ORIGINAL ARTICLE

Anti-allergic and anti-anaphylactic activities of *Dolichos biflorus*

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Background: The seeds of *Dolichos biflorus* (DB) have been traditionally used in the treatment of cough and asthma. **Aim:** Based on the traditional claim, the present study was planned to evaluate the anti-allergic and anti-anaphylactic activities of DB. **Materials and Methods:** The ethanolic extract of the seeds of DB was prepared by cold maceration process. DB was subjected to phytochemical screening, acute toxicity studies, mast cell-stabilizing activity using compound 48/80 and anti-allergic activity using milk-induced leukocytosis and eosinophilia and passive paw anaphylaxis. **Statistical Analysis:** Statistical analysis was done by using one-way analysis of variance followed by Dunnett's test. **Results:** The phytochemical investigation showed presence of proteins, flavonoids and glycosides. DB extract inhibited milk-induced leukocytosis and eosinophilia and also the compound 48/80 induced mast cell degranulation. DB extract significantly reduced passive paw anaphylaxis in a dose-dependent manner. **Conclusion**: The results demonstrated that DB extract possesses anti-allergic and anti-anaphylactic potentials that might be useful in the management of asthma.

Key words: Compound 48/80, *Dolichos biflorus*, milk, passive paw anaphylaxis

INTRODUCTION

Many diseases such as asthma, rhinitis, bronchitis, cold, cough, pain and inflammation occur due to allergy and anaphylaxis.[1] Type I hypersensitivity (immediate type of hypersensitivity) is an allergic reaction provoked by re-exposure to a specific type allergen. In type I hypersensitivity, an antigen binds to CD4 + Th2 cells specific to that antigen and stimulate B-cell production, which ultimately produce IgE antibodies specific to that antigen. These IgE antibodies then sensitize mast cells, eventually inducing release of inflammatory mediators like histamine, leukotrienes and prostaglandins. These mediators produce physiological events such as smooth muscle contraction, vasodilatation, increased vascular permeability and mucous hypersecretion.

Anti-allergic drugs stabilize mast cell and inhibit release of histamine. Traditional medicine has proven the beneficial effects for the treatment of various diseases and disorders including allergic asthma.[2] Traditionally *Dolichos biflorus* Linn. (family Fabaceae) (hereafter referred to as DB), commonly known as Horse gram, is used in the treatment of cough, oedema and asthma. The seeds of DB have been reported to show anti-oxidant activity[3] and chemomodulatory effect[4] Thus, by taking into consideration the traditionalclaims and the reported pharmacological activities of DB, the present study was designed to evaluate anti-allergic and anti-anaphylactic activity of DB.

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MATERIALS AND METHODS

Experimental Animals

Wistar rats (150-200 g) and Swiss Albino mice (20-25 g) of either sex were purchased from the National Toxicology Center, Pune. They were housed in groups of five under standard laboratory conditions of temperature (25 ± 2°C) and 12 h lightdark cycle. Animals were provided standard pellet diet (Amrut Laboratory animal feed, Sangli-Maharashtra) and water *ad libitum*. 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 the experimental protocol was approved by Institutional Animal Ethics Committee (198/99/CPCSEA).

Chemicals

All chemicals were purchased from HiMedia Lab. Pvt. Ltd., India and Sigma Aldrich, USA. Egg albumin was purchased from Central Drug House (P) Ltd New Delhi.

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Plant Material

Dried seeds of DB were purchased from commercial supplier of Pune, India. The seeds were authenticated by Agharkar Research Institute, Pune, India (Voucher no. S-137).

Preparation of Extract

About 1000 g of dried seeds of DB were coarsely powdered and defatted with petroleum ether and then subjected to maceration using 70% ethanol for 7 days occasional shaking condition. After 7 days, the mixture was filtered and the filtrate was evaporated to dryness to give the ethanolic extract of DB. The yield obtained was 2%.

Phytochemical Screening

Qualitative phytochemical screening of DB extract was performed to determine the presence of various phytochemicals such as steroid, saponin, alkaloid, flavonoid, tannin, phenolic compound and glycosides.[5]

Acute Toxicity Study

The acute toxicity study was conducted according to the OECD Guidelines, 423 (2001) using Albino rats of either sex weighing 200-250 g. The animals were given the ethanolic extract of DB in doses of 5, 50, 300 and 2000 mg/kg body weight orally. The animals were observed for 5 min every 30 min until 2 h and then at 4, 8 and 24 h after treatment for any behavioural changes/mortality. They were further bserved daily for 7 days for mortality. No mortality up to 7 days after treatment was observed with the ethanolic extract of DB and, therefore, it was found safe up to the dose of 2000 mg/kg. Doses were selected based on acute oral toxicity study.

Doses

In rats, three doses were used, i.e., 100, 200, and 400 mg/kg (1/20th; 1/10th; and 1/5th of 2000 mg/kg, the highest dose used in acute toxicity study). Whereas, in mice, the doses were 140, 280 and 560 (i.e., dose used in rat × 1.4, as suggested by Ghosh).[6]

Milk-induced Leukocytosis and Eosinophilia in Mice

Mice were divided into five groups (n = 5). Mice of the vehicle control group were administered distilled water (10 ml/kg, p.o.), whereas other groups received boiled and cooled cow milk (4 ml/kg s.c.) with or without dexamethasone (0.5 mg/kg i.p.), DB (140, 280 or 560 mg/kg p.o.). After 24 h of milk administration, the blood samples were collected from retro-orbital plexusunder light ether anaesthesia. Total leukocytes and eosinophils counts were recorded in each group.[7]

Compound 48/80-induced Mast Cell Degranulation in Rats

Rats were divided into five groups (n = 5). On the 1st day of sensitization, all the animals from each group were injected with Compound 48/80 (1 mg/kg, s. c.). Rats of the vehicle control group received distilled water (10 ml/kg, p.o.). Rats of the Keto group received ketotifen fumarate (1 mg/kg, p.o.), while rats of the test groups received 100, 200 or 400 mg/kg p.o. of DB for 15 days. On day 15th, 2 h after the assigned treatment, 10 ml of normal saline solution was injected into the peritoneal cavity and the abdomen was gently massaged for 90 s. The peritoneal cavity was carefully opened and the fluid containing mast cells were aspirated and collected in siliconised test tube containing 7-10 ml of RPMI-1640 medium (pH 7.2-7.4). The mast cells were then washed thrice by centrifugation at low speed (400-500 rev/min) and the pellets of the mast cells were taken in the RPMI-1640 medium. The mast cell suspension (approximately 1 × 10^6 cells/ml) was challenged with 5 µg/ml of compound 48/80 solution, stained with 0.1% toluidine blue and observed under high power microscope (45×). A total of 100 cells were counted from different visual areas. The numbers of intact and degranulated cells was counted and the percent protection was calculated using the formula:[8]

$$\% \text{ Protection} = [1 - (T/C)] \times 100$$

Where, T = No. of degranulated cells of test, C = No. of degranulated cells of control.

Passive Paw Anaphylaxis in Rats

Wistar rats were divided into five groups (n = 5). Antiserum to egg albumin was raised in rats by using aluminium hydroxide gel as an adjuvant. On 1st, 3rd and 5th day, the animals were given three doses of 250 µg of egg albumin (s.c.) adsorbed on 12 mg of aluminum hydroxide gel prepared in 0.5 ml of saline. On 10th day of sensitization, blood of each animal was collected from the retro-orbital plexus under light ether anaesthesia. The collected blood was allowed to clot and the serum was separated by centrifugation at 1500 rev/min. The animals were passively sensitized with 0.1 ml of the undiluted serum into the left hind paw. The right hind paw received an equal volume of saline. Rats belonging to the vehicle group were administered distilled water (10 ml/kg, p.o.). Rats of the DEXA group were administered dexamethasone (0.5 mg/kg, i.p.) whereas, the rats of the test groups were administered 100, 200 or 400 mg/kg p.o. of DB after 24 h of sensitization. After 1 hof the drug administration, the rats were challenged with 10 µg of egg albumin in 0.1 ml of saline in the subplantar region of the left hind paw, and the paw volume was measured at 0.5, 1, 2, 3 and 4 h time interval by using Plethysmometer (UGO Basile, 7140).[9] The difference in the reading prior to and after antigen challenge represents the oedema volume, and the percent inhibition of oedema was calculated by using the formula,

$$\% \text{ Inhibition} = [1 - (T/C)] \times 100$$

T = Mean relative change in paw volume in test group, C = Mean relative change in paw volume in control group.

Statistical Analysis

The results were expressed as mean ± SEM from 5 animals. Statistical analysis was done by using one-way analysis of variance (ANOVA), followed by the Dunnett's test. $P < 0.05$ was considered significant.

RESULTS

Phytochemical Screening

The phytochemical investigation of the ethanolic extract of DB showed the presence of proteins, flavonoids and glycosides.

Acute Toxicity Study

The animals showed no mortality or any adverse effect up to the dose 2000 mg/kg body weight. The present study was performed at three dose levels of ethanolic extract of DB at 100, 200 and 400 mg/kg of body weight (1/20; 1/10; 1/5 of highest dose used in the toxicity study). The dose in mice was calculated on the basis of surface area by multiplying with the factor 0.14 with LD_{50} dose required for 200 g of rat. Therefore, the regime for the DB dose in mice was 140, 280 and 560 mg/kg[6]

Effect of DB on Milk-Induced Leukocytosis and Eosinophilia in Mice

Mice treated with milk (4 ml/kg, s.c.) produced a significant ($P < 0.001$) increase in the leucocytes and eosinophil count after 24 h of its administration as compared to the control group. Mice pre-treated with dexamethasone (0.5 mg/kg, i.p) showed significant ($P < 0.01$) inhibition of milk-induced leucocytosis and eosinophilia. Mice pre-treated with DB (140 mg/kg, p.o.) did not show significant inhibition, while those pre-treated with DB (280 mg/kg and 560 mg/kg, p.o.) demonstrated significant ($P < 0.01$) inhibition of milk-induced leucocytosis and eosinophilia [Figure 1].

Effect of DB on Compound 48/80-Induced Mast Cell Degranulation

Compound 48/80 (1 mg/kg, s.c.) induced mast cell degranulation was significantly ($P < 0.01$) inhibited by rats pre-treated with ketotifen fumarate, and the percent protection was found to be 70.6%. Rats pre-treated with DB in all the three doses showed significant protection ($P < 0.01$) against degranulation as compared to the control group. The 400 mg/kg dose offered more protection (44.0%) as compared to 100 mg/kg, p.o. (5.0%) and 200 mg/kg, p.o. (19.41%) [Figure 2].

Effect of DB on Passive Paw Anaphylaxis in Rats

Anti-serum to egg albumin was injected 24 h before administration of the test drug and the standard drug dexamethasone. In the vehicle or distilled water-treated group, egg albumin increased the paw volume in the sensitized rats, which was measurable up to the time period of 4 h. In a group of rats pre-treated with dexamethasone (0.5 mg/kg, i.p.), there was significant ($P < 0.01$) reduction in the paw volume at 0.5, 1, 2, 3 and 4 h and the percentage inhibition was 52.2, 52.6, 61.2, 66.1 and 74.40%, respectively. In rats pre-treated with DB at the dose of 100, 200 and 400 mg/kg, p.o., there was significant ($P < 0.01$) reduction in the paw volume at 0.5, 1, 2, 3 and 4 h time interval. The percentage inhibition of DB at the dose of 100 mg/kg, p.o. was found to be 9.7, 20.0, 28.5, 43.4 and 59.0%, respectively. The percentage inhibition of DB at the dose of 200 mg/kg, p.o. was found to be 19.02, 20.7, 32.7, 49.6 and 57.1%, respectively. The percentage inhibition of DB at the dose of

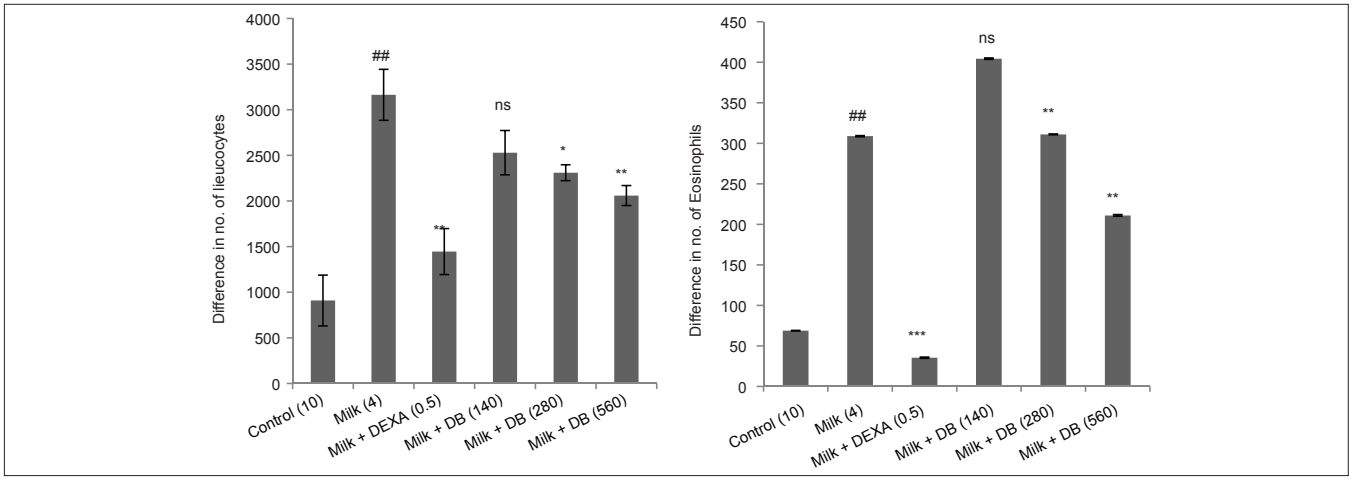

Figure 1: Effect of DB on milk-induced Leucocytosis and Eosinophilia. Data are expressed as mean ± S.E.M of 5 observations. Statistical analysis was done by using Student's *t*-test (milk-treated group was compared with the control group) ##$P < 0.01$. Group Dexa, DB-140, DB-280 and DB-560 as compared with the milk-treated group using one-way ANOVA, followed by Dunnett's test *$P < 0.05$, **$P < 0.01$. Control (10) = Distilled water (10 ml/kg, p.o.); Milk (4) = Milk (4 ml/kg, s.c.) + Distilled water (10 ml/kg, p.o.); Milk + DEXA (0.5) = Milk (4 ml/kg, s.c.) + Dexamethasone (0.5 mg/kg, i.p.); Milk + DB (140) = Milk (4 ml/kg, s.c.) + Ethanolic extract of *Dolichos biflorus* (140 mg/kg, p.o.); Milk + DB (280) = Milk (4 ml/kg, s.c.) + Ethanolic extract of *Dolichos biflorus* (280 mg/kg, p.o.); Milk + DB (560) = Milk (4 ml/kg, s.c.) + Ethanolic extract of *Dolichos biflorus* (560 mg/kg, p.o.)

400 mg/kg, p.o. was found to be 20.14, 21.5, 42.2, 50.9 and 67.1%, respectively [Figures 3 and 4].

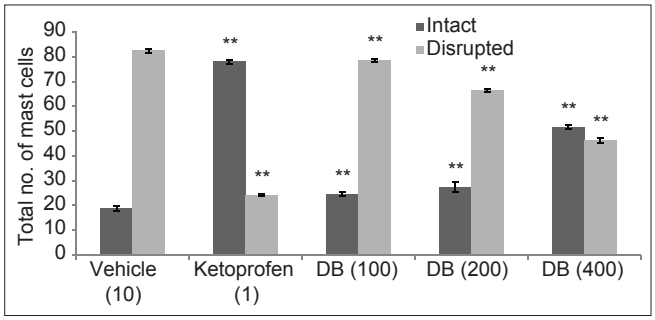

Figure 2: Effect of DB on compound 48/80-induced mast cell degranulation in rats. Data are expressed as mean ± S.E.M of 5 observations. Statistical analysis was done by using one-way ANOVA, followed by Dunnett's test. Keto and all test groups compared with vehicle treated group. $^{**}P < 0.01$. Vehicle (10) = Compound 48/80 (1 mg/kg, s.c.) + Distilled water (10 ml/kg, p.o.); Ketoprofen (1) = Compound 48/80 (1 mg/kg, s.c.) + Ketotifen fumarate (1 mg/kg, p.o.); DB (100) = Compound 48/80 (1 mg/kg, s.c.) + Ethanolic extract of *Dolichos biflorus* (100 mg/kg, p.o.); DB (200) = Compound 48/80 (1 mg/kg, s.c.) + Ethanolic extract of *Dolichos biflorus* (200 mg/kg, p.o.); DB (400) = Compound 48/80 (1 mg/kg, s.c.) + Ethanolic extract of *Dolichos biflorus* (400 mg/kg, p.o.)

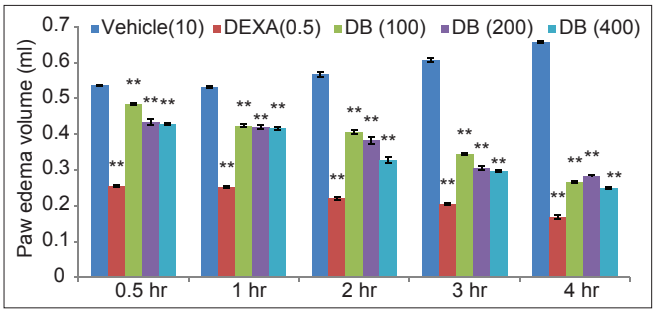

Figure 3: Effect of DB on paw oedema volume in passive paw anaphylaxis Data are expressed as mean ± S.E.M. of 5 observations. Statistical analysis was done by using one-way ANOVA, followed by Dunnett's test. DEXA and all test groups compared with the vehicle control $^{**}P < 0.01$. Vehicle (10) = Distilled water (10 ml/kg, p.o.) + Egg albumin (250 µg/kg, s.c.); DEXA (0.5) = Dexamethasone (0.5 mg/kg, i.p.) + Egg albumin (250 µg/kg s.c.); DB (100) = Ethanolic extract of *Dolichos biflorus* (100 mg/kg, p.o.) + Egg albumin (250 µg/kg, s.c.); DB (200) = Ethanolic extract of *Dolichos biflorus* (200 mg/kg, p.o.) + Egg albumin (250 µg/kg, s.c.); DB (400) = Ethanolic extract of *Dolichos biflorus* (400 mg/kg, p.o.) + Egg albumin (250 µg/kg, s.c.)

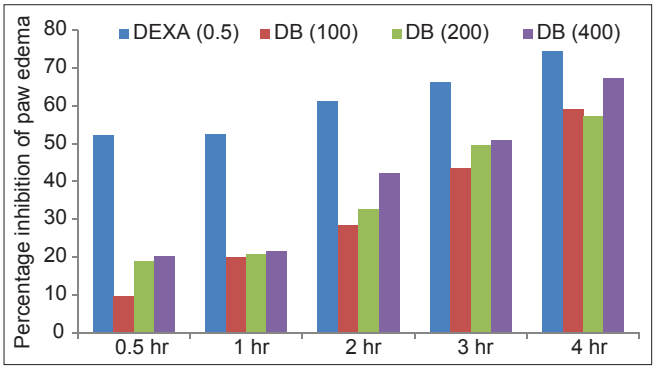

Figure 4: Effect of DB on percentage inhibition in passive paw anaphylaxis DEXA (0.5) = Dexamethasone (0.5 mg/kg, i.p.) + Egg albumin (250 µg/kg s.c.); DB (100) = Ethanolic extract of *Dolichos biflorus* (100 mg/kg, p.o.) + Egg albumin (250 µg/kg, s.c.); DB (200) = Ethanolic extract of *Dolichos biflorus* (200 mg/kg, p.o.) + Egg albumin (250 µg/kg, s.c.); DB (400) = Ethanolic extract of *Dolichos biflorus* (400 mg/kg, p.o.) + Egg albumin (250 µg/kg, s.c.)

DISCUSSION

Excessive stress or nervous debility may precipitate symptoms of asthma. There are some anti-stress drugs used in the treatment of asthma as they enable adoption to stress.[10] Adoptogens are one of medicinal drugs that develop nonspecific resistance in organism to resist stress of physical, biological or chemical nature and to develop adaptation to external challenges. During infection and inflammation, there is an increase in the release of leukocytes from the bone marrow. Physical stress and emotional stress, a variety of infections and allergic reactions can also cause leukocytosis. Milk administration leads to allergic reaction with leukocytosis.[11] Eosinophil degranulation is an important immunological mechanism leading to allergic inflammation due to cow's milk allergy.[12] The increased mast cells and basophil production in allergic condition leads to the release of eosinophil chemotactic factor that causes more eosinophils to migrate toward the inflamed allergic tissue. Thus, large number of eosinophils gets accumulated in the blood as well as in the peribronchial tissue of the lungs in the late asthmatic phase reaction to an allergen. The present study revealed that parenteral administration of milk increased the total leukocyte count that was restored by pre-treatment with DB, indicating adaptogenic activity of ethanolic extract of DB. These findings are in congruence with the observations of Maxia *et al*.[13]

Compound 48/80 is a mixed polymer of phenethylamine cross-linked by formaldehyde, which initiates the activation of signal transduction pathway leading to histamine release from mast cells.[14] Compound 48/80 can be used to study the mechanistic pathway for allergy and anaphylaxis.[15] Mast cells are the main source of initiating allergic inflammation like asthma. Compound 48/80 penetrate into mast cells and activates G protein-coupled receptors by interacting with COOH-terminal domain (C-terminus) of the α subunits of G proteins.[16,17] This decreases the intracellular cAMP concentration in mast cells that initiates the generation of superoxide anion (O_2^-) by A-kinase inactivation, leading to the opening of the Ca^{2+} channel and the release of Ca^{2+} into the cytoplasm.[18] Thus, increase in the intracellular calcium content leads to the release of histamine from mast cells that are known as degranulation of mast cells.[19] In the present study, the DB extracts significantly reduced degranulation of the mast cell in a dose-dependant manner. This effect may be due to the presence of flavonoids in the extract.[20,21]

Rat raises the antibodies to egg albumin in the plasma by challenging them with egg albumin (s.c.), and sub-plantar injection of plasma containing these antibodies leads to passive paw anaphylaxis in rats.[22] This leads to the activation of interleukin-4 (IL-4) by T lymphocytes. IL-4

stimulates B-cells to produce IgE antibodies. Binding of antigens to IgE on mast cells through FcεRI causes cross-linking of the bound IgE and the aggregation of the underlying FcεRI, leading to the degranulation and the release of mediators from the cells like histamine, leukotriene (LTC4 and LTD4) and prostaglandin. These mediators produce physiological events such as smooth muscle contraction, vasodilatation, increased vascular permeability and mucous hypersecretion.[2] The present study revealed that animals pre-treated with ethanolic extract of DB significantly reduced the paw volume at all the time intervals in the model of passive paw anaphylaxis in rats. The beneficial effect of DB could be due to the inhibition of antigen–antibody complex that may contribute to anti-asthmatic activity.

The phytochemical investigation of DB showed the presence of proteins, flavonoids and glycosides. Du *et al*.,[23] reported that Triterpene gylcosides were also inhibit the development of characteristic features in chronic experimental asthma. Flavonoids are known to possess anti-inflammatory and anti-oxidant activities.[20] Thus, the presence of these phytoconstituents in the ethanolic extract of DB may further contribute in anti-allergic and anti-anaphylactic activities in the management of asthma.

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