
Influence of Ethanolic Extract of *Tephrosia purpurea* Linn. on the Late-phase of Allergic Reaction

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The ethanolic extract of the aerial parts of *Tephrosia purpurea* administered orally at doses of 50, 100 and 200 mg/kg, significantly reduced an elevated WBC count in response to antigen challenge in sensitized mice. The extract also significantly inhibited eosinophil infiltration without any significant change in the mononuclear cell population. The extract failed to alter neutrophil adhesion to nylon fibres. However, it produced a significant inhibitory activity on enzyme lipoxygenase at concentrations of 100 and 200 µg/ml. The inhibitory effect of ethanolic extract of *T. purpurea* on late-phase allergy could be attributed to the inhibition of leukotriene synthesis.

As the type I hypersensitivity begins to subside, mediators released during the course of reaction often induce a localized inflammatory reaction called the late-phase reaction, characterized with enhanced vascular permeability and leukocyte infiltration at sites of allergen challenge¹. The localized late-phase response may also be partly mediated by cytokines released from mast cells. Both TNF- α and IL-1 increase the expression of cell-adhesion molecules on venular endothelial cells, thus facilitating the buildup of neutrophils, eosinophils and monocytes. Indeed, much of the morbidity associated with allergic asthma and other allergic diseases is thought to reflect the actions of leukocytes recruited to the affected tissues, rather than immediate consequences of the released mast cell mediators^{2,3}.

Tephrosia purpurea Linn. (Leguminosae) is a copiously branched, herbaceous perennial found throughout India. Ayurvedic texts have reported the whole plant to be used to cure asthma and bronchitis⁴. Clinical trials of entire dried plants conducted on patients with bronchitis have shown improvement in their condition⁵.

Preliminary evaluation of the ethanolic extract of aerial parts of *T. purpurea* Linn. revealed that it inhibited

rat mast cell degranulation and passive paw anaphylaxis in rats in a dose-related manner^{6,7}. Hence, it was decided to study the effect of the ethanolic extract of aerial parts of *T. purpurea* on the late-phase allergic reaction.

EXPERIMENTAL

Dried, aerial parts of *Tephrosia purpurea* Linn, obtained from Yucca Enterprises, Mumbai, India, were identified and authenticated in the Department of Pharmacognosy, University Department of Chemical Technology, Mumbai, India. A voucher specimen has been deposited in the Bombay College of Pharmacy herbarium.

Drugs used were, ketotifen fumarate (FDC Ltd., Mumbai), phenidone (Hoechst Marion Roussel India Ltd., Mumbai), heparin (Biological E. Ltd.) and egg albumin (Hi-Media Laboratories Mumbai). All other reagents used were of a high grade of purity. Ketotifen fumarate was dissolved in 0.9% NaCl Solution.

Male Wistar rats (150-200 g) and Haffkine mice of either sex (20-25 g) were used. Animals were housed under standard conditions of temperature (23 \pm 1 $^{\circ}$), relative humidity (55 \pm 10%), 12/12 -h light/dark cycle and fed with standard pellet diet (Gold Mohur brand, Lipton India Ltd.) and water *ad libitum*.

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TABLE 1 : EFFECT OF ETHANOLIC EXTRACT OF *T. PURPUREA* ON CELL NUMBER AFTER ANTIGEN CHALLENGE

Treatment	Dose mg/kg, p.o.	% Eosinophils ±S.D.	% Mononuclear cells±S.D.	WBC Count ±S.D.
Negative Control	-	3.0±0.82	93.0±0.82	26.5±2.46
Positive Control	-	20.0±1.87	62.0±3.52	51.85±0.92
Ketotifen	1	15.17±2.56*	64.3±8.37 ^{NS} .	41.58±13.8 ^{NS} .
<i>T. purpurea</i> extract	50	9.0±4.06*	70.6±11.41 ^{NS} .	31.47±2.66*
<i>T. purpurea</i> extract	100	5.25±1.5*	86.25±5.5*	26.93±3.84*
<i>T. purpurea</i> extract	200	4.33±0.58*	90.67±1.53*	30.83±1.16*

N=6 per group; Results are expressed as mean±S.D. *P<0.02 as compared to control ^{NS}Non-significant as compared to control

Preparation of Plant Extract:

The coarse powder (200 g) of the dried aerial parts of *T. purpurea* Linn. was exhaustively extracted using 85% ethanol (1000 ml) in a Soxhlet extractor. The extract was concentrated under reduced pressure at a bath temperature below 50° to yield a syrupy mass (27 g). For administration it was suspended in distilled water using 0.1% sodium carboxymethylcellulose.

Increase in cell number after antigen challenge in mice⁸:

Mice were sensitized with a solution of 1 mg/ml of egg albumin in isotonic saline subcutaneously, receiving 0.1 ml on days 1 and 2 and 0.2 ml on days 7,9 and 15. The sensitized mice were challenged on day 21 by injecting intraperitoneally, 0.2 ml of 1 mg/ml egg albumin solution. Control animals received equivalent amount of isotonic saline.

T. purpurea extract (50, 100 and 200 mg/kg, p.o.) was administered to test groups of mice 30 min prior to sensitization on day 1. A single dose of the test agent was administered subsequently everyday till day 24. The animals were sacrificed on day 25 by cervical dislocation. Saline (0.5 ml) containing 6 U/ml of heparin was injected intraperitoneally. The abdomen was gently massaged, the peritoneal cavity cut open and the fluid was drained into test tubes. Differential counts of the per-fusate were performed manually and the total WBC count was performed using an Erma PC-607 cell counter (Erma Inc., Japan).

Neutrophil adhesion test in rats⁹:

Rats were treated orally with *T. purpurea* extract at doses of 50, 100 and 200 mg/kg for 7 days. On day 7, blood samples were collected from retro-orbital plexus into heparinized vials and analyzed for total leukocyte count (TLC). The differential leukocyte count (DLC) was performed by fixing the blood smears and staining with Field Stains A and B and per cent neutrophils in each sample was determined. After initial counts, the blood samples were incubated with 80 mg/ml nylon fibers for 10 min at 37°. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and per cent neutrophils gave the neutrophil index of blood sample and per cent neutrophil adhesion was calculated.

Lipoxygenase enzyme activity *in vitro*¹⁰:

The effect of the *T. purpurea* extract on lipoxygenase enzyme activity was determined by an *in vitro* assay method using soyabean lipoxidase as enzyme and linoleic acid as the substrate. Soyabean lipoxidase was procured from Sigma Chemical Company, U.S.A. and the lipoxygenase inhibitory activity was determined according to the procedure recommended by the manufacturer¹¹.

Phenidone (100 µg/ml) was used as a reference standard for comparison. *T. purpurea* extract was studied at concentrations ranging from 10-200 µg/ml. The extract was preincubated with the enzyme buffered with 2 M borate buffer (pH 9) for 5 min before lipoxygenation was started by the addition of enzyme to linoleic acid. Enzyme activity in the presence and absence of the inhibi-

TABLE 2 : EFFECT OF ETHANOLIC EXTRACT OF *T. PURPUREA* ON 5-LIPOXYGENASE ENZYME ACTIVITY

Treatment	Concentration µg/ml	Enzyme Activity (units/mg solid)±S.D.	% Inhibition
Control	—	611.11±117.85	—
Phenidone	100	55.55±10.0*	90.91
<i>T. purpurea</i> extract	10	500.48±32.08 ^{N.S.}	18.10
<i>T. purpurea</i> extract	25	500.0±78.57 ^{N.S.}	18.18
<i>T. purpurea</i> extract	50	416.66±39.29 ^{N.S.}	31.82
<i>T. purpurea</i> extract	100	259.26±64.15*	57.58
<i>T. purpurea</i> extract	200	111.11±30.0*	81.82

N=4 per group; Results are expressed as mean±S.D. *P<0.02 as compared to control ^{N.S.}Non-significant as compared to control.

tor was determined at 234 nm using a Shimadzu UV-160A spectrophotometer.

Statistical Analysis:

Results are expressed as mean±standard deviation and the differences between means were analyzed for statistical significance using Student's 't' test. The level of significance chosen was p<0.02.

RESULTS AND DISCUSSION

Allergic reactions are characterized by two phases, an early phase involving release of primary mediators such as histamine, proteases and other chemotactic mediators and a late phase characterized by release of secondary mediators such as leukotrienes, prostaglandins, platelet activating factor and cytokines leading to cellular infiltration and chemotaxis¹. *T. purpurea* extract at 50, 100 and 200 mg/kg, p.o. significantly reduced the eosinophil infiltration without any significant change in the mononuclear cell population at lower doses. The extract significantly lowered the WBC count after antigen challenge in the sensitized mice Table 1.

Reversible adherence of leukocytes to endothelium, followed by diapedesis into the extravascular compartment, directional movement in response to chemotactic gradients and finally phagocytosis at the site of inflammation are essential events in the establishment of an inflammatory state. Thus, an *in vitro* study on neutrophil adhesion was performed using nylon fiber as an adherent surface because of its proved effectiveness in separating granulocytes from whole blood^{12,13}. However,

T. purpurea extract at the doses of 50, 100 and 200 mg/kg, p.o. was not found to have any significant effect on neutrophil adhesion to nylon fibres and thus results have not been shown.

In the present study effect of *T. purpurea* on leukotriene synthesis i.e. on enzyme 5-lipoxygenase was studied in an *in vitro* model. The study was carried out using soyabean lipoxidase as enzyme and linoleic acid as substrate, since it has been shown by Bailey and Chakrin that lipoxygenase reaction cascade occurs both in mammalian and plant tissue¹⁴. It was found that at 100 and 200 µg/ml concentration, *T. purpurea* extract showed significant dose-dependent inhibition of enzyme lipoxidase indicating its ability to inhibit synthesis of leukotrienes Table 2. Leukotrienes are important mediators that are released from mast cells and other variety of cells along with other mediators. They have direct toxic effects or recruit others cells which phagocytosize the damaged tissue and in the process lead to further release of inflammatory mediators resulting in perpetuation of this vicious cycle.

Preliminary work on the ethanolic extract of *T. purpurea* suggested that it inhibits the release of mediators of anaphylaxis from the mast cells^{6,7}. In conclusion, it appears that the ethanolic extract of *T. purpurea* is likely to have actions in preventing cellular chemotaxis into the sites of antigen challenge by virtue of its inhibitory action on release of histamine and chemotactic mediators and synthesis of leukotrienes by virtue of its lipoxygenase inhibitory activity.

REFERENCES

1. Kuby, J., In; *Immunology*, 3rd Edn., W.H. Freeman and Company, New York, 1997, 413.
2. Galli, S.J. and Lichenstein, L.M., In; Middleton, E. Jr., Reed, C.E., Ellis, E.F., Adkinson, N.F. and Yunginger, J.W., Eds., *Allergy: Principles and Practice*, 3rd Edn., C.V. Mosby, St. Louis, 1988, 106.
3. Lemanske, R.F. and Kalinear, M.A., In; Middleton, E. Jr., Reed, C.E., Ellis, E.F., Adkinson, N.F. and Yunginger, J.W., Eds., *Allergy: Principles and Practice*, 3rd Edn., C.V. Mosby, St. Louis, 1988, 224.
4. Kirtikar, K.R. and Basu, B.D., In; *Indian Medicinal Plants*, 2nd Edn., Vol 1, Lalit Mohan Basu, Allahabad, 1956, 723.
5. Karnick, C.R., *Nagarjun*, 1979, 22, 230.
6. Gokhale, A.B., Dikshit, V.J., Damre, A.S., Kulkarni, K.R. and Saraf, M.N., *Indian J. Exp. Biol.*, 2000, in press.
7. Gokhale, A.B. and Saraf, M.N., *Indian Drugs*, 2000, 37, 228.
8. Spicer, B.A., Hatt, P.A., Laycock, S.M. and Smith, H., *Int. Archs. Allergy Appl. Immunol.*, 1986, 81, 81.
9. Wilkonson, P.C., In; Vane, J.K. and Ferreria, S.H., Eds., *Handbook of Experimental Pharmacology*, Springer-Verlag, Berlin, 1978, 109.
10. Chatterjee, P. and Kashedikar, S.G., *Indian Drugs*, 1995, 32, 423.
11. Sigma Chemical Company, U.S.A. leaflet.
12. Greenwalt, T.J., Gajewski, M. and McKenna, J.L., *Transfusion*, 1962, 2, 221.
13. Djerassi, I., Kim, J.S. and Suvansri U., *Transfusion*, 1972, 12, 75.
14. Bailey, D.M. and Chakrin, L.W., *Ann. Rep. Med. Chem.*, 1981, 16, 213.