EVALUATION OF ACUTE AND SUB ACUTE`TOXICITY OF SIDDHA HERBO-MINERAL FORMULATION OF SAARANAI CHOORANAM

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Abstract: Background: The siddha system of medicine is one of the traditional medicine and having the less side effects also, because its mosltly depends on the plant herbals. My dissertation drug has been mentioned in siddha classical literature of "Agasthiyar Irandaayiram III Paagam" for the management of pitha paandu "Iron Deficiency Anaemia". Aim: To evaluate the acute and sub acute toxicity of siddha formulatory drug. It's carried out as per the OECD 423 guidelines. Study Design: Observational in vivo study. Place and Duration of Study Animal bred house, Dept of Pharmacology, Arulmigu Kalasalingam College of Pharmacy, Krishnancoil, Srivilliputtur, Tamilnadu.Acute study-14 days, Sub-acute study-28 days. Materials and Methods: In female wistar albino rats were used on acute and sub acute toxicity study, It divided into 5 groups, 3 animals are having in each group, the test drug saaranai chooranam was administered single dose at 5mg / kg, 50 mg/kg, 300mg/kg, 1000mg/kg, 2000 mg/ kg body weight of animal for 14 days all group of treated animals and toxic symptoms are also observed including behavioural changes, locomotion, convulsions and mortality.The results are assessed for detect the effect of saaranai chooranam . There is no mortality and morbidity observed in animals through the 14 days period following single dose oral administration at all selected dose levels of the Saaranai Chooranam and sub acute toxicity study carried throught out 28 days. The animals don't show any abnormal reactions and don't change in the general appearances, gait and posture, reactivity to handling sensory stimuli, grip strength also normal. It also indicating the p value is less than 0.05

Keywords: Acute and sub acute toxicity, Invivo study, Siddha drug, Trianthema portulacastrum, Traditional medicine.

I.INTRODUCTION

One such valuable siddha drug Saaranai (Trianthema portulacastrum) from siddha literature Agasthiyar Irandaayiram III part has varied uses. Distribution: It's growing throughout most tropical countries & now naturalized throughout india in cultivated fields river beds, waste ground especially in rainy season[1] Description: Root is thin, slender, tapering, and tortuous with lateral branching fibrous root 5-15 cm in length; 0.3-2.5 cm in diameter, light yellow externally, creamish white internally fractures fibrous.[2] Phyto constituents: Presence of steroids, flavanoid, fat, alkaloids, carbohyhydrates, tannins and terpens. Phytochemical constituents are Ecdysterone, Trianthenol, Leptorumol, Trianthemin, Saponin, Glycosides, 8 dimethyl flavones, 5, 2- dihydroxy- 7-methoxy-6,8-dimethyl flavone along with 5,7-dihydroxy-6,8-dimethyl chromone (leptorumol)[3] The plant is rich in iron and phosphorous but poor in calcium .The high content of oxalate affects the assimilation of calcium. Among the nutrient values, fiber was found to be the highest(430.0mg/g), followed by ash(348.0mg/g), total protein(91.9mg/dl), moisture(80.0mg/g), carbohydrate(30.2mg/g), and total lipid(20.0mg/g). Trianthema portulacastrum L is a good source of fiber, proteins, riboflavin, potassium, sodium and iron. Two toxic metals Pb and Cd were present in very minute quantities of 0.08 and 0.0006 mg/g respectively. The results suggest that Trianthema portulacastrum L is a good source of fiber, proteins, riboflavin, potassium, sodium and iron [4] . Phytochemical analysis of T.portulacastrum reveals the presence of alkaloids, flavanoids, terpenoids, saponins and phenolic compounds[5].Ethno medicinal uses: The plant cures bronchitis, heart diseases, blood disorders, anemia, inflammation, piles and ascites .The plant has been used in the indigenous system of medicine for the liver

obstruction, asthma, amenorrhoea, dropsy, edema, ascites, beri beri Root: Having Alcoholic intoxication property, antipyretic, spasmolytic, anti-inflammatory, deobstruent and curing the disease of liver and spleen.[6]

II. MATERIALS AND METHODS

2.1 Collection and Authentication

The required raw drugs for preparation : Saaranai Ver are purchased from a well reputed country shop in Nagercoil, Tamilnadu & raw drugs are identified & authenticated by the medical botanist & gunapadam experts of Govt siddha medical college & hospital, palayamkottai.

2.2 Purification and Preparation of Saaranai Cooranam

Saaranai ver should be thoroughly washed in water and soaked in cow's milk. Then it should be steamed in milk. Dried and groun into the fine powder sieved and add same quantity of indhuppu, after purification of indhuppu in buttermilk & store in a clean glass container

2.3 Toxicity study in female wistar rats

2.3.1 Acute Toxicity (14 days)

All animals were observed for any abnormal clinical signs and behavioral changes. The appearance, change and disappearance of these clinical signs, if any, were recorded for approximately 1.0, 3.0 and 4.0 hours post-dose on day of dosing and once daily thereafter for14 days. Animals in pain or showing severe signs of distress were humanely killed. The cage side observation was included changes in skin, fur, eyes and mucous membranes secretions and excretions. Autonomic activity like lacrimation, pilo erection, pupil size and unusual respiratory pattern, changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypes like excessive grooming and repetitive circling or bizarre behavior like self-mutilation, walking backwards etc were observed. At the 14th day, sensory reactivity to stimuli of different types (e.g. auditory, visual and proprioceptive stimuli) was conducted. Auditory stimuli responses were measured by clicker sound from approximately 30 cm to the rats; visual stimuli response were measured with the help of shining pen light in the eye of rats and placing a blunt object near to the eye of rats. Response to proprioceptive stimuli was measured by placing anterior/dorsal surface of animals paw to the table edge. The responses of reactions for these three exercises were normal in animals belonging to both the controls as well as drug treatment dose groups.

III. RESULTS

Table.1 Physical and behavioral examinations.

Group no.	Dose(mg/kg)	Observation sign	No. of animal affected
Group-I	5mg/kg	Normal	0 of 3
Group- II	50mg/kg	Normal	0 of 3
Group-III	300mg/kg	Normal	0 of 3
Group-IV	1000mg/kg	Normal	0 of 3
Group-V	2000mg/kg	Normal	0 of 3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $^{ns}p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, calculated by comparing treated groups with control group . Data obtained in this study indicated $^{ns}p > 0.05$ no significance physical and behavioral signs

Table.2 Home cage activity

Functional and Behavioural observation	Observation	5mg/kg Group (G-I)	50mg/kg (G-II)	300mg/kg (G-III)	1000mg/kg (G-IV)	2000mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Body position	Normal	3	3	3	3	3
Respiration	Normal	3	3	3	3	3
Clonic involuntary Movement	Normal	3	3	3	3	3
Tonic involuntary Movement	Normal	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3
Approach response	Normal	3	3	3	3	3
Touch response	Normal	3	3	3	3	3
Pinna reflex	Normal	3	3	3	3	3
Tail pinch response	Normal	3	3	3	3	3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $^{ns}p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, calculated by comparing treated groups with control group. Data obtained in this study indicated $^{ns}p > 0.05$ 05 no significance changes in Home cage activity, signs of any toxicity

Table.3 Hand held observation

Functional and Behavioral observation	Observation	Control	5 mg/kg (G-I)	50 mg/kg (G-II)	300 mg/kg (G-III)	1000 mg/kg (G-IV)	2000 mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Reactivity	Normal	3	3	3	3	3	3
Handling	Normal	3	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3	3
Lacrimation	Normal	3	3	3	3	3	3
Salivation	Normal	3	3	3	3	3	3
Piloerection	Normal	3	3	3	3	3	3
Pupillary reflex	Normal	3	3	3	3	3	3
Abdominal tone	Normal	3	3	3	3	3	3
Limb tone	Normal	3	3	3	3	3	3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $^{ns}p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, calculated by comparing treated groups with control group. Data obtained in this study indicated $^{ns}p > 0.05$ no significance changes in hand held observation and signs of any toxicity

Table.4 Mortality

Group no	Dose no(mg/kg)	Mortality
Group-I	5(mg/kg)	0 of 3
Group-II	50(mg/kg)	0 of 3
Group-III	300(mg/kg)	0 of 3
Group-IV	1000(mg/kg)	0 of 3
Group-V	2000(mg/kg)	0 of 3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group. From acute toxicity study was observed $^{ns}p>0.05$.

So No Observed-Adverse-Affect- Level (NOAEL) at SC is 2000 mg/kg.

II. 2.3.2 Sub acute toxicity

This oral toxicity study was carried through according to OECD guidelines 407.

Table.5 Study Design in dose level

Test group	Concentration / Dose to animals (ml/kg body weight /day)	No of animals
Group 1	Control	10
Group 2	Low dose of SC 200 mg/kg	10
Group 3	Mid dose of SC 400 mg/kg	10
Group 4	High dose of SC 600 mg/kg	10

The test an animal has been administered a single dose orally observed for 28 days. All the rats were observed twice in per day with a purpose of recording any abnormal signs and symptoms of behavioural changes.

Table.6 Effect of sub acute dose of SC on food intake in gram

Group	Control	Low 300g	Mid 1000g	High 2000g
1st day	12.49±0.07	13.10±0.07	14.18±0.08	15.60±0.07
7th day	13.66±0.07	14.90±0.07	16.78±0.07	16.20±0.08
14th day	14.78±0.06	15.89±0.08	17.90±0.08	18.77±0.08
21st day	15.90±0.07	17.20±0.07	28.89±0.071	19.21±0.06
28th day	16.06±0.08*	17.41±0.06*	19.05±0.05*	20.05±0.06*

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dennett's (n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group

Table.7 Effect of sub- acute dose of SC on Water Intake in ml

Group	Control	300ml	1000ml	2000ml
1st day	12.40±0.07	13.20±0.07	14.18±0.05	15.58±0.08
7th day	13.59±0.08	14.86±0.06	14.71±0.06	16.21±0.08
14th day	14.40±0.07	14.90±0.08	15.91±0.06	16.91±0.07
21st day	14.69±0.05*	15.46±0.27*	16.41±0.05*	17.50±0.09*
28th day	15.15±0.06**	16.41±0.07**	17.77±0.09**	19.59±0.08**

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dennett's (n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table.8 Efect of subacute dose of SC on body weight in grams

The body weight of each rat was recorded one weak before the start of treatment, during the course of the treatment on the day of initial, 3rd, 7th, 10th, 14th, 17th, 20th and 28th

Group	Control	Low	Mid	High
1st day	151.06±0.80	155.15±0.80	162.80±0.80	164.75±0.84
7th day	154.13±0.72	156.21±0.73	164.29±0.82	165.75±0.84
14th day	156.11±0.69	161.17±0.80	166.98±0.84	167.81±0.76
21st day	162.03±0.76	165.09±0.707	168.42±0.83	169.95±0.71
28th day	164.15±0.77*	167.14±0.70*	169.05±0.74*	171.86±0.75*

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table.9 Effect of sub acute dose of SC on organ weight in grams

Group		Control	Low	Mid	High
Heart		0.42±0.17	0.48±0.18	0.52±0.05	0.56±0.18
Liver		3.61±0.08	3.71±0.06	4.42±0.18	4.68±0.20
Lungs		0.86±0.09	0.90±0.09	1.10±0.07	1.20±0.13
Kidney	L	0.80±0.07	0.99±0.07	1.10±0.07	1.27±0.07
	R	0.88±0.04	0.99±0.04	1.10±0.044	1.25±0.044

Values are expressed as mean ± SEM Statistic significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Figure.1

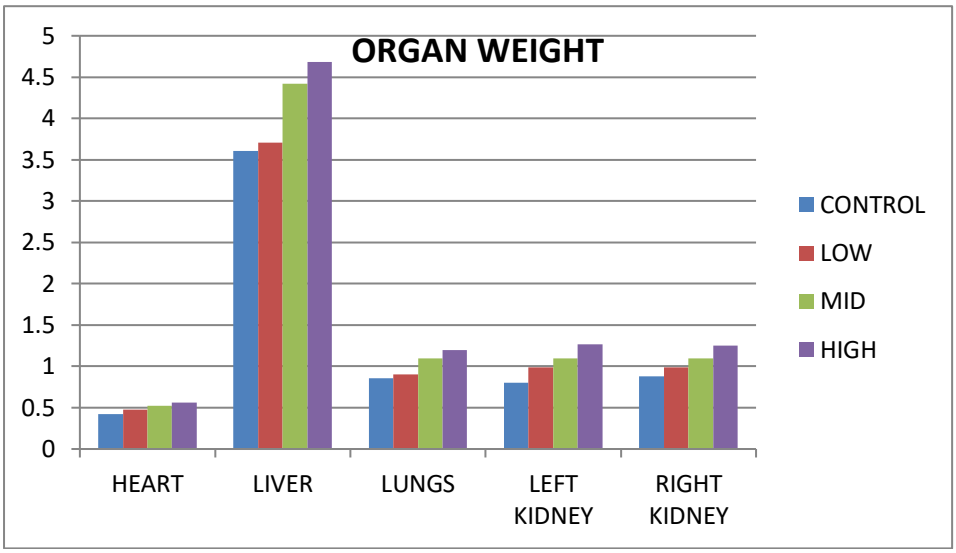

Table.10 Effect of sub acute dose of SC on haemotological parameters

Drug treat Ment	RBC 10^{12}/litre	WBC 10^{9}/litre	Haemoglobin gm/litre	Differential count %			
				Neutrophils	Eosinophils	Monocyte	Lymphocyte
Control	4.88±0.07	7.60±3.40	11.50±0.07	59.25±0.07	1.86±0.06	2.05±0.07	38.20±0.0.06
Low	4.92±0.06	7.50±3.23	11.98±0.08	56.72±0.16	1.96±0.07	2.22±0.02	40.00±0.05
Mid	4.96±0.07	7.50±3.15	12.99±0.07	56.47±0.14	2.59±0.08	3.42±0.18	39.88±0.07
High	5.00±0.07	7.90±3.45	13.45±0.07	59.12±0.65	2.81±0.06	2.60±0.07	37.04±0.02

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table.11 Effect of sub acute dose of SC on biochemical parameters

Drug Treatment	SGPT (U/L)	SGOT(U/L)	ALP(U/L)	Urea (mg/dl)	Creatinine(mg/dl)
Control	27.20±0.08	49.19±0.08	136±0.07	37.42±0.04	0.97±0.077
Low	28.51±0.25	55.71±0.02	138.77±0.06	39.62±0.07	0.98±0.06
Mid	29.62±0.55	60.84±0.61	142.16±0.72	40.44±0.49	0.99±0.08
High	31.10±0.74	63.17±0.75	145.89±0.67	42.86±0.66	1.00±0.08

Table.12 Effect of sub acute dose of SC on biochemical parameters

Group	Control	Low(300mg/kg)	Mid(1000mg/kg)	High(2000mg/kg)
Total bilirubin (mg/dl)	0.45±0.07	0.56±0.07	0.68±0.07	0.70±0.06

IV. DISCUSSION

All animals from control and all the treated dose groups survived throughout the dosing period of 28 days. The results for body weight determination of animals from control and different dose groups show comparable body weight gain throughout the dosing period of 28 days. During dosing period, the quantity of food and water consumed by animals also significantly increase. The results of hematological investigations conducted on day 29th day revealed no significant changes in the hematological values when compared with those of respective controls. This gave clear justification that bone marrow and spleen were not influenced by *SC*. The clinical biochemistry analysis was done to evaluate the possible alterations in hepatic and renal functions not influenced by the test drug. Results of Biochemical investigations conducted on days 29 and recorded in revealed the no significant changes in the values of different parameters studied when compared with those of respective controls; Urea, SGOT,SGPT, Bilirubin were within the limits.. Group Mean Relative Organ Weights are recorded Comparison of organ weights of treated animals with respective control animals on day 29 was found to be normal comparable with respective control group.

V. CONCLUSION

Acute and subacute toxicity were carried out in wistar albino rats according to OECD guidelines (423) this drug has no acute toxicity as there was no mortality seen. Sub acute toxicity is carried by repeated dose of test drug for 28 days. Mortality, the functional observation, haemotological and biochemical investigations were done. There were no significant changes in the biochemical and haematological profile. So the toxicological study of these test drug SC establish the safety of the drug for long time administration.

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