

# EFFECT OF *NARDOSTACHYS JATAMANSI* AND *RHUS SUCCEDANEA* AGAINST HISTAMINE AND SEROTONIN RESPONSES ON LUNG PERFUSION AND TIDAL AIR CHANGES\*

By

S.S. GUPTA, C.B. SETH AND G.H. BALCHANDANI

Department of Pharmacology, Gandhi Medical College, Bhopal

(Received September 23, 1962)

Alcoholic extracts of *N. jatamansi* and *R. succedanea*, the two common Ayurvedic antiasthmatic drugs, were found to inhibit histamine and serotonin induced reduction in tidal air recorded as overflow of air insufflated through the lungs in spinal dogs. Inhibitory effects of the drugs against the bronchoconstrictors was also indicated from the reduction in perfusion pressure through isolated guineapig or rat lungs. Reduction in the outflow caused by perfusion of histamine and serotonin was also antagonised by the alkaloidal fraction of *N. jatamansi*.

In a previous communication, Gupta *et al.*, (1962) reported on the inhibitory effect of *Nardostachys jatamansi* and *Rhus succedanea* against the constrictor responses of histamine, acetylcholine and serotonin on the tracheal chain preparation. The drug responses on the smooth muscles of trachea and bronchi have been found to be identical (Florey and Wells, 1931; Isogawa, 1941) as histologically also, they possess a common type of cartilage and muscle (Castillo and de Beer, 1947). It is, therefore, likely that the beneficial effects of the above drugs in bronchial asthma (Charak Samhita, 1931; Chopra *et al.*, 1956; Nadkarni, 1954; Gupta *et al.*, 1961) may also be related to the direct antagonism of the constrictor responses of histamine and serotonin on the bronchial tissue known to be responsible for the asthmatic attack (Chen and Ensor, 1949; Humphrey and Jaques, 1955). In view of the above considerations it was thought worthwhile to investigate if the drugs *N. jatamansi* and *R. succedanea* could influence the reduction in tidal air and bronchoconstriction induced by histamine and serotonin.

## METHODS

Alcoholic extract of *N. jatamansi* was prepared by extracting the dried rhizome in ethanol for 5 hrs in a Soxhlet apparatus. The extract was dried and redissolved in adequate quantity of a solvent mixture consisting of ethyl alcohol, Tween 80 and distilled water (1 : 2 : 2, to obtain a stock solution of 200 mg/ml of the drug.

---

\*Paper read in part at the 6th Annual Conference of the Association of Physiologist and Pharmacologist of India, held at Hyderabad 1960.

Alkaloidal fraction of *N. jatamansi* was extracted from the powdered rhizome after macerating with chloroform and a dilute solution of ammonia as per method reported by Bose *et al.*, (1957). The alkaloid was eluted in successive portions by 1/N hydrochloric acid. It was then separated out by treating with ammonia solution and finally extracting with successive quantities of a mixture of 3 parts of chloroform and 1 part of alcohol. It was then treated with adequate amount of 0.1/N hydrochloric acid before crystallisation.

Alcoholic extract of *R. succedanea* was obtained by extracting the dried crushed horns of the drug in ethanol for five hours, The dried residue was dissolved in the above solvent mixture to prepare a 200 mg/ml solution.

Crystalline hydrocarbon fraction of *R. succedanea* was obtained by treating the alcoholic extract successively with light petroleum ether and then with absolute alcohol as per method described by Chopra *et al.*, (1956). This was further concentrated and left for crystallisation. The crystalline residue was dissolved in an adequate amount of alcohol for preparing a 200 mg/ml stock solution.

The effects of these drug preparations were investigated against histamine and serotonin on tidal air and lung perfusion experiments.

*Tidal air experiment.*—Mongrel dogs weighing between 4.5 kg were anaesthetised with ether and the spinal cord was divided between the first and second cervical vertebrae. Respiration was maintained by a Starling's respiratory pump: The maximum ventilation pressure was kept constant at 8-10 cm of water and changes in the distensibility of the lungs were recorded by the overflow technique of Konzett and Rossler (1940). The excess air which did not enter the trachea raised a piston carrying a lever so as to record a vertical line on the slowly moving kymograph. The lever rose during insufflation and fell when insufflation ended. A reduction in the volume of air entering the lungs due to bronchoconstriction, caused the lever to rise further. Doses of histamine acid phosphate and serotonin causing a definite and appreciable reduction in tidal air were recorded. These responses were again elicited after giving doses of indigenous drugs under study. The inhibitory effect of these drugs against histamine and serotonin responses on tidal air was then compared with that caused by doses of lysergic acid diethylamide (L. S. D. 25) and antazoline respectively taken as reference standards. All the drugs were injected into femoral vein.

Effect of drugs on lung perfusion was studied by the following two techniques :—



In the first set of experiments the effect of the alcoholic extract of *N. jatamansi* and *R. succedanea* was observed on the bronchiolar pressure induced by perfusion of histamine or serotonin as per method of Luduena Euler (1957). Only one lobe of the isolated lung of a guinea pig was perfused with low calcium Von Dyke's solution containing 0.5 per cent dextrose at room temperature. The rate of flow was so adjusted that the initial pressure was 15-20 cm of water, but was raised to about 45-50 cm pressure after perfusing  $0.4 \times 10^{-5}$  histamine acid phosphate. The drug extracts in 0.1 ml quantities were then introduced into the cannula through a side tubing. Pressure changes were noted after two successive doses (10-20 mg) of the drugs, the concentrations of which were so adjusted that the same quantity was administered each time.

In the second set of experiments isolated whole lung of the guinea pig or rat was perfused with Ringer Lock solution at 30° C. The perfusion pressure, from the two aspirator bottles kept at a higher level, was maintained between 10-11 cm of water. Outflow of saline from the pin punctures made at the base of each lobe of lung was measured for one min  $V^{-5}$  at intervals of every five min for half an hr. Solution of histamine acid phosphate  $0.4 \times 10^{-5}$  in Ringers solution was then filled in the other bottle and the lungs were perfused with the solution for half an hr. Maximum reduction in the outflow was noted and the constrictor effect of histamine was washed off by perfusing Ringers solution. When the outflow had returned to the original level the alkaloidal fraction of *N. jatamansi* or the tannin free crystalline fraction of *R. succedanea* each in  $0.5 \times 10^{-3}$  concentration together with histamine ( $0.4 \times 10^{-5}$ ) were perfused in turn. The difference in the percentage reduction in the out-flow after perfusion of the constrictor agent alone and that observed in a combination of the drugs under investigation, was calculated and data statistically analysed to determine the antagonist effect of the drugs against histamine, if any. Similarly the effect of both the drug fractions was determined against the bronchoconstrictor response of  $0.25 \times 10^{-3}$  serotonin creatinine sulphate solution perfused through the isolated rat lung preparation.

#### RESULTS

Effect of the alcoholic extract of *N. jatamansi* and *R. succedanea* on tidal air changes induced by the bronchoconstrictor agents, histamine and serotonin, is shown in Fig. 1. Their inhibitory effects have also been compared with those of antistine and L. S. D. 25, the known antagonists of histamine and serotonin respectively.

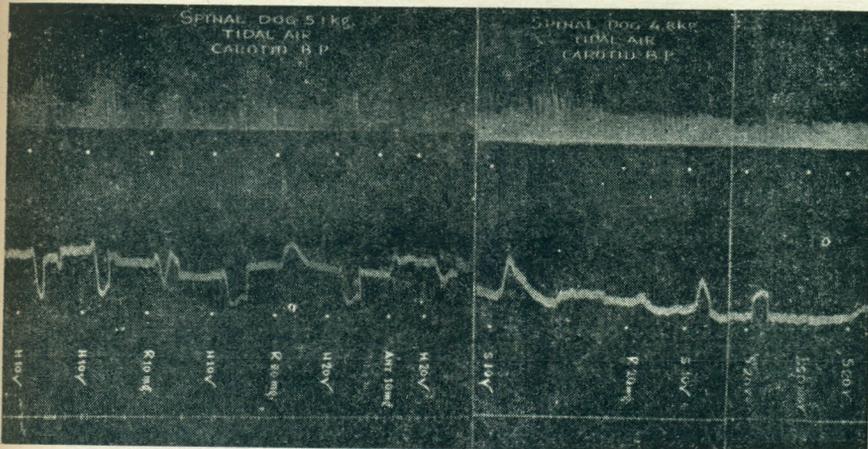
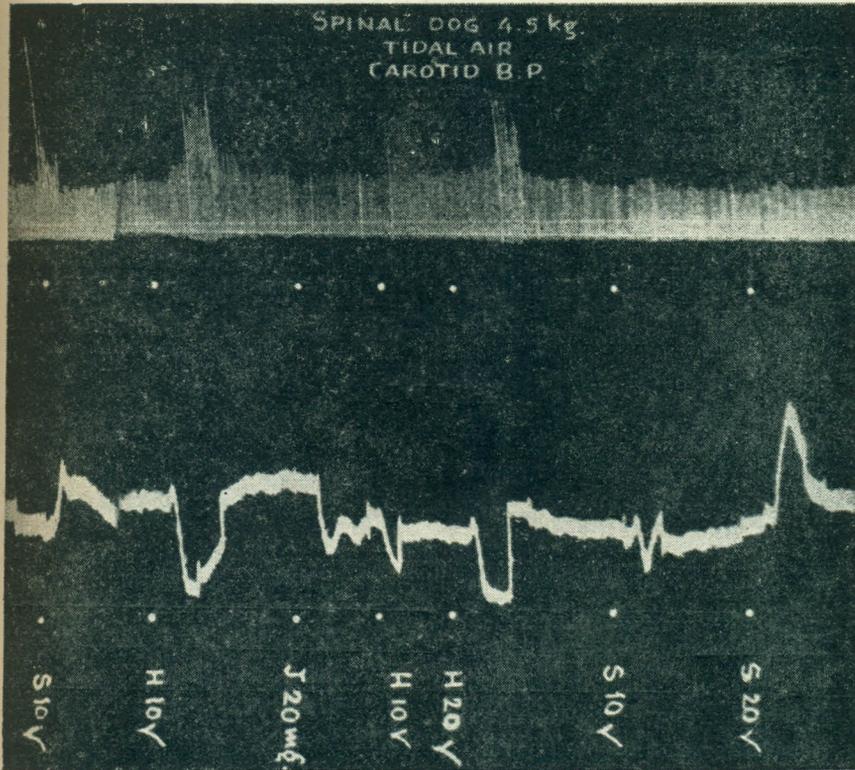


Fig. 1. Tidal air changes measured as overflow of air insufflated through lungs in a spinal dog ventilated from an artificial respiratory pump. After the constrictor agents histamine (H) and serotonin (S) injected before and after the alcoholic extracts of (a) *N. jatamansi* (J.) (b) *R. Succedanea* (R).

In a few preliminary experiments, the alcoholic extracts of *N. jatamansi* and *R. succedanea*, on perfusion through the isolated lungs of guinea pigs, were found to increase the outflow and reduce the perfusion pressure. The solvent alone, however, did not produce any significant change. Both the drug extracts were also found to inhibit the constrictor response of histamine and serotonin, as indicated by the reduction in perfusion pressure through the lungs (Table I). Effect of the alkaloidal fraction of *N. jatamansi* and that of the crystalline hydrocarbon fraction of *R. succedanea* on the outflow through the lungs perfused with the constrictor agents is summarised in Tables II and III.

TABLE I

*The Effect of alcoholic extracts of N. jatamansi and R. succedanea on the pressure of lung perfused with the constrictor agents.*

*% reduction in the pressure of lungs perfused with constrictor agents after anti-asthmatic drugs.*

| Histamine $0.4 \times 10^{-3}$ |           |               |            | Serotonin $0.25 \times 10^{-3}$ |           |               |            |            |
|--------------------------------|-----------|---------------|------------|---------------------------------|-----------|---------------|------------|------------|
| N. jatamansi                   |           | R. succedanea |            | N. jatamansi                    |           | R. succedanea |            |            |
| 10mg                           | 20mg      | 10mg          | 20mg       | 10mg                            | 20mg      | 10mg          | 20mg       |            |
| 18.18                          | 34.36     | 5.26          | 11.84      | 26.34                           | 40.0      | 10.0          | 58.33      |            |
| 23.34                          | 38.88     | 17.67         | 33.34      | 15.13                           | 53.33     | 19.12         | 27.63      |            |
| 11.76                          | 46.62     | 9.55          | 45.50      | 22.34                           | 56.42     | 32.63         | 43.12      |            |
| 17.40                          | 63.04     | 33.33         | 27.35      | 37.50                           | 58.62     | —             | —          |            |
| Mean                           | 17.67     | 45.73         | 16.60      | 29.51                           | 25.33     | 52.09         | 20.58      | 43.03      |
| S.D.                           | $\pm 4.7$ | $\pm 12.6$    | $\pm 12.4$ | $\pm 14.0$                      | $\pm 6.3$ | $\pm 8.3$     | $\pm 11.4$ | $\pm 17.0$ |

TABLE II

*The effect of the alkaloidal fraction of N. jatamansi and the crystalline fraction of R. succedanea against the broncho-constrictor response of histamine perfused through the isolated lung of guinea pigs.*

*% reduction in the outflow from lungs perfused with histamine ( $0.4 \times 10^{-5}$ ) together with the anti-asthmatic drugs.*

| No. of observations. | Control    | Jatamansi alkaloid ( $0.5 \times 10^{-3}$ ) | Change from control | Control    | R. succ. cryst. fr ( $0.5 \times 10^{-3}$ ) | Change from control |
|----------------------|------------|---|---------------------|------------|---|---------------------|
| 1                    | 43.8       | 25.0  | -18.8               | 29.3       | 28.0  | -1.3                |
| 2                    | 42.0       | 25.2  | -16.8               | 67.5       | 50.3  | -17.2               |
| 3                    | 39.1       | 30.2  | -8.9                | 22.4       | 18.2  | -4.2                |
| 4                    | 77.1       | 40.5  | -36.6               | 36.6       | 28.6  | -8.0                |
| 5                    | 43.2       | 25.0  | -18.2               | 59.5       | 46.4  | -13.1               |
| 6                    | 20.0       | 10.0  | -10.0               | 39.3       | 38.1  | -1.2                |
| Mean                 | 44.2       | 25.98                                       | -18.21              | 42.43      | 34.93                                       | -7.50               |
| S.D.                 | $\pm 11.9$ | $\pm 6.8$                                   | $\pm 9.9$           | $\pm 16.6$ | $\pm 12.2$                                  | $\pm 6.5$           |

TABLE III

Effect of the alkaloidal fraction of *N. jatamansi* and crystalline hydrocarbon fraction of *R. succedanea* on the bronchoconstrictor effect of serotonin perfused through the isolated rat lung.

% reduction in the outflow from lungs, perfused with serotonin ( $0.25 \times 10^{-3}$ ) together with the anti-asthmatic drugs.

| No. of observation | Control    | <i>N. jatamansi</i> alkaloid ( $0.5 \times 10^{-3}$ ) | Change from control | Control    | <i>R. succedanea</i> cryst. fraction ( $0.5 \times 10^{-3}$ ) | Change from control |
|--------------------|------------|---|---------------------|------------|---|---------------------|
| 1                  | 39.1       | 27.1  | -12.0               | 42.3       | 41.2  | -1.1                |
| 2                  | 70.0       | 24.7  | -45.3               | 32.5       | 27.1  | -5.4                |
| 3                  | 43.2       | 29.4  | -13.8               | 66.6       | 37.5  | -29.1               |
| 4                  | 38.9       | 8.4   | -30.9               | 54.3       | 24.5  | -19.8               |
| Mean               | 47.8       | 22.3  | -25.5               | 48.9       | 32.57   | -13.85              |
| S.D.               | $\pm 15.0$ | $\pm 9.7$   | $\pm 16.4$          | $\pm 14.7$ | $\pm 8.0$   | $\pm 12.9$          |

## DISCUSSION

The Ayurvedic drugs *N. jatamansi* and *R. succedanea* which are used in the treatment of bronchial asthma, were found to influence changes in the tidal air and the bronchial passages caused by histamine and serotonin. As shown in Fig. 1, the broncho-constrictor responses to successive doses of histamine (2-4/kg) was partly inhibited after 50-100 mg/kg doses of the alcoholic extracts of *N. jatamansi* and *R. succedanea*, while 0.15 mg/kg dose of antazoline completely inhibited the histamine response on tidal air. Inhibitory effects of the drug extracts were more marked on the serotonin responses though they were much weaker as compared to L.S.D. 25.

In another set of experiments (Table I) where broncho-constriction was measured in terms of increased perfusion pressure, the two doses of 10-20 mg of the alcoholic extract of *N. jatamansi* caused marked and proportionate reduction in the lung pressure induced by perfusion of histamine ( $0.4 \times 10^{-5}$ ) or serotonin ( $0.25 \times 10^{-3}$ ). Similar doses of the alcoholic extract of *R. succedanea* were less effective than *N. jatamansi* in causing pressure change during perfusion of the constrictor agents. A direct broncho-dilator effect of the alcoholic extracts was also indicated on perfusing the drugs alone through the isolated lungs.

Further, the bronchoconstrictor effect of histamine ( $0.4 \times 10^{-5}$ ) or serotonin ( $0.25 \times 10^{-3}$ ) as measured from the percentage reduction in perfusion outflow through pin punctures in lungs, was significantly inhibited when the alkaloidal fraction of *N. jatamansi* in  $0.5 \times 10^{-3}$  concentration was perfused together with the constrictor agents. The crystalline hydrocarbon fraction of *R. succedanea* was, however, found to be ineffective in antagonising the constrictor responses of both histamine and serotonin.

From the above data it would be realised that the total alcoholic extract of the drugs under study, besides possessing a direct bronchodilator effect, also inhibit the bronchoconstrictor responses of histamine and serotonin as observed in tidal air and lung perfusion experiments. This also seems to be substantiated by their inhibitory effects against the constrictor response of histamine on tracheal chain preparation (loc cit) as well as that reported against experimental bronchial asthma in guineapigs (Gupta *et al.*, 1961).

The anti-histaminic and anti-serotonin effects of the total extract of *N. jatamansi* can be attributed to its alkaloidal fraction in view of the fact that this fraction was found to directly antagonise the bronchoconstrictor responses of histamine and serotonin on the lung tissue. The broncho-dilator effect of *R. succedanea* may, however, be due to its volatile oil content or some other fraction (Chopra *et al.*, 1956) besides the crystalline hydrocarbon fraction since the latter did not manifest any significant effect in lung perfusion experiments.

The present investigation therefore seems to throw some light on the mechanism of action of the Ayurvedic remedies in bronchial asthma. These might as well initiate further investigations on their therapeutic possibilities.

The authors are grateful to Dr. R. P. Singh, M.S. Dean, Gandhi Medical College, Bhopal, for providing facilities for the above work. They wish to thank M/S Sandoz Product Ltd., Basle, for supplying 5-hydroxytryptamine and L.S.D. 25 for the present investigations.

#### REFERENCES

- Bose, B.C., Vijay-Vargiya, R. and Bhatnagar, J.W. (1957). *Ind. J. Med. Sci.*, **11**, 799.
- Castillo, J.C. and de Beer, E. J. (1947). *J. Pharmacol. and Exper. Therap.*, **90**, 104.
- Charak Samhita (1931). 1428 Laxmi Vanketeshwar Steam Press, Bombay.
- Chen, G. and Ensor, C.R. (1949). *J. Lab. and Clin. Med.*, **34**, 1010.
- Chopra, R.N., Chopra, I.C., Handa, K.I. and Kapoor, L.D., Chopra's *Indigenous Drugs of India*, (1956). 2nd Ed. 377 and 575. U.N. Dhar & Sons. Private Ltd., Calcutta.
- Florey, H. and Wells, A.Q. (1931). *J. Pharmacol. and Exp. Therap.*, **42**, 133.
- Gupta, S.S., Seth, C.B. and Mathur, V.S. (1961). *J. Ind. Med. Ass.*, **37**, 223.
- Gupta, S.S., Seth, C.B. and Mathur, V.S. (1962). *Ind. J. Physiol. and Pharmacol.*, **6**, 27.
- Humphrey, J.H. and Jaques, R. (1955). *J. Physiol.*, **128**, 9.
- Konzett, H. and Rossler, R. (1940). *Arch. Exp. Path. Pharmacol.*, **195**, 71.
- Isogawa, K. (1941). *Folio Endocrinol. Japan*, **17**, 13.
- Luduena Euler (1957). *Arch. Int. Pharmacodyn.*, **111**, 392.
- Nadkarni, A.K. (1954). *Indian Materia Medica*, Vol. 1, Bombay, 1062, Popular Book Depot, Bombay.