STUDY OF POTENTIAL ANTITUSSIVE ACTIVITY OF GLYCYRRHIZA GLABRA GRANULES USING A COUGH MODEL INDUCED BY SULFUR DIOXIDE GAS IN MICE

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ABSTRACT

Objective: All over world cough is a common symptom in respiratory disease. When cough becomes severe, opioids act as a potent drug, but they have various side effects such as sedation and constipation. Therefore, there is a necessity to have an effective antitussive formulation, which not revealed respiratory depressant activity. The present study was carried out to analysis the antitussive activity of granules containing Glycyrrhiza glabra L. extract using a cough model induced by sulfur dioxide (SO₂) gas in experimental mice.

Method: The antitussive effect of G. glabra granule formulation on SO₂ gas induced cough in experimental animals, which compared to standard codeine sulfate and the result was determined by statistical analysis.

Results: The antitussive activity of the granules tested in control, standard, and test animal group, respectively, it was compared to standard codeine sulfate (10, 15, and 20 mg/kg body weight [BW]). Codeine sulfate as a standard drug for suppression of cough, acts as potent antitussive agent, which produced 25.29%, 33.33%, and 47.13% inhibition in cough at a dose of 10 mg/kg, 15 mg/kg, and 20 mg/kg, respectively, whereas codeine sulfate (20 mg/kg) showed a maximum inhibition of 47.13% (p<0.01) after 60 min of experiment. The test group of mice was showed 41.17% inhibition, in cough on treatment with G. glabra granules after 60 min of an experiment. This is very significant or nearly equal to a maximum dose of codeine sulfate (20 mg/kg).

Conclusion: Statistical analysis shows very significant antitussive effects of G. glabra granules at the level of p<0.01 in inhibiting the cough reflex at 200 mg/kg BW in comparison to the control group.

Keywords: Antitussive effect, Granule formulation, Sulphurdioxide induced cough statistical analysis.

INTRODUCTION

A cough, also called as tussis, is a voluntary either involuntary carry through clear the throat as well as breathing passage of foreign particles, fluids, microbes, irritants, and mucus; its a rapid expulsion of air from the lungs, it interferes with quality of life and even cause exhaustion. Dry cough is related with eosinophilic bronchitis, irritation of airways due to several environmental pollutants, airway hypersensitivity due to infection, gastroesophageal reflux disease, and also without any related cause is mentioned to as idiopathic cough [1]. Hydration of respiratory tract by steam inhalation, demulcants are adequate to decreasing symptoms in common of cases but, for uncontrolled cough, opioidergic central cough suppressants are more preferred. Among opioids, codeine, pholcodine, noscapine, and dextromethorphan are potent, but they have certain constitutional side effects such as sedation, constipation, as well as addiction liability. Furthermore, their use in serious cough conditions like asthma is contraindicated, as they are known to more compromise the respiratory function. Hence, there is essential to have effective antitussive which can successfully improve chronic cough without side effects. Cough suppressant and antiasthmatic activities have been claimed for many medicinal plants in the literature. On the basis of this knowledge, different workers have assessed botanicals for antitussive/cough suppressant activity; for example, Ocimum sanctum, Paspalum incarnatum, Ionidium suffruticosam, Trichodesma indicum, Abies webbiana, Ficus racemosa, Lagerstroemia parviflora, Drymaria cordata, Leucas lavandulaefolia, Jussiaea suffruticia, and Asparagus racemosus. While that the different medicinal plants would work by different mechanisms of suppressing cough; there are very few studies available on the combined activity of the different medicinal plants [2].

Glycyrrhiza glabra L. is one of the most commonly recommended medicines in Ayurveda for antitussive activity. We, therefore, evaluated herbal formulations in sulfur dioxide (SO₂)-induced cough model in mice. It uses as a sweetening and flavouring agent. It is also used as an herbal remedy for gastritis and upper respiratory tract infections, the effectiveness of antihypertensive drugs (Mansoor, 2001), skin diseases such as dermatitis, eczema, and psoriasis. It was used to increases bile flow and lowers cholesterol levels, antihelminthic, anti-allergenic, antineoplastic, as well as its being tonic and demulcent laxative emollient are used in genitourinary diseases, coughs, and sore throat [3-7].

METHODS

Plant material

The dried part of plants was purchased from the renowned Ayurveda shop in Kolhapur city during the month of December 2016. It confirmed the authenticity of the plant sample using the comparison of transverse section of root and phytochemical test with the reference of literature.

Extraction of G. glabra

The roots G. glabra of (250 g.) was crushed and pulverized by a mechanical grinder to form a coarse powder then extracted using ethanol (70%/v/v) with the help of Soxhlet extractor for 24 h. The extract was dried using a water bath for 10–12 h at 45°C [8].

Phytochemical screening of extract

The standard screening test was carried out for various plant constituents such as Saponin, flavonoids, alkaloids, steroids, terpenoids, tannins, glycosides, carbohydrates, proteins, phenolic compounds, and anthraquinones [9].
Ultraviolet (UV) spectroscopy
The G. glabra L. root extracts were tested using UV spectroscopy to confirm the presence of phytochemicals in the sample with the help of standard UV range (254 nm) with the reference of literature.

Preparation of standard stock solution
The standard stock solutions of G. glabra extract were prepared by dissolving 10 mg of extract in phosphate buffer (pH 6.8) ethanol in 70:30 proportion and final volume was adjusted with the same solvent in 100 mL of the volumetric flask to get a solution containing 100 μg/mL. From the above solution concentrations of 10, 20, 30, 40, and 50 μg/ml were prepared. Working standard solutions for each solvent were scanned at the selected wavelength, and the calibration curves were constructed. The calibration curve for extract was plotted by taking absorbance at 254 nm [10].

Thin-layer chromatography (TLC)
The identification of phytoconstituents was carried out using TLC. Different reported solvent systems and spraying reagents were tried for developing a TLC system for identification of constituents on the basis of a literature survey and phytochemical screening. The solvent system chloroform:methanol:glacial acetic acid:water (8:4:1.5:1) selected as a solvent system [11].

Formulation of granules
For the preparation of granules, the accurately weighed quantities of extract and other excipients were mixed together in mortar and pestle to form homogeneous powder blend, and using wet granulation formulation was optimized, ingredient which uses for granule formulation are shown in Table 1.

Evaluation of granules
Flow properties [12-16]
Bulk density
Apparent bulk density (ρb) was determined by pouring the powder blend into a graduated cylinder. The volume bulk (Vb) and weight of powder (M) were determined. The bulk density was calculated using the formula.

\[ ρ_b = \frac{M}{V_b} \]

Tapped density
The measuring cylinder containing a weighed amount of powder blend was tapped for a fixed time. The minimum volume (Vt) occupied by powder blend after a fixed number of toppings in the cylinder and weight (M) of the blend was measured. The tapped density (ρt) was calculated using the following formula.

\[ ρ_t = \frac{M}{V_t} \]

Angle of repose
The flowability of a powdered blend of all the batches was assessed by the angle of repose. The angle of repose was determined using fixed funnel free-standing cone method. The angle of repose was determined in triplicate for all the batches using the formula

\[ θ = \tan^{-1} \left( \frac{H}{R} \right) \]

| Ingredients | Quantity (mg) |
|-------------|---------------|
| Extract     | 200           |
| HPMC K 100  | 50            |
| MCC 102     | 50            |
| Talc        | 50            |
| Mg St.      | 06            |

Table 1: Ingredient of formulation

Where “θ” is angle of repose; “H” is height between lower tip of the funnel and the base of a heap of powder; and “R” is radius of the base of heap formed [Jadhav et al., 2010].

Different ranges of flowability in terms of angle of repose are given in Table 2.

| Carr’s compressibility index (CCI) | Hausner’s ratio (HR) |
|-----------------------------------|----------------------|
| TD-BD × 100                       | TD                     |
| TDHR= BD                          |                       |

Where, TD and BD have tapped density and bulk density, respectively.

Moisture content
Control of moisture content in granulations is very important, and it could affect the physical and chemical performance of final dosage forms. Moisture content is generally measured using moisture analyzer during product development; a thin layer of sample is heated at a set temperature until it reaches a constant weight and the results are expressed as loss-on-drying. The moisture in solid can be expressed as a wet weight or dry weight. On a wet weight basis, the water content of the material is calculated as percentage of the weight of wet solid, whereas dry weight basis the water is expressed as a percentage of the weight of dry solid. The measurement of moisture in wet solid is that calculated on a dry weight basis. This value is referred to as moisture content. The following formula is used to calculate moisture content:

\[ \% \text{ Moisture content} = \frac{(\text{Weight of water in sample} \times 100)}{\text{Weight of dry sample}} \]

Experimental animals used
The experiment was carried out in Albino mice of either sex weighing between 30 and 40 g obtained from the animal house of Integral University. Animals are kept in the animal house at 26±2°C in polyacrylic cages with not more than six animals per cage and kept under standard laboratory conditions along with standard food and water ad libitum.

The mice were used for the experiment after an acclimatization period of 1 week before experimentation. Animals are divided into three groups, containing three mice each. The animal experiment was performed according to Ethical Committee Approval and guidelines BVCPK/PCSE/IAEC/01/16/2017.
Evaluation of antitussive activity

$SO_2$-induced cough

$SO_2$-induced cough method was evaluated against antitussive effect. These methods were described by Miyagoshi et al., 1986 with slight modification [17]. The 500 mg/ml concentration of sodium hydrogen sulfite with water containing 2 ml solution placed at the base of a desiccator and covered with a porcelain porous plate to serve as a platform for placement of mice as shown in Fig. 1.

The NaHSO$_3$ solution, 0.2 ml of sulfuric acid ($H_2$SO$_4$; Qualigens fine chemicals) is added using a pipette.

The reaction involved is as follows:

$$2NaHSO_3 + H_2SO_4 \rightarrow 2SO_2 + Na_2SO_4 + H_2O$$

Before 15 s, the mice were placed on the platform in the desiccator and then exposed to $SO_2$ for 20 s. The mice were pulling out from the desiccator and placed in an observation chamber for counting of bouts of cough for 5 min thereafter. Primarily the cough responses of all groups of animals were observed (0 min) by placing the animal individually in the desiccator and certain amount of $SO_2$ gas (5 ml, which was fixed throughout the experiment) was introduced. Subsequently 20 s exposure of the gas, the animal was taken out of the desiccator and the frequency of cough was observed for 5 min in an unended filter funnel. In this method, the frequency of cough was observed for all the animal groups at 0 min before the drug administration and at 60 min after the drug administration.

Scoring of bouts of cough

In this experiment, the frequency of cough was observed for all the animal groups at 0 min, before administration of any chemical or testing material. Since, it has been illustrated that cough response to a given stimulus varies from animal to animal with repeated assessments in
same animals. Thus, animals having low or high cough threshold were not entertained for further studies. A number of coughs were examined for all animal groups at 60 min after drug administration by using the same procedure.

**Drug treatment**

All drugs were administered orally. Animals were divided into five groups, containing Seven mice. Treatment to be given to the animals is shown in Table 4. Group I served as a control group and was not administered anything. Group II, Group III, and Group IV were received standard drug, i.e., Codeine sulfate 10 mg/kg, 15 mg/kg, and 20 mg/kg, respectively. Group IV received ethanol extract of *G. glabra* in a dose of 200 mg/kg.

Each animal assisted as its own control and was exposed to SO$_2$ gas twice, i.e., before and 60 min after the drug treatment.

**Statistical analysis**

Cough bouts are measured and its mean was calculated. This mean is used for calculation of percent inhibition in a number of cough bouts. The experimental results have been expressed as the mean±standard error of mean. Significance was evaluated by the Students’ t-test and p<0.05 versus control imply significance [19].

**RESULTS AND DISCUSSION**

**Physical evaluation of different solvent extracts**

The *G. glabra* L. extract was studied for physical evaluation by considering different parameters such as color, odor, pH, percentage yield, melting point, and nature of solid residue obtained after concentration of the extract. Extracts show buff color with sweet odor and the pH was found to be 5. The melting point of extract shows within range of 292–296°C, and the maximum % yield (11.10%) was found for water bath dried extract.

**Phytochemical screening**

The crude samples of *G. glabra* L. extract was prepared for phytochemical screening as per the requirement of procedure and tests were repeated for final confirmation of phytoconstituents with using laboratory reagents. Tests were performed such as lead acetate test, Dragendorff’s test, Mayer’s test, 5% Ferric chloride test, Salkowski’s test, Lieberman’s test, and Keller–Kiliani test. From the phytochemical screening, the presence of constituents such as flavoids, sterols, tannins, and phenols, and alkaloids was observed.

**UV-Analysis**

The UV spectra of *G. glabra* L. extract were observed in 254 nm in respective solvents shown in Fig. 2.

**Calibration curve**

The calibration curves of *G. glabra* L. extract were plotted in phosphate buffer (pH 6.8) by taking absorbance at 254 nm shown in Fig. 3.

**TLC**

TLC analysis was carried out using different reported solvent systems for visualization of maximum spots on the TLC plate, as per literature the standard R$^f$ value 0.79 was obtained using chloroform:methanol:glacial acetic acid:water (8:4:1.5:1) solvent system revealed in Fig. 4.

**Evaluation test of granules**

The various flow properties of granules containing *G. glabra* extract were determined and summarized in Table 5.

**Table 5: Flow properties of granules formulation batches**

| Formulation       | Angle of repose (θ)* | Bulk density (g/ml)* | Tapped density (g/ml)* | Compressibility index (%) | Hausner’s ratio | Moisture content |
|-------------------|----------------------|----------------------|------------------------|---------------------------|-----------------|------------------|
| Glycyrrhiza glabra L. granules | 31.5±4.3             | 0.67±0.14            | 0.88±0.21              | 24                        | 1.316           | 2.5              |

n=3, ± standard deviation

**Table 6: Standardization of cough induction model in laboratory condition**

| Weight of animals (g) | Exposure of SO$_2$ gas (s) | Frequency of cough bouts (In 5 min) |
|-----------------------|-----------------------------|------------------------------------|
| 26                    | 5                           | -                                  |
| 28                    | 10                          | 7                                  |
| 23                    | 15                          | 25                                 |
| 24                    | 20                          | 70                                 |
| 25                    | 25                          | 132                                |
| 22                    | 30                          | 181                                |
| 26                    | 35                          | 153                                |

SO$_2$: Sulfur-dioxide

**Standardization of cough induction model**

With the reference of Gupta et al., 2009, evaluated the antitussive activity of formulations by using the method of Miyagoshi et al., 1986, with slight modification [17,18]. He specified that a vial containing 2 ml of 500 mg/ml solution of sodium hydrogen sulfite in double distilled water was placed at the base of a desiccator and covered with wire gauze to serve as a platform for placement of mice. To the NaHSO$_3$ solution, 0.2 ml of sulfuric acid was added using a pipette. After 15 s, SO$_2$ was exposed for 35 s in mice and was placed on the platform in the desiccator. Then, mice were removed from the desiccator and placed in an observation chamber for counting of bouts of cough for 5 min thereafter. However, in laboratory condition, when the mice were placed on the for counting of bouts of cough for 5 min thereafter, it produced too much cough, even on exposing for 35 s to SO$_2$ gas show in Table 6 and demonstrated in Fig. 5.

The effect exhibited by the entire treated group on SO$_2$ induced cough in experimental animals is presented in Table 7.

In normal controls, there was no significant change in the number of cough bouts, between the two exposures. The effect of the ethanol extracts of *G. glabra* on SO$_2$ gas induced cough in experimental animals has significant effects at the level of p<0.01 in inhibiting the cough reflex at a dose of 200 mg/kg body weight (BW), in comparison with the control group.

Mice showed inhibition of 41.17%, in cough on treatment with *G. glabra*. Codeine sulfate used as a standard drug for suppression of cough, produced 25.29%, 33.33%, and 47.18% inhibition in cough at a dose of 10 mg/kg, 15 mg/kg, and 20 mg/kg, respectively. Moreover, codeine sulfate (20 mg/kg) showed a maximum of 47.13% (p<0.001) inhibition at 60 min of the experiment. The effect of the ethanol extracts of *G. glabra* on SO$_2$, gas induced cough in experimental animals also has significant (p<0.05) effects in inhibiting the cough reflex at a dose of 200 mg/kg BW, in comparison with the standard group.

The graphical representations of results are shown in Figs 6–9.

Herbs are significant contributors to the quality of human life for thousands of years. It has been assessed by the World Health Organization (WHO) that around 80% of world’s inhabitants, mainly belong to in developing countries, rely on traditional medicine, and 85% of traditional medicine consists of the use of plant extracts or their active principles [20]. Various medicinal plants have been claimed to have antitussive activity, for example, *O. sanctum*, *J. suffruticosam*, *I. suffruticosam*, *T. indicum*, *A. webbiana*, *F. racemosa*, *L. parviflor*, *I. suffruticosam*, *A. racemosus*, and *Solanum xanthocarpum* (*O. sanctum*).
CONCLUSION

It can be concluded that the *G. glabra* extract containing granules a significant antitussive effect in experimentally induced cough reflex in mice comparable to the standard drug codeine sulfate. The cough suppressant activity of *G. glabra* was 47.13% as compared to the actually of codeine sulfate. The difference between test drugs (*G. glabra*) and the standard group (codeine sulfate) was significant at the level of p<0.05.

CONTRIBUTION OF AUTHORS

The authors have participated in the work, including participation in the concept, design, analysis, writing, and revision of the manuscript.

CONFLICTS OF INTEREST

Authors declared that they have no conflicts of interest.

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| Treatment                                    | Dose (mg/kg) | No. of animals | Frequency of cough (mean±SEM) | Inhibition (%) |
|----------------------------------------------|--------------|----------------|-------------------------------|----------------|
| Control group                                | --           | 1              | 87.66±5.31                    | --             |
| Codeine sulphate                             | 10           | 1              | 65.50±6.45*                   | 25.29          |
|                                              | 15           | 1              | 58.33±6.96*                   | 33.33          |
|                                              | 20           | 1              | 46.00±7.72**                  | 47.13          |
| Granules containing extract of *Glycyrrhiza glabra* | 200          | 3              | 51.18±7.76*#                  | 41.17          |

SEM: Standard error of mean, SO\(_2\): Sulphur-dioxide
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