

Evaluation of Antitussive activity of Polyherbomineral formulation on cough reflex induced by different cough induced models in mice

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Abstract:

Now day scientists focus the research to develop the novel cough suppressant therapies of herbomineral formulation because in respiratory disease cough is an important defensive pulmonary reflex. It removes fluids, irritants, or foreign substances. When cough becomes non-productive and require suppression and opioid receptor agonists which do not have respiratory suppressant activity but opioids produce side effects such as sedation, addiction potential and constipation, and also compromise the respiratory function. The present study was carried out to evaluate the antitussive activity of polyherbomineral formulation on cough reflex induced by different cough induced models in mice. Healthy albino mice of either sex, weighing 25-30 g were divided into seven groups, (n = 6). Group I considered as control, Group II and III received lab prepared herbomineral formulation (LPHF) (250 and 500 mg/kg, p.o.), Group VI and VII treated with marketed formulation (MF) (250 and 500 mg/kg, p.o.), Group IV and V were positive control and treated with standard (10 and 20 mg/kg, p.o.) at a dose of 0.3 ml/mice, orally. Antitussive activity of LPHF and MF were studied by sulphur dioxide gas and Ammonium liquor induced cough in mice. All the formulations used showed significant antitussive activity in sulphur dioxide induced cough model. Thus, these formulations can prove to be useful for alleviating cough. Therefore, the poly herbomineral formulation contains a major piperine active constituent which induces a cough suppressant pharmacological effect and represents an attractive approach in phytotherapeutic managements.

Keywords: Antitussive activity, Sulphur dioxide gas, Ammonium liquor.

1. Introduction:

Cough is an essential most common protective and defensive mechanism of the respiratory tract whose action prevents the entry of noxious substances, mucus, and infections and clears from the larynx, trachea, and larger bronchi. On the other hand, a number of patients have dry cough or nonproductive cough often associated with eosinophilic bronchitis, irritation of airways due to several environmental pollutants, airway hyper-responsiveness due to infection, gastroesophageal reflux disease and also without any associated cause, often referred to as idiopathic cough. It may be the first overt sign of disease of airways or lungs and may significantly

contribute to the spread of airborne infections and, in some instances, may result in severe functional and structural damage to the organism. The demulcent and hydration of respiratory tract by steam inhalation are prominently effective in reducing symptoms in majority of cases but, for uncontrolled cough, opioidergic central cough suppressants are mostly used. Among cough suppressant opioids, codeine, pholcodeine, noscapine, dextromethorphan etc. are effective, but they have significant side effects like constipation, sedation, respiratory depression, dependence, drowsiness, addiction and death from this action limit their use in human. Therefore, there is need to have effective cough suppressant which can

successfully alleviate chronic cough without side effects (1, 2).

An Ayurvedic poly herbomineral formulation consists of herbal ingredients like *Zingiber officinalis* (rhizome) (3), *Emblica officinalis* (fruit, seed) (4), *Piper longum* (fruit) (5), *Cuminum cyminum* (fruit) (6), *Piper nigrum* (fruit) (7), *Terminalia bellerica* (pericarp of ripe fruit) (8), *Terminalia chebula* (mature fruit) (9), *Piper retrofractum* (stem) (10), *Coriander sativum* (fruit) (11). Literature search for each of these ingredients have showed promising cough suppressant activity (antitussive activity) but no scientific evidence was available for overall antitussive action in combination of such herbs. Hence, the present research work focused on evaluation of antitussive activity of polyherbomineral formulation on cough reflex induced by different cough induced models in albino mice.

2. Materials and Methods

2.1. Procurement of Crude Drugs

The crude herbs for the preparation of herbomineral formulation were procured from local market of Mathura, U.P., after checked, confirmed and authenticated from Botany department, BSA, College, Mathura, U.P., India. All the chemicals were used in the experiment of analytical grade. The crude herbs used in herbomineral formulation, with their botanical identities, parts used and proportions are given in Table 1.

2.2. Preparation of Herbomineral formulation:

According to the procedure of Ayurvedic Sarsangrah (12,13), the LPHF were prepared. All the ingredients were powdered separately, passed through sieve number 80#; weighing separately each powder ingredient and mix

together in specified proportions to get homogeneous blend of LPHF.

2.3. Preparation of extract of LPHF and MF

As per the standard procedures of Ayurvedic Pharmacopoeia of India (14,15), the extraction of LPHF and MFs (Marketed Formulations) were carried out. The more yields of produced extracts were collected by the same procedure. The extracts were kept in sterile container (bottles) under refrigerated conditions, until further use. The extracts were preserved and used for further investigations.

2.4. Gas chromatography-Mass spectrometry (GC-MS) analysis

GC-MS study was conducted using an Agilent Technologies (Santa Clara, CA) 7890A gas chromatograph and an Agilent7683 Bauto injector coupled with a 5975 C VL Agilent mass selective detector. The injection volume of sample was 1 μ L, and the mass spectral scan rate 2.86 scans per second. The GC was operated split less mode with a carrier gas (helium grade 5), flow rate at 0.7mL/min, and a column head pressure of 10 psi. The mass spectrometer was operated on the electron impact (EI) mode using an ionization voltage of 70 eV and a source temperature of 230°C. The GC injector was maintained at 250°C and the transfer line at 280°C. The temperature program used consisted of an initial temperature hold at 70°C for 1min, ramped up to 250°C at a rate of 30°C/min followed by a hold at 250°C for 30 min. It operated in scan mode in 40-400 m/z range The mass spectra reported were obtained by background subtraction and are the average of at least five scans. The chromatographic separations (and collection of retention data) were carried out on a 30 m \times 0.25 mm-i.d. column coated with 0.25 μ m 100%

dimethyl polysiloxane (Rtx-1) purchased from Restek Corporation (Bellefonte, PA)(16).

2.5. Experimental animals (1, 2, 7, 17, 18, 19)

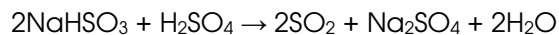
The *in-vivo* Antitussive activity was carried out in Albino mice of either sex weighing between 25–30 g by using sulphur dioxide induced cough method and Ammonium liquor induced cough method. The protocol of the present work was approved by Institutional Animal Ethical Committee (IAEC), Kota College of Pharmacy, Kota, Rajasthan, India (Ref.no.KCP/1291/09/ac/CPCSEA). The animals were procured from the Defence Research and Development Organisation (DRDO) Gwalior, M.P. In the departmental animal house, the mice were group housed in poly acrylic cages (38x23x10 cm) with not more than four animals per cage and maintained under standard laboratory conditions with natural dark and light cycle (14 hrs light/10 hrs dark) at 27±2°C and relative humidity (RH) 44-56%. They were allowed free access to standard diet (Golden Feeds, India) and tap water *ad libitum* for one week before and during the experiments. The animals were acclimatized one week before start the activity in laboratory.

2.5.1. Pharmacological Antitussive activity study

(a) *In-Vivo* Sulphur dioxide induced cough activity:

The *in-vivo* antitussive activity against Sulphur dioxide (SO₂) induced cough was evaluated by the method as describe by Miyagoshi et al., 1986 (12), as modified and simplified A solution 2 ml of 500mg/ml of sodium hydrogen sulfite (NaHSO₃, Qualikems, Fine Chemicals, Pvt. Ltd.) in double distilled water was placed in a vial at the base of a dessicator and then covered with wire gauze to act as a platform for placement of mice. Mice was exposed to sulphur dioxide gas as per the experiment model shown in Figure 1 for induce the cough. Introduce the concentrate sulphuric

acid (H₂SO₄; CDH, New Delhi, India) into a sodium hydrogen sulphite solution to evolve the sulphur dioxide gas according to the reaction as follow-



The mice were placed on the wire gauze platform in the dessicator after 15 seconds and exposed to SO₂ for 45 sec. The mice were removed from the dessicator and placed in an observation chamber for counting of cough bouts for 5 minutes thereafter. In the same fashion above procedure repeat for all the mice of the treated groups, the frequency of cough bouts was measured. Frequencies of cough bouts were counted by using stopwatch.

Initially placed the animal individually of all groups in the desiccators and noted down the cough bout responses (zero min) after exposing to certain amount 5ml of SO₂ gas. After 45 seconds exposure of gas, the animal was removed from the desiccator and observed the frequency of cough bout for 5 min. In the same way, the frequency of cough bout observed for all the animal groups at zero min before the administration of herbal formulation and at 1 hr after the administration of LPHF and MF.

Scoring of Cough Bouts:

Before administration of drug, the frequency of cough bout was observed for all the animal groups at 0 min. It has been discussed that cough bout response to a given stimulus varies from animal to animal but fairly reproducible if repeat the measurements within the same animals. So, low or high cough bout threshold in animals were not entertained for further studies. The frequency of cough bouts was observed for all animal groups at 1 hr after administration of standard drug, LPHF and MF by using same procedure and then percentage inhibition of frequency of cough bout was calculated by the formula-

$$\% \text{ percentage inhibition of frequency of cough} = (1 - T_a / C_a) \times 100$$

Where,

T_a = Frequency of Cough bout in tested herbal formulation treated animal

C_a = Frequency of Cough bout in control group treated animal

Drug treatment-

The LPHF and MF were administered orally (p.o.). The mice were divided into seven groups, each group contain six animals of either sex. The animals received the treatment as represented in table 1. Group I serve as a control, Group II and Group III received standard drug i.e. Codeine phosphate 10 mg/kg, and 20 mg/kg, p.o. respectively. Group IV and Group V administered LPHF in a dose of 250 mg/kg and 500 mg/kg p.o. Group VI and Group VII received MF in a dose of 250 mg/kg and 500 mg/kg p.o.

(b) *In-Vivo* Ammonium liquor induced cough activity:

The *in-vivo* antitussive activity against Ammonium liquor (NH_3) induced cough was evaluated. Healthy mice were selected and divided into seven groups: Group I considered as a Control, Group II and III received standard (10 and 20 mg/kg, p.o.), Group IV and V treated with LPHF (250 and 500 mg/kg, p.o.) and Group VI and VII treated with MF (250 and 500 mg/kg, p.o.). After 1h of oral administration of test drug, Individually all mice of each group was placed on wire gauze platform in desiccator and exposed to 0.3 ml NH_4OH (25%) generated by a nebulizer for 45 second. After 45 second the mice were removed from the dessicator and placed in an observation chamber for monitoring of frequency of cough bouts for 5 minutes thereafter. In the same way, above procedure repeat for all the mice of the treated groups, the frequency of cough bouts

was measured. Frequencies of cough bouts were counted by using stopwatch.

Statistical analysis

The results of pharmacological studies were expressed as Mean \pm S.D. The total variations present in data were evaluated by using Graph Pad Prism 5 project software one way ANOVA (analysis of variance) followed by Student's t-Test. The result were considered statistically significant when P- value less than 0.05 ($P < 0.05$) vs control

Result and Discussion:

Gas chromatography-Mass spectrometry (GC-MS) analysis

The identification of the compounds was done by using data base of National Institute Standard and Technology (NIST) having more than 62000 patterns and compared the spectrum of the unknown component with the spectrum of the known component stored in the NIST library. The retention time, percent area and composition percentage of the compound (piperine) was recorded and shown in figure 2. That is used in treatment of cough and cold as cough suppressant (antitussive agent).

Pharmacological Antitussive activity study

The codeine phosphate (20 mg/kg, and 10 mg/kg, p.o.), a prototype cough suppressant, administered to animals produced 77.87%, 58.10% and 72.75%, 52.67% inhibition of frequency of cough bout induced by sulfur dioxide gas and Ammonium liquor after 60 min respectively. The extract of LPHF and MF in multiple doses showed dose-dependent cough suppressant activity, showed in Table 1 and Table 2. The extract of LPHF and MF (500 mg/kg, p.o.) exhibited significant activity i.e. 68.77% and 65.02% respectively, but lower dose (250 mg/kg, p.o.) of these showed less

activity i.e. 37.30% and 34.19% respectively, inhibition in sulfur dioxide gas induced cough. While on the other hand, LPHF and MF extract (500 mg/kg, p.o.) exhibited significant activity i.e. 64.54% and 61.51% respectively, but lower dose (250 mg/kg, p.o.) of these showed less activity i.e. 34.02% and 28.65% respectively, inhibition in Ammonium liquor induced cough. All the anticough LPHF and MF formulations showed a significant inhibition in frequency of cough bouts albeit not to the extent shown by codeine phosphate Table 1, 2 and figure 3,4,5,6.

In control group, it was observed and investigated that animals behaved normal and in codeine phosphate group animals were sedated while in rest groups (mice treated with LPHF and MF) mice were active as a control group. There was no apparent sedation and no animal mortalities in all the groups.

A lot of medicinal plants have been claimed to have cough suppressant (antitussive activity). For

like *Zingiber officinalis* (rhizome) (3), *Embllica officinalis* (fruit, seed) (4), *Piper longum* (fruit) (5), *Cuminum cyminum* (fruit) (6), *Piper nigrum* (fruit) (7), *Terminalia belerica* (pericarp of ripe fruit) (8), *Terminalia chebula* (mature fruit) (9), *Piper retrofractum* (stem) (10), *Coriander sativum* (fruit) (11) etc. are major components of household cough and cold remedies worldwide, in the form of teas, decoctions etc.

We combined some of these herbal drugs in a single LPHF. To the best of our knowledge, no report illustrates use of polyherbomineral formulation as cough suppressant till date. Sulphur dioxide gas and Ammonium liquor induced cough model are widely used for evaluating antitussive activity of a candidate compound. Thus LPHF formulation with herbal extracts are effective for cough as antitussive agent but there is need for carrying out studies to determine additional benefits and underlying mechanisms (2).

Table 1: Effect of LPHF and MF formulation on cough frequency in sulfur dioxide gas induced cough mice

Experimental Group	Treatment	Dose (mg/kg, p.o.)	No. of Animals	Frequency of cough Bout	Percent Inhibition of Cough Bout
Group I	Control	Normal control	6	84.33±1.47	-
Group II	LPHF	250	6	52.66±1.27*	37.30
Group III	LPHF	500	6	26.33±1.32***	68.77
Group IV	MF	250	6	55.50±1.41*	34.19
Group V	MF	500	6	29.00±1.19**	65.02
Group VI	Codeine phosphate	10	6	35.33±1.11*	58.10
Group VII	Codeine phosphate	20	6	18.66±1.17***	77.87

Values are Mean ± SEM, n=6, No. of animals in each group.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for comparison of treated groups vs control

Table 2: Effect of LAHF and MF formulation on cough frequency in Ammonium liquor induced cough mice

Experimental Group	Treatment	Dose (mg/kg, p.o.)	No. of Animals	Frequency of cough Bout	Percent Inhibition of Cough Bouts
Group I	Control	Normal control	6	81.34±0.82	-
Group II	LPHF	250	6	53.67±1.17*	34.02
Group III	LPHF	500	6	28.84±1.32***	64.54
Group IV	MF	250	6	58.50±0.72*	28.65
Group V	MF	500	6	31.50±1.08**	61.51
Group VI	Codeine phosphate	10	6	38.56±1.05*	52.67
Group VII	Codeine phosphate	20	6	22.17±1.17***	72.75

Values are Mean ± SEM, n=6, No. of animals in each group.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for comparison of treated groups vs control

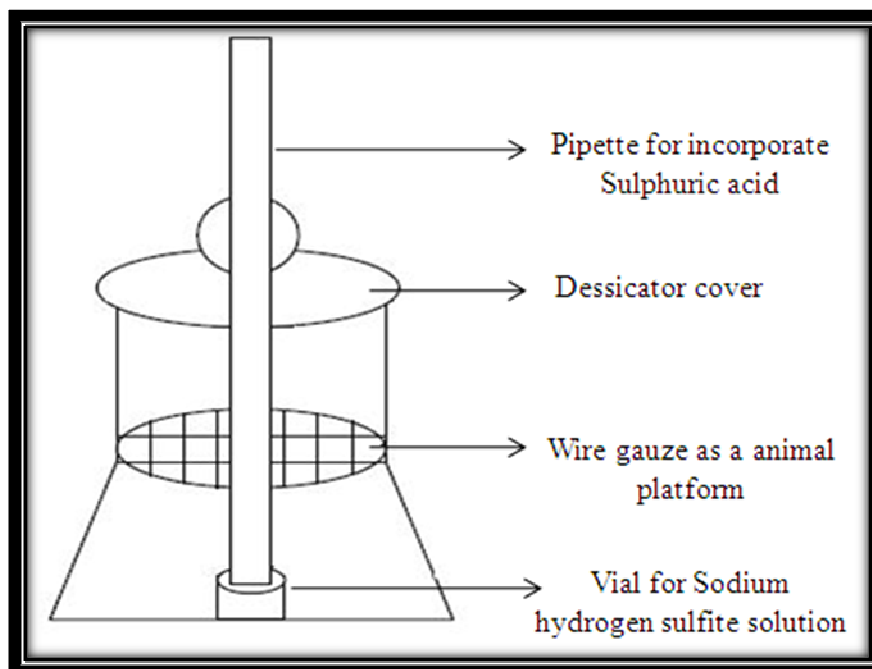


Figure 1: Schematic representation for sulfur dioxide gas (SO₂) induced cough bout model (2)

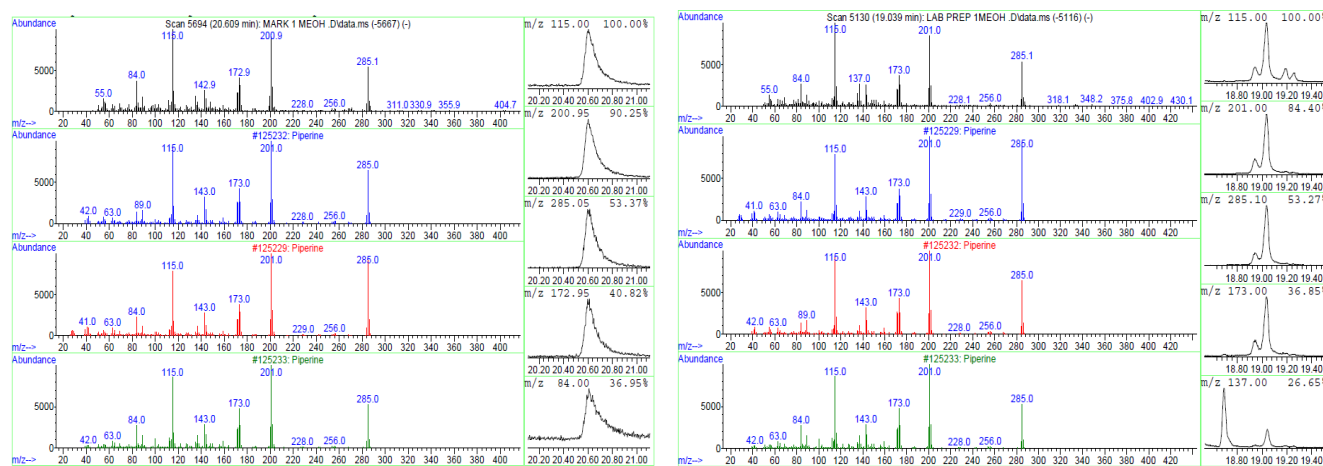


Figure 2: GC-MS spectrum of LPHF (A) and MF (B)

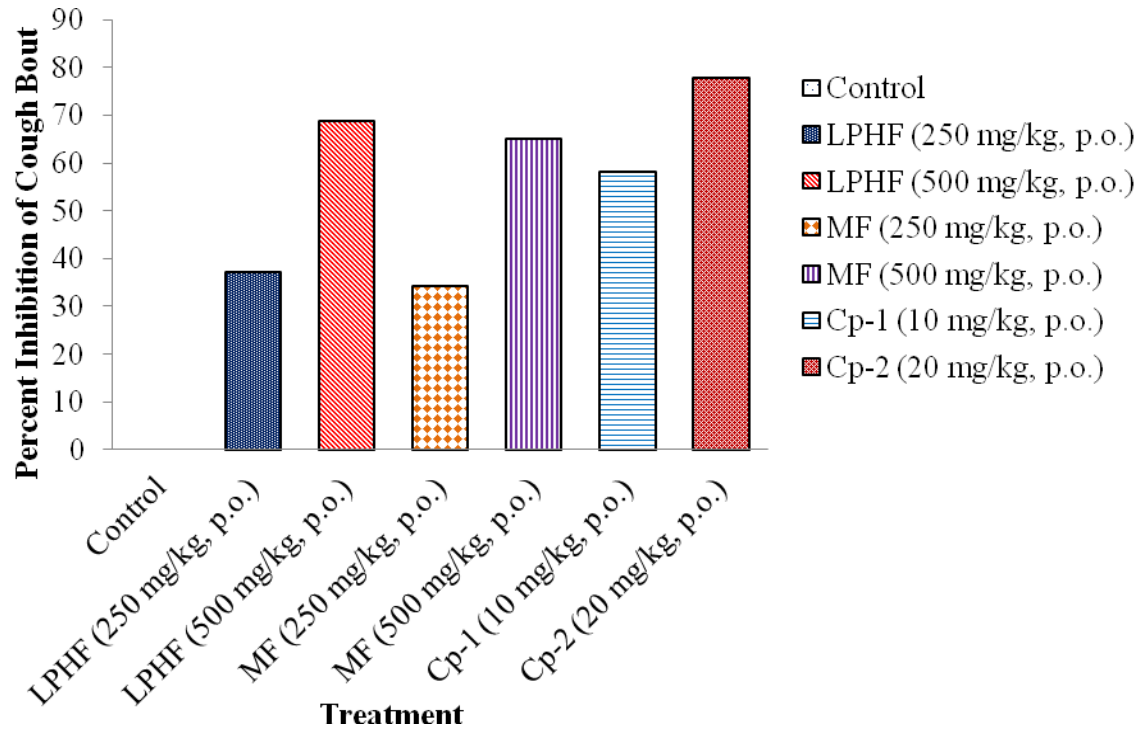


Figure 3: Comparative study of percent inhibition of cough bout of sulphur dioxide (SO₂) induced model on treatment with LPHF (Lab prepared herbomineral formulation), MF (Marketed formulation) and CP-1, (Codeine phosphate 10 mg/kg, p.o.), CP-2 (Codeine phosphate 20 mg/kg, p.o.) as a standard.

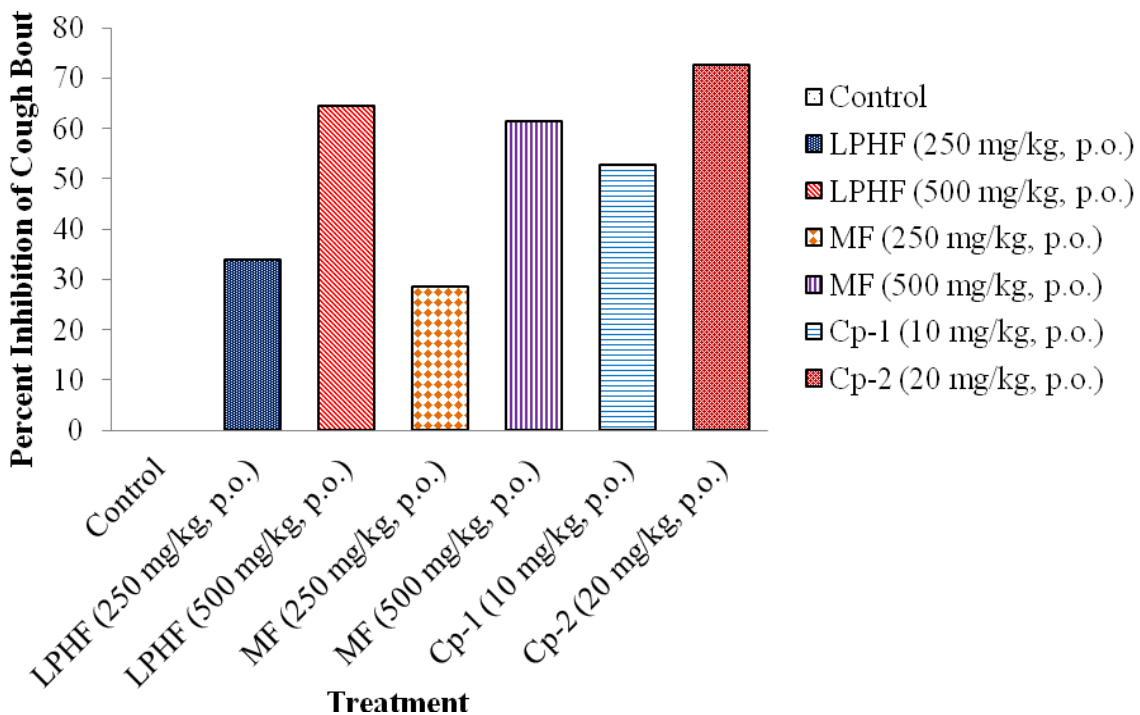


Figure 4: Comparative study of percent inhibition of cough bout of ammonia (NH₃) induced model on treatment with LPHF (Lab prepared herbomineral formulation), MF (Marketed formulation) and CP-1, (Codeine phosphate 10 mg/kg, p.o.), CP-2 (Codeine phosphate 20 mg/kg, p.o.) as a standard.

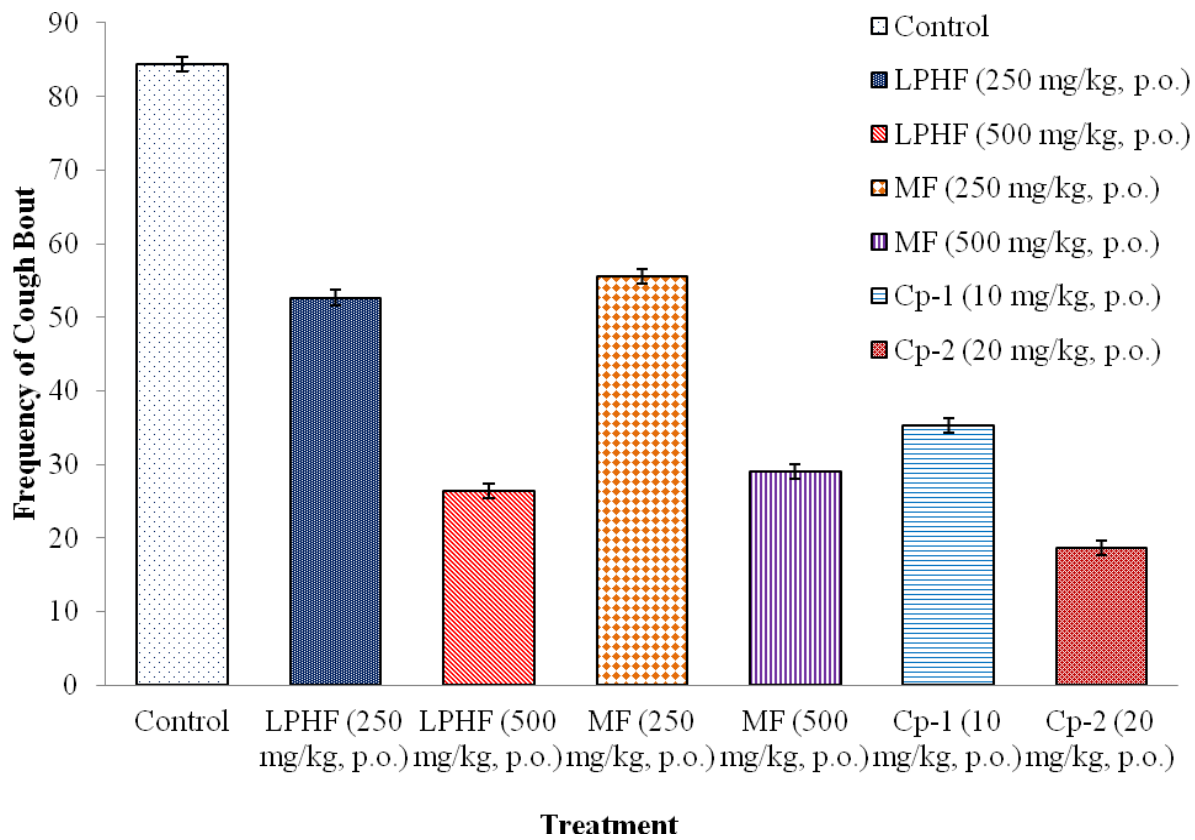


Figure 5: Comparative study of frequency of cough bout of sulphur dioxide (SO₂) induced model on treatment with LPHF (Lab prepared herbomineral formulation), MF (Marketed formulation) and CP-1, (Codeine phosphate 10 mg/kg, p.o.), CP-2(Codeine phosphate 20 mg/kg, p.o.) as a standard.

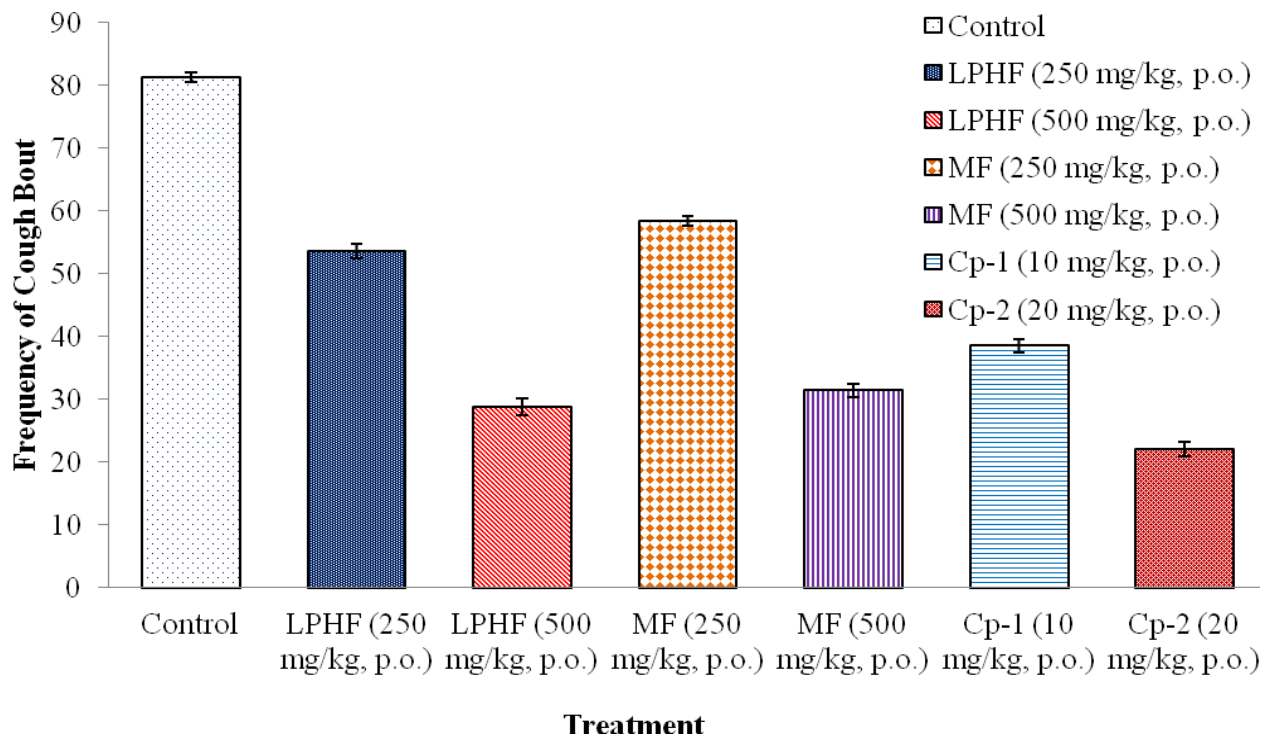


Figure 6: Comparative study of frequency of cough bout of ammonia (NH₃) induced model on treatment with LPHF (Lab prepared herbomineral formulation), MF (Marketed formulation) and CP-1, (Codeine phosphate 10 mg/kg, p.o.), CP-2(Codeine phosphate 20 mg/kg, p.o.) as a standard.

Conclusion

To conclude, our study indicate that the antitussive LPHF and MF formulation exerted significant ($p < 0.05$) antitussive effect in experimentally induced cough reflex in mice comparable to the codeine phosphate as a standard drug and provides pharmacological evidence for the traditional use of LPHF as antitussive agents. Hence, additional research work relating to evaluation of their mechanism of action for antitussive effect should be carried out (2,16).

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