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Antitussive Activity of Herbal Cough Medicine on Guinea Pigs (*Cavia cobaya*)

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ABSTRACT

This study aims to investigate the antitussive activity of herbal cough medicine containing combined herbal extracts of *Echinacea purpurea, Sambucus nigra* (Black Elderberry), *Glycyrrhiza glabra* (licorice), *Vitex trifolia* (Indian wild pepper), and *Zingiber officinale (*ginger). Antitussive assays were conducted on male Dunkin-Hartley albino guinea pigs (*Cavia cobaya*), which are divided into 5 groups, each with 8 individuals. These 5 groups were assigned for negative control group (treated with CMC-Na 0.5%), a positive control group (treated with Dextromethorphan 20 mg/kg BW), and three groups receiving herbal medicine with 3 different doses (Group I: 145 mg/kg BW, Group II: 290 mg/kg BW; Group III: 580 mg/kg BW, respectively). The antitussive activity was evaluated by using a citric acid-induced cough assay. For baseline level, 20 % m/v liquid citric acid was exposed using a nebulizer for 10 min, then the number of coughs in 15 min was counted. Sixty (60) minutes after oral administration of the tested herbal cough medicine, the number of citric acid-induced coughs was counted again to see the effect of herbal cough medicine to reduce cough. The number of coughs before and after herbal administration was calculated to obtain antitussive activity, represented by the percentage of cough suppression (PCS). Average PCS for each treatment group is then compared to control positive Dextromethorphan 20 mg/kg BW and control negative CMC-Na 0.5%. Data are analyzed by applying the one-way ANOVA method, which is followed by conducting a Tukey's Test to discover differences between groups at 95% level of confidence. In terms of the percentage of cough suppression (PCS), treatment doses at 145mg/kg BW, 290 mg/kg BW and 580 mg/kg BW result in 58.48% ± 8.60% (Group I); 58.69% ± 7.96% (Group II); 59.21% ± 8.79% (Group III) PCS, respectively, which insignificantly differ to treatment with Dextromethorphan dose (66.99% ± 9.63 with $p > 0.05$, implicating that the herbal cough medicine has comparable effect with dextromethorphan in the doses used in this study.

Keywords: cough; antitussive; dextromethorphan; citric acid assay

INTRODUCTION

According to Weinberger (2005), cough is an explosive expiration that provides a normal protective mechanism for cleaning tracheobronchial branches from secretions and foreign substances. Cough can occur due to irritation to infections, such as upper and lower respiratory tract infections, cigarette smoke, ash, and animal hair, especially cats. Other causes of respiratory disease are asthma, postnasal drip, chronic obstructive pulmonary disease, bronchiectasis, tracheitis, croup, and interstitial fibrosis (McGowan, 2006). Excessive and disturbing cough is the most common complaint that causes patients to go to the doctor. This is because cough makes discomfort and disturbs normal activity (Ikawati, 2014).

Medications used for cough vary depending on the type of cough. There are several types of cough medicine which is antitussive, expectorant, and mucolytic. Antitussive is a cough medicine that can suppress cough. Antitussive drugs work by two mechanisms based on their site of action: central nervous system (CNS) or peripheral. Peripheral antitussive drugs act by reducing the sensitivity of cough receptors in the lungs. Central antitussive drugs act at the cough center located in the medulla. Central antitussive drugs are divided into non-narcotics and narcotics antitussive. However, non-narcotic antitussive that currently works is less effective, whereas narcotic antitussive had undesirable side effects, for example giving addictive side effects (Reynolds, 2003).

The new antitussive drugs are needed to overcome the problems arising from the side effects of existing antitussive drugs. One way to find these medicines is from nature or using herbal medicines. Indonesia is famous for its diversity of plants, including medicinal plants. Local people usually use these herbs for health needs such as

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cough medicine. Innovations on the use of natural herbal combinations can be used as antitussive herbal cough medicine. This herbal cough medicine containing combined herbal extracts of dried *Echinacea purpurea* extract, *Sambucus nigra* (black elderberry) fruit extract, *Glycyrrhiza glabra* (licorice) root extract, *Vitex trifolia* (Indian wild pepper) extract, and *Zingiber officinale* (ginger) extract.

The combination of these five herbs is expected to provide synergistic effects as cough therapy. For this reason, it is necessary to do a preclinical test of these herbal combinations for cough therapy. Previously, in their study, Wisastra and Arifin (2020) found that the herbal cough medicine containing *E. purpurea* herba dry extract, *S.nigra* fructus extract, *G.glabra* radix extract, *V. trifolia* folium extract, *Z.officinale* rhizoma extract is a mucolytic agent. This herbal cough medicine had also acted as mucoactive (Arifin *et al*., 2020).

The objective of this study was an evaluation of the antitussive activity of herbal cough medicine containing combined herbal extracts of dried *E. purpurea* extract, *S.nigra* (black elderberry) fruit extract, *G.glabra* (licorice) root extract, *V.trifolia* (Indian wild pepper) extract, and *Z.officinale* (ginger) extract on guinea pig animal model induced with citric acid.

METHODOLOGY

Materials

Experimental animals

This preclinical testing was conducted at the Pharmacology and Toxicology Laboratory of Faculty of Pharmacy UGM Yogyakarta. In the antitussive test, the experimental animals used were albino male Dunkin-Hartley guinea pigs from the Animal Care Unit (UPHP) of PT Biofarma Bandung, who was 8-10 weeks old, weighing between 500-570 grams. Before being treated, guinea pigs were acclimatized for 5-7 days to adjust to the conditions of the cage and laboratory. The condition of the cage was maintained at a temperature of 22°C ± 3°C and with a relative humidity of around 55 ± 15%. The light source came from the lamp (artificial light), with a duration of 12 hours of bright light and 12 hours of dark hour. The research protocol in this research has been approved by the Ethics Commission for Preclinical Research of the Integrated Research and Experimental Laboratory (*Laboratorium Penelitian dan Pengujian Terpadu* – LPPT), Gadjah Mada University, Yogyakarta, Indonesia.

Test Substance

The herbal cough medicine was provided by PT SOHO Industri Pharmasi. A commercially available Dextromethorphan was used as a positive control. The citric acid and other chemicals used in the study were of analytical grade.

Methods

Sample Preparation

The herbal cough medicine was provided by the manufacturer in tablet form. The supplement was then powderized and prepared in 0.5% Sodium Carboxy Methyl Cellulose (CMC-Na) carrier as suspension.

Dose Administration

The dose used was converted from the estimated dose in humans, which is 3 caplets a day which is equivalent to 3744 mg / day with a conversion factor for guinea pigs (400 grams) of 0.031, to 116 mg / 400 g BW. The dose becomes the middle dose. The positive control dose of dextromethorphan was converted from the therapeutic dose in humans, namely 20 mg. The dosage ranges to be used are: Dosage I: ½ x 116 mg / 400 grams = 58 mg / 400 grams=145 mg/kg BW; Dosage II: 116 mg / 400 gram = 290 mg/kg BW; Dosage III: 2 x 116 mg / 400 grams = 232 mg / 400 grams= 580 mg/kg BW.

Test on antitussive effect

The antitussive test method referred to Song (Song *et al*., 2015). At the treatment, experimental animals were given oral administration of the tested herbal product following the treatment allocation. After 60 minutes, guinea pigs were exposed to 20% w/v citric acid through a nebulizer and kept in a conscious state for 10 minutes. The frequency of cough was counted for 15 minutes. The antitussive activity was shown as a percentage of cough suppression (PCS) in each animal, which was the comparison of baseline cough frequency and treatment. The average PCS in each treatment group was then compared with the positive and negative control groups. The guinea pigs' airways possess the needed afferent nerves and can produce cough like in human because this guinea pig is used as an animal model (Aggrawal *et al*,1991).

Calculation of Antitussive Effects

The antitussive effect was calculated using the formula below:

$$\mathbf{PCS} = [\mathbf{1} - (\boldsymbol{C2}/\boldsymbol{C1}) \times \mathbf{100}]$$

PCS = Percentage of cough suppression; C1 = Baseline cough frequency; C2 = Cough frequency after treatment.

G.glabra (licorice) root extract, *V.trifolia* (Indian

Table I. Percentage of Cough Supression from Herbal Cough Medicine (HCM)

Treatment	Baseline Cough Frequency X±SEM	Treatment Cough Frequency X±SEM	Percentage of cough suppression X±SEM
Negative Control CMC-Na 0,5% 1 ml/kg	13.88 ± 0.85	12.75 ± 0,98	7.98 ± 5.38
Positive Control Dextromethorphan 20 mg/kg BB	16.88 ± 1,16	5.25 ± 1,25	66.99 ± 9.63*
Dose I HCM- 145 mg/kg BW	16.63 ± 1,29	7.00 ± 1,46	58.48 ± 8.60*
Dose II HCM - 290 mg/kg BW	17.88 ± 1.34	7.50 ± 1,61	58.69 ± 7.96*
Dose III HCM - 580 mg/ kg BW	16.88 ± 1,16	8.25 ± 2,19	49.59 ± 15.04*

**significantly different from the negative control based on statistical tests (p = 0.05)*

Statistical Analysis

The data obtained were stated as mean ± SEM. Statistical analysis was performed first by using the normality test with the Shapiro Wilk Test with a significance level of 95%. Data were considered normal if P≥0.05. Then the data were proceeded by homogeneity test with a significance level of 95%. Data were considered homogeneous if P≥0.05. Then, the data were analyzed with one-way ANOVA. Furthermore, the data were analyzed further using the Tukey HSD test. The difference between the treatment and control groups was considered statistically significant if the P-value ≤ 0.05

RESULT AND DISCUSSION

The antitussive activity was shown as a percentage of cough suppression (PCS) in each experimental animal, which was the comparison of baseline cough frequency and the treatment. As shown in Table I, the herbal cough medicine was able to significantly reduce the frequency of cough compared to the negative control group at a dose of 145 mg/kg BW, 290 mg/ kg BW, and 580 mg/kg BW with a PCS value (percent reduction in cough) of 58.48 % ± 8.60%; 58.69% ± 7.96%; 49,59 ± 15,04. The PCS values were not significantly different from that of dextromethorphan in the dose of 20 mg/kg BW, which was 66.99% ± 9.63%. It was interesting that increasing the dose of the herbal cough medicine did not significantly increase its antitussive effect. Dose I which was equivalent to 1.5 caplets of human dose could already provide antitussive effects which were not statistically significantly different from the positive control of dextromethorphan, which had an antitussive effect of 66.99% ± 9.63%. These results showed that the herbal cough medicine containing combined herbal extracts of dried *E.purpurea* extract, *S.nigra* (black elderberry) fruit extract, wild pepper) extract, and *Z.officinale* (ginger) extract had the potential as antitussive herbal medicine.

The several herbals of this combination are thought to work synergistically. *Vitex trifolia* leaf extract is known to have *tracheospasmolytic*, anti-allergic, and anti-inflammatory effects to relax the trachea muscles and reduce inflammation of the airways (Ikawati *et al*., 2009). The anti-allergic effect from *Vitex trifolia* leaf extract is playing an important role in the dry cough suppression caused by an allergen. Although the tracheospasmolytic and anti-inflammatory effect from the *Vitex* extract is indirectly involved in the antitussive mechanism of action, the thracheospasmolytic could help to elevate the spasm or trachea caused by persistent dry cough, whereas anti-inflammatory properties of *Vitex* extract could help to reduce the inflammation that also accompanies a dry cough (Alam, 2002). Therefore, having the *Vitex* extract in the combination gives an additional benefit for a comprehensive dry cough. The extract has also been tested in humans and has been shown to decrease the effect of allergic rhinitis symptoms (Herdwiani, 2011). Ginger is known to have pharmacological activities that also lead to the respiratory tract, especially its activity as an antitussive and anti-inflammatory (Rehman *et al*., 2010).

According to Suekawa *et al* (1984) (6)-Shogaol a compound isolated from ginger showed an intense antitussive effect compared with dihydrocodeine phosphate While, Echinacea is a popular herb that is widely used as an immune system booster, which works by triggering the complement pathway and increasing the number of leukocytes, stimulating phagocytosis, production of T-cell lymphocytes, increasing lymphocytic activity, and cellular respiration

(Hudson, 2012). Black elderberry has a significant effect in healing flu-like symptoms and inhibits bronchitis virus infection (Chen *et al*, 2014), while liquidity apposite and liquidity isolated from licorice have antitussive effects (Kuang *et al*, 2018).

The mechanism of action of this herbal cough medicine should be interesting for further investigation especially the sinergism of *Echinacea purpurea, Sambucus nigra, Glycyrrhizae glabrae, Vitecis trifoliae,* and *Zingiberis officinale* but the development of antitussive herbal medicines as an alternative to dextromethorphan has its advantages considering that dextromethorphan is now a medicine that is often misused.

CONCLUSION

From the results of the study, it can be concluded that the herbal cough medicine containing combined herbal extracts of *E. purpurea*, *S. nigra* (black elderberry), *G.glabra* (licorice), *V.trifolia* (Indian wild pepper), and *Zingiber officinale* (ginger) at therapeutic doses in humans (1-3 caplets a day) has an antitussive effect comparable to dextromethorphan at the therapeutic dose.

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