



Evaluation of the Anti-asthmatic and Expectorant potentials of *Dregea volubilis*

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Abstract

Aerial part of *Dregea volubilis* has been documented as an effective medicinal plant for the management of asthma in traditional medicine. The present study was designed to evaluate Anti-asthmatic and expectorant activity of methanolic extract of *Dregea volubilis*.

Acute toxicity studies of methanolic extract of *Dregea volubilis* were performed in accordance with OECD guidelines. The sensitized guinea pigs were screened out and divided into control, extract and standard treated groups. Anti-asthmatic activity of methanolic extract was evaluated by using in vitro goat tracheal chain preparation model and in vivo broncho-protective test method using aminophylline as a standard drug. Expectorant activity was evaluated by phenol red secretion experiments. Methanolic extract of *Dregea volubilis* was found to be safe up to 2000 mg/kg, body weight. The extract showed presence of carbohydrates, flavonoids, triterpenoids, alkaloids and steroids.

Dose response studies of methanolic extract of *Dregea volubilis* were conducted at 100 µg/ml in vitro and 200, 400 mg/kg p.o. in vivo models. The treatment with methanolic extract of *Dregea volubilis* at different dose level showed that significant anti-asthmatic activity. Methanolic extract of *Dregea volubilis* also showed significant expectorant activity compared with the control in phenol red secretion experiments. The results of the present study provide evidence that methanolic extract of *Dregea volubilis* can be used as an anti-asthmatic and expectorant herbal medicine.

Keywords: *Dregea volubilis*, Anti-asthmatic, Expectorant

Introduction

Chronic bronchitis, Emphysema and Asthma are the major inflammatory diseases related to respiratory system, they are responsible for mucus production and increased airway hyper responsiveness, which leads to episodes of shortness of breath, coughing and wheezing.^[1] Currently for the management of these problem corticosteroids as anti-inflammatory in combination with bronchodilators like beta 2 adrenergic agents frequently used.^[2] However, these drugs show serious side effects in some patients. Worldwide incidence of Asthma and chronic bronchitis in adults is about 10 % and 35 % in children.

So, the high incidence of the diseases among the individuals and side effects of present therapy needs research on medications for chronic obstructive lung diseases (COLD).

The use of medicinal plants for the treatment of human diseases has increased considerably worldwide. Now a day, herbal drugs a part of complementary and alternative medicine, has gained popularity in the management of chronic obstructive lung diseases.^[3,4]

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Factors responsible for the increased use include cost of orthodox drugs, fewer adverse effects, cultural beliefs and practices and possibly most importantly, aggressive marketing by manufacturers. The ethnomedicinal plant *Dregea volubilis*, belongs to the kingdom of Plantae, family of Apocynaceae, class of Dicotyledons and is distributed widely in tropical countries and South East Asia.^[5] *Dregea volubilis* is commonly known as Harandodi in Marathi and Jukti in Bengal and is used by different Indian tribal communities. In Indian Ayurvedic medicine different parts of the plant have been traditionally used for the treatment of various diseases and disorders like diabetes mellitus, boils, inflammation, piles, tumors, eye ailments, stomach ache, leukoderma, rat bite, and urinary problems.^[6] Earlier studies on plant *Dregea volubilis*, explore the biological activities of root, fruit, flower and leaf extracts of *Dregea volubilis*,^[7,8] till now there is no scientific data available on role of *Dregea volubilis* in the management of chronic obstructive lung diseases, hence the present study has been attempted.

Material and Methods

Collection of plant material:

The fresh Aerial part of *Dregea volubilis* used in this study, were collected at the flowering stage in the month of September from local Irrigation part of Pusad District Yavatmal Maharashtra, India. Plant was identified and authenticated at Post Graduate Teaching Department of Botany R. S. T. M. Nagpur University Nagpur India, where a voucher specimen of the plant has been kept in the herbarium.

Preparation of Extract

The fresh Aerial part of *Dregea volubilis* were shade dried for 15 days and then pulverized to coarse powder and passed through sieve no. 20. Coarsely dried powder was first defatted with petroleum ether between 60°C to 80°C for 72 h to remove fatty materials and then extracted with methanol using soxhlet apparatus, the extract was collected and concentrated in vacuum under reduced pressure and the dried crude extract was stored at 4°C to 6°C for further study.

Preliminary Phytochemical screening

Methanolic extract of *D. volubilis* was subjected to phytochemical tests for the identification of the

phytochemicals present in the methanolic extract using standard procedures.^[9]

Experimental Animals

Guinea pigs of either sex (150–200 g) for anti-asthmatic experiments and Swiss albino mice of either sex (25–35 g) for expectorant experiment were obtained from animal house of Sudhakar Rao Naik Institute of Pharmacy, Pusad, India. Protocol or the experimental procedure approved from animal ethical committee of the institute (SNIOP/CPCSEA/IACE/CP-PL/2020/01).

Animals under the study were maintained, before the start of experiment and during the experiment according to standard procedure for animal house, guidelines given by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animals were housed in Poly propylene cages and maintained at 24°C ± 2°C under 12h light/ dark cycle and were feed *ad libitum* with standard pellet diet and had free access to water.

Acute oral toxicity study

The acute oral toxicity study was evaluated as per the 425 guidelines of OECD on Swiss albino mice (25 to 35 g) of either sex. Before the start of experiment, animals were fasted overnight with water *ad libitum*. Five animals were selected and given a single dose of 2000 mg/kg body weight of methanolic extract of *Dregea volubilis* by p.o. Animals were observed individually for any sign of behavioral changes, toxicity and mortality after dosing. Special attention was given during the first 4 h, and thereafter for 24 h, for a total period of 14 days.^[10]

Anti-asthmatic Activity of Extracts

In vitro method

Isolated goat trachea chain preparation:

Isolated adult goat tracheal tissue was obtained from slaughter house of Pusad, districts Yavatmal. Tracheal ring chain was prepared by cutting individual rings of trachea and tied together in series to form a chain. Krebs solution was used to suspend trachea in bath and was continuously aerator at 37°C. Dose response curve of histamine in presences of plane Krebs solution and in 100 µg/mL methanolic extract of *Dregea volubilis* was taken. Graph of percentage of maximum contractile response on ordinate and concentration of histamine on abscissa was plotted to record

DRC of histamine, in absence and in presence of methanolic extract of *Dregea volubilis*.^[11, 12]

In vivo method

To screen the sensitivity, guinea pigs were placed in a glass chamber and sprayed with the mixture of 0.1% histamine and 2% acetylcholine chloride (1: 1, v/v) under the average pressure of 450 ± 50 mm Hg for 15 s. During an aerosol challenge the onset of respiratory distress in seconds (Preconvulsive) was carefully measured for each animal. The guinea pigs with preconvulsive time of more than 120 seconds were considered to be insensitive and not included in the study. Selected animals were divided in to four groups; control group (received 5 ml/kg of 0.1% carboxy methyl cellulose (CMC), standard group (received aminophylline 10 mg/kg, body weight) and test group (received methanolic extract of *Dregea volubilis* 200 mg/kg and 400 mg/kg body weight). All the treatments were given by the oral gavage route for three days before to the bronchial challenge, while the last dose of the treatment was administered before 60 minutes of the bronchial challenge. The delitescence of convulsion and tumble numbers for each guinea pig within 6 minutes interval of exposure were observed.^[13]

Expectorant Activity of Extracts

For evaluation of expectorant activity of *Dregea volubilis*, Swiss albino mice of either sex were randomly divided in to four groups, each group contains five mice. Control group (received 5 ml/kg of 0.1% carboxy methyl cellulose (CMC), standard group (received Ammonium chloride 1000mg/kg, body weight) and test group (received methanolic extract of *Dregea volubilis* 200 mg/kg and 400 mg/kg body weight). Treatments were given for 5 days (single dose daily) and the last dose was given 1 h before IP injection of phenol red solution (5% in saline solution, w/v, 0.1 mL/10 g body weight). After 30 min of phenol red application, the mice were anesthetized with pentobarbital at the dose of 75mg/kg body weight and exsanguinated by cutting the abdominal aorta.

Table 1: Effect of methanolic extract of *D. volubilis* on histamine induced contraction in goat tracheal chain preparation

Sr. No.	Histamine Dose (10 µg/ml)	Negative molar Conc. of Histamine	Maximum Percentage contractions	
			Control	<i>D. volubilis</i> Extract (100 µg/ml)
1	0.1	7.08	21.85 ± 1.03	$10.41 \pm 0.56^*$
2	0.2	6.79	29.67 ± 1.12	$15.72 \pm 0.54^*$
3	0.4	6.48	45.80 ± 1.58	$26.04 \pm 0.63^*$

The trachea was removed from the thyroid cartilage to the main stem bronchi and put into 1mL normal saline. After ultrasonic for 15min, 1mL NaHCO₃ solution (5%, w/v) was added to the saline and optical density of the mixture was measured at 558 nm using UV-visible.^[14]

Statistical Data

The statistical analysis was performed by using one-way analysis-of-variance (ANOVA) Followed by Dennett's test for individual comparison of groups with control.

Results and Discussion

Phytochemical screening

Preliminary phytochemical investigation of methanolic extract of *Dregea volubilis* revealed the presence of carbohydrates, tannins, alkaloids, triterpenoids, glycosides, polyphenols, flavonoids, and proteins.

Acute oral toxicity study

Acute toxicity studies of methanolic extract of *Dregea volubilis* were performed as per the guidelines of OECD 425. The findings of acute toxicity study showed that the extract, exhibit a great margin of safety up to dose of 2000 mg/kg, BW; there was no change in the behavioral pattern and no sign of toxicity and mortality was observed during the overall toxicity studies. Based on the results of acute toxicity testing the doses of 200 and 400 mg/kg p.o. were chosen for further experiments.

Goat tracheal chain preparation model

The findings of this study showed that methanolic extracts of *Dregea volubilis* inhibits the histamine induced contraction in goat tracheal chain preparation. Histamine (10 µg/mL) was taken in different dose level and dose response curve was plotted in presence and in absence of methanolic extract of *D. volubilis*. Results of present study showed that methanolic extract of *D. volubilis* significantly inhibits (* $p < 0.05$, ** $p < 0.01$) percentage contraction at 100 µg/mL in goat tracheal chain preparation. (Table 1)

4	0.8	6.18	64.47 ± 1.68	34.42 ± 0.42**
5	1.6	5.88	73.48 ± 1.22	38.23 ± 0.72**
6	3.2	5.58	79.96 ± 1.23	39.41 ± 0.38**
7	6.4	5.23	100.0 ± 1.12	49.12 ± 0.48

(n=5); Values are in mean ± SEM; Statistical analysis done by using Student's 't'-test (* $p < 0.05$, ** $p < 0.01$), significantly different from control.

Table 2: Bronchoprotective effect of methanolic extracts of *Dregea volubilis* against histamine and acetylcholine induced bronchospasm

Parameters	Control Group	Standard (Aminophylline 10 mg/kg)	Methanolic extract of <i>D. volubilis</i> (200 mg/kg)	Methanolic extract of <i>D. volubilis</i> (200 mg/kg)
Latency Score (Seconds)	60.50 ± 3.32	153.00 ± 4.0**	120.48 ± 3.8**	140.50 ± 5.5**
Tumble Number	14.46 ± 1.20	07.20 ± 0.44**	06.10 ± 0.20**	05.36 ± 0.25**
% Bronchoprotection	00	43.30	38.11	42.12

Values are presented as mean ± SEM, (n=5) ** $P < 0.01$, compared with control group.

Table 3: Effect of methanolic extract of *D. volubilis* on the volume of phenol red in mice's tracheas

Sr. No.	Treatments groups	Phenol Red output (µg/ml)
1	Control Group	0.41±0.12
2	Standard (ammonium chloride 1000mg/kg)	3.10±0.28**
3	Methanolic extract of <i>D. volubilis</i> (200 mg/kg)	1.02±0.06*
4	Methanolic extract of <i>D. volubilis</i> (400 mg/kg)	2.12±0.18**

Values are presented as mean ± SEM, (n=5) * $P < 0.05$, ** $P < 0.01$, compared with control group.

In-vivo Anti-asthmatic Effects

The effects of methanolic extract of *D. volubilis* in guinea pigs exposed to 0.1% histamine and 2% acetylcholine chloride mixture spray were shown in Table 2. The preconvulsive times of guinea pigs in all groups had no difference before administration. After administration, the preconvulsive times (Latency score) were 60.50±3.32 for control group animals, 153.00±4.0 standard group animals and 120.48±3.8, 140.50 ±5.5 for 200 mg/kg and 400 mg/kg dose of methanolic extracts of *Dregea volubilis*. Comparing with control group, there was significant differences in standard group and extract treated groups. Methanolic extracts of *Dregea volubilis* was also found to exhibit a potential broncho protection with reduced tumble numbers ($P < 0.05$) compared with control group. The percentage bronchoprotection was found to

be 43.30% for standard drug aminophylline and 38.11%, 42.12% respectively for 200mg/kg and 400mg/kg dose of *Dregea volubilis*.

Expectorant Effects

As shown in Table 3, ammonium chloride treated, 400 mg/kg methanolic extract of *D. volubilis* treated group showed highly significant ($P < 0.01$) and 200 mg/kg methanolic extract of *D. volubilis* showed significant ($P < 0.05$) tracheal phenol red output.

Mast cells, epithelial cells, neutrophils and T-lymphocytes like cellular elements play important role in asthma pathophysiology. [15] The currently used drugs like corticosteroids, NSAIDs and bronchodilators for the management of asthma are far from satisfactory as they provide only symptomatic relief and lose effectiveness on prolong use. These agents produce several side effects like hypokalemia and muscle tremors are major adverse effects of β_2 receptor agonist. Theophylline requires monitoring as it has narrow therapeutic index. Fluid retention, osteoporosis, increased cell mass, weight gain, capillary fragility, hypertension are the adverse effects of corticosteroids. [16]

The present study was design to evaluate the anti-asthmatic and expectorant activities of methanolic extract of *Dregea volubilis*. Preliminary phytochemical investigation of *Dregea volubilis* methanolic extract revealed the presence of carbohydrates, tannins, alkaloids, triterpenoids, glycosides, polyphenols, flavonoids, and proteins. 14 days acute oral toxicity study of methanolic extract of *Dregea volubilis* proves the non-toxic nature of the *Dregea volubilis*.

Histamine and acetylcholine plays very important role in asthma attack. Histamine via H₁ receptors leads to contraction and obstruction of bronchial smooth muscle, whereas acetylcholine leads to bronchoconstriction mediated by IP-3 and calcium. It has been reported that histamine H₁ receptor and acetylcholine blockade results in bronchodilation, which plays important role in treatment of asthma. [17,18]

Spasmogens such as histamine and acetylcholine activate release of calcium and utilization processes that accentuate contraction of bronchial smooth muscle. [19, 20] The findings of this study showed that methanolic extracts of *Dregea volubilis* inhibits the histamine induced contraction in goat tracheal chain preparation. These effects seem accounted for at least partly by its ability to block the inflow of calcium into airway smooth muscles. Methanolic extracts of *Dregea volubilis* contains flavonoids which are known to have bronchodilatory effects. [21, 22] Flavonoids also inhibit histamine release from mast cells. [23]

On the histamine and acetylcholine chloride-induced bronchoconstriction in guinea pigs, the methanolic extracts of *Dregea volubilis* increased significantly the latency score in asthma relieving (Table 2). Methanolic extracts of *Dregea volubilis* was also found to exhibit a potential bronchoprotection with reduced tumble numbers ($P < 0.05$) compared with control group. The percentage bronchoprotection was found to be 43.30% for standard drug aminophylline and 38.11%, 42.12% respectively for 200mg/kg and 400mg/kg dose of *Dregea volubilis*. Previous studies on many plants with similar phytoconstituents as Methanolic extracts of *Dregea volubilis* have also exhibited anti-asthmatic properties. [24,25]

Additionally, the Methanolic extracts of *Dregea volubilis* enhanced phenol red secretion into the airway with standard expectorant drug ammonium chloride *in vivo* (Table 3), which indicated that the expectorant action may be related to its ability to increase tracheobronchial mucus secretion and, thus, may decrease viscosity of mucus. [26]

Conclusion

In conclusion, our study indicated that the methanolic extracts of *Dregea volubilis* demonstrated the significantly anti-asthmatic and expectorant effects *in-vitro* as well as *in vivo* studies. These effects were the important evidence for the traditional use of *Dregea volubilis* as anti-asthmatic remedy.

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