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Toxicity Profile Of Herbo Marine Siddha Drug Sangu Parpam

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ABSTRACT

The present study was carried out to investigate the toxicity profile of Herbo marine Siddha formulation Sangu Parpam. Three types of purification was done on three samples of raw sangu and sangu Parpam was prepared from each purified sample and named as SP I,II,III. Acute and Sub-acute toxicity study was conducted as per the OECD guidelines 423 and 407 respectively. Acute toxicity study revealed Sangu Parpam I, II, III did not produce drug- related toxicity. The maximum tolerable dose obtained from the acute toxicity study is about 2000mg/kg b.wt. Sub acute toxicity study revealed Sangu Parpam I, II, III can be considered as safe and did not produce any toxic effects over a period of 28 days. Histopathological study shows normal architecture of oragans. Thus the Sangu Parpam I, II, III are safe in prescribed dose level as per the literature.

Key Words: Siddha, Sangu Parpam, OECD, Purification, Herbo marine drug

INTRODUCTION

The Siddha Medical System is based upon the teachings of the Siddhas. A great deal of the Siddha Medical System comes to us from the selfless work of untiring souls who preferred obscurity and austerity. Those who attained or achieved the above-mentioned powers were known as Siddhas1. It is a one-of-a-kind system that has existed among the Tamil people of South India for more than five thousand years, serving humanity in combating diseases and maintaining physical, mental, social, and spiritual health2.Unlike contemporary medicine, the Siddha system uses more than one ingredient for preparing medicines because of their synergistic activity and lower toxicity. These drugs possess increased bioavailability as the mineral drugs are treated with herbal juices, which leads to a reduction in particle size up to the nano level. As a result, even the smallest doses provide increased potency.

In this study, acute and sub-acute toxicity studies were done on *Sangu Parpam,* a herbo-mineral Siddha drug that is extensively used by traditional medicine practitioners. Three various types of purifications were done on raw sangu and from each purified sangu, Sangu parpam was prepared. The same preparation was followed to prepare the Sangu parpam. It has high therapeutic value in treating diseases like Peptic Ulcer, Gastrointestinal Disorders, Cough, Piles, etc.

MATERIALS AND METHODS

Preparation of Sangu Parpam

Purification of Sangu [5]

Process I (Spu I) [5]

35g of Sangu (1 palam) was soaked in 175g of Juice of Ilaikkalli (Common Milk Hedge –*Euphorbia ligularia*) and let to dry in sunlight from morning to evening. This process was repeated for another 3 times with fresh juice.

Process II (Spu II)[5]

Sangu was processed in thaalithal method (Heating process**)** by covering it with Karchunnam (limestone).

Process III (Spu III)[5]

Equal parts of Karchunnam (limestone) and Uvarmann (Alkaline earth) was mixed up with 8 parts of water and the clarified water was collected. Sangu was processed by heating with this clarified water. After heating, Sangu was washed with water and dried.

Preparation process [6]

100g of purified Sangu from each purification process (Spu I,II,III) was covered up by ground paste of Uthamani (*Pergularia damea*) and kept in the mud lid and closed by another mud lid. Cotton ribbon soaked in wet clay was winded over the rims of both mud lids and let to dry in sun light for 8 hours. Then this set up was subjected to Ganapudam. (100 cow dung cakes were used). After cooling the set up was taken out and the calcinated Sangu was taken out ground well and stored in an airtight container. Sangu Parpam prepared from above mentioned purified process (Spu I, II, III) were named as SP I, II, III respectively.

Acute toxicity study of SP I, II, III

The complete protocol of the animal experiments has been approved by the Institutional Animal Ethics committee, National Institute of Siddha, Chennai. The IAEC approval number is **1248/AC/09/CPCSEA -9/Dec 2013/8.** Acute toxicity study of Sangu Parpam was evaluated in rats as per the Method - OECD 423 - Acute toxic class method[7].The animals were fasted overnight with water *ad libitum.* The Sangu Parpam I, II, III was administered in four different dose levels i.e. 50mg, 300mg, 1000mg & 2000mg/kg b.wt. as OECD guidelines insist the stepwise administration first 50mg/kg b.w of Sangu parpam I, II, III was given as a single oral dose. As no death was noticed, 300mg was administered. Likewise, 1000mg and 2000mg/kg b.wt was administered from group II to Group 13. Group I was served as control which received vehicle i.e. ghee (2ml/200gm). After drug administration, all animals were observed for 14 days. Observations were made and recorded systematically and continuously observed as per the guidelines after substance administration. On day 15, the overnight fasted animals (water allowed *ad libitum*) were sacrificed and examined for gross pathological changes in the major internal organs. All the animals were observed at least two times a day to record abnormal behavior. In clinical signs of toxicity, they should be observed daily for 14 days.

Sub-Acute Toxicity Study Of SP I, II, III 28 days Repeated Dose Oral Toxicity

Study were conducted as per the Method - OECD 407- Sub-Acute Toxicity study (Repeated Dose 28-Day Oral Toxicity Study in Rodents)[8]The IAEC approval number is **1248/AC/09/CPCSEA -9/Dec 2013/8.** Animals were divided into 4 groups and each group contains 5 animals per sex. The control animals were administered ghee 2ml/200gm. Group II received SP I, Group III received SP II, Group IV received SP III with low dose of 100mg/kg b.w, middle dose of 200mg/kg b.w and high dose of 300mg/kg b.w respectively. Administration was by oral for 28 consecutive days. Experimental animals were kept under observations throughout the study for the following, clinical signs and mortality, body weight, food and water consumption, Haematological parameters and Biochemical parameters. All the animals were sacrificed on day 29. Necropsy of all animals were carried out and routine histopathological examination was done. The various organs collected were fixed in 10% neutral buffer formaline. They were dehydrated through a series of graded alcohol, fixed in paraffin, and routinely processed for histopathological assessment. The tissues cut into 4-5µm thick sections and stained with Haematoxylin-eosin. Tissue slides were examined

and photographs were taken by using N-400ME photomicroscope (CELL-TECH diagnostics, Hamburg, Germany) in X40, X100 and X400 objectives.

The mean changes in body weight, daily food and water intake, organ weight relative to body weight, and biochemical and haematological parameters were statistically analyzed and significant differences within groups were calculated using the one-way ANOVA test followed by Dunnett's test to compare mean differences of control and test drug treated groups. The results were expressed as the mean ± SD. Statistical analysis was performed using the SPSS version 18.

RESULTS

TABLE I - PHYSICAL AND BEHAVIOURAL EXAMINATIONS FOR SP I, II, III

Group no.	Drug	Dose (mg/kg)	Observation signs	No. of animal affected.
I	Ghee	Control	Normal	0 of 3
II	SP I	50mg/kg	Normal	0 of 3
III	SP I	300mg/kg	Normal	0 of 3
IV	SP I	1000mg/kg	Normal	0 of 3
V	SP I	2000mg/kg	Normal	0 of 3
VI	SP II	50 mg/kg	Normal	0 of 3
VII	SP II	300 mg/kg	Normal	0 of 3
VIII	SP II	1000mg/kg	Normal	0 of 3
IX	SP II	2000mg/kg	Normal	0 of 3
X	SP III	50 mg/kg	Normal	0 of 3
XI	SP III	300mg/kg	Normal	0 of 3
XII	SP III	1000mg/kg	Normal	0 of 3
XIII	SP III	2000 mg/kg	Normal	0 of 3

GROUP	O day	7^{th} day	14^{th} day	21^{st} day	28^{th} day
CONTROL	125±2.76	133±3.09	143±2.47	152±2.86	161±2.044
S.P I-LOW DOSE	128±2.49	136±2.5	143±1.69	157±1.93	161±1.86
S.P I-MID DOSE	128±3.20	136±3.22	147±3.23	157±3.58	165±2.52
S.P I-HIGH DOSE	139±2.17	149±2.53	159±2.43	168±3.26	174±2.49

S.P II-LOW DOSE	146±3.79	153±5.05	145±5.69	147±3.93	158±2.61
S.P II-MID DOSE	145±2.02	148±2.5	149±2.82	155±5.09	164±3.24
S.P II-HIGH DOSE	147±4.23	142±3.14	151±2.80	146±2.79	159±4.35
S.P III-LOW DOSE	149±4.42	146±1.37	151±3.30	152±2.85	163±2.64
S.P III-MID DOSE	141±2.47	141± 3.41	153±5.43	153±4.55	163±3.08
S.P III-HIGH DOSE	149±2.66	136±2.90	158±4.0	161±1.71	158±3.45

TABLE II- EFFECT OF SP I, II, III ON BODY WEIGHT

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's $^{c}P< 0.001$, $^{b}P< 0.01$, $^{a}P < 0.05$ calculated by comparing treated group with CONTROL group.

Table III –EFFECT OF SP I, II, III ON HAEMATOLOGICAL PARAMETERS

GROUP	**WBC cells/mm³**	**POLY MORPHS**	**RBC cells/lt**	**HB g/dl**	**PCV g/dl**	**LYMPHO CYTES cells /µlt**	**MONO CYTES cells /µlt**	**EOSINO PHILS cells /µlt**	**MCH Picogm/cell**
CONTROL	10±0.79	7±1.53	4±1.14	15±0.58	48±2.41	84±1.52	3±0.63	6±1.15	26±0.59
S.P I-LOW DOSE	12±0.83	9±1.85	6±0.27	15±0.80	45±2.47	81±1.67	3±0.76	6±0.58	24±0.79
S.P I-MIDL DOSE	13±0.63	4±1.85	5±0.07	13±0.15	41±1.70	87±1.87	3±0.76	4±0.88	24±0.50
S.P I-HIGE DOSE	12±0.55*	5±0.33	5±0.22	15±0.56	45±1.06	84±0.73	4±0.56	5±0.88	28±0.32
S.P II-LOW DOSE	11±0.34	5±1.20	5±0.25	14±0.67	40±2.17	77±3.83	3±0.36	3±0.66	22±0.67
S.P II-MIDL DOSE	10±0.35	6±1.33	5±0.24	15±0.78	49±6.07	73±3.15	4±0.21	4±1.15	26±1.19
S.P II-HIGE DOSE	11±1.02	6±1.53	5±0.15	14±0.54	42±4.69	77±4.20	4±0.56	4±1.16	17±1.69
S.P III-LOW DOSE	10±0.35	7±0.67	6±0.20*	14±0.75	46±7.55	81±2.01	5±0.36	5±0.88	20±2.03
S.P III-MIDL DOSE	12±0.79	6±0.58	6±0.21*	14±0.52*	41±1.88	88±1.26	4±0.36	5±1.76	21±2.03

S.P III-HIGE DOSE	12±0.59	7±0.67	5±0.27	15±0.73	40±1.51	77±3.48	3±0.76	4±0.88	23±1.48

Values are expressed as the mean ± S.D; No significant difference between SP I, II, III when compared with control group

TABLE IV - EFFECT OF SP I, II, III ON ORGAN WEIGHT IN GMS

GROUP	BRAIN	HEART	LUNGS	LIVER	TESTIS
CONTROL	1.12±0.06	0.89±0.02	2.31±0.21	6.86±0.43	2.95±0.29
S.P I-LOW DOSE	1.20±0.12	0.89±0.03	1.01±0.10	5.04±0.45	2.39±0.23
S.P I-MIDL DOSE	1.65±0.09	0.82±0.03	1.32±0.16	5.53±0.59	2.00±0.19
S.P I-HIGE DOSE	1.49±0.23	1.0±0.05*	2.06±0.14	6.05±0.12	2.57±0.21
S.P II-LOW DOSE	1.52±0.23	1.08±0.08*	1.71±0.19	6.42±0.39	2.44±0.16
S.P II-MIDL DOSE	1.08±0.07	0.96±0.03	1.69±0.23	5.78±0.55	1.37±0.15
S.P II-HIGE DOSE	1.02±0.03	0.97±0.02	1.41±0.15	6.78±0.59	3.04±0.20
S.P III-LOW DOSE	1.29±0.10	0.92±0.04	1.46±0.20	6.43±0.69	3.27±0.19
S.P III-MIDL DOSE	1.31±0.04	0.87±0.02	1.548±0.2	6.42±0.19	2.79±0.47
S.P III-HIGE DOSE	1.38±0.09	0.94±0.05	2.06±0.10	6.43±0.93	3.25±0.15

Values are expressed as the mean ± S.D; control vs SP I High dose * P< 0.05 control vs SP II Low dose * P<0.05

TABLE V - EFFECT OF SP I, II, III ON ORGAN WEIGHT IN GMS

GROUP	KIDNEY (L)	KIDNEY (R) WEIGHT	UTERUS WEIGHT
CONTROL	1.05±0.09	1.04±0.04	0.49±0.02
S.P I-LOW DOSE	0.69±0.014	0.75±0.03	0.56±0.06
S.P I-MID DOSE	0.69±0.03	0.68±0.01	0.52±0.05
S.P I-HIGH DOSE	0.95±0.09	0.89±0.03	0.72±0.02
S.P II-LOW DOSE	0.82±0.08	0.81±0.08	0.51±0.04
S.P II-MID DOSE	0.98±0.06	0.94±0.03	0.48±0.06
S.P II-HIGH DOSE	0.81±0.08	0.79±0.08	0.54±0.12
S.P III-LOWDOSE	0.91±0.08	0.79±0.07	0.43±0.07

S.P III-MID DOSE	0.79±0.07	0.78±0.07	0.41±0.02
S.P III-HIGH DOSE	0.95±0.16	0.87±0.11	0.43±0.08

Values are expressed as the mean ± S.D; No significant difference between SP I, II, III when compared with control group

Table VI - EFFECT OF SP I, II, III ON BLOOD GLUCOSE LEVEL

GROUP	**BLOOD GLUCOSE LEVEL mg/dl**	
	Male	**Female**
CONTROL	78±3.12	79±4.02
S.P I-LOW DOSE	83±1.64	95±3.45
S.P I-MID DOSE	75±6.63	83±1.87
S.P I-HIGH DOSE	92±7.62	92±1.46
S.P II-LOW DOSE	92±5.93	79±4.10
S.P II-MID DOSE	91±8.10	93±2.95
S.P II-HIGH DOSE	90±4.10	78±4.93
S.P III-LOW DOSE	87±3.31	89±4.02
S.P III-MID DOSE	94±9.02	90±6.28
S.P III-HIGH DOSE	89±4.39	92±2.35

Values are expressed as the mean ± S.D; No significant difference between SP I, II, III when compared with control group

TABLE VII – EFFECT OF SP I, II, III ON LIPID PROFILE

GROUP	**TOTAL CHOLESTEROL (mg/dl)**	**TRIGLYCERIDES (mg/dl)**	**HDL-CHOLESTEROL(mg/dl)**
CONTROL	37±2.89	101±1.59	8±0.99
S.P I-LOW DOSE	38±1.99	98±2.09	8±1.48
S.P I-MID DOSE	37±3.97	96±10.89	8±0.93
S.P I-HIGH DOSE	39±2.54	96±9.59	8±2.60
S.P II-LOW DOSE	36±2.69	100±3.83	7±0.42

S.P II-MID DOSE	37±3.61	99±2.22	8±0.13
S.P II-HIGH DOSE	37±1.65	98±1.83	7±0.092
S.P III-LOW DOSE	36±6.38	97±2.56	7±0.24
S.P III-MID DOSE	37±3.29	101±3.65	8±0.40
S.P III-HIGH DOSE	38±1.52	99±3.31	8±0.15

Values are expressed as the mean ± S.D; No significant difference between SP I, II, III when compared with control group

TABLE VIII - EFFECT OF SP I, II, III ON SGOT, SGPT, ALP LEVELS

GROUP	SGOT (U/L)	SGPT(U/L)	ALP(U/L)	TOTAL BILIRUBIN (g/dl)
CONTROL	79±5.56	62±6.88	297±7.87	0.53±0.08
S.P I-LOW DOSE	81±17.53	61±5.99	301±13.18	0.75±0.13
S.P I-MID DOSE	77±4.79	56±9.44	267±17.32	0.64±0.03
S.P I-HIGH DOSE	80±0.83	59±1.56	254±2.30	0.69±0.06
S.P II-LOW DOSE	83±15.30	52±1.33	322±20.45	0.55±0.09
S.P II-MID DOSE	82±4.74	68±1.42	210±4.40	0.66±0.09
S.P II-HIGH DOSE	71±0.86	61±1.79	260±6.07	0.72±0.06
S.P III-LOW DOSE	85±30.4	64±3.27	314±24.85	0.64±0.03
S.P III-MID DOSE	69±1.08	59±5.36	288±1.47	0.75±0.02
S.P III-HIGH DOSE	63±4.56	6 2±3.08	268±10.08	0.73±0.08

Values are expressed as the mean ± S.D; No significant difference between SP I, II, III when compared with control group

TABLE IX - EFFECT OF SP I, II, III ON UREA, URIC ACID, CREATININE LEVELS

GROUP	UREA(mg/dl)	URIC ACID (mg/dl)	CREATININE (mg/dl)
CONTROL	33±4.42	1±0.08	0.33±0.01
S.P I-LOW DOSE	32±2.49	0.94±0.16	0.28±0.04
S.P I-MID DOSE	38±0.61	1±0.03	0.19±0.02

S.P I-HIGH DOSE	29±1.91	1±0.08	0.31±0.03
S.P II-LOW DOSE	45±4.75	8±4.39	0.36±0.04
S.P II-MID DOSE	37±1.52	0.89±0.10	0.42±0.05
S.P II-HIGH DOSE	38±5.79	0.61±0.11	0.34±0.04
S.P III-LOW DOSE	40±2.5	1±0.17	0.40±0.06
S.P III-MID DOSE	33±1.31	1±0.16	0.37±0.02
S.P III-HIGH DOSE	45±0.96	0.68±0.04	0.26±0.01

Values are expressed as the mean ± S.D; No significant difference between SP I, II, III when compared with control group

Acute toxicity study

The trial drugs sangu parpam I, II, III were tested for their acute toxicity study as a step wise procedure. The different doses did not produce any mortality and morbidity throughout the study period. The animals were healthy and they had gained weight throughout the study period. There was no body weight changes noted. No animals in the groups showed significant variation in food and water intake. After the completion period, there was no grass pathological change noted in all the group of animals. The animals did not show any mortality and morbidity up to 2000 mg/kg body weight. So, the maximum tolerable can be obtained as 2000 mg/kg body weight was shown in (Table I)

28 days repeated oral toxicity study

The repeated oral administration of Sangu parpam II in three different dose levels did not produce any significant changes in the body weight (Table II) when compared to normal control animals.

There was no significant difference was noticed in the food intake as well as the water intake.

The animals treated with sangu parpam of different doses did not produce any significant variations in the haematological parameters like WBC, polymorphs, RBC,HB, PCV& MCH (Table III)

The organs which were collected for histopathological studies were weighed and the mean organ weights were tabulated. There was no significant variation noted in the major organs like brain, heart, lungs, liver, testis kidney and uterus (Table IV, V)

The animals treated with sangu parpam of different doses did not produce any significant variations in the Blood glucose level. (Table VI)

The animals treated with Low Middle and High dose of SP I, II, III did not produce any significant changes in HDL cholesterol level, Total cholesterol, Triglyceride (Table VII).

The animals treated with Sangu parpam of different doses did not produce any variation in Total Bilirubin level, SGOT, SGPT and ALP (Table VIII) when compared with the control group animals.

Various dose treatment of Sangu parpam after 28 days did not produce any significant deviation in and urea, Uric Acid and Creatinine (Table IX) levels when compared to the normal control levels. There were no significant observation in histopathology examination and revealed normal architecture in comparison with control and treated animal (Fig I).

Control	SP I Higher Dose (300mg/kg)	SP II Higher Dose (300mg/kg)	SP III Higher Dose(300mg/kg)
Stomach			
Heart			
Kidney			
Liver			
Lung			
Spleen			

FIG I: HISTOPATHOGY SLIDES OF CONTROL & SP I, II, III HIGHER DOSE LEVEL

DISCUSSION

The oldest form of healthcare in the world is the use of herbs as a medicine[9].·During the past decade, developed and developing countries were highly accepting the natural therapy. Because of poverty and limited access to allopathy medicine, about 80% of the world's population, uses traditional system of medicines as their source of healthcare[10].Most of the herbal preparations are safe for consumption, some herbs like most biologically active substances could be toxic[11] Mostly, without any proper scientific

evaluation herbal products are launched into the market. And also there is no effective tool to regulate manufacturing practices and standards of drug.

The standardization of herbal drug is very essential for the global acceptance. In this study Sangu Parpam which is highly recommended for the disease Peptic Ulcer in Siddha Literature is taken up for standardization methods. Proper standardization techniques, toxicological and pharmacological evaluation on these medicines to meet the criteria will support its use worldwide. Therefore, in this study an attempt has been made to evaluate the toxicological analysis of a Herbo-marine Siddha drug **Sangu Parpam.** Sangu (Conch) is a Marine origin drug. Loads of research has been conducted in drugs of plant origin but very little amount research work done in the marine origin drugs. This may help to reveal the quality and safety of the drug and will lead to universal acceptance of the drug Sangu parpam for the disease Peptic Ulcer.

Acute toxicity

SANGU PARPAM I, II, III was administered single time at the dose of 50 mg/kg, 300mg/kg, 1000mg/kg and 2000mg/kg and observed for consecutive 14 days after administration. All animals were observed daily once for any abnormal clinical signs. Weekly body weight and food consumption were recorded. No mortality was observed during the entire period of the study. Data obtained in this study indicated no physical and behavioral signs of any toxicity due to administration of SANGU PARPAM I, II, III at the doses of 50mg/kg, 300mg/kg, 1000mg/kg and 2000mg/kg.

14 days, all animals were observed for functional and behavioral examination. In functional and behavioral examination, home cage activity, hand held activity were observed. Home cage activities like body position, respiration, clonic involuntary movement, tonic involuntary movement, palpebral closure, approach response, touch response, pinna reflex, sound responses, and tail pinch response were observed. Handheld activities like reactivity, handling, palpebral closure, lacrimation, salivation, piloerection, papillary reflex, abdominal tone, limb tone were observed. Both functional and behavioral examination was normal in all treated groups.

Sub-acute toxicity

All the animals from control and all the treated dose groups up to 300mg/kg survived throughout the dosing period of 28 days. No signs of toxicity were observed in animals from different dose groups during the dosing period of 28 days. Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days. Thus food consumption and water intake of control and treated animals was found to be comparable throughout the dosing period of 28 days. Haematological analysis conducted at the end of the dosing period on day 29, revealed no abnormalities attributable to the treatment. Biochemical analysis conducted at the end of the dosing period on day 29, no abnormalities attributable to the treatment. Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls. Mild changes observed in the SP I high dose and SP II low dose. No significant changes was observed in organ weight during the treatment. But the range is with in the laboratory limit. Histopathological examination revealed normal architecture in comparison with control and treated animal.

CONCLUSION

Acute toxicity study revealed Sangu Parpam I, II, III at the doses of 50mg/kg, 300mg/kg, 1000mg/kg and 2000mg/kg to the rats did not produce drug- related toxicity. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance physical and behavioral signs of any toxicity. The maximum tolerable dose obtained from the acute toxicity study is about 2000mg/kg b.wt. Sub-acute toxicity study revealed **Sangu Parpam I, II, III** can be considered safe, as it did not cause either any lethality or adverse changes with general behaviour of rats and also there were no

observable detrimental effects (100 to 300mg/kg body weight) over a period of 28 days. Both acute and Sub-acute toxicity studies of various preparation of Sangu parpam revealed safe in animals tested. Thus the Sangu Parpam I, II, III are safe in prescribed dose level as per the literature.

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