

MAST CELL STABILIZING POTENTIAL OF THE ETHANOLIC EXTRACT OF *TEPHROSIA PURPUREA* (LINN.) IN THE MANAGEMENT OF ASTHMA

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ABSTRACT

The present study was carried out to evaluate mast cell stabilizing potential of the ethanolic extract of *Tephrosia purpurea* (Linn.) in the management of asthma using experimental animal models. Ethanolic extract of aerial parts of *Tephrosia purpurea* (L.) (EETP) was prepared and mast cell stabilizing potential evaluated against compound 48/80 and clonidine induced mast cell degranulation in adult wistar albino rats. The result of present study revealed that, compound 48/80 and clonidine treatment produced 75.83% and 73.67% of mast cell degranulation respectively. EETP at a concentration of 250, 500, and 750 µg/ml showed dose-dependent significant reduction in mast cell degranulation ($P < 0.01$) as compared to the compound 48/80 and clonidine-treated animals. However, its effect was less than the dexamethasone and disodium cromoglycate, potent mast cell stabilizers. To conclude, the EETP possesses good mast cell stabilizing properties and therefore can be a candidate for the antiasthmatic treatment. Future research should focus on the molecular mechanism of responsible constituent for mast cell stabilizing potential.

Key words: Mast cell stabilizing, Anti-asthmatic, *Tephrosia purpurea*, Ethanolic extract.

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INTRODUCTION

Tephrosia purpurea (Linn.) Pers. (Fabaceae) commonly known in Sanskrit as Sharapunkha is a highly branched, sub-erect, herbaceous perennial herb. It is one of the excellent plants for human being made and gifted by the nature having composition of all the essential constituents that are required for normal and good human health.¹

In Ayurvedic system of medicine various parts of this plant are used as remedy for impotency, asthma, diarrhoea, gonorrhoea, rheumatism, ulcer and urinary disorders. In the Ayurvedic system of medicine, the whole plant has been used to cure tumours, ulcers, leprosy, allergic and inflammatory conditions such as rheumatism, asthma and bronchitis.² The phytochemical investigations on *Tephrosia purpurea* have revealed the presence of glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, and sterols.³ Recent investigations have shown that the LD₅₀ of the ethanolic extract of aerial parts of *Tephrosia purpurea* (Linn.) is 5.12g/kg and it inhibits type I hypersensitivity

reactions.^{4,5} The extract also inhibited the late inflammatory phase of an allergic reaction.⁶ Bronchial asthma is an inflammatory disorder of the airways characterized by various airway obstructions, airway inflammation and bronchial hyperresponsiveness.⁷ Asthma affects about 300 million people worldwide, a total that is expected to rise to about 400 million over the next 15-20 years. Large number of drugs belonging to β_2 adrenergic agonists, corticosteroids, mast cell stabilizers, methylxanthins, leucotriene antagonists and others are in use for treating asthma.⁸

Tephrosia purpurea (Linn.) seems to be a promising plant for treatment of bronchial asthma because of its reported anti-oxidant, immunomodulatory and anti-inflammatory activity.⁹⁻¹¹ There are hardly any reports on anti-asthmatic and mast cell stabilizing activity of this plant. Hence, in the present study, we have investigated mast cell stabilizing activity of ethanolic extract of *Tephrosia purpurea* on experimental animal models.

MATERIAL AND METHODS**Collection of Plant and its authentication**

Tephrosia purpurea (Linn.) Pers. was collected from Anand Agriculture University and the field around it in the month of January- February' 2011, during flowering stage. The plant, *Tephrosia purpurea* (Linn.), was authenticated by **Dr. Minoo H. Parabia**, advisor of Shri R. M. Dhariwal Ayurved College and Research Centre, Waghaldhara, Valsad, Gujarat. And voucher specimen submitted to herbarium department of this college.

Preparation of ethanolic extract

Aerial parts of the plants were collected, cleaned and dried in shade. After 7-days of drying, the dried plant was powdered by grinding and sieved with a 40# sieve. The powder was extracted in soxhlet apparatus for 24 hours with 95% ethanol. The extract was filter and concentrated in vacuum under reduced pressure using a rotary flash evaporator (yield obtained: 5.1 % w/w). The concentrated mass was used.

Procurement of Animals

Albino rats (Wistar strain) of either sex weighing 200-250 g were used for studies. The albino rats were obtained from animal house of Jai Research Foundation, Vapi, Gujarat. They were housed in polypropylene cages with standard pellet chow and water *ad libitum*. Permission was obtained, prior to the start of experiments, from Institutional Animal Ethics Committee (IAEC) of *Rofel*, Shri G. M. Bilakhia college of Pharmacy as per CPCSEA guidelines (Reg. no.: 403/01/a/CPCSEA; Rofel/2011/01).

Drugs and solvents

Compound 48/80 was obtained as a gift sample from Zydus Pharmaceutical Ltd., Ahmedabad (Gujarat), Clonidine, Disodium cromoglycate, Dexamethasone and Toluidine blue were procured from Priya enterprise (Sigma), vapi (Gujarat).

Mast cell degranulation induced by Compound 48/80 in rats

Aim: To evaluate mass cell stabilizing activity of EETP against Compound 48/80 induced degranulation.

Rationale: Both compound 48/80 and clonidine act through the dynamic expulsion of granules without damaging cell wall. Drugs stabilizing mast cells will inhibit mast cell degranulation.

Procedure: Male albino rats were sacrificed by cervical dislocation. The animals were immediately injected with 15 ml of pre-warmed (37°C) buffered salt solution (NaCl 137 mM; KCl 2.7 mM; MgCl₂ 1 mM; CaCl₂ 0.5 mM; NaH₂PO₄ 0.4 mM; Glucose 5.6 mM; HEPES 10 mM) into the peritoneal cavity, and massaged gently in this region for 90 s, to facilitate cell recovery. A midline incision was made and the peritoneum was exposed. The

pale fluid was aspirated using a blunted plastic Pasteur pipette, and collected in a plastic centrifuge tube. The fluid was then centrifuged at 1000 rpm for 5 min, and the supernatant discarded to reveal a pale cell pellet. The cell pellets were re-suspended in fresh buffer and re-centrifuged. Aliquots of the cell suspension were incubated with the ethanolic extract of *Tephrosia purpurea* (250, 500 and 750 µg/ml) and disodium cromoglycate (20 µg/ml), before challenge with compound 48/80 (0.1ml, 10 µg/ml). The aliquots were carefully spread over glass slides and the mast cells were stained with 1% toluidine blue and counterstained with 0.1% light green. The slides were dried in air and the mast cells counted from randomly selected high power objective fields (X450). The effect of ethanolic extract of *Tephrosia purpurea* (EETP) on mast cells was studied by incubating the mast cells for 10 min with the above formulation in a concentration of 250, 500 and 750 µg/ml. In another set of experiments the mast cells which were pre-incubated with EETP were exposed to the mast cell degranulator, compound 48:80 (10 µg/ml) and the incubation continued for a further 10 min. Then, the mast cells were carefully spread over glass slides. The percent degranulation of the mast cells was calculated. Disodium cromoglycate (DSCG) (20 µg/ml) was included in one of the study group for comparison. The mast cells were examined under the high power of light microscope. One hundred mast cells were observed and the mast cells showing degranulation was counted. The percentage of protection against degranulation was computed.^{12,13}

Mast cell degranulation induced by Clonidine in rats

Procedure: Four ml of normal saline (containing 5 units/ml of heparin) was injected in the peritoneal cavity of rats lightly anaesthetized with ether. After a gentle abdominal massage, the peritoneal fluid containing mast cells were collected in centrifuge tubes placed over ice. Peritoneal fluid of 5 - 6 rats was collected and pooled. Mast cells then washed by centrifugation at a low speed (400 rpm-500 rpm) for 10 min. Supernatant solution was discarded and the cells were washed twice with saline and re-suspended in 1 ml of saline. 0.1 ml of the peritoneal cell suspension was transferred to 6 test tubes (TT) and was treated as follows.

- I. Negative control- TT no. 1 - Saline
- II. Positive control- TT no. 2- Saline
- III. Test group – TT no. 3 - 0.1 ml of 250µg/ml EETP in Saline
- IV. Test group – TT no. 4 - 0.1ml of 250µg/ml EETP in Saline
- V. Test group – TT no. 5 - 0.1 ml of 250µg/ml EETP in Saline

VI. Standard TT no. 6 - 0.1 ml of 10 µg/ml of Dexamethasone

Each test tube was incubated for 15 min at 37°C and then Clonidine (0.2 ml, 80 µg/ml) was added to each test tube except test tube no. 1. After further incubation for 10 min. at 37°C, the cells were stained with 0.1% toluidine blue solution made in distilled water and examined under the high power of light microscope. One hundred mast cells were observed and the mast cells showing degranulation was counted. The percentage of protection against degranulation was computed.¹⁴

Statistical analysis

All values were expressed as Mean± SEM for both the models. Data was analyzed by Student's *t*-test and One way ANOVA followed by Dunnett's *t*-test. The results were considered to be statistically significant when $p < 0.05$.

RESULT

The results of the present study revealed that ethanolic extract of *Tephrosia purpurea* (EETP) possesses significant mast cell stabilization action. In the mast cell degranulation induced by compound 48/80 paradigm (Figure 1), 33% of mast cells were degranulated in the control group. Addition of DSCG (20 µg/ml), and the EETP (250, 500 and 750 µg/ml) reduced the percentage of mast cell degranulation ($P < 0.01$) compared to the control group. Compound 48/80 (10 µg/ml) produced about 75.83% degranulation of mast cells. Pretreatment with DSCG (20 µg/ml) and the EETP (250, 500 and 750 µg/ml) significantly reduced ($P < 0.01$) degranulation of mast cells as compared to compound 48/80-treated control group (Table 1). The protection given by them at higher concentrations was comparable to that of DSCG, a potent mast cell stabilizing agent. These results suggest that EETP protects mast cells from compound 48/80-evoked degranulation.

In the mast cell degranulation induced by clonidine paradigm, 73.67% of mast cells were degranulated in the positive control group. Addition of Dexamethasone (10 µg/ml) - potent mast cell stabilizer, and the EETP (250, 500 and 750 µg/ml) reduced significantly ($p < 0.01$) of mast cell degranulation compared to the control group (Figure 2).

As shown in table 2, Clonidine induced mast cell degranulation was significantly ($p < 0.001$) inhibited by Dexamethasone (10 µg/ml) and percent protection was found to be 72.06%. There was significant ($p < 0.01$) protection of mast cells was observed in the pre-treated groups with EETP (250, 500, 750 µg/ml) and the percent protection was 24.40, 41.80, and 54.85 % respectively. The protection given by them at higher concentrations was comparable to that of Dexamethasone (10µg/ml), a

potent mast cell stabilizing agent. It shows that EETP possesses dose dependent effect.

DISCUSSION

Bronchial asthma is commonly characterized by increased airway reactivity to spasmogens. An initial event in asthma appears to be the release of inflammatory mediators (e.g. Histamine, Tryptase, Leukotrienes and prostaglandins). Some of these mediators directly cause acute bronchoconstriction, airway hyper-responsiveness and bronchial airway inflammation. Spasmolytic drugs like beta adrenergic agonists, xanthine derivatives and anticholinergics relax the airway smooth muscles and are used as quick relief medications in acute asthmatic attacks. Beta adrenergic agonists promote bronchodilation by direct stimulation of beta adrenergic receptors in the airway smooth muscle, that lead to relaxation of bronchial smooth muscle by rapid decrease in airway resistance in vivo. Specific β_2 agonists like salbutamol, salmeterol etc. are used since long for symptomatic relief in asthma.^{15, 16}

Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens.¹⁷ Once binding of allergen to cell-bound IgE occurs, mediators such as histamine; eosinophil and neutrophil chemotactic factors; leukotrienes C_4 , D_4 and E_4 ; prostaglandins; platelet-activating factor; and others are released from mast cells which are responsible for development of airway inflammation and bronchoconstriction. An attempt was made to find out whether ethanolic extract of *Tephrosia purpurea* has any effect on the rate of disruption of mast cells following exposure to compound 48/80 and clonidine, an agent which causes histamine release.¹⁸ It has been assumed that the process leading to histamine secretion may be mediated by calcium release from an intracellular store of mast cells.¹⁹

Adhatoda vasica, *Albizia lebeck*, *Coleus forskohlii*, *Tylophora asthmatica* etc. are several well known drugs from indigenous plant sources used in asthma and have been reported to have mast cell stabilizing activity.²⁰⁻²³

In this study, EETP offered significant protection against Compound 48/80 and clonidine induced mast cell degranulation by stabilizing it, which is responsible for the decreasing airway inflammation by preventing release of various inflammatory mediators.

CONCLUSION

It can be concluded from the results obtained that EETP possesses significant mast cell stabilizing activity against Compound 48/80 and Clonidine induced mast cell degranulation in rats. Hence it may be used in prophylaxis and/or management of asthma.

However, further studies on other experimental models are needed to support the hypothesis. A detailed study needs to be conducted to evaluate the phytoconstituent responsible to produce above result and their clinical efficacy in the treatment of asthmatic patients.

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Table 1: Effect of EETP on mast cell degranulation induced by compound 48/80

Sr. No.	Treatment	Concentration (µg/ml)	% Mast cells degranulation ± S.E.M.	% Inhibition of degranulation
1.	Negative control (Saline)	0.9 % w/v	4.67 ± 1.23 ^{ns}	-
2.	Positive control (Comp 48/80)	10 µg/ml	75.83 ± 3.22 ^{***}	-
3.	EETP	250 µg/ml	54.33 ± 4.22 ^{***}	26.72*
4.	EETP	500 µg/ml	40.50 ± 3.31 ^{**}	45.47 ^{**}
5.	EETP	750 µg/ml	29.17 ± 2.96*	60.74 ^{***}
6.	DSCG	20 µg/ml	19.50 ± 2.88*	73.94 ^{***}

N=6, Values are in Mean ± SEM. -Ve control and +Ve control: group of animals in absence and in presence of compound 48/80. EETP (250, 500 and 750 µg/ml): ethanolic extract of *Tephrosia purpurea* (Linn.). DSCG: Disodium cromoglycate. Statistical analysis was done by using ANOVA followed by Dunnett's t-test. * p<0.05, ** p<0.01 and *** p<0.001 compared with positive control.

Table 2: Effect of EETP on mast cell degranulation induced by Clonidine:

Treatment	Concentration (µg/ml)	% Mast cells degranulation ± S.E.M.	% Inhibition of degranulation
Negative control (Saline)	0.90%	3.17 ± 1.08 ^{ns}	-
Positive control (Clonidine)	80 µg/ml	73.67 ± 2.65 ^{***}	-
Dexamethasone	10 µg/ml	20.83 ± 2.60*	72.06 ^{***}
EETP 250	250 µg/ml	56.00 ± 4.03 ^{***}	24.40 ^{ns}
EETP 500	500 µg/ml	43.17 ± 3.24 ^{**}	41.80*
EETP 750	750 µg/ml	33.50 ± 3.06*	54.85 ^{**}

N=6, Values are in Mean ± SEM. -Ve control and +Ve control: group of animals in absence and in presence of clonidine. EETP (250, 500 and 750 µg/ml): ethanolic extract of *Tephrosia purpurea* (Linn.). Statistical analysis done by using student's t-test where ns: non-significant, *p<0.05, **p<0.01 and ***p<0.001 compared with positive control.

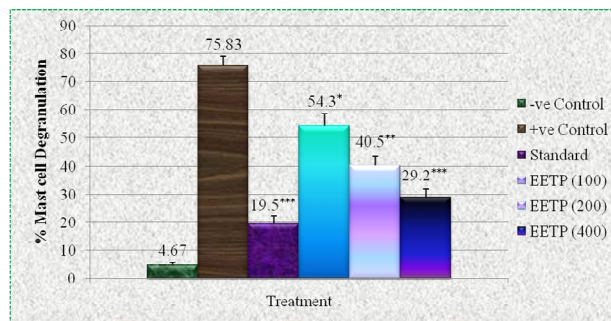


Figure 1: Effect of ethanolic extract of *Tephrosia purpurea* (Linn.) on mast cell degranulation induced by compound 48/80.

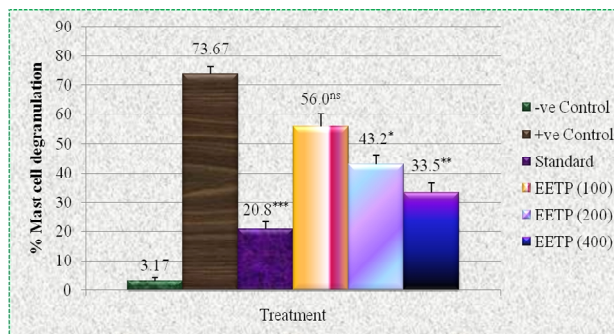


Figure 2: Effect of ethanolic extract of *Tephrosia purpurea* (Linn.) on mast cell degranulation induced by Clonidine.

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