CENTRAL NERVOUS SYSTEM EFFECTS OF SOME INDIGENOUS ANTI-ASTHMATIC DRUGS

By

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A few of the Ayurvedic remedies, *Nardostachys jatamansi* and *Rhus succedanea* mentioned to be useful in epilepsy, hysteria, hiccough and vomiting (6, 16) have been observed to possess mild anti-histaminic and anti-serotonin effects (12, 13). The former drug has also been reported to protect animals against histamine aerosols induced bronchial asthma. (11). The anti-convulsant and sedative effects of *N. jatamansi* have also been confirmed by the reports of Arora *et al.* (2), Arora and Madan (3) and Bose *et al.* (4). These neuro-pharmacological effects (anti-epileptic, anti-emetic, sedative etc.) seems to be common with other psychotropic agents (7), a few of which have been reported to prevent experimental asthma in guinea pigs and reduce the plasma histamine and serotonin levels in anaphylatic shock (21). It may therefore be of interest to investigate, if the above Ayurvedic remedies also possess psychotropic effect which may possibly supplement the week antihistaminic and anti-serotonin effects in their anti-asthmatic action. With this idea in view, psychotropic and neuro-pharmacological effects of *N. jatamansi* and *R. succedanea* have been investigated in experimental animals.

MATERIALS AND METHODS

The alcholic extracts of the drugs, *N. jatamansi* and *R. succedanea* were prepared by extracting the dried powered rhizomes and horns respectively in ethanol for five hours in a glass soxhlet apparatus. The extract was dried and dissolved in a mixture of ethanol, Tween 80 and distilled water (1:2:5) so as to prepare 100 *mg/ml.* solution for intra-peritonial administration. Reserpine and diphenylhydantoin sodium (Dilantin sodium) used for comparison were also dissolved in the above solvent mixture so that the quantity of the solvent injected with each drug was the same. Fresh solution of hexobarbital sodium (100 *mg/ml.*) was prepared in distilled water just before injecting the drugs.

Investigation on the alcoholic extracts of *N. jatamansi* and *R. succedanea* were conducted through a battery of pharmacological tests detailed below :-

(a) *Potentiation of narcosis* was studied as per technique of Brodie *et al.* (5) on adult albino rats of Norweigian strain weighing between 100-200 *gms.* Rats of group I were injected 0.3 *ml/kg.* of the solvent only to serve as control while Group II, III and IV rats were administered the alcoholic extracts of *N. jatamansi* (30 *mg/kg*), *R. succedanea* (30 *mg/kg*) and reserpine (5 *mg/kg*) respectively. This was followed after 10 minutes by intra-peritoneal administration of hexobarbital sodium (70 *mg/kg*) in all the four groups of rats. Time for loss of righting reflex and recovery of the erect posture was noted in each case to measure the duration of narcosis. Cross over tests were performed every week so that each group received the three drugs or the solvent in turn in four sets of observations.

(b) *Serotonin* content of brain was determined in four groups of rats (150-200 *gms*) maintained on a synthetic diet as reported previously (10). Group I, was given only the solvent to serve as control, while Group II, III were given the alcoholic extracts of *N. jatamansi* and *R. succedanea* 30 *mg/kg* each respectively. Reserpine was injected in 5 *mg/kg* doses to Group IV rats for comparison. All the drugs were given intra-peritoneally. After an interval of one hour, the animals were decapitated. The whole brain excluding the cerebella was removed by a rapid blunt dissection. The brains of each group were pooled and extracted with acetone and petroleum ether as per method of Graven (9). The brain extracts thus prepared were assayed on oestrous uterus of rat suspended in 20 *ml* bath containing oxyginated and atropinised de Jalon's solution at 30°C after the method of Amin, Crawford and Gaddum (1). Oestrous was induced in virgin rats by injecting subcutaneously Stilboestrol (0.1 *mg/kg* in arachis oil) a day before. Two doses of the brain extracts were assayed against the standard doses of serotonin creatinine phosphate. The serotonin content (*mg/gm*) of brain tissue was determined in the treated and control rats.

(c) *Studies on reaction time* : Four groups of sensitive rats were made to learn to escape out through a narrow tunnel of the Columbia obstruction box on subjecting them to a continuous alarming sound from a electric bell fitted in the chamber A, covered with a glass top. No sooner the hind leg of the escaping rat entered the tunnel, mild electric shocks (5 *ma*) were delivered through the basement grids so that the rat did not remain seated inside the tunnel but ran out to the next chamber B. The time from the onset of the buzzer to the exit of the rat at the other end of the tunnel was determined in each of the four daily trials during 15 days control observation period. After training the average pre-treatment reaction time (T1) for the last five days for the each group of rats was not found to differ significantly from each other. On 16th day, the alcoholic extract of *N. jatamansi R. succedanea* each 300 *mg/kg* and reserpine 5 *mg* was injected intra-peritoneally respectively to Group II, III, IV, while solvent in equivelant amount was administered to Group I to serve as control. One hour after the drugs, the rats were again subjected to the test four times and average reaction time (T2) was determined as before. Percentage change in the average reaction time after the treatment with drugs was calculated according to the formula. $\left(\frac{T_2-T_1}{T_2}\right) 100.$

(d) *Conditioned avoidance studies* were undertaken as per technique of Wada *et al.* (20). Three cats were trained over one and a half months to perform 90-100 per cent avoidance by jumping across a partition in a jump box in response to conditioning signal (CS) of 10 seconds clicking sound. If the animal did not jump to the other side of the midline barrier within 20 seconds of the onset of C.S, it was shocked by a mild 50 volts current (SS) passed through electrified grid so as to stimulate it to escape to the otherside and the response was classifide as 'Escape'. Further, if the cat failed to avoid shock, the CS and SS lasted until it crossed the barrier, but discontinued if the animal failed to escape within 40 seconds after the onset and the response was classified as 'No response'. The conditioned response was again tested after administration of the alcoholic extract of *N. jatamansi* given intra-peritoneally in doses of 5, 10, 25 and 50 *mg/kg* to respective cats at every 30 minutes for two hours. Cross over test with the solvents and other graded doses of the drugs was performed at weekly intervals. Peformance of individual cats was recorded as 'conditioned avoidance', escape and 'no response'.

(e) *Effects on Maximal seizure pattern as per technigue of Goodman et al.* (8) *was observed* :- Seizure's were induced by supramaximal alternating current (60 cycle,

150 *ma*) delivered through corneal electrodes for 0.2 seconds in rats and the ability of the drugs to abolish hind limb tonic extension was considered to be a positive anti-convulsant effect. For compartive studies, graded doses of reserpine, *N. jatamansi*, *R. suceedanea* and Dilantin sodium were injected in groups of 5 rats and the response of individual rats to MES was observed 2 hours after administration of the drug.

(f) *Neurological deficit occuring after the drugs* was determined in the same rats before subjecting to maximal seizure on their ability to hold on for a minute on a horizontal rod (12 inches) rotating at a speed of six revolution per minute as per method detailed by Fink and Swinyard (7). The treated and control rats were also observed for another 24 hours for mortality, if any, in a particular group. Percentage of animals protected (absence of tonic phase of M.E.S.) for those manifesting neurological toxicity or mortality after the graded doses of the drugs was calculated and E.D. 50, T.D. 50 determined per method of Karber. (15)

OBSERVATIONS

Effect of the alcoholic extract of *N. jatamansi*, *R. succedanea* and reserpine on the duration of the narcosis induced by hexobarbital in different groups of rats is shown Table I and compared in Fig. 1.

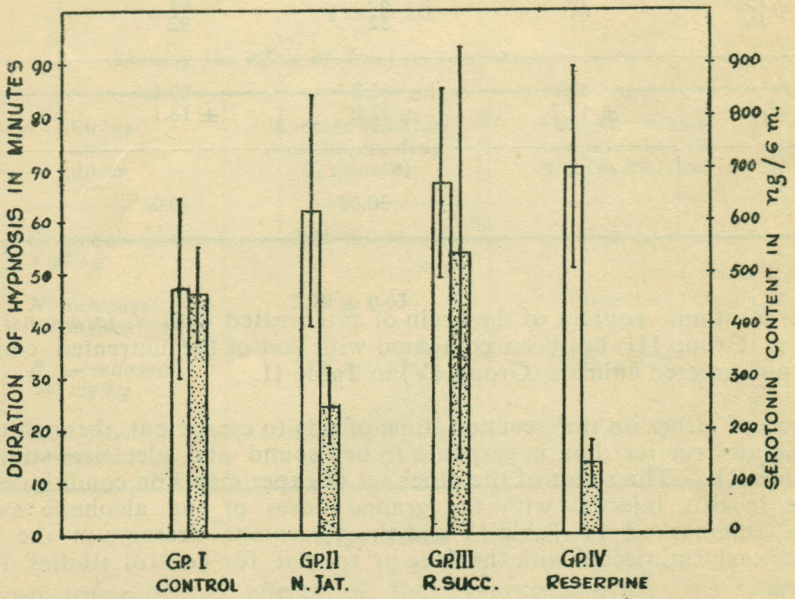

Fig. 1. Showing the duration of hexobarbital narcosis (blank columns) and brain serotonin content (shaded columns) of rats treated with *N. jatamansi*, *R. succedanea* and reserpine.

TABLE I

Showing the effect of N. Jatamansi, R. succedanea and Reserpine on the duration of hexobarbital (70 mg/kg) narcosis in albino rats

No. of sets	Rat No.	Duration of Hexobarbital narcosis (in minutes) after the following drugs			
		Control (solvent) Group I.	N. jatamansi 30 *mg/kg* Group II	R. succedanea 30 *mg/kg* Group III	Reserpine 5 *mg/kg* Group IV
1st	1	68	77	73	113
	2	53	47	90	84
	3	25	104	45	58
	4	49	67	60	57
2nd	5	54	64	68	66
	6	54	38	72	64
	7	90	96	87	62
	8	52	58	55	54
3rd	9	34	39	82	87
	10	33	36	30	30
	11	21	92	77	93
	12	33	45	53	74
4th	13	28	48	82	64
	14	33	54	45	66
	15	76	85	63	87
	16	33	52	92	66
Average		47.9 ± 17.7	62.2 ± 22.0	67.1 ± 18.1	70.3 ± 19.2
Percentage Potentiation		—	30.68	40.08	46.76

The serotonin content of the brain of rats treated with *N. jatamansi* (Group II), *R. succedanea* (Group III) has been compared with that of the untreated control (Group I) and reserpine treated animals (Group IV) in Table II.

Effect of drugs on the reaction time of rats to escape out through the tunnel of the Columbia obstruction box in response to bell sound and electrical stimulus is compared in Table III. The result of the other set of experiments on conditioned avoidance performance in cats injected with the graded doses of the alcoholic extract of *N. jatamansi* is summarised in Table IV and the percentage decrease in the conditioned perforance of each catinjected with the drug or solvent for control studies is shown in Fig. 2.

The effect of the drugs on toxicity and the maximal electroshock seizures in rats has been summarised in Table IV.

TABLE II

Showing the effect of N. jatamansi, R. succedanea and reserpine on the serotonin content of brain of albino rats

Serotonin content *mg/gm* of brain tissue of rats.				
Grp. I (Solv) 0.5 *ml/kg*		Grp. II N. jata. 30 *mg/kg.*	Grp. III R. Succ. 30 *mg/kg.*	Grp. IV reserpine 5 *mg/kg.*
	495	124	400	125
	512	192	464	174
	384	280	670	120
	409	350	273	132
	500	279	880	
	458	232		
Mean	459.7	242.8	537.4	137.8
S. D.	87.5	71.8	386.3	42.7.
% change from control		—47.18	+17.30	—20.01
T. Value		5.504	0.3541	3.913
P. Value		<0.01	>.05	<0.05

TABLE III

Showing the effect of drugs on reaction time in rats.

Group	Drugs and doses	Average reaction time before drug (T_1 minute)	Average reaction time 1hr. after the drug (T_2 minutes)	% change after drugs $\left(\frac{T_2-T_1}{T_2}\right)100$
I	Cont. Solvent 3 *ml/kg*	2.84 ± 0.91	1.85 ± 1.12	—32.1
II	*N. jatamansi* 300 *mg/kg*	2.79 ± 0.63	3.70 ± 1.48	+32.1
II	*R. succedanea* 300 *mg/kg*	2.68 ± 0.63	2.03 ± 0.32	—25.9
IV	Reserpine 5 *mg/kg*	3.26 ± 1.11	5.00 ± 1.15	+51.5

DISCUSSION

Psycho-pharmacological effects of the Ayurvedic drugs *N. jatamansi* and *R. succedanea*, used in the treatment of bronchial asthma were investigated through a battery of tests. The alcoholic extract of both the drugs given in 30 *mg/kg.* doses caused potentiation of hexobarbital narcosis. The duration of narcotic effect in

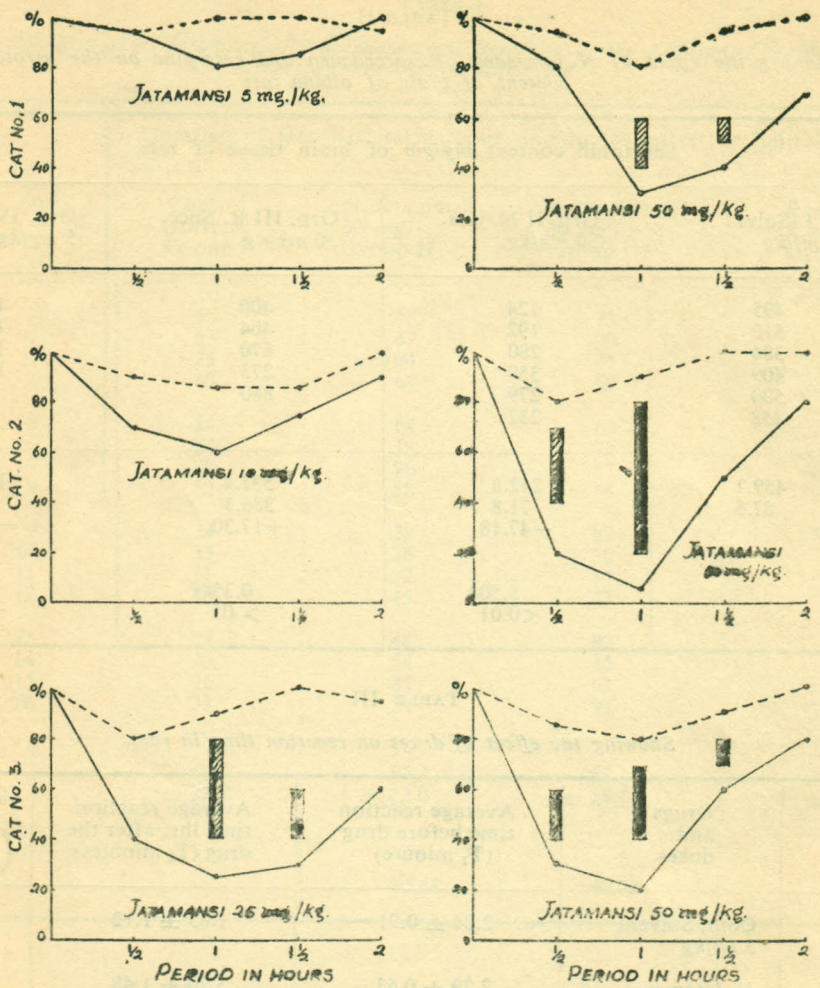

Fig. 2. Showing the percentage decrease in the conditioned avoidance response in cats injected *N. jatamansi* intraperitoneally.

the drug treated animals, was found to be significantly ($P<0.05$) higher than that in controls given the solvent only. This test though not very specific for the psychotropic action (18) yet cannot be neglected in view of the ability of the drugs to influence narcosis in quite small doses (30 *mg/kg*, being only 1/10 of ED 50 and TD 50 doses approximately). The brain serotonin content also was found to be markedly decreased in 30 *mg/kg* and 5 *mg/kg* doses of *jatamansi* and reserpine respectively. *R. succecanea* however, did not cause any significant change in brain serotonin content as compared to the controls given the solvent only. The narcosis potentiating effect of reserpine and *N. jatamansi*, seems to be partly related to the depletion of brain serotonin content. Similarlity in both these drugs were also observed to inhibit the adrenal ascorbate depletion caused by adreno-pituitary activating agents as reported by Gupta and Variyar, (14).

TABLE IV

Showing the effect of N. jatamansi on condition avoidance response in cats.

Gat. No.	Drug and doses.	Percentage of conditioned avoidance and no response (in brackets) after				peripheral manifestations
		½ hr.	1 hr.	1½ hrs	2 hrs	
1	Solvent 0.5 *ml/kg*	95	100	100	95	Scratching
	N. *Jat.* 5 *mg/kg*	95	80	85	100	Scratching
	Solvent 0.5 *ml/kg*	95	80	95	100	
	N. jatamansi 50 *mg/kg*	80	30 (20)	40 (10)	70	Drowsy
2	Solvent 0.1 *ml/kg*	90	85	85	100	
	N. jatamausi 10 *mg/kg*	70	60	75	90	Salivation
	Solvent 0.5 *mg/kg*	80	90	100	100	
	N. jatamansi 50 *mg/kg*	20 (30)	5 (60)	50	80	Asleep, Salivation
3	Solvent 0.25 *ml/kg*	80	90	100	95	
	N. Jat. 25*mg/kg*	40	25 (40)	30 (20)	60	Vomiting, Salivation, panting
	Solv. 0.5 *ml/kg*	85	80	90	100	
	N. Jat. 50 *mg/kg*	30 (20)	20 (30)	60 (10)	80	Sedation Defecation

The studies on reaction time of the trained rats to escape through the narrow tunnel on being subjected to electric bell sound and then mild shock in a Columbia obstruction box, indicated that the average reaction time in each of the four groups was reduced by training during first 10 days of control period to approximately same extent in all the groups, so that the pre-treatment average reaction time for the last five days did not differ significantly ($P>0.05$) from each other. From Table III it would be observed that the average reaction time after intraperitoneal injection of the solvent (Group 1) and *R. succedanea* (Group III) was reduced further. On the other hand, there was a marked increase in reaction time (32,1% and 51.5%) after 300 *mg/kg* and 5 *mg/kg* doses of *N. jatamansi* and reserpine respectively.

Similarly in the conditioned avoidance studies in cats treated with graded doses of *N. jatamansi* as shown in Table IV, there has been a marked and significant decrease in conditioned performance in treated cats after 25 to 50 *mg/kg* doses, when compared with their performance after solvent injection during control cross over test. Increase percentage of no response in higher doses (50 *mg/kg*) in one of the cats (No. 2) may be partly attributed to the marked sedation. The other peripheral effects *i.e.* salivation,

TABLE V

Showing the relative toxicity and potency of drugs as measured by maximal electroshock seizure (MES) test in rats.

Drugs	Time test hours	Lethality (L.D. 50) *mg/kg*	Neurotoxicity (T.D. 50) *mg/kg*	MES absence of tonic extensor response (E.D. 50) *mg/kg*
Reserpine	4	13.5*	9.5	>25.0
N. jatamansi	2	352.9	242.1	288.4
R. succedanea	2	301.1	283.6	<400.4
Diphenyl hydantoin sodium.	2	138.2	97.8	12.6

* Percentage mortality was observed in the same rats subjected to MES test hence L.D. 50 is too low.

vomiting, defecation seem to indicate the para-sympathominetic effect, which however need confirmation after atropinization. In general, cats in comparison to rats seem to be more sensitive to the psychotropic response of *N. jatamansi*.

As regards the anti-convulsant effects, the data presented in Table V. would show that the alcoholic extract of *N. jatamansi* influenced the maximal seizure pattern in doses which also caused neurological deficit. This effect was much less and non-specific as compared to diphenylhydantoin sodium which abolished the tonic extensor phase in 50% animals in 12.68 *mg/kg* doses. *R. succedanea* in doses upto 400 *mg/kg* and reserpine upto 25 *mg/kg* doses had no significant effect on the maximal seizure pattern, though the former drug in the above dosage proved fatal to 60% of rats. Postictal depression was found to be prolonged both after *R. succedanea* and reserpine. The latter drug, however, increased the severity of the seizures and enhanced mortality.

The psycho-pharmacological effects as observed in the present experiments though mild, (but as these are also associated with anti-histaminic, anti-serotonin properties) are likely to contribute to their benefical effects in bronchial asthma, in view of the fact that psychotropic agents like-lysergide and chlorphenaramine have been shown to influence experimental asthma in guinea pigs (20) and reduce plasma histamine and serotonin levels during anaphylactic shock (Supra). This seems to be further substantiated by the fact that reserpine has also been shown to produce beneficial effect in bronchial asthma (21). Thus these interesting observations on two other Ayurvedic drugs also seem to throw light on the role of psycho—somatic influences in bronchial asthma as emphasised by Rackemann (17).

SUMMARY

(1) Psycho-Pharmacological effects of *N. jatamansi* and *R. succedanea* have been investigated in albino rats through a battery of tests involving potentiation of hexobarbital narcosis, brain serotonin content, reaction time to run out, conditioned

avoidance performance, maximal electrical seizure pattern and neurological deficit response.

(2) Alcoholic extracts of both *N. jatamansi* and *R. succedanea* in 30 *mg/kg* potentiated the duration of hexobarbital narcosis to the extent of 30.68% and 40.03%, though the effect was less as compared to that caused by 5 *mg/kg* dose of reserpine.

(3) The brain serotonin content was found to be decreased significantly in animals treated with 30 *mg/kg* of *N. jatamansi* and 5 *mg/kg* of reserpine in rats. *R. succedanea* however, caused some increase which was not found to be significant ($P > 0.05$).

(4) Reaction time of trained rat to escape out through the tunnel in a Columbia obstruction box was markedly increased after 300 *mg/kg* dose of *N. jatamansi* and 5 *mg/kg* of reserpine, though the effect of latter was only significant. *N. jatamansi* however significantly decreased the conditioned avoidance performance in cats after 25-50 *mg/kg* doses.

(5) Alcoholic extract of *N. jatamansi* in 288.5 *mg/kg* doses caused abolition the tonic extensor response in 50% rats subjected to maximal electro shock seizures, but this effect was much less to that of diphenyl-hydantoin sodium and was associated with signs of neurological deficit.

(6) The psycho-pharmacological effects of *N. jatamansi* seem to supplement its peripheral anti-histaminic anti-serotonin and anti-acetylcholine effects on lung tissues for its anti-asthmatic action.

ACKNOWLEDGEMENTS

Authors are grateful to the Dean, Gandhi Medical College, Bhopal for providing facilities for the above work. One of them (S.S.G.) is also greatly indebted to Dr. P.L. McGeer and John Wada of Kinsmans Neurological Research laboratory, University of British Columbia, Canada for permitting to carry out part of psycho-pharmacological studies in their laboratories. They also thank M/s Sandoz Products Ltd., Basle, Switzerland and Parke Davis (India) Ltd., Bombay for supplying serotonin creatinine phosphate and diphenyl-hydantoin sodium respectively, for the present investigations.

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