ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 10, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

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

MAYURI SHITOLE*, PAWAR VT

Department of Pharmaceutics and Chemistry, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra, India.
Email: mayurishitole0612@gmail.com

Received: 07 May 2019, Revised and Accepted: 05 July 2019

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 (SO_2) gas in experimental mice.

Method: The antitussive effect of *G. glabra* granule formulation on SO_2 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.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i10.33967

INTRODUCTION

A cough, is 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, demulcents 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*, *Passiflora incarnata, Ionidium suffruticosam*, *Trichodesma indicum*, *Abies webbiana*, *Ficus racemosa*, *Lagerstroemia parviflora*, *Drymaria cordata*, *Leucas lavandulaefolia*, *Jussiaea suffruticosa*, 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 herbals formulations in sulfur dioxide (SO_2)-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.

$$\rho b=\frac{M}{Vb}$$

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 (ρb) was calculated using the following formula,

$$\rho b=\frac{M}{Vt}$$

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

$$\theta=\tan^{-1}\left(\frac{H}{R}\right)$$

Table 1: Ingredient of formulation

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

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) and Hausner's ratio (HR)

Powdered blend of all the batches was evaluated for CC) and HR. Bulk density apparatus was used for tapping (Lab Hosp, Mumbai, Maharashtra, India). Different ranges Consolidation Index show in Table 3.

$$CCI=\frac{TD-BD}{TD}\times 100$$

$$HR=\left(\frac{TD}{BD}\right)$$

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:

% Moisture content = (Weight of water in sample × 100)/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/CPCSE/IAEC/01/16/2017.

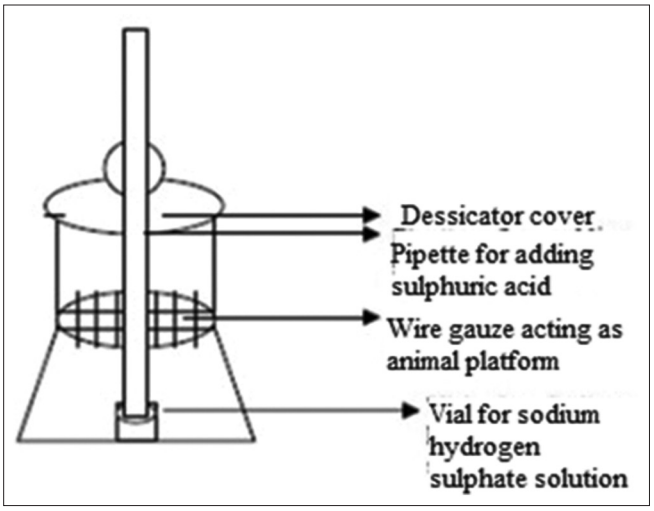

Fig. 1: Setup for sulfur dioxide induced cough [18]

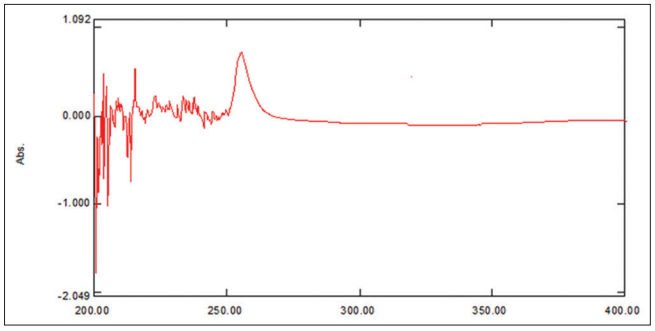

Fig. 2: Ultraviolet spectra *Glycyrrhiza glabra* L. extract

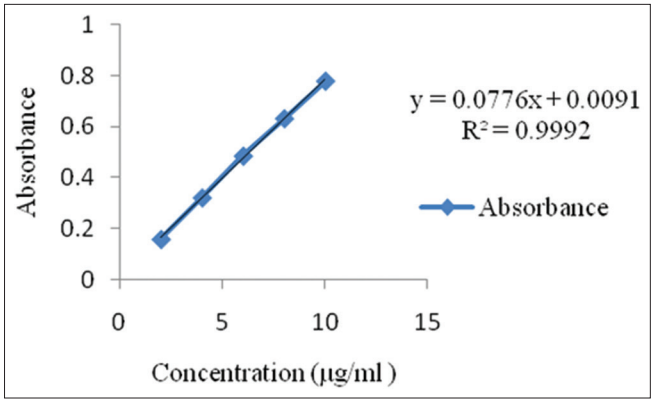

Fig. 3: Calibration curve *Glycyrrhiza glabra* L. extract

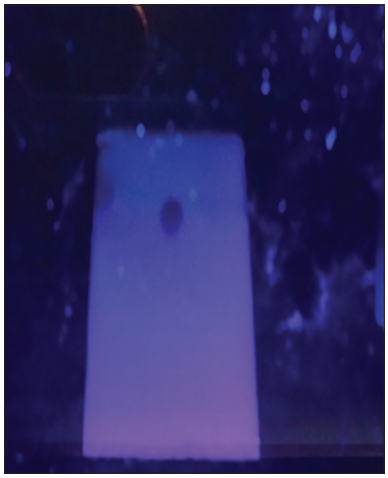

Fig. 4: Thin-layer chromatography analysis of *Glycyrrhiza glabra* L. extract

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 place 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_2SO_4; Qualigens fine chemicals) is added using a pipette.

The reaction involved is as follows:

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

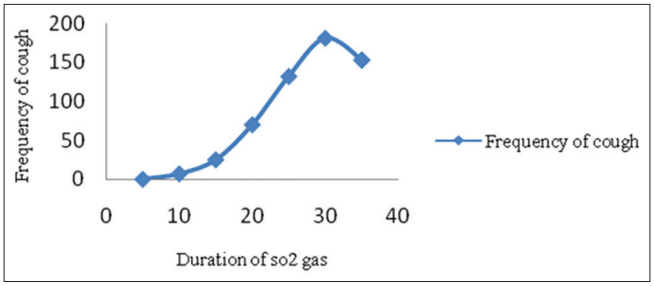

Fig. 5: Standardization of cough induction model in laboratory condition

Table 2: Relationship between the angle of repose (θ) and flow properties

The angle of repose (θ) (degrees)	Flow
<25	Excellent
25–30	Good
30–40	Passable
>40	Very poor

Table 3: Powders for their flow properties according to Carr's index

Consolidation index (%)	Flow
5–12	Excellent
12–16	Good
18–21	Fair to passable
23–35	Poor
33–38	Very poor
>40	Very very poor

Table 4: Treatment to be given to the animals

Group	Number of animals	Treatment to be given
I	1	Normal control
II	1	Treated with standard drug codeine sulfate (10 mg/kg)
III	1	Treated with standard drug codeine sulfate (15 mg/kg)
IV	1	Treated with standard drug codeine sulfate (20 mg/kg)
V	3	Treated with test drug *Glycyrrhiza glabra* L. (200 mg/kg)

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

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	31.54±0.3	0.673±0.14	0.886±0.21	24	1.316	2.5

n=3, ± standard deviation

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 *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 reagent. 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–Killani test. From the phytochemical screening, the presence of constituents such as flavoinds, 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 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
27	20	70
24	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 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 47.13% 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 and 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*, *I. suffruticosam*, *T. indicum*, *A. webbiana*, *F. racemosa*, *L. parviflora*, *J. suffruticosa*, *A. racemosus,* and *Solanum xanthocarpum* (*O. sanctum*

Table 7: Effect exhibited by the entire treated group on SO_2-induced cough in experimental animals

Effect of drugs and formulation on the cough reflex induced by SO_2 gas in mice				
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

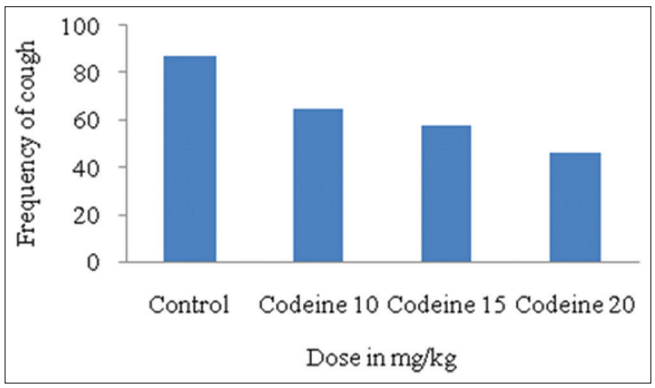

Fig. 6: Frequency of cough on treatment with codeine

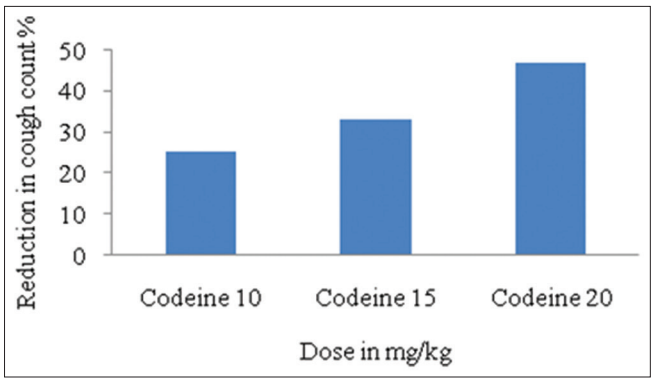

Fig. 7: Percent inhibitions in cough on treatment with codeine

[tulsi], ginger, *Viola odorata* [banafsha], *G. glabra* [licorice/mulethi], *Foeniculum vulgare* [fennel], *Justicia adhatoda* [vasaka] leaves, etc.) [20-23]. Worldwide main components of household cough and cold therapies, in the form of decoctions, teas, etc., *G. glabra* is potent as an expectorant and demulcent in inflammation of bronchi tubules [24]. The glycyrrhetic acid interferes with mucopolysaccharid synthesis as well as bronchial secretion or reduces its viscosity, facilitating its removal by coughing.

Some isolated experimental, as well as clinical studies, have been carried out on these agents for cough. The preliminary investigation indicated promising effects as an antitussive and expectorant activity; this aspect has been further investigated so that these herbs can be established individually as a standard antitussive and expectorant drug. The model used in this study is a modification of Gupta *et al*., 2009 [25-30]. In this study, the quantification of SO_2 generated has not been attempted; it is expected that the not only quantity but also saturation level in the chamber would be the similar in all the exposures, as the other conditions were kept the same. The present data indicate that the granules of *G. glabra* extracts of the plant possess obvious antitussive activity against a chemically induced cough in mice. The antitussive activity of granules of the plant was tested and the results showed significant activity in this animal model which supports the use of the

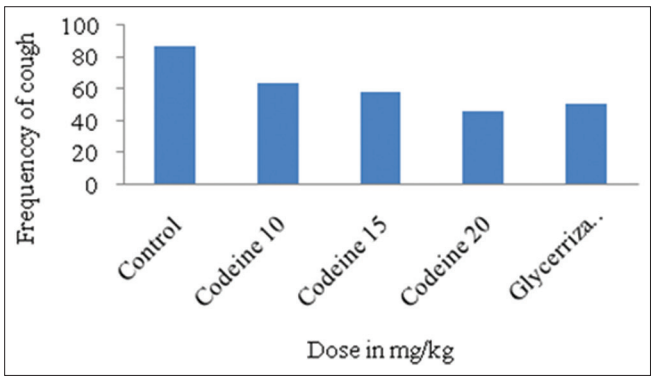

Fig. 8: Frequency of cough on treatment with *glycyrrhiza glabra* granules

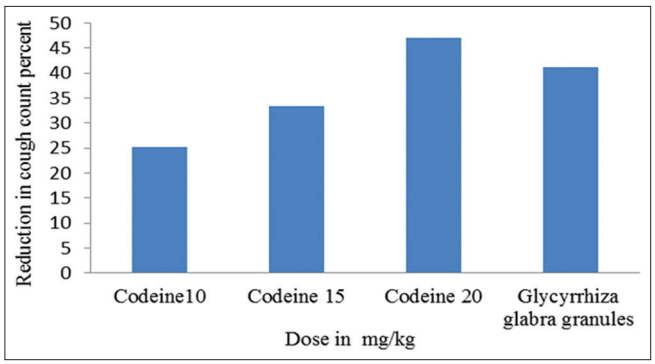

Fig. 9: Comparative study of percent inhibition on cough on treatment with test drug formulation

plant in traditional medicine. The granules at dose levels of 200 mg/kg (*G. glabra* extract*)* showed significant activity after 1 h. as far as the frequency of cough as well as inhibition of cough reflex is concerned.

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 actually of *G. glabra* was 47.13% as compared to the actually of codeine sulfate. The difference between test drugs (*G. glabra*) and the control group was very significant at the level of $p<0.01$. Moreover, the difference between test drugs (*G. glabra*) and 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.

REFERENCES

1. Chung KF, Pavord ID. Prevalence, pathogenesis, and causes of chronic cough. Lancet 2008;371:1364-74.
2. Patwardhan B, Hooper M. Ayurveda and future drug development. Int J Altern Complement Med 1992;10:109-10.
3. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi, India: Indian Council of Scientific and Industrial Research; 1956.
4. Gibson MR. *Glycyrrhiza* in old and new perspectives. Lloydia 1978;41:348-54.
5. Baker ME. Endocrine activity of plant-derived compounds: An evolutionary perspective. Proc Soc Exp Biol Med 1995;208:131-8.
6. Norman HA, Pillai P, Baker ME. Licorice derived compounds inhibit linoleic acid desaturation in soybean chloroplasts. FEBS Lett 1995;368:135-8.
7. Dermarderosian A, Beutler JA. The Review of Natural Products. Illinois: Facts, and Comparisons; 2001. p. 369-70.
8. Tanaka A, Horiuchi M, Umano K, Shibamoto T. Antioxidant and anti-inflammatory activities of water distillate and its dichloromethane extract from licorice root (*Glycyrrhiza uralensis*) and chemical composition of dichloromethane extract. J Sci Food Agric 2008;88:1158-65.
9. Sharma V, Pandey S. Phytochemical screening and determination of anti-bacterial and anti-oxidant potential of *Glycyrrhiza glabra* root extracts. J Environ Res Dev 2013;7:1552-8.
10. Patil SK, Salukhe VR, Mohite SK. Development and validation of UV spectrophotometric method for estimation of glycyrrhetinic acid in hydroalcoholic extract of *Glycyrrhiza glabra.* Int J Pharm Chem Biol Sci 2012;2:617-21.
11. Syed YH, Khan M, Bhuvaneshwari J, Sayyed M. Comparative analysis and standardization of *Glycyrrhiza glabra* roots using chromatographic fingerprinting. Int J Adv Pharm Med Bioallied Sci 2014;2:30-4.
12. Shah R, Tawakkul M, Khan M. Comparative evaluation of flow for pharmaceutical powders and granules. AAPS PharmSciTech 2008:9:250-8.
13. USP. <616> Bulk Density and Tapped Density. USP30 NF 25. USP; 2007.
14. Carr RL. Evaluating flow properties of solids. Chem Eng 1965;72:69-72.
15. Hausner HH. Friction conditions in a mass of metal powder. Int J Powder Metall 1967;3:7-13.
16. Jagtap S, Pawar A, Jadhav K. Comparative study of reverse wet granulation with conventional wet granulation in solubility enhancement of simvastatin. Int J Pharm Pharm Sci 2015;7:264-72.
17. Miyagoshi M, Amagaya S, Ogihara Y. Antitussive effects of L-ephedrine, amygdalin, and makyokansekito (Chinese traditional medicine) using a cough model induced by sulfur dioxide gas in mice. Planta Med 1986;4:275-8.
18. Gupta YK, Katyal J, Kumar G, Mehla J, Katiyar CK, Sharma N, *et al.* Evaluation of antitussive activity of formulations with herbal extracts in sulphur dioxide (SO2) induced cough model in mice. Indian J Physiol Pharmacol 2009;53:61-6.
19. Woodson RF. Statistical Methods for the Analysis of Biomedical Data. Wiley Series in Probability and Mathematical Statistics. New York: Wiley; 1987. p. 315-6.
20. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. Medicinal plants in therapy. Bull World Health Organ 1985;63:965-81.
21. Nadig P, Laxmi S. Study of anti-tussive activity of *Ocimum sanctum* Linn in guinea pigs. Indian J Physiol Pharmacol 2005;49:243-5.
22. Boominathan R, Devi BP, Mandal SC. Evaluation of antitussive potential of *Ionidium suffruticosum* Ging. (*Violaceae*) extract in albino mice. Phytother Res 2003;17:838-9.
23. Srikanth K, Murugesan T, Kumar ChA, Suba V, Das AK, Sinha S, *et al.* Effect of *Trichodesma indicum* extract on cough reflex induced by sulphur dioxide in mice. Phytomedicine 2002;9:75-7.
24. Yasmeen J, Siddiqui H. Study of antitussive potential of *Glycyrrhiza glabra* and *Adhatoda vasica* using a cough model induced by sulphur dioxide gas in mice. Int J Pharm Sci Res 2012;3:1668-74. Available from: http://www.ijpsr.com 1674.
25. Nayak SS, Ghosh AK, Srikanth K, Debnath B, Jha T. Antitussive activity of *Abies webbiana* Lindl. Leaf extract against sulphur dioxide-induced cough reflex in mice. Phytother Res 2003;17:930-2.
26. Bhaskara Rao R, Murugesan T, Pal M, Saha BP, Mandal SC. Antitussive potential of methanol extract of stem bark of *Ficus racemosa* Linn. Phytother Res 2003;17:1117-8.
27. Mazumder A, Saha BP, Basu SP, Mazumder R, Boominathan R, Devi BP, *et al.* Evaluation of antitussive activity of *Lagerstroemia parviflora* leaf extract. Phytother Res 2004;18:780-2.
28. Murugesan T, Ghosh L, Mukherjee PK, Pal M, Saha BP. Evaluation of antitussive potential of *Jussiaea suffruticosa* Linn. Extract in albino mice. Phytother Res 2000;14:541-2.
29. Bector NP, Puri AS. *Solanum xanthocarpum* (Kantakari) in chronic bronchitis, bronchial asthma and non-specific unproductive cough. (An experimental and clinical co-relation). J Assoc Physicians India 1971;19:741-4.
30. Djarot P, Badar M. Formulation and production of granule from *Annona muricata* fruit juice as antihypertensive instant drink. Int J Pharm Pharm Sci 2017;9:18.