Research Article

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PRECLINICAL TOXICOLOGICAL INVESTIGATION OF SIDDHA FORMULATION NAGA SANGU PARPAM BY ACUTE AND SUB-ACUTE REPEATED ORAL TOXICITY STUDIES IN WISTAR RATS

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ABSTRACT

Siddha, an ancient traditional practise originating from the southern region of India, encompasses a profound philosophy formulated by the esteemed siddhars, renowned ancient physicians. Siddha preparations are of several categories of which parpam based formulation grabs greater attention due to its nano size range of particles and its been widely used for management of dreadful disease in humans. The main aim of the present study is to carry out the toxicity profile of the formulation NSP using acute (OECD 423) and sub-acute (OECD 407) repeated oral toxicity studies in both male and female wistar rats in accordance with regulatory guidelines. In the acute study, a single dose of 2000 mg/kg was orally administered and experimental animals were monitored for 14 days. In the sub-acute study, repeated doses of the test drug NSP at low, mid and high dose (100, 200 & 400 mg/kg) were administered for the period of 28 days and biochemical, hematological and histopathological parameters were evaluated. Results of the acute toxicity study indicates that there was no mortality up to a maximum dose of 2000 mg/kg of NSP administered rats. No significant changes in bodyweight and other Clinical signs like skin color change, fecal consistency, gait analysis, urine analysis, sensory responses, animal behavior abnormalities, neuro muscular coordination of the NSP treated rats. In sub-acute study there was a no significant increase in liver and kidney function profile of experimental animals treated with NSP at all three dose levels. No significant pathological difference was observed in the histological examination of all the vital organs includes kidney, brain and spleen of rats treated with NSP at all three dose levels. It was concluded from the results of the acute or sub-acute oral administration of the test drug NSP that this drug may be considerably safe and may render clinical benefits in patients upon short and long term usage.

KEY WORDS: ***Siddha, Nicotiana tabacum, Naga Sangu Parpam, Toxicity profile, OECD, Acute, Sub-acute toxicity, Histopathological parameters***

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1. Introduction

Siddha system of medicine. During the passage of time it interacted with the other streams of medicines complementing and enriching them and in turn getting enriched. The materia medica of Siddha system of medicine depends to large extent on drugs of metal and mineral origin in contrast to Ayurveda of earlier period, which was mainly dependent upon drugs of vegetable origin. Siddha system also follows ashtanga concept with regards to treatment procedures, majority of the siddha preparations are derived of herbs, metals, minerals and of other animal origin [1]. These siddhars meticulously categories medicines based on their inherent properties, therapeutic requirements, and distinctive characteristics. Based on the siddha nomenclature, it is postulated that the manifestation of disease may arise from an imbalance in the fundamental humours, namely vatham, pitham, and kabam. The restoration of equilibrium holds significant potential for the amelioration of the disease and the advancement of human welfare [2-4]. The majority of siddha preparations operate based on this principle, thus raising doubts about the likelihood of disease recurrence.

Safety pharmacology is a subdivision of pharmacology which focuses on identification and characterization of pharmacological activities that affect the clinical safety of a drug. The guideline recommends assessing effects on functions of cardiovascular, central nervous and respiratory systems, which are referred as the core test battery of safety pharmacology [5]. The target organ of toxicity most frequently involved in systemic toxicity is Kidney, Liver and Spleen. Next in order of frequency of involvement in systemic toxicity are the circulatory system; the blood and hematopoietic system. Muscle and bone are least often the target tissues for systemic effects [6]. With substances that have a predominantly local effect, the frequency with which tissues react depends largely on the portal of entry (skin, gastrointestinal tract, or respiratory tract). The safety of using most of siddha preparations are not well established due to its complexity in composition, although most of this information comes from case reports rather than systematic investigations. Hence the present research work aimed at evaluating the short and long term safety of the siddha formulation Naga Sangu Parpam by acute and sub-acute toxicity studies in suitable rodent model.

2. Materials and Methods

2.1. Animal

Healthy adult Wistar albino rats were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air supported by air handling unit (AHU). A 12 light / dark cycle were maintained. Room temperature was maintained between 22 ¬± 2˚C and relative humidity 50–65%. They were provided with standard pelleted feed and water ad libitum. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of C.L.Baid Metha college of pharmacy, Chennai, Tamil Nadu, India with the IAEC approval number: IAEC NO:02/31/PO/Re/S/01/CPCSEA/dated 06/04/2022

2.2. Acute toxicity Study

The animals were fasted overnight (08- 12 hrs) with free access to water. Study was conducted with single oral administration of study drug Naga Sangu Parpam (NSP) the dose of 2000mg/kg (p.o) to experimental rats. The animals were observed continuously for first 72 h and then 14 days for emerging signs of behavioral changes, body weight changes and for mortality.

Occurrence of toxicity in animals were observed continuously for the first 4 to 24 h and observed periodically for the next 14 days. Observation includes the change in skin, fur, eyes and mucus membrane. Appearance of C.N.S, C.V.S and A.N.S related toxicity such as tremors, convulsions, sedation, steric behavior, respiratory distress, cardiovascular collapse, response to sensory stimuli, salivation, diarrhea, lethargy, sleep, coma and mortality were observed with special attention [7]. Body weight was recorded periodically. At the end of the experiment all animals were subjected

to gross necropsy and observed for pathological changes.

2.3. Sub-Acute toxicity Study

Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the start of treatment. The female rats used for the study were nulliparous and non-pregnant. The animals were randomly divided into low, mid and high dose treated groups of 18 wistar albino rats (09 males and 09 females) were selected and divided into three groups. Each group consist of 06 animals (03 Males and 03 Females). First group served as a control and other three group were treated with test drug NSP (100,200 and 400 mg/kg/day) for 28 days.

The rats were weighed periodically and observed for signs of toxicity pertains to C.N.S, C.V.S, A.N.S including behavioral changes, food - water intake and morphological changes. At the end of 28th day, the animals were fasted for overnight with free access to water. On 29th day the animals were sacrificed with excess dose of anesthesia as listed in the CPCSEA annexure. Blood samples were collected from aorta and stored in EDTA (ethylenediamine –tetra acetate) for Hematological analysis and for serum generation for biochemical analysis. The vital organs were harvested and carefully examined for gross lesions. The organs were preserved in 10% formalin for histopathological assessment and interpretation [8].

2.4. Hematological analysis

Blood samples were analyzed using established procedures using automated mindray hematology analyzer 2800. Parameters evaluated includes Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White blood cell count (WBC), Platelet Count, Hemoglobin (Hb), Mean cell Haemoglobin Concentration (MCHC), Mean Red Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean platelet volume (MPV), Neutrophils, Eosinophil's, Basophils, Lymphocytes and Monocytes.

2.5. Biochemical analysis [9]

Serum samples were analyzed for High Density Lipoprotein (HDL), Low density Lipoprotein (LDL), Very low density Lipoprotein (VLDL), Triglycerides (TGL), Total Cholesterol, Blood urea nitrogen (BUN), Creatinine, Albumin, Total Protein, Glucose, Uric acid, Aspartate Transaminase (AST), Alanine amino Transaminase (ALT) and Alkaline Phosphatase (ALP) using mind ray auto analyzer model BS 120.

2.6. Histopathological evaluation [10]

Vital organs were harvested and the histological slides of organs were made and observed under the microscope. The pathological observations of cross section of these organs were performed on gross and microscopic analysis. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological changes.

2.7. Statistical analysis [11]

The statistical analysis will be carried by one-way ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean ± standard error. A statistical comparison was carried out using the Dunnet's test for the control and treatment group. P-values less than 0.05 were set as the level of significance.

3. Results

3.1. Assessment of clinical signs in rats treated with Naga Sangu Parpam on Acute toxicity study

The dose of Naga Sangu Parpam used for acute toxicity study is 2000mg/kg is higher than the normal therapeutic dose. No mortality observed at this dose level, further no significant change with respect to clinical signs on acute toxicity observed for (24-48 h) and a long period (14 days). The results were tabulated in Table 1.

Table 1: Clinical signs in rats on Acute toxicity study

S.No	Group Control	Observation	Group Test Group	Observation
1	Body weight	Normal	Body weight	Normally increased
2	Assessments of posture	Normal	Assessments of posture	Normal
3	Signs of Convulsion	Normal	Signs of Convulsion Limb paralysis	Absence of sign (-)
4	Body tone	Normal	Body tone	Normal
5	Lacrimation	Normal	Lacrimation	Absence
6	Salivation	Normal	Salivation	Absence
7	Change in skin color	No significant color change	Change in skin color	No significant color change
8	Piloerection	Normal	Piloerection	Normal
9	Defecation	Normal	Defecation	Normal
10	Sensitivity response	Normal	Sensitivity response	Normal
11	Locomotion	Normal	Locomotion	Normal
12	Muscle gripness	Normal	Muscle gripness	Normal
13	Rearing	Mild	Rearing	Mild
14	Urination	Normal	Urination	Normal

3.2. . Quantitative data on the body weight of rats treated with Naga Sangu Parpam in Acute toxicity study

No significant change was observed in body weight of female rats treated with SC at the dose of 2000mg/ kg. The results were tabulated in Table 2.

Table 2: Body weight of rats in Acute toxicity study

DOSE	DAYS		
	1	7	14
CONTROL	186.6± 2.75	189.2± 3.87	194.2 ± 7.62
2000 mg/kg	182.5± 4.08	184.2± 2.16	187.4 ± 2.67
P value (p)*	NS	NS	NS

N.S- Non Significant,**(p > 0.01), *(p >0.05), n = 03 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.3. Quantitative data on the food and water intake of rats treated with Naga Sangu Parpam for in acute toxicity study

No statistically significant differences were recorded in food and water intake observation of rats treated with Naga Sangu Parpam at low and high dose of 100 and 200 mg/ kg b.w. The results were tabulated in Table 3.

Table 3: Food and water intake of rats in Sub-acute toxicity study

DOSE	Water intake (ml/day) DAYS			Food intake (gm/day) DAYS		
	1	7	14	1	7	14
CONTROL	28.5 ± 2.74	30.0± 9.13	32.4 ± 3.13	23.56±3.36	28.60±2.42	31.61±5.46
***Naga Sangu Parpam* 2000 mg/kg**	30.4±2.33	36.6±1.11	38.9 ± 2.19	22.42±1.64	29.31±1.22	32.22±3.24
P value (p)*	NS	NS	NS	NS	NS	NS

N.S- Non Significant,**(p > 0.01), *(p >0.05), n = 03 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.4. Effect of Naga Sangu Parpam on Body weight of Rats in Sub-acute toxicity study

No significant change was observed in body weight of both male and female rats treated with Naga Sangu Parpam at low, mid and high dose of 100, 200 and 400 mg/kg b.w.The results were tabulated in Table 4.

Table 4: Body weight of rats in Sub-Acute toxicity study

DOSE	DAYS				
	1	7	14	21	28
CONTROL	165.6± 2.76	166.4 ± 3.42	167.7 ± 3.26	169.2 ± 3.73	170.7 ± 1.31
LOW DOSE	170.2 ± 2.12	172.7 ± 3.64	174.4± 1.51	175.2 ± 1.66	176.42± 2.76
MID DOSE	176.6± 1.64	177.3 ± 2.74	179.4 ± 8.12	182.1 ± 3.36	183.7 ± 3.12
HIGH DOSE	187.4± 6.74	189.6 ± 3.72	192.6 ± 2.46	187 ± 6.81	191.92 ± 2.49
P value (p)*	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 06 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.5. Quantitative data on the food intake of rats treated with Naga Sangu Parpam for 28 days in Sub-acute toxicity study

No statistically significant differences were recorded in food intake observation of rats treated with Naga Sangu Parpam at low, mid and high dose of 100, 200 and 400 mg/kg b.w. The results were tabulated in Table 5.

Table 5: Food intake of rats in Sub-acute toxicity study

DOSE	DAYS Food intake (gm/day)				
	1	7	14	21	28
CONTROL	37.12 ±5.37	38.5±3.22	39.5±3.37	38.5±3.37	37.12±3.12
LOW DOSE	43.7±2.98	45.3±1.22	45.1±1.18	45.4±2.12	45.6±2.42
MID DOSE	47.2±3.75	47.2±3.60	47.2±4.25	47.4±2.68	49.2±2.44
HIGH DOSE	46.2±2.34	46.2±2.64	49.6±2.66	48.2±3.20	48.0±3.62
P value (p)*	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 06 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.6 Quantitative data on the water intake of rats treated with Naga Sangu Parpam for 28 days in Sub-acute toxicity study

No statistically significant differences were recorded in water intake observation of rats treated with Naga Sangu Parpam at low, mid and high dose of 100, 200 and 400 mg/kg b.w. The results were tabulated in Table 6.

Table 6: Food intake of rats in Sub-acute toxicity study

DOSE	DAYS Water intake (ml/day)				
	1	7	14	21	28
CONTROL	31.5 ± 8.95	32.0 ±6.23	28.5±6.23	29.12±8.19	31.5±3.96
LOW DOSE	28.5±3.31	26.4±3.62	26.7±3.02	22.2±3.29	34.9±3.13
MID DOSE	26.7±4.33	26.3±2.11	27.1±2.43	28.4±2.11	32.4±2.34
HIGH DOSE	30.1±1.32	30.2±2.13	32.7±2.13	35.2±1.73	38.4±2.65
P value (p)*	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 06 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.7. Effect of Naga Sangu Parpam on Hematological parameters of rats in Sub-acute oral toxicity study

No statistically significant differences were recorded in hematological parameters of rats treated with Naga Sangu Parpam at low, mid and high dose of 100, 200 and 400 mg/kg b.w. The results were tabulated in Table 7
tabulated in Table 5.

Table 7: Hematological parameters of rats in Sub-acute oral toxicity study

Category	Control	Low dose	Mid dose	High dose	P value (p)*
Haemoglobin (g/dl)	14.8±1.88	13.88±1.66	14.94±0.66	15.28±0.96	N.S
Total WBC (×10³ l)	10.91±2.59	11.25±3.73	11.48±3.91	12.20±3.17	N.S
Neutrophils(%)	32.65±1.06	33.23±2.14	35.61±1.36	35.40±2.20	N.S
lymphocyte (%)	69.34±2.48	72.12±3.12	72.48±2.66	73.10±3.16	N.S
Monocyte (%)	0.78±0.17	0.79±0.09	0.82±0.03	0.84±0.06	N.S
Eosinohil(%)	0.64±0.09	0.68±0.02	0.70±0.06	0.72±0.04	N.S
Platelets cells10^3/µl	687.17±8.76	702.71±8.16	725.18±9.0	726.16±9.74	N.S
Total RBC 10^6/µl	7.99±0.12	7.82±0.57	8.82±0.59	8.38±0.72	N.S
PCV%	37.79±0.6	43.35±1.13	45.2±1.68	46.82±2.54	N.S
MCHC g/dL	33.6±2.23	35.09±1.29	35.98±1.22	36.03±1.24	N.S
MCV fL(µm³)	49.17±3.64	50.20±1.22	52.28±1.24	53.24±1.44	N.S

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 06 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.8. Effect of Naga Sangu Parpam on Serum Bio-chemistry profile of rats in sub-acute toxicity study

No statistically significant differences were recorded in serum biochemistry parameters of rats treated with Naga Sangu Parpam at at low, mid and high dose of 100, 200 and 400 mg/kg b.w. The results were tabulated in Table 8

Table 8: Serum Bio-chemistry profile of rats in Sub-acute oral toxicity study

BIOCHEMICAL PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
GLUCOSE (R) (mg/dl)	76.45±13.4	78.16±8.44	78.26±11.20	78.42±11.6	N.S
T.CHOLOSTEROL(mg/dl)	115.26±1.83	115.45±1.83	116.42±1.78	116.22±1.73	N.S
TRIGLY(mg/dl)	46.35±1.48	46.32±1.48	44.58±1.30	45.66±1.33	N.S
LDL	72.81±2.13	71.24±2.14	72.8±2.14	71.64±4.32	NS
VLDL	15.2±2.44	15.42±4.64	15.44±6.64	15.64±4.36	NS
HDL	26.66±6.88	26.86±2.24	26.68±4.66	31.78±2.22	NS
Ratio 1(T.CHO/HDL)	4.42±2.44	4.16±3.14	4.34±8.44	4.46±2.22	NS
Ratio 2(LDL/HDL)	2.83±4.22	2.84±2.22	2.86±2.20	2.96±6.02	NS
Albumin(g/dL)	3.63±0.17	3.43±0.12	3.14±2.02	3.24±6.86	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 06 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.9. Effect of Naga Sangu Parpam on renal function profile of rats in sub-acute toxicity study

No statistically significant differences were recorded in renal function parameters of rats treated with Naga Sangu Parpam at low, mid and high dose of 100, 200 and 400 mg/kg b.w. Mild increase in the uric acid level were observed in rats treated with mid dose of the test drug. The results were tabulated in Table 9.

Table 9: Serum Renal function profile of rats in Sub-acute oral toxicity study

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
UREA (mg/dl)	13.35±0.99	14.31±0.16	13.06±1.08	13.48±1.12	N.S
CREATININE(mg/dl)	0.28±0.08	0.36±0.06	0.52±0.04	0.66±0.02	N.S
BUN(mg/dL)	15.02±0.10	16.10±0.60	16.22±0.44	18.10±2.12	NS
URIC ACID(mg/dl)	5.17±0.35	5.31±0.43	5.72±1.25*	5.58±0.23	S

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 06 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.10. Effect of Naga Sangu Parpam on liver function profile of rats in sub-acute toxicity study

No statistically significant differences were recorded in liver function parameters of rats treated with Naga Sangu Parpam at at low, mid and high dose of 100, 200 and 400 mg/kg b.w. Mild increase in the ALP level were observed in rats treated with mid and high dose of the test drug. The results were tabulated in Table 10

Table 10: Serum Liver function profile of rats in Sub-acute oral toxicity study

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
T BILIRUBIN(mg/dl).	0.48±0.07	0.53±0.06	0.51±0.08	0.48±0.05	N.S
SGOT/AST(U/L)	79.95±1.39	78.35±0.51	76.01±1.53	81.55±1.03	N.S
SGPT/ALT(U/L)	31.23±1.28	30.91±1.59	28.34±1.48	34.32±0.68	N.S
ALP(U/L)	143.25±8.70	142±16.17	147.16±24.07*	149.33±14.65*	S
T.PROTEIN(g/dL)	5.32±0.38	6.48±0.34	7.01±0.23	7.53±0.46	N.S

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 06 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.11. Effect of Naga Sangu Parpam on Histopathological changes of Male rat in Sub-acute oral toxicity study

Microscopic observation of vital organs (Kidney, Liver and Spleen) belongs of rats treated with Low, Mid and High dose of the test drug NSP representing the following architecture as shown in figure1.

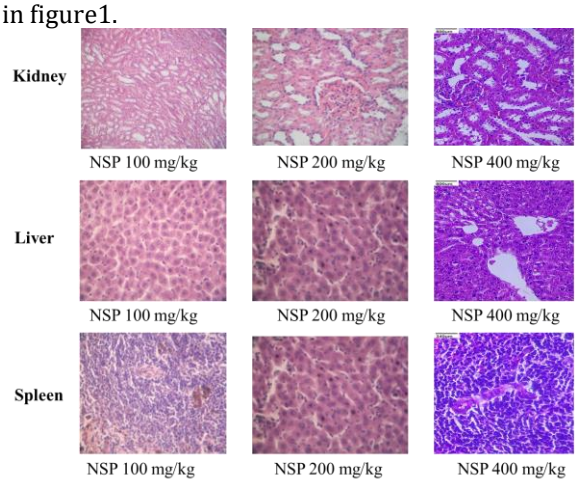

Figure 1: Histopathology of vital organs belongs to Low, Mid and high dose treated groups

4. Discussion

Ensuring safety is a key consideration for pharmaceuticals that impact human health. This emphasis on safety is also highly advocated by the World Health Organisation (WHO), prioritising it over efficacy. The primary objective of the toxicity study is to determine the safety margin of the drugs in rodents, as the siddha preparations have been widely prescribed to a large population for several years [12]. It has become imperative for researchers to provide evidence of safety in both humans and animals in order to comply with regulatory requirements.

To assess the potential health risks associated with the inherent chemical components in siddha formulations, toxicity studies are frequently employed. These studies aim to examine the adverse effects that may arise from the plant-based ingredients [13]. The early stages of toxicological assessments for herbal remedies involve the examination of acute toxicity and calculation of LD50. These evaluations yield extensive data that contribute to the toxicological categorization of traditional medicines [14].

Acute toxicity study was carried out in accordance with OECD guideline 423 by which the test drug Naga Sangu Parpam (NSP) administered at the dose of 2000mg/kg. Results of the study revealed that there is no mortality in the treated rats after post administration period of 14 days. Further there is no significant change in any of the observed parameters like body weight, food intake, water intake, social behavior, sensory and motor coordination, muscle strength, exploratory behavior etc. Sub-acute studies provide information on dosage regimens, target organ toxicity, and identify observable adverse effect that may affect the average life span of experimental animals. Consequently, in this study, the siddha drug NSP were evaluated in rats at doses of 100, 200 and 400 mg/kg for 28 days. A drastic change in body weight is a critical evaluator of toxicity and may serve as a sensitive indication of the general wellbeing of animals [1516]. The mean body weight gained by the animals in all the treatment groups may be an indication that the test drug NSP did not interfere with their normal metabolism as closely supported by the non-significant difference in this parameter when compared with the control group. The increase in body weight could be attributed to the nutritive components in their feed and the palatability of the test drug NSP.

Investigation on the haematological parameters can be used to determine the extent of the deleterious effect of foreign compounds in plant extracts on the blood constituents of an animal [17,18]. RBC and HGB counts could be an indication of toxicity appears in the blood. This implies that the morphology and osmotic fragility of the RBC, as well as HGB incorporation into the RBC. This index suggests that the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissues following treatment with the test drug NSP intact. In the present study treatment with NSP at three dose levels viz 100, 200 and 400 mg/kg reveals no significant change in any of the hematological parameters.

Renal alterations are prone to manifest in preclinical toxicity investigations due to the administration of large dosages and the role of kidney and liver in excreting several medications and their metabolites [19,20]. No statistically significant differences were recorded in renal function parameters of rats treated with Naga Sangu Parpam at low, mid and high dose of 100, 200 and 400 mg/kg b.w. Mild increase in the uric acid level were observed in rats treated with mid dose of the test drug.Serum enzymes, namely aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP), serve as valuable indicators for the detection and assessment of liver damage. The

enzymes discussed are predominantly present in the liver, red blood cells, heart, pancreas, kidneys, and biliary ducts of the liver. Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are utilised in the diagnostic process to assess the presence of tissue damage, particularly in the cardiac and hepatic systems [21].In the present investigation there is no statistically significant differences were recorded in liver function parameters of rats treated with Naga Sangu Parpam at at low, mid and high dose of 100, 200 and 400 mg/kg b.w. Mild increase in the ALP level were observed in rats treated with mid and high dose of the test drug. Microscopic observation of vital organs (Kidney, Liver and Spleen) belongs of rats treated with Low, Mid and High dose of the test drug NSP representing normal architecture with no significant pathological difference in comparison with control rats.

5. Conclusion

Based on the empirical data derived from the current research study, it can be inferred that the siddha formulation known as Naga Sangu Parpam does not exhibit toxicity across all dosages (low, mid, and high) examined in this investigation. Furthermore, no discernible symptoms were observed in the acute and sub-acute oral toxicity assessments. The histological analysis did not find any significant alterations in the internal organs of both the control and treatment groups. In addition, the data pertaining to acute and sub-acute toxicity tests conducted on this formulation were acquired with the aim of enhancing the level of assurance of its safety for human use in the context of traditional pharmaceutical development.

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