Table 1. Incidence of DMD/BMD in different caste groups of Uttar Pradesh

Caste	Population* per cent in UP	No. of affected families (patients)	Gene deletion observed in families	Gene deletion in-frame/out-frame
Brahmin	11	46 (60)	33	14/19
Kshatriya	09	19 (20)	10	2/8
Vaishya	05	21 (28)	13	3/10
BC/SC	50	51 (60)	26	5/21
Muslims	16	17 (18)	07	1/6
Others	09	02 (03)	00	0/0

*% Population of different castes was obtained from *India Today*, 15 May 1996.

Various factors have been attributed for population-based variations in the dystrophin gene mutations. Presence of repetitive elements like d*A*–d*T* stretches and transposon-like sequences have been implicated in the high frequency of deletional mutations at the central hot spot region of the dystrophin gene[16,17]. It is possible that due to phenomena like genetic drift, local DNA environment might be different in various caste groups. Such differences can probably account for variations in occurrence of pathogenic mutations at the DMD locus. It would be of interest to further explore specific haplotypes for Xp[21] locus (location of dystrophin gene) in different caste groups. These observations may have implications for genetic counselling programmes in our country.

1. Emery, A. E. H., *Neuromuscular Disord.*, 1991, **1**, 19–20.
2. Harper, P. S., *The Metabolic Basis of Inherited Disease* (eds Soriver, C. R., Beaudet, A., Sly, W. S. and Valle, D.), McGraw Hill, New York, 1989, pp. 2896–2902.
3. Roddie, A. and Bundey, S., *J. Med. Genet.*, 1992, **29**, 555–557.
4. Danieli, G. A., Mioni, F., Mueller, C. R., Vitiello, L., Mostacciuolo, M. L. and Grimm, T., *Hum. Genet.*, 1993, **91**, 342–346.
5. Singh, V., Sinha, S., Mishra, S., Chaturvedi, L. S., Pradhan, S., Mittal, R. D. and Mittal, B., *Hum. Genet.*, 1997, **99**, 206–208.
6. Sinha, S., Mishra, S., Singh, V., Mittal R. D. and Mittal, B., *Clin. Genet.*, 1996, **50**, 327–331.
7. Pradhan, S., *Pediatr. Nerol.*, 1994, **11**, 298–300.
8. Pradhan, S. and Mittal, B., *Clin. Neurol. Neurosurg.*, 1995, **97**, 134–138.
9. Sinha, S., Pradhan, S., Mittal, R. D. and Mittal, B., *Indian J. Med. Res.*, 1992, **96**, 297–301.
10. Mittal, B., Singh, V., Mishra, S., *et al.*, *Indian J. Med. Res.*, 1997, **105**, 32–38.
11. Das, S. K., Mukherejee, B. N., Malhotra, K. C. and Majumdar, P. P., *Ann. Hum. Biol.*, 1978, **5**, 25–31.
12. Mukherjee, B. N., Majumdar, P. P., Malhotra, K. C. and Das, S. K., *J. Hum. Evol.*, 1979, **8**, 567–570.
13. Kiliman, M. W., Pizzuti, A., Grompe, M. and Caskey, C. T., *Hum. Genet.*, 1992, **89**, 253–258.
14. Mishra, S., Ph D. Thesis, submitted to Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, 1996.
15. Manaco, A. P., Bertelson, C. J., Lieehti-Gallati, S., Moser, H. and Kunkel, L. M., *Genomics*, 1988, **2**, 90–95.
16. Krawezak, M. and Cooper, D. N., *Hum. Genet.*, 1991, **86**, 425–441.
17. Love, D. R., England, S. B., Speer, A., *et al.*, *Genomics*, 1991, **10**, 56–67.

Received 6 November 1996; revised accepted 14 February 1997.

Studies on antianaphylactic activity of fractions of *Albizzia lebbeck*

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Two fractions (F079 and F080) of the hot aqueous extract of stem bark of *Albizzia lebbeck* have been evaluated for antianaphylactic/antiallergic activity *in vitro* and *in vivo*. Both fractions inhibited antigen-induced contraction of the sensitized guinea pig ileum (Schultz–Dale phenomenon). The bronchoconstriction induced by the antigen egg albumin in presensitized guinea pig was also inhibited by these fractions in a dose-dependent manner. The fractions, however, did not possess any bronchodilatory effect *per se* in nonsensitized animals.

THE treatment of bronchial asthma is far from satisfactory. A potent drug against bronchial asthma is still wanting. However, there are claims in the traditional systems of medicine for the treatment of bronchial asthma. *Albizzia lebbeck* is a major constituent of the traditional medicines used against bronchial asthma[1]. The decoction of the bark of *A. lebbeck* was found to protect guinea pigs against antigen-induced challenge and there was a marked inhibition of Schultz–Dale phenomenon[2]. Chronic treatment with the bark decoction also protected the sensitized guinea pigs against antigen challenge[3]. It has been observed that the aqueous extract of the plant possesses antiallergic activity. In a previous study we have observed that the hot aqueous extract of the plant showed promising antiallergic activity in PCA (passive cutaneous anaphylaxis) and mast cell stabilizing activity[4]. The most active fraction from this extract was further chromatographed and fractions F079 and F080 showed maximum activity. The antiasthmatic/

antiallergic activities of these fractions cannot be wholly due to smooth muscle relaxant, antihistaminic or antispasmodic activity[2]. To validate the traditional use of the plant, the present study deals with the effect of these fractions on Schultz–Dale phenomenon, egg albumin-induced bronchoconstriction and bronchodilatory activity *per se* (Konzett and Rossler experiment).

The present study was conducted with the aqueous decoction of the stem bark of *A. lebbeck*. Coarsely powdered bark of the tree (1 kg) was heated with water (2 l) over a boiling water bath for 4 h. The aqueous extract was collected by decantation. The process was repeated four times. The combined extract was evaporated to dryness under vacuum at 70°C, leaving a residue (150 g). The dried extract was not fully soluble in water and did not show any clean spots on thin layer chromatogram. A portion of the dried extract (5 g) was macerated with water (4 × 10 ml) and the insoluble residue (1.5 g) was filtered off. The soluble portion was concentrated and charged on a column of XAD – 2 resin (20 g). Elution was carried out successively with water, 20% aq. methanol, 40% aq. methanol, 60% aq. methanol, 80% aq. methanol and methanol, 100 ml each. Eluates of water, 20% aq. methanol, and 40% aq. methanol were combined together to give a water-soluble fraction F079, whereas the eluates of 60% aq. methanol, 80% aq. methanol and methanol were combined together to give a methanol-soluble fraction F080.

Male guinea pigs (350–450 g) were sensitized with egg albumin (EA 10 mg/kg i.p.) on day 1 and 3. On the day 14, animals were sacrificed and ileal strips from each animal were mounted in isolated organ bath using Tyrode's solution at 37 ± 0.5°C. Tissue sensitivity was tested with histamine hydrochloride (0.05 µg/ml). Antigen was added to the 30 ml bath (EA 3 µg/ml) and responses recorded for 90 s. Various concentrations of *A. lebbeck* fractions (F079/F080) were added to see the inhibition or potentiation of antigen-induced concentration of ileal strips. IC_{50} (inhibitory concentration) were calculated.

Male guinea pigs (350–450 g) were sensitized with antigen on day 1, 3 and 5 (EA, 100 mg i.m.). After 4 weeks, the animals were transferred to the aerosol chamber and a fine mist of the antigen (EA 5%) w sprayed into the chamber under a constant pressu (280 mmHg) till the preconvulsive breathing starte The time was noted down. *A. lebbeck* (F079/F080) fra tions were administered orally (50 mg/kg) and disodi cromoglycate (50 mg/kg i.p.) 1 h prior to challenge a the time for showing preconvulsive breathing was r corded. Per cent protection from anoxic convulsion w

Table 1. Inhibition of antigen-induced contraction (Schultz–Dale phenomenon) by fraction of *A. lebbeck* ($n = 3$). (Dose of egg albumin = 3 µg/ml)

Fraction	Conc. (µg/ml)	Per cent inhibition of contraction (Mean ± SD)
F079	1.5	24.5 ± 5.19
	2.0	41.85 ± 8.17
	2.5	71.9 ± 7.67
F080	1.0	25.16 ± 5.10
	1.5	61.36 ± 3.27
	2.0	79.66 ± 1.52

Table 2. Per cent protection from egg albumin induced anoxi convulsion by *A. lebbeck* ($n = 3$)

Fraction	Dose (mg/kg p.o.)	Per cent protection from anoxic convulsion
F079	50	62.69 ± 1.99*
F080	50	46.86 ± 1.95*
DSCG	50 (i.p.)	68.33 ± 3.21*

*Significant activity ($p < 0.05$).

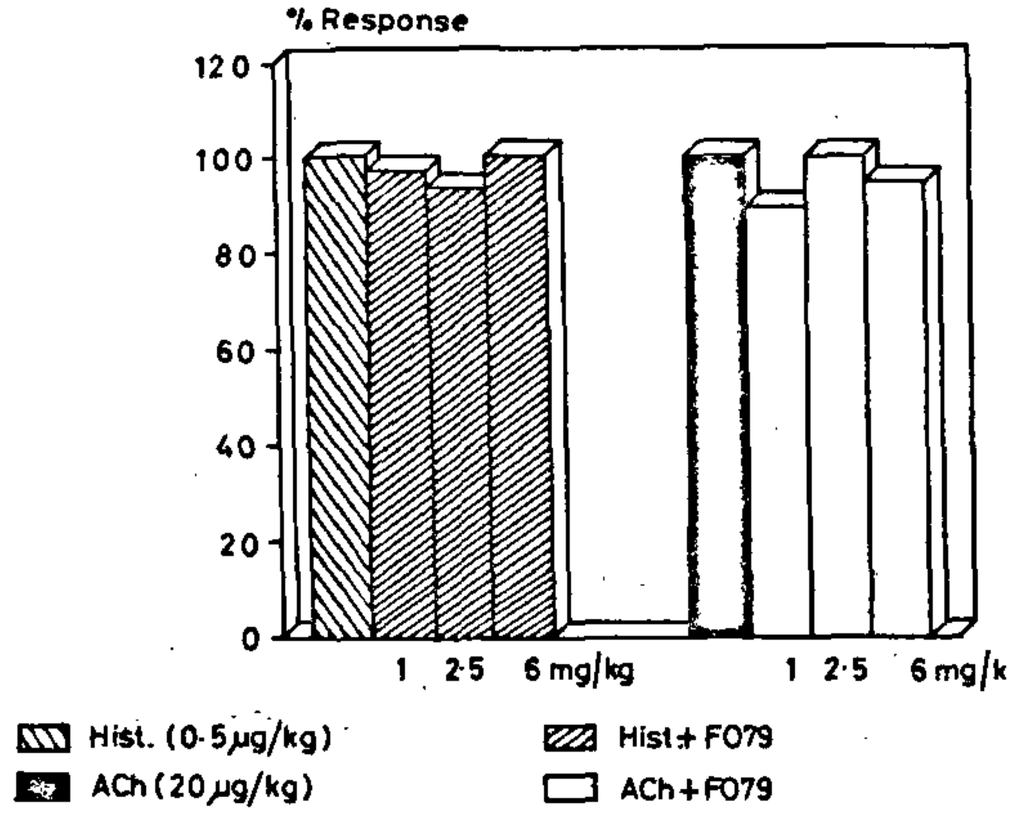

Figure 1. Effect of *A. lebbeck* fraction F079 on bronchoconstricti in guinea pig Konzett–Rossler preparation (Not significant); $n = 3$.

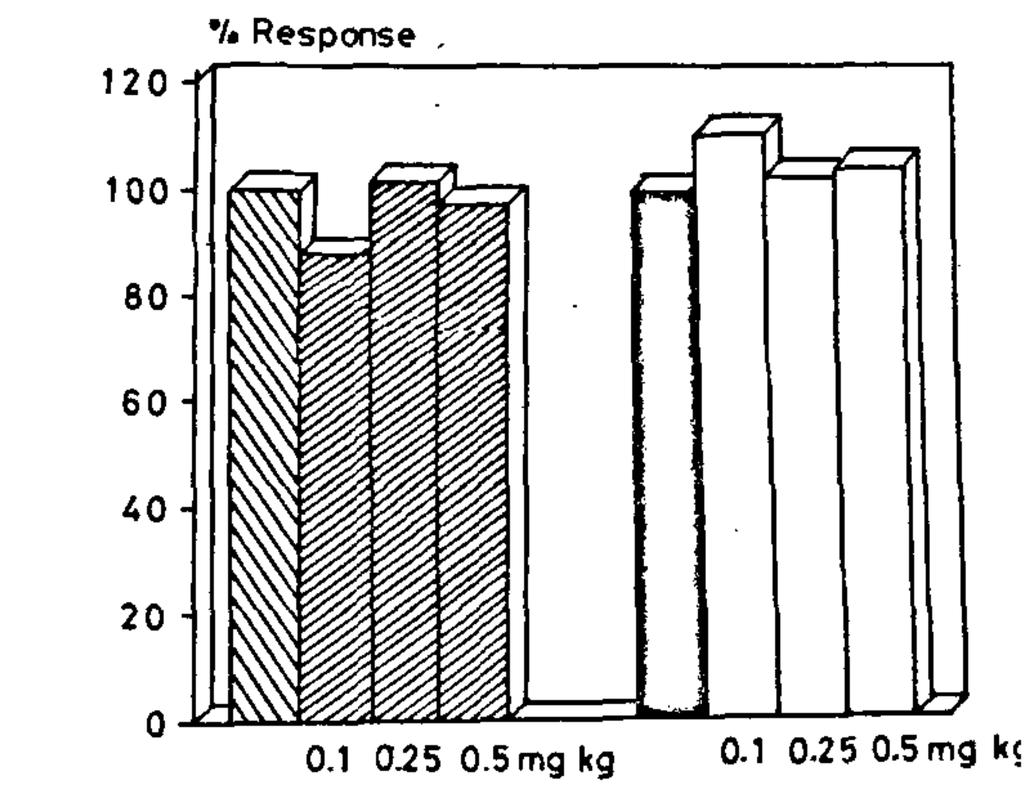

Figure 2. Effect of *A. lebbeck* fraction F080 on bronchoconstricti in guinea pig Konzett–Rossler preparation; $n = 3$.

calculated by the formula $(1 - T_1/T_2 \times 100)$ where T_1 is the mean of preconvulsion time 2 days before treatment and T_2 is the mean of preconvulsion time 2 days after the treatment[5].

In Konzett preparation, normal male guinea pigs (350–450 g) were used. They were anaesthetized with urethane (0.6 ml/100 g, 25% solution) and tied on the operating table. The trachea was cannulated and constant volume of air (6–10 cc) from the respirator was passed through the tracheal cannula[6]. The bronchoconstriction was induced by histamine (0.5 µg/kg) or acetylcholine (20 µg/kg). Thereafter, varying concentrations (0.1 to 5 mg/kg) of *A. lebbeck* (F079/F080) were injected intravenously to see the effect on spasmogen-induced bronchoconstriction.

Statistical analysis was done using Student's *t* test. The effect of *A. lebbeck* on Schultz–Dale phenomenon in guinea pig is summarized in Table 1. It was seen that *A. lebbeck* treatment significantly inhibited antigen-induced contraction in a dose-dependent manner while the response to histamine was maintained. IC_{50} (inhibitory concentration) was also calculated, which was 2.2 and 1.3 µg/ml with F079 and F080 respectively.

When *A. lebbeck*-treated presensitized guinea pigs were exposed to antigen in the aerosol chamber, there was a significant protection from anoxic convulsion. The results are summarized in Table 2. The fraction F079 showed 62 per cent protection which is comparable to the standard drug disodium cromoglycate, whereas with the fraction F080, it was not very significant.

In the Konzett preparation, *A. lebbeck* fractions (F079/F080) did not produce any inhibition or potentiation of histamine-induced bronchospasm. Fraction F079 (0.5, 2.5 and 5 mg/kg) and fraction F080 (0.1, 0.2 and 0.5 mg/kg) did not antagonize histamine and acetylcholine-induced bronchospasm (Figures 1 and 2).

The beneficial effect of *A. lebbeck* bark in bronchial asthma is not due to any significant bronchial smooth muscle activity[3]. In the studies from our laboratory on the mast cells of albino rats, it was found that it has cromoglycate-like action, inhibiting degranulation of sensitized mast cells when exposed to the antigen. In studies on the Schultz–Dale reaction in guinea pigs, there was dose-dependent inhibition of antigen-induced contraction of sensitized ileum treated with *A. lebbeck*. This effect could be due to stabilization of guinea pig intestinal mast cell membrane, inhibiting antigen-induced histamine release or due to inhibition of phenomenon of sensitization[7]. In the peritoneal mast cells of sensitized albino rats treated with *A. lebbeck*, there is inhibition of degranulation when exposed to antigen *in vitro*[2]. Thus *A. lebbeck* has the mast cell-stabilizing activity. This may be responsible for inhibition of the release of mediators in Schultz–Dale reaction in guinea pigs also.

Similarly, when the sensitized guinea pigs were exposed to the antigen spray in the aerosol chamber, there was either prevention of anaphylaxis or prolongation of the latent period of preconvulsive breathing. The protection from preconvulsion is comparable to the standard drug disodium cromoglycate. In previous studies when normal animals were exposed to the histamine aerosol[2], neither the fractions nor the standard drug could protect the animals from anoxic convulsion. These results indicate that *A. lebbeck* protects the sensitized guinea pigs from anoxic convulsions in a manner similar to disodium cromoglycate.

In the Konzett Rossler preparation, the plant fraction does not protect the normal guinea pigs from histamine and acetylcholine-induced bronchospasm even in high doses.

Thus, the present study shows that *A. lebbeck* fractions can protect the sensitized guinea pigs against antigen-induced bronchospasm, inhibit Schultz–Dale phenomenon of guinea pigs, but are devoid of any bronchodilatory activity. The fractions, therefore, contain the active constituents responsible for antiasthmatic activity of the plant. Further study is in progress to isolate and characterize the active principle(s) and to establish their mode of action.

1. Ray, P. and Gupta, H. N., *Caraka Samhita – A Scientific Synopsis*, Indian National Science Academy, New Delhi, 1980, p. 73.
2. Tripathi, R. M., Sen P. C. and Das, P. K., *J. Ethnopharmacol.*, 1979, **1**, 397–406.
3. Tripathi, R. M. and Das, P. K., *Indian J. Pharmacol.*, 1977, **9**, 189–194.
4. Baruah, C. C., Gupta, P. P., Nath, A., Patnaik, G. K., Kulshrestha, D. K. and Dhawan, B. N., Paper presented at the Ist Annual Conference of Indian Society of Chemist and Biologist, held at CDRI, Lucknow, March 23–24, 1996, Abst. No. PB 12.
5. Armitage, A. K., Boswood, J. and Large, B. J., *Br. J. Pharmacol.*, 1961, **16**, 59–76.
6. Konzett, H. and Rossler, H., *Naunyn. Sch. Arch. Exp. Pathol. Pharmacol.*, 1940, **195**, 71–74.
7. Tripathi, R. M., Biswas, M. and Das, P. K., *J. Res. Indian Med. Yoga and Homeo.*, 1977, **12**, 37–41.

ACKNOWLEDGEMENT. We thank Mr Amar Nath for technical assistance.

Received 23 October 1996; revised accepted 7 February 1997