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Research Article

ANTIASTHMATIC ACTIVITY OF METHANOLIC EXTRACT OF DELPHINIUM DENUDATUM WALL

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ABSTRACT

Delphinium denudatum Non L. (Ranunculaceae) commonly known as *Jadwar*. It is used for various ailments in Indian system of traditional medicines. Methanolic extract of aerial parts was evaluated for antiasthmatic activity. Histamine induced bronchospasm in guinea pigs and egg albumin induced bronchospasm in guinea pig model were performed and various parameters were investigated like PCD time, serum bicarbonate level, differential leukocytes count and histopathological changes in lung. Biochemical estimation like glutathione (GSH) level, malondialdehyde (MDA) level and total proteins were also checked. The result showed that it significantly increased preconvulsion dyspnoea (PCD) time, decreased differential leukocyte counts and serum bicarbonate level. It decreased the oxidative burden by reducing the MDA level and increasing the GSH level which was imbalanced in asthmatic condition, also shown significant action on total protein level. The drug also reduced the inflammation and dilated the bronchioles. All these data suggested that *Delphinium denudatum* is having promising antiasthmatic activity.

Keywords: Delphinium denudatum, Histamine, Antiasthmatic, Ranunculaceae and Bronchospasm

INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, in particular, mast cell, eosinophils, T-lymphocytes, macrophages, neutrophils, and epithelial cells[1].The pathophysiological changes occur in asthma is like airway obstruction due to combination of various factors include spasm of airway smooth muscle, edema of airway mucosa, increased mucus secretion, cellular infiltration of the airway walls and injury and desquamation of the airway epithelium [2,3] .

Delphinium denudatum Wall (Ranunculaceae), It is one of the important drugs used as Indigenous medicine in India, especially in Unani medicine[4][5]. Its vernacular name is *Jadwar*. The entire plant are reported to be useful in a variety of ailments such as aconite poisoning, brain diseases, fungal asthma infection, piles and toothache as analgesic and astringent[6][7][8]. A number of studies have been done on its phytochemical and pharmacological properties[9][10].Its use in opium addiction is mentioned in some classical literature[11][12], which has been verified and validated in morphine induced physical dependent de-addiction studies.[13][14][15][16][17][18][19][20][21]. Considering the ayurvedic uses and reported activities of the drug, it is proposed to investigate its antiasthmatic potential using various experimental models.

MATERIAL AND METHODS

All chemicals and solvents used were of analytical grades. Ketotifen fumarate was obtained as a gift sample from FDC Ltd., Mumbai. Histamine hydrochloride was purchased from Chitichem, Baroda. Egg albumin, sodium dodecyl sulfate, acetic acid, hydrochloric acid, thiobarbituric acid (TBA), trichloroacetic acid, disodium hydrogen phosphate, 5, 5'-dithiobis-2-nitrobenoic acid, sodium-potassium tartrate, sodium hydroxide, sodium carbonate, Folin-phenol reagent were of analytical grade.

Plant material and Extraction

Fully grown, fresh entire herb of *Delphinium denudatum*. was collected in the month of December from Vallabh Vidyanagar. The herb was authenticated by a taxonomist at the Department of Bioscience, Sardar Patel University, and Vallabh Vidyanagar. A voucher specimen (no argh-7) was deposited in the Department of Pharmacognosy, A. R. College of Pharmacy, and Vallabh Vidyanagar. The aerial parts were cleaned, dried at room temperature, powdered to coarse powder (60 #) and stored in air tight container. 5 kg of dried aerial parts of *Delphinium denudatum* was exhaustively extracted by subjecting to reflux below 80^{0}C (3 X 5 L). The combined methanol extract was distilled and evaporated to dryness (25 %w/w). A weighed quantity of the extract was taken and suspension was prepared using Tween 80 and was used for further study.

Animals

Guinea pigs (450-600 gms) were procured from Animal Vaccine Institute, Gandhinagar. All animals were housed at ambient temperature (21±10C) and relative humidity (55±5 %) with fixed 12h/12h light/dark cycle. Animals had free access to standard pellet diet and water given *ad libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Histamine induced bronchospasm in guinea pig

The guinea pigs (Animal breeding centre) were kept in a closed chamber and exposed to an aerosol of 0.1 percent histamine hydrochloride and time for preconvulsion dyspnoea was noted. As soon as preconvulsion dyspnoea (PCD) commenced, animals were removed from the chamber and placed in fresh air to recover. This time for PCD was taken as basal value. After 15 days of wash out period the same animals were randomly divided into five groups each containing six animals. Group I received distilled water (normal control); Group II received Ketotifen (1 mg/kg, P.O.) (Standard control) and Group III, IV and V received methanolic extract of *Delphinium denudatum* (MDD group) in divided doses 150, 300 and 450 mg/kg respectively. The suspension of drugs was administered orally. Two hours after the drugs treatment, animals were exposed to histamine aerosol and times for PCD were noted. The effect of drug was calculated by the following formula [22]

$$\% \text{ increase in PCD time} = [1 - T1/T2] \times 100$$

Where, T1 = time for PCD onset on day 0, T2 = time for PCD onset after drug treatment.

Table 1: Effect of *Delphinium denudatum* on PCD time in guinea pigs after acute treatment

Treatment group	% increase in time of PCD
Normal control group	5.17 ± 2.54
Ketotifen group (1mg/kg)	87.35±3.80@
MDD group (150mg/kg)	64.26±2.62@
MDD group (300mg/kg)	36.34±4.8@
MDD group (450mg/kg)	29.63±3.9@

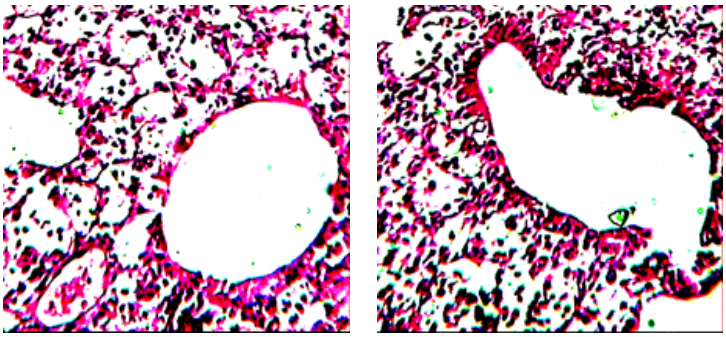
@Significantly different from control group ($p < 0.05$); MDD-Methanolic extract of aerial part of *Delphinium denudatum*

Egg albumin induced bronchospasm in conscious Guinea pigs

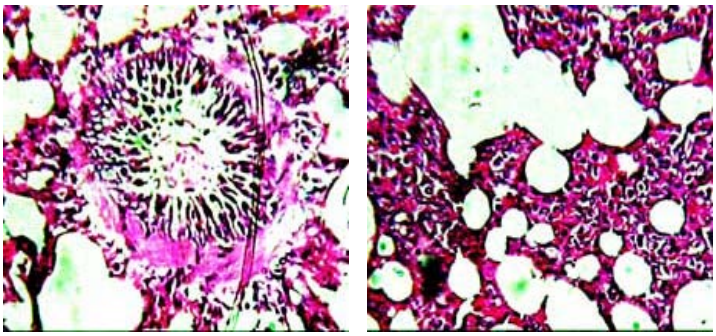
Hartley strain Guinea pigs of either sex weighing 400-600 gm were selected. The animals were examined for the following parameters:

Eosinophil and macrophages count in the bronchoalveolar fluid
Serum bicarbonate level
Measurement of MDA level
Measurement of GSH level
Measurement of total protein level
Histopathology

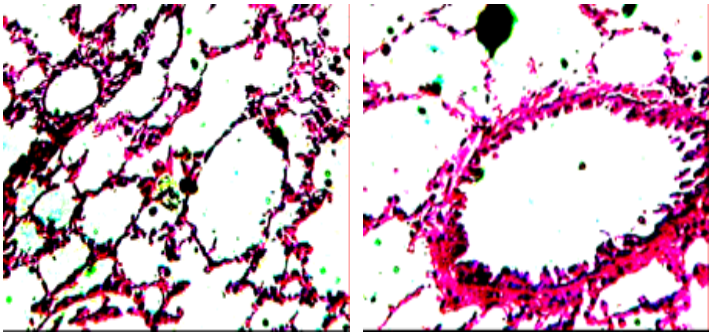
The animals were randomly divided into three groups each containing six animals. Group I received distilled water (normal control), Group II received egg albumin(sensitized group) and Group III received methanolic extract of *Delphinium denudatum* (MDD) group) (150mg/kg).



Normal group



Sensitized group



MSI group

Fig. 1: Histopathological studies

Eosinophil and macrophages count in the bronchoalveolar fluid

The suspension of drugs was administered orally. The guinea pigs of all the groups except Group I were sensitized with egg albumin (1 ml, 10% w/v).The animals of Group III was dosed once daily for fifteen days with the suspension of drug. Two hours after the last dose of drug administration on fifteen days all the animals except Group I animals were challenged with egg albumin (0.5 ml, 2% w/v) through saphenous vein. After 3 hours of the challenge of the egg albumin or just prior to death of animals, whichever was earlier, the tracheobronchial tree was lavaged with 10 ml of saline and the fluid so collected was centrifuged at 2000 rpm for 5 minutes and the pellet was resuspended in 0.5 ml saline. 0.2 ml of geimsa stain in buffered saline (pH 6.5) was added to it. After 5 minute the number of each type of leukocytes in 0.5 ml fluids was determined under the microscope 450 X magnifications. The results obtained were compared with unsensitized and untreated egg albumin sensitized guinea pig.[23]

Serum bicarbonate level

Serum is suitable specimens for measurement of bicarbonate level. The whole blood should be collected and handled anaerobically to minimize exposure to air. Serum bicarbonate is stable for one hour when stored under anaerobic conditions in an ice bath. Lable one test tube or cuvette for a reagent blank. Calibrator, controis and unknown sample. Add 1 ml of completely dissolved reagent to each tube or Cuvette. Add 0.01 ml of H_2O to the reagent blank and 0.01 ml of calibrator, controls and unknown sample to the appropriately labeled tube. Mix and incubate for 5min and measure the absorbance at 380 nm.[24]

Table 2: Effect of MDD on differential leukocyte counts of the bronchoalveolar lavage (BAL) fluid

	Normal control group	Sensitized group	MDD group (150mg/kg)
TLC/cmm	7134.16± 101.79	13440.16 ± 315.9#	11422.5 ± 537.18$
Polymorphs count/cmm	20.5 ± 0.83	37.74 ± 0.93#	34.09 ± 1.29$
Lymphocyte count/cmm	49.79 ± 0.66	58.86 ± 0.76#	52.18 ± 1.44$
Eosinophil count/cmm	6.21 ± 0.31	14.05 ± 0.62#	8.98 ± 0.57$
Monocyte count/cmm	4.83± 0.33	13.41 ± 0.87#	7.01 ± 0.91$

Significantly different from control (p<0.001); $ Significantly different from sensitized (p<0.05); MDD - Methanolic extract of aerial part of *Delphinium denudatum*

Table 3: Effect of MDD on serum bicarbonate level

	Serum bicarbonate level (meq/l)
Normal control group	40.44 ± 0.70 meq/l
Sensitized group	51.31 ± 1.02 meq/l
MDD group (150mg/kg)	39.89 ± 1.20# meq/l

Significantly different from sensitized (p<0.05); MDD - Methanolic extract of aerial part of *Delphinium denudatum*

Measurement of Malondialdehyde (MDA) level

One gm of lung tissue homogenized in 10 ml ice cold Phosphate buffer. The prepared homogenates were centrifuged and used for the determination of antioxidant parameters. Supernatant (0.2 ml) was mixed with 0.2 ml of 8 percent W/V sodium dodecyl sulfate, 1.5 ml of 20 percent acetic acid in 0.27 M hydrochloric acid, 1.5 ml freshly prepared of thiobarbituric acid (TBA) (1percent W/V) and 0.6 ml of distilled water. The mixture was heated in a water bath at 95^0C for 45 minute, cooled and 2 ml of the mixture was mixed with 2 ml of 10 percent trichloroacetic acid. The resulting mixture was centrifuged at 1000 rpm for 5 min. the intensity of pink color developed was read against blank at 532 nm. The amount of (MDA) (thiobarbituric acid reactive material) was calculated and reported as µg of MDA / mg protein.[25, 26]

Measurement of GSH level

The supernatant (2 ml) was mixed with 10 percent chilled trichloroacetic acid. The mixture was kept in ice bath for 30 min and centrifuged at 1000 g for 10 min at 4 ^{0}C. Supernatant (0.5ml) was mixed with 2.0 ml 0.3 M disodium hydrogen phosphate and 0.25 ml 5, 5'-dithiobis-2-nitrobenoic acid (40 mg/100 ml in 1 percent sodium citrate) was added just before measuring the absorbance at 412 nm. Standard curve for GSH was prepared using glutathione. Results are expressed as µg of GSH /mg protein.

Measurement of total protein level

0.2 ml of crude homogenate was added to 4.0 ml of solution – C (solution A- 2 g of sodium hydroxide, 10g of sodium carbonate, 0.1 g of sodium-potassium tartrate in 500 ml of distilled water; solution B- 0.5 g of cuprous sulphate in 100 ml of distilled water; Solution C – (mixture of 10 ml of solution A and 0.2 ml of solution B) and 0.6 ml distilled water was added and allowed to stand for 15 min at 37 C. Folin-phenol reagent 0.4 ml was added and incubated for 30 min. Absorbance was read at 540 nm. Amount of protein was calculated in mg/ml of tissue homogenate from the graph of standard albumin.

Histopathology of Lung

Histopathological study of bronchi and bronchioles were carried out to study the effects of MDD on bronchocontractive effects brought about by sensitization of the animals.

Statistical Analysis

All the values were expressed as mean ± SEM of six observations. The statistical analysis was performed using Student-t-test. Value of p less than 5 % ($p < 0.05$) was considered statistically significant.

RESULTS

MDD in different concentrations were tested for its antiasthmatic activity in two different *in vivo* models and various parameters were checked.

Guinea pigs were administered with Ketotifen (1mg/kg) and MSI (150, 300, 450 mg/kg) and PCD time was recorded. With histamine aerosol, the percentage increase in PCD time of *Delphinium denudatum* gives significant result in a dose of 150 mg/kg (64.26 ± 2.64) ($p < 0.05$). (Table 1)

Table 4: Effect of MDD on total protein, malondialdehyde and glutathione level

	Protein (mg/ml)	MDA (µg/mg/protein)	GSH (µg/mg/protein)
Normal control group	6.80 ± 0.056	0.076 ± 0.0021	16.17 ± 0.019
Sensitized group	9.57 ± 0.017$	0.14 ± 0.0002$	2.7 ± 0.006$
MSI group (150mg/kg)	6.76 ± 0.037#	0.064 ± 0.0017#	11.92 ± 0.08#

Significantly different from sensitized (p<0.05); $Significantly different from control (p<0.001) (student's t test); MDD- Methanolic extract of aerial part of *Delphinium denudatum*

The smear of the bronchoalveolar lavage (BAL) fluid showed different types of White Blood Cells. The total leukocyte count, eosinophil, monocyte, polymorphs and lymphocyte count revealed a change after sensitization of guinea pigs with egg albumin. There was significant ($p < 0.001$) increase in the total leukocyte, polymorph, eosinophil, monocyte and lymphocyte counts in the sensitized animals (13440.16 ± 315.9), (37.74 ± 0.93), (14.05 ± 0.62), (13.41 ± 0.87), (58.86 ± 0.76) as compared to control animals (7134.16 ± 101.79), (20.5 ± 0.83), (6.21 ± 0.31), (4.83 ± 0.33), (49.79 ± 0.66) respectively. The treatment with MSI produced a significant ($p < 0.05$) decrease in the elevated total leukocyte, polymorph, eosinophil, monocyte and lymphocyte counts (11422.5 ± 537.18), (35.77 ± 1.50), (8.98 ± 0.57), (7.01 ± 0.91), (52.18 ± 1.44) as compared to sensitized animals respectively. (Table 2)

Significant ($p < 0.001$) increase in the serum bicarbonate level in the sensitized animals (51.31 ± 1.02 meq/l) as compared to control animals (40.44 ± 0.70 meq/l). The treatment with MSI produced a significant ($p < 0.05$) decrease in the elevated serum bicarbonate level (39.89 ± 1.20 meq/l) as compared to sensitized animals. (Table 3)

In Guinea pig sensitized with egg albumin showed significant (0.14 ± 0.0002) ($p<0.001$) increase in level of the lipid peroxidation product

malondialdehyde (MDA) compared to normal control group which indicated high oxidation procedure in asthma stage. While sensitized animals treated with MSI showed significant (0.064 ± 0.0017) ($p<0.05$) decrease in level of the lipid peroxidation product malondialdehyde compare to sensitized animal. (Table 4)

Further sensitized animals showed significant (2.7 ± 0.006) ($p<0.001$) decrease in the level of glutathione which was reversed by the treatment with MSI significantly (11.92 ± 0.08) ($p<0.05$) increases the GSH level. Moreover elevated level in protein in sensitized animals were reduced by (Table 4)

In histopathological study of lungs of sensitized guinea pigs showed retained of normal vasculature and cellular structure with dilated blood vessels and very few of chronic inflammatory cells compared to sensitize guinea pigs which showed pneumonic patch formed by mixed inflammatory cells and showed bronchoconstriction. (Fig. 1)

DISCUSSION

Bronchial asthma is commonly characterized by increased airway reactivity to spasmogen. The MDD was found to significantly increase the PCD time against aerosol as compared to normal control animals indicating its bronchodilating activity. The total leukocyte, eosinophil, polymorph, monocyte and lymphocytes counts were increased after allergen challenge in the BAL fluid, thus gave an indication of inflammatory asthma[27, 28]. In the sensitized plus treated animals with MDD it was found that extract of *Delphinium denudatum* significantly reduced total leukocyte, neutrophil, lymphocyte counts, and eosinophil and monocyte counts. This indicated the protective effect by preventing release. Of several preformed mediators there by preventing direct damage of airway epithelium. In our study, oxidative stress has been increased in sensitized group of animal. The increased oxidative burden is result both of increased oxidative stress as evidenced by increased MDA and of the deceased antioxidant capacity as evidence by the lowered glutathione while in case of treated with MSI decreased the oxidative burden by reducing the MDA level and increasing the GSH level which is imbalanced in asthmatic condition[29]. In asthmatic condition bicarbonate level was increased due to increased tension of CO_2 in lung. Our drug significantly decreased the increased bicarbonate level which is beneficial in asthma. Further in histopathological study of lung, the treatment with the drug prevented inflammation and bronchoconstriction. Our all findings supported the use of *Delphinium denudatum* as traditional medicine in asthma.

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