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Evaluation of anti-asthmatic activity of *Glycyrrhiza glabra*

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ABSTRACT

Asthma is a chronic disease associated with allergic reaction in the body. The allergic reaction is due to the production of histamine secreted by the mast cells of the human body. *Glycyrrhiza glabra* Linn. is a plant of family Fabaceae. The rhizome, stem and seeds of the plant are used as anti-inflammatory drug in the tribal areas. In this research, the crude extract was obtained from the parts of *Glycyrrhiza glabra* by the process of cold percolation and Soxhlet method. It's different fractions were purified by column chromatography and thin layer chromatography. The purified samples were identified by spectral analysis including HNMR, CNMR and Mass spectroscopy. The swiss variety of albino rats were induced asthma by triple antigen. Purified saponin fraction of the extract of *Glycyrrhiza glabra* was injected to the infected rats. The result obtained shows that the saponin fraction is effective in triple antigen sensitized albino rats as anti-asthmatic agent. The inhibition on mast cell degranulation took place up to 62% at 25 mg/Kg body weight.

Key words: *Glycyrrhiza glabra*, saponin, anti-asthmatic activity.

INTRODUCTION

Asthma is a disorder of airways in which inflammation is considered to provide the basis for altered structure and function that leads to bronchial hyper-responsiveness (BHR) and variable airflow obstruction. Human mast cells (HMCs) play a central roll in these changes by releasing mediators that cause exaggerated bronchoconstriction, induce human airway smooth muscle (HASM) cell proliferation and recruit and activate inflammatory cells. The mast cells are part of the lining of the air passages; they are part of the immune system, reacting immediately to allergens and other obnoxious stimuli. The mast cells release many different substances when they are stimulated, including histamine, which is a chemical responsible for most allergic reactions including the airway inflammation of asthma. The number of HMCs present on asthmatic HASM is increased compared with that on nonasthmatic HASM. Asthma is caused by various agents like dust particles, insects, pollen grains, smoke, animal hairs, fungal and mucilaginous substances which are called allergens and change in the climatic conditions.

Ethno medicinal survey among the tribal groups in India yielded new uses of some less known plant species. There are some less known herbal remedies popular among the people of Betul district of Madhya Pradesh, India for anti-asthmatic activities. An attempt has been made to find out the anti-asthmatic active principles in *Glycyrrhiza glabra*, commonly known as 'Mulethi' which is quite popular for cough, cold and respiratory ailment among the tribals of Betul district of Madhya Praesh, India. Perado (2003) from Amazon has discussed the uses of medicinal plants for the local development of indigenous communities and conservation of natural resourses. Some prominent

references in the field of active formulation include anti-inflammatory and analgesic activity of Hibiscus species (Gore et al, 2005). Use of *Achyranthes aspera* to overcome the skin problems, wound and asthma have been reported by Kumar and Kumari (2000). Tuzlaci and Tolon (2000) reported fourty three folk medicinal plants from Turkey. Among them thirty five were wild species used in the treatment of eczema, diabetes, wounds and asthma. Dongmo et al (2001) observed the anti-inflammatory and analgesic properties of the stem bark extracts of *Erythrophleum suaveolens*. Asthma is a disease or condition in which the bronchial airways temporarily constricted so that it becomes difficult to breath. Such difficulty may arise due to muscles spasm in the bronchi of the lungs. The spasm is produced by the mast cell. This is caused by the degranulation of mast cells. Several workers have worked on the degranulation property of the mast cells (Saxena, 2003; Soni, 2007; Soni et al, 2006). Moreira et al, 2002 also reported anti-asthmatic and anti-inflammatory activity of extract and fractions of the leaves of *Gochnatia polymorpha*. They used ethanolic and aqueous extracts of the leaves of this plant and their fractions of the ethanolic extract showing anti-inflammatory activities, which may be a good tool for anti-asthmatic also. Mimaki et al (1998) isolated two new steroidal saponins from *Ruscus aculeatus* which are quite useful in asthma. Agarwal et al (2003) reported the mast cell stabilizing activity of *Achyranthes aspera* and *Lepidium sativum* in the treatment of asthma. By reviewing the literature on medicinal plants, it was thought important to investigate the biologically active compound from *Glycyrrhiza glabra* which may be used as anti-asthmatic substance.

MATERIAL AND METHODS

The plant *Glycyrrhiza glabra* Linn. (Family: Fabaceae) was collected from Betul District. It was identified by the botanist of J. H. College, Betul and a voucher specimen was produced in the herbarium record of Pest Control and Ayurvedic Drug Research Laboratory, Vidisha (M. P.).

Phytochemical study for the detection of active principles

Extraction

The powdered plant material was extracted by the method of cold-percolation and soxhlet method. Since saponin is a polar compound, they are extracted with polar solvent such as methanol, ethanol and water.

Cold-percolation method

250 gm powdered plant material was taken in conical flask. The cold percolation was stirred thoroughly over the magnetic stirrer in methanol and water respectively for 24 hours. The extract was filtered by Whatman filter paper no. 1 and evaporated under vacuum evaporator with low temperature and high pressure.

Soxhletion method

The powdered plant material was extracted in Soxhlet apparatus using ethanol and water as solvent. The extraction was done for 48 hours duration and up to 8 cycles of extraction. The crude extract was concentrated in a Rotavapour below 40º C. After that crude extract evaporated on water bath to get dryness. The extract obtained with solvent was weighed. It's percentage was calculated as compared to the initial weight of plant material.

Preliminary phytochemical analysis

Preliminary phytochemical screening for the presence of saponin was carried out using standard test procedure.

Frothing test for saponins

0.5 ml filtrate sample was taken in a test tube, added 5 ml distilled water and shaken. Presence of persistent foams above the liquid surface confirms the presence of saponin.

Isolation and Purification of the crude extract

The crude extracts obtained from the plant were analyzed through column chromatography and thin layer chromatography.

Column Chromatography

In column chromatography, the packing material used was silica gel. The different fractions were collected in small glass vials using four solvent systems named as solvent system I, II, III, and IV at the ratio of chloroform: methanol: water (2:1:0.2), chloroform: methanol: water (2:1:0.5) and chloroform: methanol: water (2:1:0.6) and chloroform: carbon tetra chloride: acetone (2:2:1),

Thin Layer Chromatography

The fractions obtained from plant extract were tested for their purity by using thin layer chromatography method. The column purified fractions were assessed on thin layer chromatography. The TLC plates were made of silica gel 'G' and activated by heating in the chromatographic oven at 100º C for 5 min. The purified samples were applied with the help of a capillary tube as a minute spot at the start line marked at 1 cm from the edge of the plate. The spots were allowed to dry and kept carefully in large glass bottles containing the solvent system. After the run of the solvent on the plates up to the marked line, they were taken out from the bottle and the plates were exposed in UV chambers and measured the run of the solvent with a centimeter scale to determine the Rf values.

$$Rf = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

The fraction collected from column chromatography was subjected to acid hydrolysis and methylation for further analysis.

Spectral Analysis

For characterization of biological active principle, the plant extract was sent to Central Drug Research Institute, Lucknow for spectral analysis by Mass Spectroscopy and Nuclear Magnetic Resonance Spectroscopy. The spectrums obtained from CDRI, Lucknow were analyzed for various functional groups by organic chemist of Govt. P. G. College, Betul and Govt. M. V. M. College, Bhopal.

Anti-asthmatic study of plant extract on experimental animal

Swiss variety of albino rats had been reared at Pest Control and Ayurvedic Drug Research Laboratory, S. S. L. Jain P. G. College, Vidisha, India. The rats were given asthmatic drug intraperitonially for 15 days period to induce asthma. The rats were sacrificed to get the mast cells for histological preparation. The experiments were conducted mainly on the albino rat's connective tissue. Mast cells of experimental rats were taken out from the mesenteries and areolor connective tissues of the sacrificed rats. They were fixed in the fixative used for general histological preparation (aqueous bouins) and sections were cut of paraffin block at 8 mm thickness over rotary microtome.

The herbal extract isolated and purified from *Glycyrrhiza glabra* has been tested for it's detailed pharmacological properties on anaphylactic albino rats. The herbal extract was injected intraperitonially to the albino rats. The amount of plant extract actually needed in experiment are quite small often as little as 10 mg or less. For albino rats, 100mg/Kg body weight of the extract was injected for 15 days period. The areolar connective tissues were taken out from the experimental animals for histological observation of mast cell degranulation.

RESULTS

Extraction

For any phytochemical investigation especially for medicinal chemistry of the plant for herbal formulation, it is very essential to see percentage yield of the plant material in various solvents.

Cold Percolation & percentage yield

The yield of *Glycyrrhiza glabra* in various solvents using cold percolation by using 250 gm of dry material is as under:

S. No.	Solvent	Volume of Solvent	Weight of Plant extract	Yield %
1.	Petroleum Ether	250 ml	6.20 gm	2.48 %
2.	Methanol	250 ml	6.58 gm	2.63 %
3.	Water	250 ml	3.68 gm	1.47 %

Percentage yield using Soxhlet method

The yield of *Glycyrrhiza glabra* in various solvents using soxhlet method by using 250 gm of dry material is as under:

S. No.	Solvent	Volume of Solvent	Weight of Plant extract	Yield %
1.	Petroleum Ether	250 ml	16.13 gm	6.45 %
2.	Methanol	250 ml	19.11 gm	7.64 %
3.	Water	250 ml	7.80 gm	3.12 %

Frothing Test

For qualitative chemical analysis of saponin, frothing test was done. Persistent foam was observed during the test which confirms the presence of saponin in *Glycyrrhiza glabra*.

Column Chromatography

Column Chromatography was performed using 4 solvent systems and the weight of fractions obtained and its colour is tabulated below.

S. No.	Solvent Used	Fractions	Weight of Fraction (mg)	Colour of fraction
1.	$CHCL_3$:MeOH:H_2O(2:1:0.2)	GF_1	0.39	Brown
		GF_2	0.09	Dark Brown
		GF_3	0.58	Light Brown
		GF_4	0.39	Reddish
2.	$CHCL_3$:MeOH:H_2O(2:1:0.5)	GF_1	0.82	Dark Reddish
		GF_2	0.14	Pinkish
		GF_3	0.74	Brown
		GF_4	0.83	Shiny Brown
3.	$CHCL_3$:MeOH:H_2O(2:1:0.6)	GF_1	0.79	Dark Orange
		GF_2	0.74	Light Red
		GF_3	0.22	Red
		GF_4	0.30	Transparent reddish
4.	$CHCL_3$:CCl_4:CH_3COCH_3(2:2:1)	GF_1	0.98	Dark Reddish
		GF_2	0.36	Light Transparent
		GF_3	0.21	Pinkish
		GF_4	0.78	Light Red

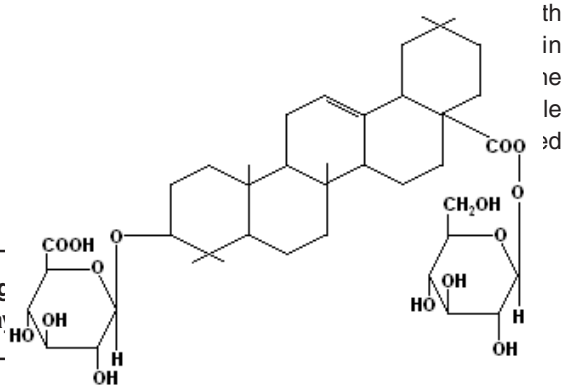
Thin Layer Chromatography

TLC was performed with the fractions obtained with Solvent 3 i.e. $CHCL_3$:MeOH:H_2O (2:1:0.6).The values of R_f obtained by TLC for all fractions varied form 0.40 to 0.93. Maximum value of R_f was 0.93 with fraction GF_4 which appeared Light Red, Red and Red under Visual light, UV Light and Iodine Chamber respectively and minimum value of R_f was 0.40 with fraction GF_1 which appeared Yellowish, Greenish and Light Brown under Visual light, UV Light and Iodine Chamber respectively.

S. No.	Fractions	Colour of Fraction			Length of Spot	R_f value
		Visual Light	UV Light	Iodine Chamber		
1.	GF_1	Yellowish	Greenish	Light Brown	6.0 / 15.0	0.40
2.	GF_2	Orange	Light Orange	Reddish	7.3 / 15.0	0.49
3.	GF_3	Light Pink	Yellowish Green	Reddish Brown	11.0 / 15.0	0.73
4.	GF_4	Light Red	Red	Red	14.0 / 15.0	0.93

Spectral Analysis

By the spectral analysis of the sample of *Glycyrrhiza glabra* at CDRI Lucknow, H-NMR and C-NMR Spectrum for the sample were obtained. Analysis of these epctrum by organic chemist of Govt. P. G. College, Betul and Govt. M. V. M. College, Bhopal confirmed the following structure of the Triterpenoid saponins of *Glycyrrhiza glabra*.



Effect of Herbal Drugs on Mast Cells Degranulation in sensitized albino rats

Mast Cells play an important role in anaphylaxis and inflammation. Inhibition of mast cells degranulation which in turn inhibits release of mast cells mediators is also considered as one way of treating asthma. In order to study the mast cell stabilizing activities, the herbal drug was tried in vivo in albino rats and its effect was studied.

Two weeks after sensitization, the antigen degranulated about 76 % of the mast cells. When the sensitized animals were treated with *Glycyrrhiza glabra* in different concentrations (25, 50, 75 & 100

S. No.	Treatment	Dose (mg for 15 da		
1.	Control	—		
2.	A-1	25	31	62
3.	A-2	50	56	42
4.	A-3	75	68	30
5.	A-4	100	71	27
6.	Standard Drug Prednisolone	10	76	24

DISCUSSION

Ethnomedicine is a new branch of enthnobotany, which is mainly based on the knowledge of the plants used in the traditional medicine for diseases in the modern world. In the present study, it has been found that highest yield of *Glycyrrhiza glabra* during isolation is obtained using Soxhlte method with methanol as solvent. Frothing test confirmed the presence of saponin in

the extract and the structure of the saponin was investigated using spectral analysis techniques. Finally, effect of *Glycyrrhiza glabra* on Mast Cells Degranulation in sensitized albino rats was studied and it was confirmed that this herbal medicine is effective against the asthama disease and in higher concentration, it is comparable with the Standard Drug Prednisolone.

This study highlights the potential capability of the *Glycyrrhiza glabra* for the treatment of asthma. Detailed study on the possible side effects, scope of mass production, comparison with other standard drugs and cost-benefit analysis may be carried out in future to ascertain the commercial viability in establishing *Glycyrrhiza glabra* as a practical medicine for treating asthma.

REFERENCES

1. Dongmo A. B., Kamanyi A., Anchang M. S., Nkeh B. C. A., Njamen D., Ngelefack T. B., Nole T., Wagner H., Anti inflammatory and analgesic properties of the stem bark extracts of Erythrophleum suaveolens (Caesalpiniaceae). *J. Ethnopharmacol.;* **77**(3): 137-141 (2001).
2. Gore V. K., Rao K. A., Guruswamy M. N., Physiological studies with Tylophora asthmatica in bronchial asthma. *Ind. J. Med. Res.,* **71**: 144-148 (2005).
3. Kumar S., Kumari B., Use of Achyranthes aspera as an alternative to baking soda. *Biosci. Res. Bull*; **16**(2): 107-109 (2000).
4. Mimaki Y., Kuroda M., Yokosuka A., Sashida Y., Two new bis-desmosidic steroidal saponins from the underground parts of Ruscus aculeatus. *Chem. Pharm. Bull.*, **46**(5): 879-881 (1998).
5. Moreira A. S., Spitzer V., Schapoval E. E., Schenkel E. P., Anti-inflammatory activity of extracts and fractions from the leaf of Gochnatia polymorpha. *Phyt. Res.*; **14**: 638-640 (2000).
6. Saxena R. C., Anti-histaminic activity of the saponin isolated from *Achyranthes aspera* Linn. Paper presented in 3rd world congress on medicinal and aromatic plants (WOCMAP III) from 3-7 Feb. Chiang Mai University, Thailand: 137 (2003).
7. Soni K., Anti-histaminic and spasmolytic effect of certain herbal extracts on guinea pigs. Ph. D. thesis in zoology. Barkatullah University, Bhopal (2007).
8. Soni K. K., Saxena R. C., Anti-histaminic effect of Tephrosin extract isolated from Tephrosia purpurea on histamine induced broncho constriction in isolated trachea of guinea pig. *Ind. J. Appl. Life Sci.,* **1**(1): 75-76 (2006).
9. Tuzlaci E., Tolon E., Turkish folk medicinal plants part III: sile (Istanbul). *Fitoterapia;* **71**(6): 673-685 (2000).