EVALUATION OF ANTIHISTAMINIC ACTIVITY OF *DOLICHOS BIFLORUS*

ANUPAMA A. SURALKAR1* AND SANJAY B. KASTURE2

1Padmashree Dr. D.Y Patil Institute of Pharmaceuticals Science and Research, Pimpri, Pune.
2Department of Pharmacology, Sanjivani College of Pharmaceutical Education & Research, Kopargaon.

ABSTRACT

*Dolichos biflorus* Linn. (Family- Fabaceae) is commonly known as ‘Horse gram’. The seeds are used in the treatment of piles, pain, constipation, wounds, urinary calculi, cough, edema, and asthma. The present study was therefore designed for evaluation of antihistaminic activity of seeds of *Dolichos biflorus* in the management of asthma. Antihistaminic activity of ethanolic extract of *Dolichos biflorus* seeds (DB) was evaluated by using histamine induced contraction on goat tracheal chain preparation and histamine induced bronchoconstriction in Guinea pigs. The ethanolic extract of *Dolichos biflorus* seeds (DB) significantly inhibited histamine induced contraction of isolated goat tracheal chain preparation and significantly protected the guinea pigs against histamine induced bronchospasm as indicated by delay in the preconvulsive dyspnoea time (PCT) following the exposure of histamine aerosol. The results suggest that ethanolic extract of *Dolichos biflorus* seeds (DB) has antihistaminic activity and may prove beneficial in the management of asthma.

**KEYWORDS:** *Dolichos biflorus*, Horse gram’, Antihistaminic activity, asthma
1. INTRODUCTION

In many countries like India traditional medicinal system practice has been still followed as better option due to its cost effectiveness as it is comparatively economical and easy availability of genuine raw material, free from the hazardous side effects and toxicity. An ancient system of Indian medicine has recommended a numerous plants which can be used for the treatment of bronchial asthma and allergic disorders. Dolichos biflorus Linn. (Family-Fabaceae) is commonly known as 'Horse gram'. The seeds are used in the treatment of piles, pain, constipation, wounds, urinary calculi, cough, edema, and asthma. The seeds of D. biflorus have been reported to show Antioxidant activity, Chemomodulatory effect, antilithiatic, antihepatotoxic and hypolipidemic activity.

Histamine, an important mediator of Type-1 Hypersensitivity reaction is responsible for allergic inflammatory reactions like asthma. Prevention of histamine release from mast cells or by the use of histaminergic receptor antagonists has been proved beneficial antihistaminic therapy in treatment of asthma. Therefore, the present study was designed for evaluation of antihistaminic activity of seeds of Dolichos biflorus which can be useful in management of asthma.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Dunkin-Hartley Guinea pigs weighing between 350 to 400gm were purchased from National Toxicology Center, Pune. They were housed in groups of five under standard laboratory conditions of temperature (25 ± 2°C) and 12/12 hr light/dark cycle. Animals had free access to standard pellet diet and water ad libitum. Goat tracheal chain was obtained from slaughter house. The distribution of animals in the groups, the sequence of trials and the treatment allotted to each group were randomized, throughout the experiment. Laboratory animal handling and experimental procedures were performed in accordance with the guidelines of CPCSEA and experimental protocol was approved by Institutional Animal Ethics Committee (198/CPCSEA).

2.2 Chemicals

Histamine and Chlorpheniramine maleate was purchased from Research Lab Fine Chem. Industries, India.

2.3 Plant Material

Dried seeds of Dolichos biflorus were purchased from commercial supplier of Pune, India. The plant was authenticated Agharkar Institute of India, Pune, India (Voucher no. 3/187/Adm.-3007).

2.4 Preparation of Extract

About 1000gm of seeds of Dolichos biflorus (DB) were dried under shed and coarsely powdered. Seeds were defatted with petroleum ether and then subjected to maceration process by using 70% ethanol for 7 days shaking occasionally. After 7 days mixture was filtered and filtrate was evaporated to dryness to give an ethanolic extract of Dolichos biflorus (DB). The yield obtained was 18 g.

2.5 Preliminary phytochemical screening

After obtaining of dry extract, qualitative preliminary phytochemical screening was performed to find out the presence of various phytochemicals such as steroids, saponins, alkaloids, flavonoids, tannins, phenolic compounds, and glycosides.

2.6 Acute toxicity Study (OECD Guidelines, 423, 2001)

Albino rats of either sex weighing 200-250 gm were used in the study. Acute oral toxicity study was performed as per Organization for Economic Co-operation and Development (OECD)-423 guidelines. The animals were divided in 3 groups (n=3) and were fasted overnight prior to drug administration. Following the period of fasting, the animals were weighed and the test substance was administered. The
animals were given ethanolic extract of *Dolichos biflorus* (DB) in the doses of 5, 50, 300 and 2000 mg/kg body weight orally. The animals were observed for 5 min every 30 min till 2 hr and then at 4, 8 and 24 hr after treatment for any behavioral changes/mortality. They were further observed daily for 7 days for mortality.

**METHODS**

**Isolated Goat tracheal chain preparation**

The goat tracheal tissue was obtained immediately after slaughter of animals. Pieces of trachea were collected in freshly prepared ice-cold oxygenated Kreb’s solution (Composition (mM): NaCl, 115; KCl, 4.7; CaCl$_2$, 2; NaHCO$_3$, 25; KH$_2$PO$_4$, 1.2; MgCl$_2$, 1.2; glucose, 11.5). Goat trachea was then cut into individual rings and tied together in series to form a chain. It was suspended in bath containing Kreb’s solution and maintained at 37 ± 1°C, a stream of air was bubbled through the organ tube (1 bubble/sec). One end of the tracheal muscle was attached to S-shaped aerator and the other attached to isotonic frontal writing lever to a drum. The tissue was allowed to equilibrate for 45 min under a load of 1000 mg. A dose response curve for histamine was recorded at variant molar concentrations by maintaining 15 min time cycle. After obtaining dose response curve of histamine (30 µg/ml) on trachea, the DB (10 µg/ml) was added to reservoir and same doses of histamine were repeated. Graph of percentage of maximum contractile response on ordinate and negative log of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and in presence of DB and standard drug Chlorpheniramine maleate (1 µg/ml).

**Histamine induced bronchconstriction in guinea pigs**

Overnight fasted guinea pigs were randomly divided into five groups (n=5). Prior to drug treatment, each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The Preconvulsion dyspnoea time (PCD) was noted for each animal. The Preconvulsion dyspnoea time is the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion. As soon as preconvulsive dyspnoea commenced, animals were removed from the chamber and placed in fresh air to recover from dyspnoea for 24 hours. This time for preconvulsive dyspnoea was recorded as basal value. After 24 hours, animals belonging to group I served as control and were administered with phosphate buffer (1ml/kg, p.o.); Animals belonging to group II were administered with Chlorpheniramine maleate (2 mg/kg, i.p.) while group III to V were received respective doses of DB. These animals were again subjected to histamine aerosol later at an interval of 1 hr, 4 hr and 24 hr and to determine Preconvulsion dyspnoea time (PCD). The protection offered by the treatment was calculated by using the following formula

% Protection= (1-T1/T2) ×100

Where, T$_1$ = the mean of PCT before administration of test drugs, T$_2$ = the mean of PCT after administration of test drugs at 1hr, 4hr and 24 hr.

**Statistical Analysis**

The results have been indicated in terms of mean ± SEM, (n=5). Difference between the groups was statistically determined by One way ANOVA with Dunnett’s test. The level of significance was set at **p<0.01.

**3. RESULTS**

**3.1 Phytochemical Screening**

Preliminary phytochemical investigation of ethanolic extract of *Dolichos biflorus* (DB) showed the presence of glycosides, flavonoids and proteins.

**3.2 Acute toxicity Study**

The animals were showed no mortality and safe up to the dose 2000 mg/kg body weight. Dose was selected by using acute toxicity study
As no sign of toxicity was observed, $\frac{1}{10}$th dose of above dose as 200mg/kg was taken as safe dose. These doses were then converted in to guinea pig doses by using conversion factor (i.e. dose used in 200 gm of rat x 1.74, as suggested by Ghosh, 2005)\(^{12}\). Three different doses (88, 175 and 350 mg/kg, p.o) of DB were later chosen for this study based on the acute toxicity testing. Dose of 10µg/ml for isolated goat tracheal chain preparation was selected by our laboratory trials.

### 3.3 Isolated goat trachea chain preparation

Histamine produced dose dependant contraction in goat tracheal chain preparation at the concentration 30 µg/ml. This was significantly inhibited by modified PSS incubated with Chlorpheniramine maleate (CPM) (1 µg/ml). The modified physiological salt solution containing ethanolic extract of DB (10 µg/ml) significantly inhibited (p<0.01) the contractile effect of histamine. (Graph 3.1)

**Graph 3.1**

*Effect of DB on histamine induced contraction in goat tracheal chain preparation.*

![Graph 3.1](image)

**p<0.01 compared to control group

Control = D.R.C. of histamine (30 µg/ml) in absence of test drug;
CPM= D.R.C. of histamine (30 µg/ml) in presence Chlorpheniramine maleate (1 µg/ml);
DB = D.R.C. of histamine (30 µg/ml) in presence DB (10 µg/ml).

### 3.4 Effect of Dolichos biflorus Extract on Histamine Induced Bronchoconstriction in Guinea Pigs

The guinea pigs when exposed to 0.2 % w/v histamine aerosol showed signs of progressive dyspnoea leading to convulsions. Chlorpheniramine maleate (2 mg/kg, i.p) significantly prolonged (p<0.01) the preconvulsive dyspnea at 1\textsuperscript{st}, 4\textsuperscript{th} and 24 hr following exposure to histamine aerosol. DB at doses of 88 mg/kg did not significantly prolong the preconvulsive dyspnea at 1st hr; but significantly prolonged (p<0.01) the preconvulsive dyspnea at 4th and 24 hr as compared to control. DB at doses of 175 and 350 mg/kg, p.o significantly prolonged (p<0.01) the preconvulsive dyspnea at 1st, 4th and 24 hr as compared to control following exposure to histamine aerosol. (Graph 3.2)
Effect of test drug on Preconvulsive dyspnea time in histamine induced bronchoconstriction in guinea pigs.

Graph 3.2

Graph 3.3

3.5 Percentage protection against Histamine Induced Bronchoconstriction in Guinea pigs at different time interval

The Standard drug Chlorpheniramine maleate (2 mg/kg, i.p.) offered protection at 1\textsuperscript{st} hour (66.9\%), 4\textsuperscript{th} hour (70.9\%), and 24\textsuperscript{th} hour (70.6\%). DB at dose of 88 mg/kg offered less protection at 1st hour (1.68\%) as compared to 4\textsuperscript{th} (17.02\%) and 24\textsuperscript{th} Hr (5.64\%). DB at dose of 175 mg/kg offered protection at 1\textsuperscript{st} hour (58.30\%), 4\textsuperscript{th} hour (66.06\%), and 24\textsuperscript{th} hour (59.05\%). DB at dose of 350 mg/kg offered protection at 1\textsuperscript{st} hour (68.43\%), 4\textsuperscript{th} hour (68.87\%), and 24\textsuperscript{th} hour (70.41\%). (Graph 3.3)
DISCUSSION

The contraction of tracheal or bronchial smooth muscle in vitro has often been utilized to study the contractile / dilator responses of agonists as well as antagonist. Both goat tracheal chain and strip preparations are suitable for screening the activity of a drug on respiratory smooth muscles as spasmogens such as histamine produce dose dependent contraction of goat tracheal chain preparation. Histamine is one of the important mediators of allergy, inflammation and bronchoconstriction. Antihistaminic therapy in asthma can be achieved either by prevention of histamine release from mast cells or by the use of histaminergic receptor antagonists. The goat tracheal muscle has H₁, M₃ and β₂ receptors. Goat tracheal smooth muscles are contracted by histamine by the stimulation of H₁-receptor. This leads to activation of IP₃ and DAG pathway. This increased IP₃ release of calcium ions from storage vesicles. Elevated intracellular calcium binds to calmodulin, calcium-binding protein, which in turn activates myosin light chain kinase (MLCK). This MLCK then causes phosphorylation of actin-myosin fibers of goat trachea causing the contraction.

In the present study, the ethanolic extract of *Dolichos biflorus* significantly inhibited the histamine-induced contraction of isolated goat tracheal chain preparation by shifting DRC to the right side; thus competitively antagonized the action of histamine on H₁-histaminergic receptors on isolated goat tracheal chain preparation. This is indicating antihistaminic activity of *Dolichos biflorus* which further contribute to Antiasthmatic activity. Histamine is a central mediator in the pathogenesis of allergic and inflammatory disorders and inhalation of which causes immediate airway constriction and late airway reactivity. Bronchoconstriction effect of Histamine may be due to direct H₁-receptor activation and also by a naturally mediated bronchoconstrictor effect via vagal reflexes. Histamine has been shown to activate action potentials in intrapulmonary vagal afferents. In the present study of Histamine induced bronchoconstriction in guinea pigs, when guinea pigs exposed to histamine aerosol showed signs of progressive dyspnoea leading to convulsions. The ethanolic extract *Dolichos biflorus*...
significantly protected the guinea pigs against histamine-induced bronchospasm and significantly prolonged the latent period of convulsions (PCT) as compared to control following the exposure to histamine aerosol. The action of the ethanolic extract of *Dolichos biflorus* indicating the utility in the treatment of asthma and bronchitis by virtue of its H\(_1\)-receptor blocking or bronchodilating activity. Therefore, the protection against histamine induced bronchospasm in vivo may be either due to smooth muscle relaxant or anti-histaminic activity. The similar findings were suggested by Kumar et al, (2011)\(^{16}\), Gopumadhavan et al, (2005)\(^{17}\). Thus *Dolichos biflorus* have anti-histaminic by blocking H\(_1\)- receptor or bronchodilating activity which suggestive of its potential in prophylaxis and management of asthma.

**ACKNOWLEDGEMENT**

Authors are grateful to Dr. S. S. Chitlange, Principal, Padm. Dr. D. Y. Patil, Institute of Pharmaceutical Sciences and Research, Pimpri, Pune-18, Maharashtra, India, for providing laboratory facilities. Authors are grateful to Agharkar research Institute, Pune for authentification of plant material.

**REFERENCES**


