore, around one in four of all prescription drugs dispensed by western pharmacists are likely to contain ingredients derived from plants. Some well-known medicines such as Reserpine from the root of *Rauwolfia serpentina*, used for lowering blood pressure and as a tranquilliser; L-Dopa from a tropical legume *Mucuna deeringiana*, used for treating Parkinson's disease; Ephedrine, a decongestant, from the Chinese shrub *Ephedra sinica* and Picrotoxin from *Anamirta cocculus*, used as a nervous system stimulant and in cases of barbiturate poisoning, have been derived from plants (BGCI Fact Sheet, 2000).

Sulphur dioxide induced cough is widely used as a model for evaluating antitussive activity for test samples (Bhaskara et al, 2003; Kakali et al, 1997). In this study, the reference standard, codeine, treated animals predictably had a significantly reduced number of cough bouts compared to the test formulation. The cough inhibition was generally higher for Herbigor Honey & lemon syrup both at 30 min (24.63%) and 60 min (30.38%) after drug administration in comparison to sample V which is currently in clinical use. However, the market sample showed a higher cough inhibition at 30 min (24.39%) than at 60 min (14.35%) after drug administration. The opposite effects observed with Herbigor Honey & lemon syrup is an indication of a slower onset of pharmacological activity though more efficacious compared to the market sample (sample V). Both formulations are honey based and studies have also shown that honey is an effective treatment for cough in children aged 2-18 yrs than dextromethorphan, an opiate-derived antitussive commonly found in over-the-counter cough and cold preparations (Paul et al, 2007; Warren et al, 2007). Furthermore, World Health Organization encourages the use of safe antitussives such as lemon juice and honey for the treatment of cough if treatment is desired by the patient and family (WHO, 2001).

Honikof syrup exhibited high cough inhibition at 60 minutes (30.4%) just like the Honikof tablets (37.4%) an indication of slow onset of pharmacological effects. However, the market sample currently in clinical use (Sample H) exhibited slightly higher activity at 30 minutes (26.02%) than at 60 min (23.4%). Both

Honikof tablets and syrup exhibited higher activity at 60 min than sample H syrup. These results show that both formulations have better delayed therapeutic effects than the market product. Honey which is part of the ingredients in both Honitus and Honikof has been found to have topical demulcent effects that contribute to its benefits in management of cough. Herbal cough products are a better option given the relatively low adverse effect profile and potential benefit.

Many medicinal plants have been claimed to have antitussive activity and have also been screened for this purpose, singly. Medicinal plants such as *Ocimum sanctum*, ginger, *Glycyrrhiza glabra* (licorice), which are constituents in the Honikof syrup, sample H syrup and probably in Honikof tablets, and others are major components of household cough and cold remedies worldwide. Some isolated experimental and clinical studies have also been carried out on these agents for cough (Nadig and Laxmi 2005; Chang and Butt, 1986; Suekawa et al, 1984). It is evident that the different medicinal plants would work by different mechanisms in suppressing cough and combined activity of the different medicinal plants are promising antitussive products. It was also noted that all the herbal formulations were devoid of sedative effects unlike the reference drug and this absence of sedation allows the patient to get on with normal working day without drowsiness.

5. Conclusion

It can be concluded that the antitussive products exerted significant ($p < 0.05$) antitussive effect in experimentally induced cough reflex in rats comparable to the standard drug codeine phosphate and this provides pharmacological evidence in support of these herbal products as antitussive agents. Hence, additional work relating to evaluation of their mechanism of action for antitussive effect should be carried out.

Conflict of Interest declaration

The authors declare no conflict of interest

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