EFFECT OF *NARDOSTACHYS JATAMANSI* AND *RHUS SUCCEDANEA* AGAINST CONSTRICTOR RESPONSES OF HISTAMINE, ACETYLCHOLINE AND SEROTONIN ON SMOOTH MUSCLES

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The aloholic extract of *N. jatamansi* and *R. succedanea* were found to inhibit the constrictor response of histamine, serotonin and acetylcholine on the isolated smooth muscles of trachea, colon, uterus and intestine. Their inhibitory effect was found to be qualitatively similar though much weaker to antazoline, lysergic acid di-ethylamide or atropine, the known synthetic antagonist of the constrictor agents. The drug *N. jatamansi* was also found to possess direct papavarine like spasmolytic effect against barium chloride induced intestinal spasm. These effects seem to support the use of the indigenous remedies in asthma, cough, diarrhoea and colicky pains as prevalent in *Ayurvedic* medicine.

Nardostachys jatamansi and *Rhus succedanea* have been used in indigenous medicine for quite a long time in the treatment of asthma, cough and colicky pains(Charak Samhita 1931;Chopra *et al.*,1956)but as yet scientific data seems to be inadequate to rationalise their use in these conditions.Neuro-circulatory, anti fibrillatory and smooth muscle relaxant effects of *N. jatamansi* were reported by Vakil and Dalal (1955), Arora and Madan (1956), Bose *et al.* (1957) and more recently Gupta *et al.* (1961) reported on the protective effects of *N. jatamansi* against histamine aerosol induced asthma in guinea-pigs. Benificial effects of *Rhus succedanea* in relieving cough, vomiting and diarrhoea have also been mentioned by Chopra *et al.* (1956). It is, however, not clear whether these effects are mediated through the inhibition of histamine, serotonin and acetylcholine which have been reported to be associated with asthma, colicky pains and rapid evacuation of the bowels (Chen and Ensor 1949; Humphery and Jaques 1955; Bülbring and Lin, 1957; Erspamer, 1952; Brittain and Collier, 1958). In view of these observations it was thought worthwhile to assay the inhibitory effect of these drugs, if any, against histamine, serotonin and acetylcholine responses on the smooth muscles.

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METHODS

Experiments were conducted on the isolated smooth muscles of guinea-pig's tracheal rings, intestine, uterus and rat's colon for assaying the effect of the drug against histamine, serotonin and acetylcholine responses. The drug extracts used were prepared by soxhlet extraction of the powdered rhizomes of *N. jatamansi* and horn of *R. succedanea* in ethanol for 5 hours. The dried residue was dissolved in a mixture of ethyl alcohol and propylene glycol (1:2) to prepare 100 mg/ml stock solution. Further dilutions were made in normal saline for investigating the effect of drugs directly on the isolated tissues. The blank solvent in equivalent concentrations as contained in the doses of the drug extracts was also assayed side by side against the constrictor responses of the local hormones as detailed in the following set of experiments.

In the first set of experiments, effect of the drug extracts was investigated against the broncho-constrictor responses of histamine acid phosphate and acetylcholine bromide on the tracheal chain preparation suspended in 20 ml oxygenated Tyrode bath at 37°C as per method of Castello and de Beer (1947) modified by Akcasu (1952). Effect of the two doses of the alcoholic extracts of *N. jatamansi* and *R. succedanea* added to the bath 30 sec before the standard constrictor doses (0.1 mg) of histamine acid phosphate and acetylcholine bromide was recorded for $1\frac{1}{2}$ min on a slowly moving kymograph. The inhibitory responses of two doses of the drug extracts were compared with that caused by antazoline methane-sulphonate and atropine sulphate taken as reference standards for their anti-histaminic and anti acetylcholine potencies respectively.

In the second set of experiments, effect of these drugs was investigated against serotonin responses on oestrous uterus following the technique of Erspamer (1952) as modified by Amin, Crawford, and Gaddum (1954). Virgin rats weighing 160-200 g were injected with stilboesterol (0.1 mg/kg in arachis oil) subcutaneously 24 hrs before being sacrificed. Middle two centimeters of the uterine horn was dissected out and suspended in an aerated 20 ml bath containing atropinised (10^{-6}) de Jalon's solution at 30°C as per method of Gaddum, Pert and Vogt (1949). The lever magnification was x10 and tension 1 g weight. Constrictor responses of the standard doses (1 μg) of serotonin were assayed against two doses of *N. jatamansi, R. succedanea* and lysergic acid diethylamide (LSD 25) added to the bath 30 secs before the constrictor agents. Similarly their inhibitory effects were also assayed against serotonin responses on rat's colon, suspended in a 20 ml aerated and atropinised Tyrode bath at 22°-24°C (Bhattacharya and Lewis, 1956).

In the third set of experiments inhibitory effect of these drugs was observed against the constrictor responses of acetylcholine on guineapig's ileum as per technique of Gaddum, (1936). Inhibitory effect of the two doses of *N. jatamansi* and *R. succedanea* added to the Tyrode bath 30 secs before the standard doses (1 μg) of acetylcholine was recorded for half minute and was compared to that caused by two doses of atropine sulphate similarly added to the bath before acetylcholine bromide.

The drugs were also assayed on the isolated strips of rat's intestine suspended in aerated Tyrode bath at 37°-38° C for their anti-spasmodic effect against the spasmogenic response of barium chloride. The effect was compared with that of atropine sulphate and papavarine hydrochloride for the spasmolytic activity.

Percentage inhibition of the contractile responses of standard doses of histamine, acetycholine, and serotonin caused by two doses of the drug extracts or the reference standards was worked out in each case. Log-dose percentage inhibition curve was then plotted on a centimeter graph for calculating the dose of the drugs causing 50 per cent inhibition (ID_{50}) of the constrictor responses.

RESULTS

Inhibitory effect of the alcoholic extract of *N. jatamansi* and *R succedanea* against the constrictor responses of histamine acid phosphate and acetylcholine bromide on tracheal chain preparation is shown in Fig. I. The doses

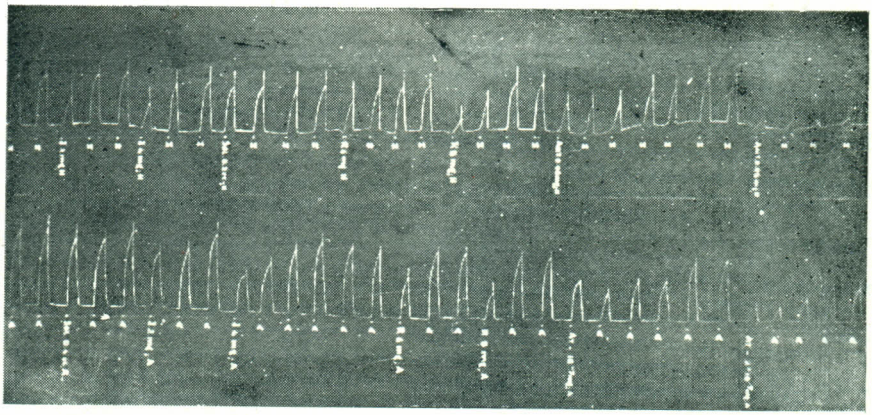

Fig. 1. Guineapig tracheal chain preparation : Constrictor responses (upper) standard doses (0.1 mg) of histamine (H) and (lower) acetylcholine (A) against inhibitoy doses of *N. jatamansi* (J), *R. succedanea* (R), antazoline (ANT) and atropine (AT) in 20 ml Tyrode bath.

of the drugs extracts causing 50 per cent inhibition have been compared with the inhibitory doses of antazoline methane sulphonate and atropine sulphate in Table I.

TABLE I

Inhibitory effect of N. jatamansi and R. succedanea against the constrictor responses of histamine and acetylcholine on isolated tracheal chain of guineapig

Drugs	Inhibition of constrictor response to					
	Histamine 0.1 mg/20 ml			Acetylcholine 0.1 mg/20 ml		
	Inhibitor dose mg/20 ml	% Reduction	I D_{50} dose mg/20 ml	Inhibitor dose mg/20 ml	% Reduction	I D_{50} dose mg/20 ml
N. jatamansi	2.00	45.0	2.88	2.00	30.0	3.64
	4.00	55.0		4.00	53.3	
R. succedanea	6.00	33.3	6.92	6.00	38.5	6.92
	8.00	72.2		8.00	61.5	
Antazoline methane sulphonate	0.06	33.3	0.07	—	—	—
	0.08	72.2				
Atropine sulphate	—	—	—	0.002	34.6	—
				0.004	73.9	0.0026

The antiserotonin effects of the alcoholic extract of *N. jatamansi* and *R. succedanea* on the smooth muscles of uterus and colon is shown in Fig. 2 and compared with the known serotonin antagonist lysergic acid diethylamide in Table II.

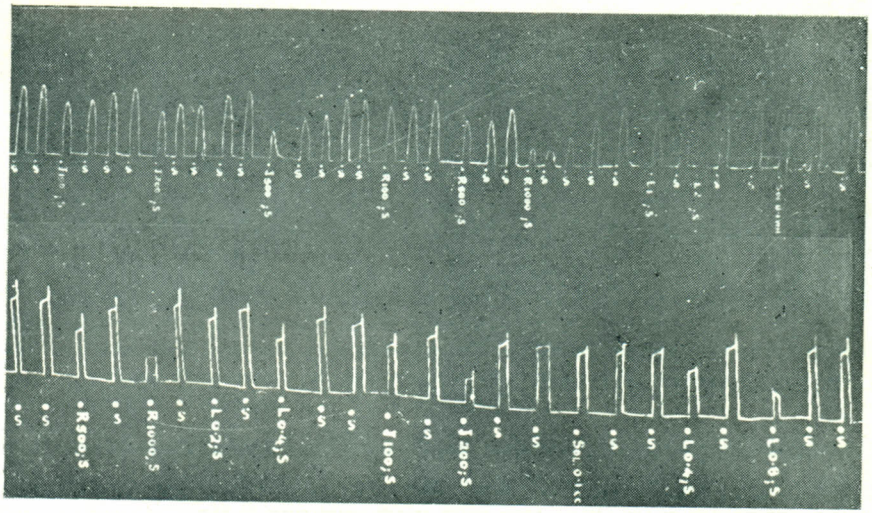

Fig. 2. Isolated rat uterus (upper) and rat colon (lower) : Constrictor responses of standard doses (1/μg) of serotonin (S) against the inhibitory doses (in/μg) of *N. Jatamansi* (J), *R. succedanea* (R) and (LSD) 25 (L) in 20 ml bath.

TABLE II

Inhibitory effect of N. jatamansi and R. succedanea against the constrictor serotonin responses on rat uterus and colon

Drugs	Inhibition of the serotonin (0.01 mg) constrictor responses on					
	Rat uterus			Rat colon		
	Inhibitors dose mg/20 ml	% Reduction.	I D_{50} dose mg/20 ml	Inhibitor dose mg/20 ml.	% Reduction	I D_{50} dose mg/20ml
N. jatamansi	0.200	36.3	0.300	0.100	28.0	0.178
	0.500	63 6		0.200	55.5	
R. succedanea	0.500	35.0	0.670	0.500	37.0	0.672
	1.000	70.0		1.000	68.4	
LSD 25	0.001	26.3	0.0013	0.0004	33.3	0.0006
	0.002	68.4		0.0008	61.1	

Effect of the alcoholic extracts of *N. jatamansi* and *R. succedanea* against acetylcholine responses on guineapig ileum has been compared with atropine sulphate in Fig. 3. The percentage inhibition and their ID_{50} doses are shown in Table III.

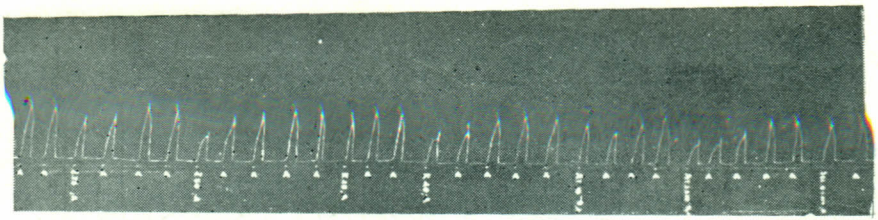

Fig. 3. Isolated guinea pig ileum : Constriction responses of the standard doses (1/μg) of acetycholine (A) against inhibitory dose (μg) of *N. jatamansi* (J) *R. succedanea* (R) and atropine (AT) in 20 ml bath.

TABLE III

Inhibitory effect of N. jatamansi and R. succedanea against the constrictor responses of acetylcholine on isolated guineapig ileum

Drugs	Inhibition of acetylcholine (1 μg) constrictor response		
	Inhibitor dose μg/20 ml	% Reduction	I D_{50} dose μg/20 ml
N. jatamansi	25	35.7	38.0
	50	60.7	
R. succedanea	20	23.5	30.0
	40	61.5	
Atropine sulphate	0.0002	23.0	0.0003
	0.0004	61.5	

The anti-spasmodic effect of the alcoholic extract of *N. jatamansi* against barium chloride induced contraction of rat ileum is shown in Fig. 4. The constrictor tone of the isolated strip of rat ileum induced by barium chloride (10^{-4} and 2×10^{-4}) was completely relaxed by the equivalent concentrations of the alcoholic extract of *N. jatamansi*. Response to subsequent dose of barium chloride was also reduced after the drug. This anti-spasmodic effect of the drug was found to be similar to that caused by 0.2×10^{-4} and 0.4×10^{-4} concentrations of atropine sulphate. The anti-spasmodic potency of the drug compared well with 10^{-5} concentration of papavarine hydrochloride, *R. succedanea*, however, was not found to relax the constrictor tone of the intestine in $0.2\text{x}10^{-4}$ to $0.2\text{x}10^{-2}$ concentrations tried against barium chloride (10^{-4}).

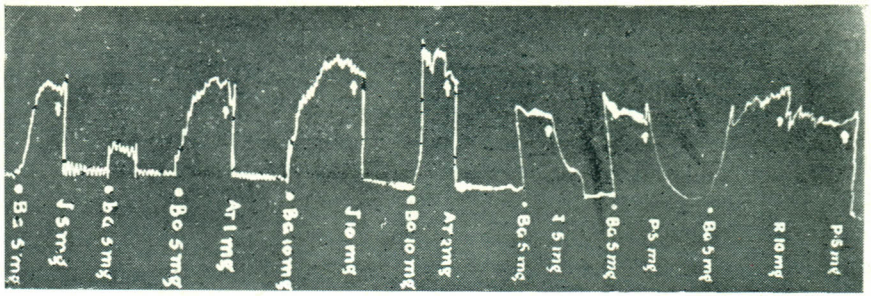

Fig. 4. Isolated rat intestine, antispasmodic effect of *N. jatamansi* (J), atropine (A) papavarine (P) and *R. succedanea* (R) against spasmodic constrictor response of barium chloride (Ba) in 50 ml bath.

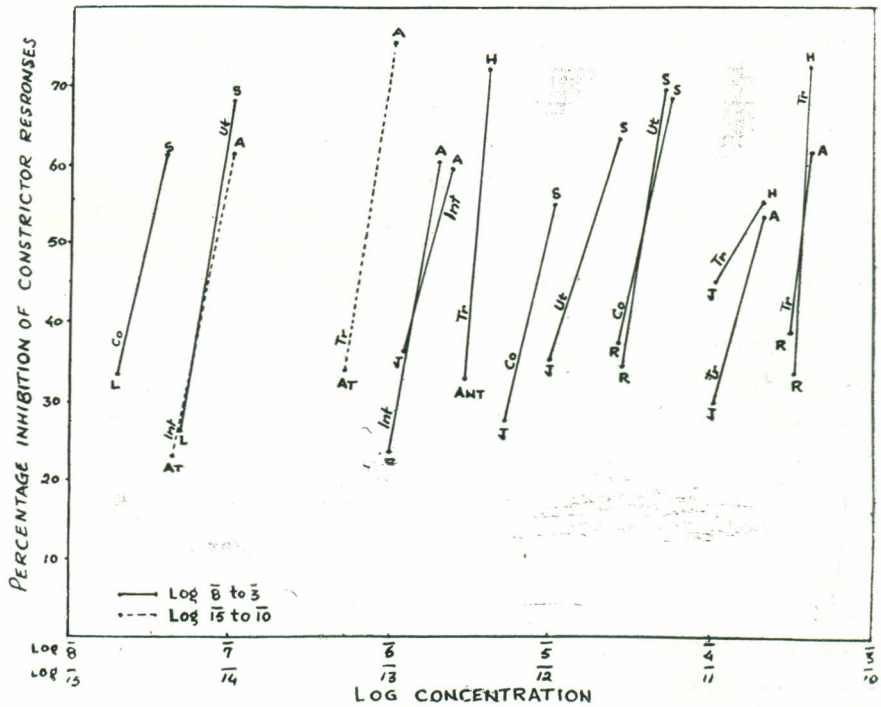

Fig. 5. Log dose inhibition curve for *N. jatamansi* (J), *R. succedanea* (R), antazoline (ANT), atropine (AT), LSD 25 (L) against constrictor responses of histamine (H), acetylcholine (A) serotonin (S), on trachea (Tr), uterus (Ut), colon (Co) and intestine (Int).

Percentage inhibition of the constrictor responses of histamine, serotonin and acetylcholine on the different tissues caused by the two doses of the

drug extracts has been compared with that caused by antazoline methane sulphonate, atropine sulphate and lysergic acid diethylamide in Fig. 5.

DISCUSSION

Inspection of the data presented in Figs. 1 to 3 would reveal that the drug extracts under investigation caused appreciable inhibition of the tissue responses of the standard doses of the constrictor agents. Their inhibitory effect qualitatively was found to be of similar nature as caused by the known synthetic antagonists antazoline, lysergic acid diethylamide and atropine. Further as the blank solvent in quantity as present in the doses of the drug extract did not cause any appreciable inhibition of the tissue responses, direct comparison of the inhibitory doses of the drug extract could thus be possible.

Analysis of the data presented in the Table I would show that the two doses *N. jatamansi* and *R. succedanea* caused a proportionate inhibition of the constrictor response of histamine and acetylcholine on the guineapigs tracheal chain preparation. Comparative doses of the drugs producing 50 per cent inhibition of the constrictor responses of histamine (0.5×10^{-5}) on the smooth muscle of trachea was found to be 2.8 mg, 6.8 mg and 0.07 mg for *N. jatamansi*, *R. succedanea* and antazoline respectively. The anti-histaminic effect of *N. jatamansi* and *R. succedanea* thus would work out to be 0.02 and 0.01 of antazoline respectively. The drug, *N. jatamansi* was also found to be more effective in inhibiting acetylcholine responses on tracheal chain as compared to *R. succedanea*. On comparing the ID_{50} doses, *N. jatamansi* would be found to be nearly twice as active as *R. succedanea* though both these drugs would work out to be much weaker than atropine sulphate as regards the anti-acetylcholine activity.

On comparing the anti-serotonin effects of *N. jatamansi* with *R. succedanea* on rat uterus as shown in Table II, the former would appear to be nearly twice more potent than the latter. This inhibitory effect of the drugs is in the same order as their anti-histaminic potency observed on guineapig trachea. The anti-serotonin effect of *N. jatamansi* on rat colon was much more marked than that observed on uterus, though the effcct of *R. succedanea* was not affected. Both these drugs were however, found to be two to four hundred times weaker than LSD 25 as serotonin antagonist.

Inhibitory effect of both *N. jatamansi* and *R. succedanea* against constrictor responses of acetylcholine on rat ileum was much more marked than on other tissues (Fig. 3). Comparative doses producing 50 per cent inhibition of the constrictor response of acetylcholine (1 μg) were found to be 38.0 μg,

30 μg and 0.32 μg for *N. jatamansi*. *R. succedanea* and atropine sulphate respectively as shown in Table III. The drugs were found to cause prompt inhibition of acetylcholine responses and their effect, like atropine persisted for considerable period. Their anti-acetylcholine activity in comparison to atropine was however, much weaker.

It was interesting to note the marked anti-spasmodic effect of the alcoholic extract of *N. jatamansi* against barium chloride induced tonic spasm of rat ileum. Comparative doses of *N. jatamansi*, atropine sulphate and papavarine hydrochloride causing complete relaxation of barium chloride (10^{-4}) induced constrictor tone in 50 ml Tyrode bath, were found to be 5 mg, 1 mg and 0.5 mg respectively. The anti-spasmodic effect of *N. jatamansi* would therefore work out to be about 1/10 that of papavarine, and nearly 1/5 of atropine on intestine. *R. succedanea*, however, was found to be ineffective in causing relaxation of the barium chloride induced tonic spasmodic contraction of intestine, though it markedly inhibited acetylcholine constrictor response on this tissue.

From the above observation it would be apparent that both *N. jatamansi and R. succedanea*, possess mild degree of anti-histaminic, anti-serotonin and anti-acetylcholine effects as compared to the known synthetic antagonists of the local hormones. However, in view of much larger doses of the crude drugs used in therapy, the beneficial effects of *N. jatamansi* and *R. succedanea* in bronchial asthma could partly be attributed to their inhibition of histamine response on tracheal chain preparation. This seems to be further substantiated by our previous observation on the protective effect of the aerosol of *N. jatamansi* against histamine aerosol induced experimental asthma in guineapigs (Gupta *et al.*, 1961.) These drugs have also been observed to prevent histamine and serotonin induced broncho-constriction in lung perfusion and tidal air experiment reported elsewhere (Gupta *et al.*, unpublished). The anti-serotonin and central sedative psychotropic effects of the drugs (Arora, 1960 ; Gupta *et al.*, 1961) might also be helpful in asthma in view of the fact that psychotropic agents have been reported to influence bronchial asthma by decreasing plasma serotonin and histamine concentration (Waser and Itzbicki, 1959).

Similarly, the beneficial effects of *R. succedanea* in vomiting may be attributable to its sedative, anti-serotonin and anti-histaminic effects, which like chlorpromazine (Gujral and Lahiri, 1957 ; Gyermek *et al.*, 1956 ; Berger *et al.* 1957 ; Ducrot and Decourt, 1951) might influence the condition directly

(Glaviano and Wang, 1954) or through the chemorecepter trigger zone (Courvoisier *et al.*, 1953). Moreover, direct antagonism of the drug to serotonin effects as observed in the present experiment might be effective in inhibiting cough in view of the fact that injection of serotonin has been reported to induce cough in man (Page, 1954). Further the anti-acetylcholine effect of these drugs on bronchial tissues as observed on tracheal chain preparation, might also contribute to their beneficial effect in asthma and cough, since increased vagal activity has been shown to influence these conditions (Chen and Ensor, 1949; Boyd and Lapp, 1946).

The beneficial effect of the drugs in the gastro-intestinal irritability and spasmodic conditions seem to be also related with the inhibitory effects of the drugs against acetylcholine and serotonin. The atropine like anti-cholinergic as well as direct anti-spasmodic activity of the extract of *N. jatamansi* as observed in the present investigation seems to be responsible for the beneficial effects in gastrodynia and colicky pains (Nadkarni, 1954). The anti-serotonin and anti-acetylcholine effects of *R. succedanea* might also influence gastro-intestinal motility in checking diarrhoea in view of the fact that both serotonin and acetylcholine have been known to initiate peristalsis and rapid evacuation of bowels. Moreover, the astringent nature of the drug (Chopra *et al.*, 1956) might as well substantiate these effects.

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