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Anti-ulcer potential and toxicological evaluation of two siddha formulations

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ABSTRACT

The two Siddha formulations, Nillavari Choornam and Thamarai Parpam were investigated for anti-ulcer activity and their toxicological effects in rats. Nilaavari Choornam and Thamarai Parpam are procured from the Indian Medical Practitioners Cooperative Pharmacy and Stores Limited, Thiruvanmiyur, Madras-41 (IMPCOPS 1989). Nilaavari Choornam and Thamarai Parpam were investigated at the dose levels of 200mg/kg and 250 mg/kg body weight respectively. The methods of pylorus ligation induced ulcer model, aspirin induced ulcer model and gastric lesions induced by stress in rats were used to investigate the anti-ulcer activity and to evaluate their possible mechanism of action. The toxicological effects of the two Siddha formulations were studied at the dose levels of 100, 200, 400,800 and 1000 mg/kg body weight of the animals. Both, Nilaavari Choornam and Thamarai Parpam were found to posses significant anti-ulcer activity in all the above models. The possible mode of action might be by anti-secretary effect and neutralization of acids. Also it was found that even at the higher dose levels of 1000mg/kg body weight the two preparations were devoid of any significant toxic effects. Thus it can be concluded that the two Siddha formulations, Nilaavari Choornam and Thamarai Parpam posses significant anti-ulcer activity and have no toxic effects unless given continuously at higher dose levels.

Key Words: Siddha**,** Nilaavari Choornam, Thamarai Parpam, anti-ulcer activity, Toxicology.

INTRODUCTION

The two siddha formulations, Nilaavari chooornam and Thamarai Parpam were used to study their anti ulcer activity and toxicological effects. Nilaavari chooornam has been used for hiccup, vomiting, constipation, gaseous digestion of stomach, biliousness and as a mild laxative. Thamarai Parpam has been used in diseases of vitiated kapha given with honey, in bilious

disease and tuberculosis given with ghee, in skin diseases given with sugar, in venereal diseases and delirium given with tender coconut water. Due to lack of animal experimental data in pertaining to pharmacological and toxicology evaluation of these preparations an effort has been made here to study the anti-ulcer activity and toxicological effects.

MATERIALS AND METHODS

A. The two siddha formulations, Nilaavari choornam and Thamarai parpam were procured from the Indian Medical Practitioners Cooperative Pharmacy and Stores Limited, Thiruvanmiyur, Madras-41 (IMPCOPS 1989) and were subjected for pharmacological and toxicological studies. Aspirin (Germen Remedies, Mumbai), Ranitidine (Unique JB Chemicals, Mumbai,) Atropine sulphate (German Remedies, Mumbai), carboxymethyl cellulose (Thomas Baker, Mumbai) were also used in the study.

B. Animals - Albino rats of either sex weighing 150-220 gms were acclimatized for a period of seven days in laboratory under standard husbandry conditions i.e. room temperature 26 ± 2^0 C, relative humidity 45-55% and light/dark cycle12/12 hours. All the animals were fed with a standard diet (Gold Mohr, Lipton India Ltd., Bangalore) and water was supplied *ad libitum* under strict hygienic conditions. All the experimental protocols were approved by Institutional Ethical Committee.

C. Pharmacological Studies:

C.1 Anti-ulcer activity:

Pylorus ligation induced ulcer model[1]:

Albino rats of either sex weighing 150 to 220 gm were divided into 6 groups of 6 animals each. All the animals were fasted for 24 hours prior to the experiment but water allowed ad libitum However, no water was supplied during the experiment. Under light ether anaesthesia the abdomen was opened by a small midline incision below the xiphoid process. Group I receives 0.5% carboxy methyl cellulose (1mg/kg p.o) served as control, Group II, III and IV recieves Atropine sulphate (1 mg/kg p.o) Ranitidine (27 mg/kg p.o.), omeprazole (2 mg/kg p.o.) served as positive control, Group V and and Group VI receives Nilaavari choornam (200 mg/kg p.o.) and Thamarai Parpam (250 mg/kg p.o.) respectively and served as test. The procedure followed by Shay H et. was followed for assessing anti-ulcer activity and free and total acidity were analysed. The severity of ulcer was scored using a binocular stereomicroscope[2] [i.e. The severity of the ulcers scoring: 0 = normal colored stomach, 0.5 = red coloration, 1 = spot ulcer, 1.5 = hemorrhagic streaks, 2 = ulcers of 3 mm but < 5 mm, 3 = ulcers > 5 mm]

In order to calculate the difference between the control and treated group animals, the results were subjected to student's "t" test.

Aspirin induced ulcer model[3, 4]:

Albino rats of either sex weighing 150 to 220 gm were divided into 6 groups of 6 animals each and are dosed according to earlier. The animals were fasted for 24 hours prior to the experiment but water allowed ad libitum however; no water was supplied during the experiment. Drugs were administered daily once for two days by oral route. On second day aspirin is administered at a dose of 200mg/kg orally in a suspension of 1% carboxy methylcellulose for 1 hour prior to

pylorus ligation. (The time interval between standard drugs and aspirin should be 1 hour). At the end of 4 hours of ligation animals were sacrificed. The stomach was washed and severity of the ulcers were observed and scored as earlier.

Gastric lesions induced by stress in Rats[5].
Albino rats of either sex weighing 150 to 220 gm were divided into 6 groups of 6 animals each and were dosed as earlier. After 1 hour administration of the drugs, each rat was immobilized in a cylindrical cage and vertically immersed in water till the level of the xiphoid for 17 hours at temperature of 25°C. After this period the animals were sacrificed under ether anaesthesia and their stomach was excised and opened along the greater curvature. They were washed and stretched on cork plates and severity of the ulcers were examined and scored as earlier.

C.2 Toxicological studies:
Sub acute toxicity[6, 7] was determined by using 11 groups of albino rats each comprising of 6 animals weighing 150 to 220 Gms of either sex. Five groups of animals received Nilaavari choornam at dose levels of 100, 200, 400, 800 and 1000 mg/kg body weight and other five groups received Thamarai Parpam at similar dose levels. The 11th group was kept as solvent control which received only the vehicle. The drugs are administered for 30 days once daily. Before the drug administration, the blood parameters were analysed for their normal values. The mortality rate was recorded on 10th, 20th, and 30th, day of drug administration. Weights of the animals were recorded on zero and 30th day. The haemotological studies[8] include estimation of Haemoglobin (using Sahli's haemoglobinometer), Total R.B.C. count (using Haemocytometer), Total W.B.C. count (using Haemocytometer) and differential count (using Leishmann's stain) on zero, 10th, 20th and 30th day of drug treatment. For Haemotological estimation the blood samples were collected from retro-orbital route[9]. Finally the animals were sacrificed on the 30th day and vital organs like liver, spleen and kidney were isolated and stored in 10% formalin and examined for gross pathological abnormalities if any.

RESULTS

Anti ulcer activity:
Nilaavari choornam and Thamarai Parpam showed significant anti ulcer activity in all the three experimental models by reducing the ulcer scores. In pylorus ligation model gastric volume was reduced and pH of the gastric contents was increased (table 01). The total low ulcer score was reduced, thus supports its anti ulcer activity. The ulcer score was also reduced significantly in aspirin induced ulcer model (table 02) and stress induced ulcer model (table 03).

Sub acute toxicity studies:
There was no significant increase in the body weight of the animals till the end of 30th day (table 04). In thirty days observation period Nilaavari choornam at the dose level of 1000mg/kg showed the mortality rate of 16.67%, where as Thamarai parpam at the dose levels of 800mg/kg and 1000mg/kg showed the mortality of 16.67%and 33.33% respectively (table 05).

Table 01. Data showing the effect of the two-siddha formulations, Nilaavari Choornam and Thamarai Parpam in experimentally induced gastric ulcers in rats by pylorus ligation model

Sl. No.	Groups	Dose (mg/kg)	Average weight (in grams)	No. of animals	Gastric volume in ml	pH	Volume of NaoH consumed in ml		Ulcer score
							Free acidity	Total acidity	
1	Solvent control 0.5%cmc	1ml/kg	185.85	6	14.25 ±0.550	2.9 ±0.100	46.0 ±0.500	104.23 ±0.2304	6.0
2	Atropine sulphate	1mg/kg	192.2	6	11.90 ±0.300	3.7 ±0.100*	18.6 $\pm0.100^{++}$	96.76 $\pm0.707^{+}$	5.2
3	Ranitidine	27mg/kg	178.65	6	11.20 ±0.400*	4.0 $\pm0.0^{+}$	17.5 $\pm0.100^{++}$	94.43 $\pm0.4301^{+}$	4.4
4	Omeprazole	2mg/kg	190.85	6	10.20 ±0.200**	3.95 ±0.070**	18.45 $\pm0.050^{++}$	92.15 $\pm0.100^{++}$	3.0
5	Thamarai Parpam	250mg/kg	180.36	6	9.8 ±1.000**	3.65 $\pm0.3122^{+}$	35.33 $\pm0.100^{+}$	86.70 $\pm0.2828^{++}$	3.5
6	Nilaavari Choornam	200mg/kg	175.9	6	9.8 ±1.000**	3.8 ±0.0**	29.60 $\pm0.707^{++}$	76.53 $\pm0.3162^{++}$	4.0

$*p < 0.05$, $**p < 0.02$, $+ p < 0.01$, $++ P < 0.001$.

Table 02. Data showing the effect of the two formulations, Nilaavari Choornam and Thamarai Parpam in aspirin induced gastric ulcers in rats.

Sl. No.	Groups	Dose (mg/kg)	Average body weight (in grams)	No. of animals	Ulcer score
1	Solvent control 0.5%cmc	1ml/kg	176.85	6	6.0
2	Atropine sulphate	1mg/kg	189.2	6	5.5
3	Ranitidine	27mg/kg	178.65	6	5.0
4	Omeprazole	2mg/kg	190.85	6	4.5
5	Thamarai Parpam	250mg/kg	185.36	6	3.5
6	Nilaavari Choornam	200mg/kg	195.9	6	4.0

Table 03. Data showing the effect of the two formulations, Nilaavari Choornam and Thamarai Parpam in gastric lesions induced by stress in rats.

Sl. No.	Groups	Dose	Average body weights (in grams)	No. of animals	Ulcer score
1	Solvent control 0.5%cmc	1ml/kg	189.45	6	6.0
2	Atropine sulphate	1mg/kg	172.8	6	5.0
3	Ranitidine	27mg/kg	175.00	6	4.5
4	Omeprazole	2mg/kg	190.30	6	3.0
5	Thamarai Parpam	250mg/kg	186.36	6	3.5
6	Nilaavari Choornam	200mg/kg	178.90	6	4.0

Haematological parameters:

Nilaavari choornam did not showed any significant change in haemoglobin values at the maximum dose levels where as Thamarai Parpam at higher doses showed a slight increase in haemoglobin values at the end of 30^{th} day when compared to zero day observation.

Nilaavari choornam and Thamarai Parpam did not showed any significant change in the R.B.C. count at the maximum dose levels till the end of 30^{th} day.

Nilaavari choornam did not showed any significant change in W.B.C. count at the maximum dose levels where as Thamarai Parpam (1000mg/kg) showed a slight increase in W.B.C. count at the end of 30th day when compared to zero day observation.

Nilaavari choornam did not showed any significant change in differential leucocyte count at the maximum dose levels where as Thamarai Parpam (1000mg/kg) showed a significant increase in differential leucocyte count at the end of 30th day when compared to zero day observation. Results are tabulated in table no 06 and 07 for Nilaavari choornam and Thamarai parpam respectively.

Table 04. Quantitative data on the body weight of rats (6 in each group) treated with 30 days oral administration of Siddha formulations (Nillavari Choornam and Thamarai Parpam)

Groups	Treatment and dose mg/kg/day for 30 days	Pre treatment (average body weight ± SEM) Zero day	Post treatment (average body weight ± SEM) 30th day
I	Thamarai Parpam (100mg/kg)	207.5±3.570	217.5±2.803
II	Thamarai Parpam (200mg/kg)	191.25±1.314	202.75±1.345
III	Thamarai Parpam (400mg/kg)	207.5±2.327	218.75±2.277
IV	Thamarai Parpam (800mg/kg)	162.5±2.872	171.75±2.812
V	Thamarai Parpam (1000mg/kg)	167.5±2.363	174.25±2.239
VI	Solvent control (0.5% cmc) 1 mg/kg	200.0±3.464	213.025±3.765
VII	Nilaavari choornam (100mg/kg)	200.0±2.697	210.50±2.686
VIII	Nilaavari choornam (200mg/kg)	220.0±2.684	226.2±2.683
IX	Nilaavari choornam (400mg/kg)	217.5±1.588	223.0±1.494
X	Nilaavari choornam (800mg/kg)	210.0±2.365	217.75±3.875
XI	Nilaavari choornam (1000mg/kg)	201.5±3.589	207.75±0.793

Table 05. Data on mortality observed in rats by sub acute toxicity studies on oral administration of Nilaavari Choornam and Thamarai parpam Siddha preparations

Groups	Treatment and dose mg/kg/day for 30 days	No. of rats treated	Mortality during treatment in days			Total No. of dead animals	Percentage lethalty %
			10	20	30		
I	Thamarai Parpam (100mg/kg)	6	0	0	0	0	Nil
II	Thamarai Parpam (200mg/kg)	6	0	0	0	0	Nil
III	Thamarai Parpam (400mg/kg)	6	0	0	0	0	Nil
IV	Thamarai Parpam (800mg/kg)	6	0	0	1	1	16.67
V	Thamarai Parpam (1000mg/kg)	6	0	0	2	2	33.33
VI	Solvent control (0.5% cmc 1mg/kg)	6	0	0	0	0	Nil
VII	Nilaavari choornam (100mg/kg)	6	0	0	0	0	Nil
VIII	Nilaavari choornam (200mg/kg)	6	0	0	0	0	Nil
IX	Nilaavari choornam (400mg/kg)	6	0	0	0	0	Nil
X	Nilaavari choornam (800mg/kg)	6	0	0	0	0	Nil
XI	Nilaavari choornam (1000mg/kg)	6	0	0	1	1	16.67

Table 06. Haematological parameters obsereved on zero and 30th day of Nilaavari Choorrnam treatment at different dose levels

Group	Dose in mg/kg		Total R.B.C x 10^6/mm^3	Total W.B.C x 10^6/mm^3	Haemoglobin Gm%	Differential W.B.C. count			
						Neutrophils %	Lymphocytes %	Monocytes %	Eosinophils %
I	100 mg/kg	Zero day	6.4100±0.563	8.225±0.788	12.6±1.189	32.0±3.360	62.5±0.867	4.0±0.816	1.5±1.290
		30th day	7.253±0.900	9.450±1.020	13.8±0.850	30.5±0.519	64.0±0.856	3.25±0.695	2.25±0.500
II	200 mg/kg	Zero day	6.45±0.408	8.0125±0.992	12.85±1.063	28.5±0.500	67.25±0.876	2.5±2.081	1.75±0.957

		30th day	6.948±2.100	8.900±0.818	14.0±1.023	32.25±3.862	62.25±0.856	3.25±0.957	2.25±0.957
III	400 mg/kg	Zero day	8.095±0.082	9.300±3.097	15.35±0.500	24.0±2.943	69.5±0.939	4.0±0.816	2.5±0.578
		30th day	8.703±0.451	9.821±0.633	15.4±0.883	29.5±0.613	64.5±1.291	3.75±0.957	2.25±0.957
IV	800 mg/kg	Zero day	7.1475±1.000	7.225±2.941	13.6±2.065	32.5±0.645	62.5±0.645	3.25±2.173	1.75±0.500
		30th day	8.008±1.376	7.725±1.009	14.0±0.414	34.0±0.637	63.75±0.434	3.25±0.500	1.5±0.577
V	1000 mg/kg	Zero day	7.3275±0.893	8.575±0.105	14.65±1.500	28.5±2.380	67.0±2.934	3.0±2.160	1.5±0.577
		30th day	7.565±1.912	9.812±0.973	15.4±0.417	31.5±0.634	62.5±0.500*	3.5±0.577	2.25±0.500
VI	1ml/kg	Zero day	7.23±0.425	7.8125±0.220	14.2±0.963	28.0±0.529	65.5±0.479	4.0±0.281	2.5±1.290
		30th day	8.005±0.793	7.600±0.994	15.2±0.590	31.25±0.554	62.0±0.826	4.0±0.655	2.75±0.645

**indicates p < 0.05*

Table 07. Haematological parameters on zero and 30th day of Thamarai Parpam treatment at different dose levels
** indicates p < 0.05*

Group	Dose in mg/kg		Total R.B.C x 10^6/mm^3	Total W.B.C x 10^6/mm^3	Haemoglobin Gm%	Differential W.B.C. count			
						Neutrophils %	Lymphocytes %	Monocytes %	Eosinophils %
I	100 mg/kg	Zero day	6.3445±0.555	7.0375±0.640	11.95±1.207	30.5±0.519	65.00±0.621	3.0±0.381	1.5±0.577
		30th day	6.840±2.990	8.063±0.618	13.2±1.409	29.75±0.624	63.25±0.905	5.0±0.816	2.00±0.816
II	200 mg/kg	Zero day	6.6750±0.562	7.4875±0.374	13.35±1.360	24.75±2.219	70.50±1.732	2.5±1.464	2.00±0.577
		30th day	7.233±0.336	8.500±0.310	14.6±0.907	29.25±0.957	63.75±0.898	1.75±0.557	2.5±0.557
III	400 mg/kg	Zero day	6.935±0.516	7.750±1.201	13.90±1.000	32.25±0.830	60.50±0.624	3.5±0.891	3.5±1.290
		30th day	7.40±0.436	8.763±3.310	15.0±0.990	31.5±2.380	62.75±0.850	3.75±0.189	2.25±0.957
IV	800 mg/kg	Zero day	5.4875±0.619	6.2125±1.023	10.75±1.473	31.00±0.559	64.0±0.594	3.0±2.189	1.75±0.288
		30th day	5.955±0.551	7.063±0.890	11.8±3.756	31.25±1.708	62.5±0.888	3.75±0.866	2.0±0.816
V	1000 mg/kg	Zero day	5.9245±0.695	7.675±0.890	11.45±0.680	24.0±0.692	70.00±0.583	4.0±0.861	2.0±1.290
		30th day	6.438±0.685	8.082±0.892	13.0±0.685	32.25±0.622	60.5±0.858*	4.75±0.957	2.5±0.665
VI	1ml/kg	Zero day	7.2300±0.425	7.8125±0.220	14.2±0.963	28.0±0.529	65.5±0.479	4.0±0.281	2.5±1.290
		30th day	8.005±0.793	7.600±0.994	15.2±0.590	23.75±0.554	70.0±0.826	4.0±2.655	2.25±0.645

CONCLUSION

In the present study using various positive controls to assertain the mode of action of these drugs, it was observed in the pylorus ligation rat model, it was clear that these formulations have produced the anti-secretary effect. But the free acidity, which was the direct indication of H+ ions was not decreased, showed that they may be neutralize the total acidity. This was also evidenced by the decreased ulcer score in aspirin and stress induced ulcer models.

The two Siddha formulations, Nillavari Choornam and Thamarai Parpam may produce toxic effects on the vital organs like kidney, liver and spleen after continuous administration of higher doses where as the haematological parameters are not influenced at these higher dose levels.

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