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RESEARCH ARTICLE
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ACUTE AND SUBACUTE TOXICITY OF SIDDHA FORMULATION SOODHA VALLATHI URUNDAI

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ABSTRACT

Background: Soodha vallathi urundai (SVU) is a Siddha formulation indicated for the treatment of chronic ulcers, skin diseases, arthritis, varied pains and cancers. Though this formulation is clinically effective, the degree to which substances are toxic (poisonous) for humans, animals or the environment has not yet been established. Therefore inorder, to investigate the mechanism of toxicity of this formulation, the present study was designed to evaluate the acute and sub acute toxicity of *Soodha vallathi urundai* according to OECD guidelines.

Materials and Methods: For acute toxicity rats were divided into 2 groups, with 6 rats per group. Group 1 served as the control (5% Tween-20) and the other group was treated with soodha vallathi urundai (2000mg/kg) suspended in 5% Tween-20 through oral gavage. The limit test ws performed with soodha vallathi urundai (2000mg/kg). For sub acute toxicity, the animals were divided into four groups of 6 rats in each group. Group-1 animals served as a control. Group II animals received low dose of herbal mixture 200mg/kg, Group III animals received middle dose 400 mg/kg and Group IV animals received high dose of 600 mg/kg (orally)once daily for 28 days respectively.

Results: The assessment study results showed that, no significant changes were observed in the haematological and biochemical parameters, relative organ weight, gross necropsy and histopathological examination with *Soodha vallathi urundai*

Conclusion: The rWHesults of the present study suggest that *Soodha vallathi urundai* is safe and non-toxic at its prescribed therapeutic dosage..

Key words: *Soodha vallathi urundai,* Siddha, Herbomineral formulation, Sub acute-toxicity study

INTRODUCTION

Siddha system of medicine is the ancient form of health care system known to mankind. Nearly 80% of the world's population utilize the benefits of herbal medicine [1]. Indeed, about 25% of prescription drugs contain at least one active ingredient derived from plant material [2, 3]. Plant derived medicines are used in all civilizations and cultures and, hence, plants have always played a key role in health care systems worldwide. In most developing countries, the indigenous modes of herbal treatment are parts of the culture and the dominant method of healing therapy. Though these remedies, have a considerable extent of effectiveness, and are socially accepted and economically viable and, mostly, are the only available source [4, 5].

As a continuum, more scientific research are being directed towards the identification and isolation of bioactive principles from natural sources. The different process variables employed in the development of such products resulted in qualitative or quantitative changes in the chemical profiles of the plant material causing the alteration of the biological profile [1, 2].Despite the development of successful drugs from natural products, it is always questionable about the side effects due to lack of scientific validation.Most of the researches focus on optimizing and advancing the extraction methods so as to extract maximum biological active material. However, assessing the toxicity profile of such products is of vital importance [3]. It becomes particularly essential since most people have a general belief that all natural products are safe and free to access and consume since most of these products are marketed as supplements [4]. Hence it is imperative to have proper chemical, toxicological, and safety data for the usage of plants with traditional claims on health benefits.

Hence in this research, the study drug *Soodha vallathi urundai was subjected to* acute oral toxicity and sub acute toxicity to shed light on the general safety of a substance. In acute toxicity study, generally, a single high dose of the material is administered to animals and they are observed for behavioral, motor-neuronal changes and mortality [5].

The median lethal dose (LD50) could be calculated so as to decide the safety window for usage. This present study is aimed at evaluating the acute oral toxicity of Siddha formulation namely *soodha vallathi urundai*. In Sub acute oral toxicity, the low dose, mid dose and high dose of SVU was fixed based on the dose conversion of animal dose from human dose and the toxicity was observed for a period of 28 days.

Materials and methods Animal Housing

Eight-week-old albino wistar rats of both sexes, weighed 150–180 g for acute oral toxicity and 180 200 g for sub acute toxicity [6] Animals were purchased from Animal house, K.M.College of Pharmacy,Madurai. All rats were acclimatized to the laboratory condition for a period of one week prior to dosing. The rats were housed in air conditioned room at 24 ± 2 ◦C on a 12/12h light-dark cycle. Three rats were housed per polycarbonate cage and had free access to rodent chow (Hindustan chow ltd Bangalore) and water *ad libitum*. All the animal experimental protocols were approved (IAEC/REVATHY M/TNMGRMU/Ph.D/M.D(S)/KMCP/137/2021-22) by Institutional Animal ethical committee(IAEC), K.M.College of Pharmacy,Madurai. All efforts were made to minimize suffering and distress of rats.

Grouping of animals Acute Toxicity Study

The rats were divided into 2 groups, with 6 rats per group. Group 1 served as the control, and the other group was treated with Siddha formulation namely *Soodha vallathi urundai* at a dose of 2000mg/kg. The control group was given 5% Tween-20, while the treatment groups received Siddha formulation namely *Soodha vallathi Urundai* at 2000mg/Kg body weight (BW) suspended in 5% Tween-20 through oral gavage. A 2000mg/Kg BW dose of Siddha formulation namely *Soodha vallathi Urundai* was chosen for the limit test in accordance with Organization for Economic

Cooperation and Development (OECD) guidelines for the Testing of Chemicals [7]. The administered volume was adjusted to 3 mL/Kg BW for every rat. The vehicle and the Siddha formulation namely *Soodha vallathi Urundai w*ere administered only once (on day 0) at the start of the experiment, and the rats were monitored for 14 days.

Sub acute toxicity study (28 days repeated oral toxicity study)

Experimental animals were divided into four groups of 6 rats were placed in cages. Set 1 served as control (i.e. the rats were fed without ayurvedic preparation), then the groups 2to 4 were daily fed by oral administration of Siddha formulation *Soodha vallathi Urundai* at different doses (200, 400 & 600 mg/kg) for 28 days. On day 28, the rats were anaesthetized using ether after fasting for the night while blood samples were taken for biochemical and haematological analyses using both EDTA and plain vials while the brain,heart,kidney and liver were harvested for histological assessment.The study was done according to the OECD guidelines 407 [8]

Observations

Cage Side Observation and Body Weight.

The general behavior, body weight, and feed-water intake of the rats were observed during the acclimatization period. After administration of the Siddha formulation namely soodha vallathi urundai, each rat was continuously monitored for the first hour followed by every 2 hours till 12 h and then every day for 14 days in acute toxicity study and 28 days in subacute toxicity study. The monitored parameters included properties of skin and fur, eyes, respiratory pattern, autonomic nervous system features such as salivation, diarrhea, and urination, central nervous system features such as tremors, ptosis, relaxation, changes in the level of activity, gait, and posture, and any other abnormal behavior[9] The food and water intake pattern of the animals was observed throughout the study period. The weight of the animal was recorded on alternate days in acute oral toxicity and weekly basis in subacute toxicity and the individual record on all observations was maintained for each rat.

Relative organ and body weights study

The changes in body weights were recorded on a weekly basis, while the organs (the liver, spleen, kidneys, brain and heart) were weighed using standard weighing balance to calculate relative organ weight for the different sets on the sacrifice day[10].

Relative organ weight (%) = [Absolute weight of organ (g)/weight of rat on sacrifice day (g)] x100
The following Hematological, biochemical and Histopathological analysis was cariied out in both acute and sub acute toxicity to assess its safety.

Hematological Analysis

At the end of the study, the animals were fasted overnight but were allowed water *ad libitum*. The blood drawn by cardiac puncture method was collected into plain tubes and Vacutainer coated with EDTA.The blood sample collected to EDTA coated tube was used in analysis of hematological parameters.

Biochemical Analysis

At the end of the study, the blood sample collected into the plain tubes was left at 4∘C for 3 h and then was centrifuged at 3000 rpm for 10 minutes to separate the serum. Biochemical studies were carried out using standard methods for aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and conjugated bilirubin, total protein, albumin, urea and creatinine. The serum

electrolytes (Sodium, potassium, chloride and bicarbonate ions) were estimated using an automated ion selective electrode machine (Audicom electrolyte analyser).

Histopathology

The rats were painlessly killed under ether anesthesia and the organs (liver and kidney) were harvested for histopathological examination. The weight of the organs was recorded after being washed with normal saline and dried using blotting paper. The relative weight index of each organ to its body weight was calculated by the formula: (weight of organ/bodyweight of rats on the day of sacrifice) × 100%[10].The organs were then fixed in 10% formalin. The fixed tissues were embedded and cut into 5 μm thick sections. The hematoxylin and eosin stained sections were observed under light microscope.

For sub acute toxicty study, on 28th day, the brain, heart, kidney and liver excised from the sets administered with the Siddha formulation Soodha vallathi urundai and the control groups were collected and weighed and quickly set in 10% neutral buffered formalin at pH 7.4 and developed for histological studies. Following fixation, tissues were cleansed in graded series of alcohol, washed in xylene, inserted into paraffin, segmented by a microtome (5-µm thin) and tainted with dye in glass slides. Segments were viewed by a standard microscope (at X 100 and X 400) magnification. Statistical Analysis. One-way analysis of variance (ANOVA) was performed using GraphPad Prism V 3.0 (GraphPad Software Inc., San Diego, CA, USA); Newmann keuls multiple range tests was chosen as *post hoc* analysis method where $p \leq 0.05$ was considered to be statistically significant. The results were expressed as mean ± SEM[11].

RESULTS Acute Toxicity Study

All the treatment group rats recorded normal behavioral, motor, and neuronal functions for the administered Siddha formulation namely *Soodha Vallathi Urundai* with no mortality observed.

Table 1: Effect of Siddha formulation SVU on body weight(gm) in rats during 14-day oral acute toxicity study.

Groups	Treatment	Day- 1	Day- 7	Day- 14
G1	Normal control	182.35±4.55	194.40±4.40	218.20±5.15
G2	SVU (2000mg/kg)	185.70±4.75	208.15±4.70	230.20±5.35

Data are expressed as mean ± standard error of the mean (SEM)

Table 2. Effect of Siddha formulation SVU on food intake(g/rat/day) in rats during 14-day oral acute toxicity study.

Groups	Treatment	Day- 1	Day -(2-7)	Day -(8-14)
G1	Normal control	133.5±3.50	157.5±4.20	162.50±4.40
G2	SVU (2000mg/kg)	129.5±3.35	151.35±3.75	158.40±4.65

Data are expressed as mean ± standard error of the mean (SEM) and analyzed by one-way ANOVA, followed by newmann keuls multiple range tests ($p < 0.05$) as compared to that of control groups.

Table 3. Effect of Siddha formulation SVU on water intake(ml/rat/day) in rats during 14-day oral acute toxicity study.

Groups	Treatment	Day- 1	Day -(2-7)	Day -(8-14)
G1	Normal control	87.8±2.20	89.0±2.25	91.5±2.40

G2	**SVU (2000mg/kg)**	81.4±1.95	86.5±2.12	88.0±2.10

Data are expressed as mean ± standard error of the mean (SEM) and analysed by one-way ANOVA, followed by newmann Keuls multiple range tests ($p < 0.05$) as compared to that of control groups.

Table 4. Effect of Siddha Formulation SVU on haematological parameters in rats during 14day oral acute toxicity study.

Parameters	**Unit**	**Normal control**	**SVU (2000mg/kg)**
WBC	10^9/L	5.95 ± 0.68	6.12 ± 0.80
RBC	10^{12}/L	5.50 ± 0.65	5.65 ± 0.80
PLT	10^9/L	925.8 ± 50.15	882.35± 53.75
LYM	10^9/L	5.25 ± 0.50	5.45 ± 0.65
Hb	g/dL	12.45 ± 0.40	12.25 ± 0.40
HCT	%	40.20 ± 2.35	37.9 ± 2.10
MCV	fL	66.80 ± 2.80	66.70 ± 3.15
MCH	pg	23.20 ± 1.15	22.8 ± 1.10

Data are expressed as mean ± standard error of the mean (SEM) and analysed by one-way ANOVA, followed by newmann Keuls multiple range tests ($P < 0.05$) as compared to that of control groups.

Table 5. Effect of Siddha Formulation SVU on serum biochemical parameters in rats during 14-day oral acute toxicity study.

Parameters	**Unit**	**Normal control**	**SVU**
Urea	(mmol/L)	6.38 ± 0.34	5.95± 0.35
Creatinine	(μmol/L)	44.25 ± 1.40	43.45 ± 1.25
Glucose	(mmol/L)	10.32 ± 0.75	9.90 ± 0.65
Total protein	g/L	54.20 ± 2.15	53.80 ± 1.90
ALT	(U/L)	46.40 ± 1.88	47.45 ± 2.15
AST	(U/L)	182.7 ± 4.75	185.4 ± 4.70
Bilirubin	(mg/dL)	0.485 ± 0.22	0.445 ± 0.12

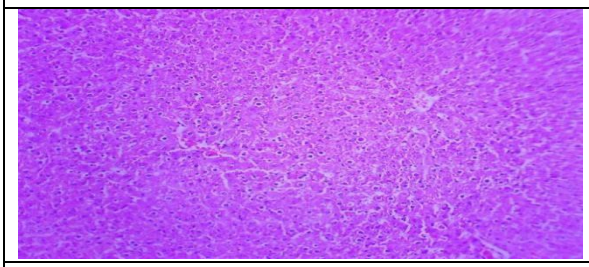
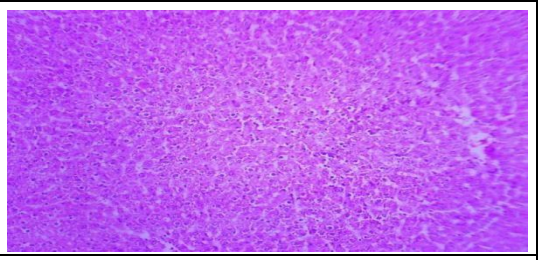
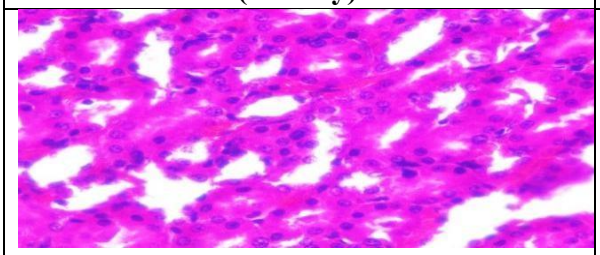
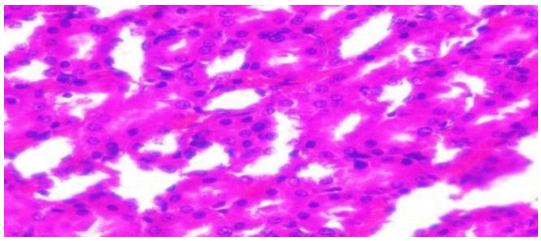
Data are expressed as mean ± standard error of the mean (SEM) and analysed by one-way ANOVA, followed by newmann Keuls multiple range tests ($p < 0.05$) as compared to that of control groups.

Table 6. Effect of siddha formulation *SVU* on relative organ weight(gm) in rats during 14-day oral acute toxicity study.

Organs	**Normal control (gm)**	**SVU(gm)**
Liver	4.98 ± 0.47	4.85 ± 0.40
Kidney	0.83 ± 0.12	0.79 ± 0.10
Heart	0.155±0.018	0.145±0.011
Stomach	1.12 ± 0.10	0.97 ± 0.05
Spleen	0.228±0.045	0.230±0.054
Testis	0.93 ± 0.12	0.95 ± 0.12
Ovary	0.45 ± 0.04	0.54 ± 0.08

Data are expressed as mean ± standard error of the mean (SEM) and analysed by one-way ANOVA, followed by newmann Keuls multiple range tests ($p < 0.05$) as compared to that of control groups. The microscopic evaluation of the tissue sections of SVU (2000mg/kg) treated rats did not show any lesions or abnormal histopathological changes as compared to their respective control groups. The organs retained normal texture and appearance on gross examination.

Figure-1. Effect of SVU on histopathology of liver and kidney in Acute toxicity study

Normal control (Liver)	**Treatment control (Liver)(SVU 2000MG/KG)**
	
Normal control (Kidney)	**Treatment control (Kidney)**
	

Subacute toxicity study

The subacute toxicity study of the tested Siddha formulation Soodha vallathi urundai was determined as per OECD guideline 407. All study animals given Siddha formulation Soodha vallathi urundai daily at all study doses (SVU 200,400&600 mg/kg) survived the entire 28-day period.

Table7-Effects of the Siddha formulation SVU on food and water intakes in subacute toxicity study.

S.No	Treatment	Average food intake (g/d)	Average water intake (ml/d)
1.	NormalSaline 10ml/kg	14.85± 1.60	18.55±2.70
2.	SVU (200mg/kg)	19.25± 2.20	23.50±2.85
3.	SVU (400mg/kg)	18.30± 1.80	19.40 ±2.60
4.	SVU (600mg/kg)	17.95± 1.65	22.30 ±2.90

Values are expressed as mean ± standard deviation; $n = 6$. No significant difference between Siddha formulation Soodha vallathi urundai treatment groups and control group ($P > 0.05$)

Table 7 depicts the effects of the Siddha formulation Soodha vallathi urundai on the food and water intakes in subacute treatment. The single daily administration of the Siddha formulation Soodha vallathi urundai at study doses (200,400 and 600 mg/kg) for 28 d caused no significant changes ($P > 0.05$) in food and water intakes when compared with the control group.

Table-8.Effects of the Siddha formulation SVUon the relative weight of organs in rats

Treatment	Relative organ weight (%)					
	Heart	Liver	Lungs	Kidney	Spleen	brain
Normal saline(10ml/kg)	0.90±0.75	8.30±0.85	1.25±043	2.36±0.90	0.70±0.28	1.28±0.11
SVU (200mg/kg)	0.93±0.13	9.15±1.2	1.16±0.35	2.22±0.75	0.65±0.25	1.45±0.20
SVU (400mg/kg)	0.95±0.17	9.05±1.4	1.30±0.48	2.24±0.79	0.68±0.30	1.35±0.15
SVU (600mg/kg)	0.88±0.12	9.30±1.5	1.25±0.45	2.28±0.75	0.74 ±0.31	1.31±0.12

Values are expressed as mean ± standard deviation; $n = 6$. No significant difference between Siddha formulation Soodha vallathi urundai treatment groups and control group ($P > 0.05$)

Table -9. Effects of the Siddha formulation SVU on the body weights.

Treatment	Body weight (Gm)				
	0 Days	7 Days	14 Days	21 days	28 Days
Normal saline(10ml/kg)	186.20±4.20	190.80±4.65	193.40±4.90	197.30± 4.80	205.30±5.20
SVU (200mg/kg)	197.90±4.55	198.40±4.75	205.30±5.25	215.60 ±5.35	225.45 ±5.40
SVU(400mg/kg)	189.60±4.35	195.35±4.50	198.20±4.70	212.45 ±4.90	222.30 ±5.10
SVU (600mg/kg)	194.50±4.50	197.45± 4.70	203.50±4.90	213.90 ±5.05	225.35 ±5.20

Values are expressed as mean ± standard deviation; $n = 6$. No significant difference between Siddha formulation Soodha vallathi urundai treatment groups and control group ($P > 0.05$)

Table-10. Effects of the Siddha formulation SVU on haematological parameters of treated rats

Treatment	Control	SVU(200mg/kg)	SVU (400mg/kg)	SVU (600mg/kg)
WBC($\times 10^9$/L)	9.18 ± 1.07	9.32 ± 1.18	9.55 ± 1.25	10.65 ± 1.35
RBC($\times 10^{12}$/L)	7.82 ± 0.41	7.95 ± 0.48	8.14 ± 0.55	8.20 ± 0.58
Hb (g/dL)	14.90 ± 1.38	15.55 ±1.47	14.80± 1.28	15.70± 1.50
MCV (fL)	70.70 ± 2.80	67.25 ± 2.65	64.25 ± 2.40	66.80 ± 2.55
MCH (pg)	18.80 ± 1.45	18.95 ±1.60	18.90 ± 1.85	19.20 ± 1.58
PCV ($\times 10^9$/L)	753.15±21.30	777.40 ± 21.35	773.35 ± 20.20	763.25 ± 21.40
Neutrophils ($\times 10^9$/L)	0.45 ± 0.12	0.62 ± 0.15	0.65 ± 0.24	0.71 ± 0.25
Lymphocytes ($\times 10^9$/L)	6.35 ±0.60	6.52 ± 0.98	6.45 ± 0.88	6.55 ± 0.95
Haematocrit (L/L)	0.61 ± 0.12	0.56 ± 0.12	0.57 ± 0.11	0.64 ± 0.15

Values are expressed as mean ± standard deviation; $n = 6$. No significant difference between Siddha formulation Soodha vallathi urundai treatment groups and control group ($P > 0.05$)

Table-11. Effects of the ayurvedic preparation Siddha formulation SVU on some serum biochemical parameters in sub-acute toxicity study of treated rats

Treatment	Control	SVU(200mg/kg)	SVU (400mg/kg)	SVU (600mg/kg)
Sodium (mmol/L)	135.40 ± 4.40	143.70 ± 5.15	142.35 ± 4.70	147.85 ± 5.40
Chloride (mmol/L)	108.45 ± 3.75	105.30 ± 3.50	106.80 ± 3.70	109.75 ± 3.45

Urea (mmol/L)	4.38 ± 0.37	4.80 ± 0.55	4.63 ± 0.44	4.84 ± 0.62
Creatinine (µmol/L)	33.35 ± 1.80	32.75± 1.53	31.85 ± 1.40	34.10 ± 1.95
Glucose (mmol/L)	5.22 ± 0.64	4.93 ± 0.48	5.23 ± 0.77	5.40 ± 0.80
Calcium (mmol/L)	2.63 ± 0.20	2.64 ± 0.30	2.68 ± 0.35	2.55 ± 0.20
Magnesium (mmol/L)	0.96 ± 0.07	1.10 ± 0.20	1.24 ± 0.25	1.22 ± 0.24
Uric acid (mmol/L)	0.20 ± 0.06	0.26 ± 0.15	0.24 ± 0.11	0.27 ± 0.15
Total protein (g/L)	53.50 ± 2.90	53.50 ± 2.94	54.35 ± 3.22	54.35 ± 3.25
Albumin (g/L)	19.70± 0.46	19.45 ± 0.40	18.75 ± 0.45	19.65 ± 0.54
Total bil (µmol/L)	16.80 ± 3.45	16.35 ± 3.60	16.95 ± 3.35	16.24 ± 3.10
ALT (U/L)	61.80 ±3.25	61.65 ± 3.55	63.70 ± 3.40	63.12 ± 3.75
AST (U/L)	163.40 ±4.25	159.45 ±4.22	162.25 ±4.10	158.25 ±4.15
ALP (U/L)	253.40 ±5.40	259.30 ±5.80	258.35 ±5.70	264.40 ±5.85
Total cholesterol(mmol/L)	1.12 ± 0.10	1.18 ± 0.21	1.22 ± 0.24	1.24 ± 0.25
TAG (mmol/L)	2.35 ± 0.48	1.87 ± 0.35	1.98 ± 0.43	1.93 ± 0.42
HDL cholesterol(mmol/L)	0.75 ± 0.09	0.84 ± 0.15	0.87± 0.21	0.91 ± 0.19

Values are expressed as mean ± standard deviation; $n = 6$. No significant difference between Siddha formulation Soodha vallathi urundai treatment groups and control group ($P > 0.05$)

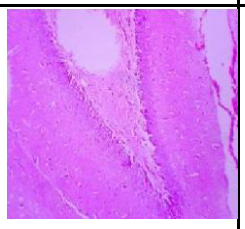
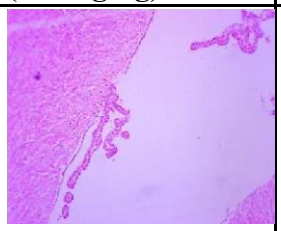
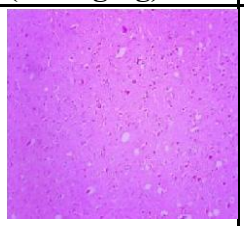
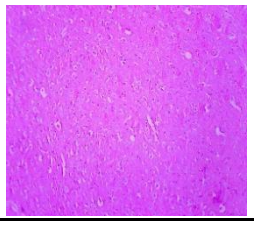
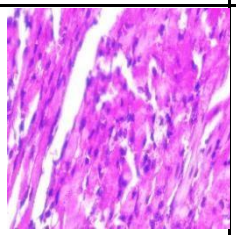
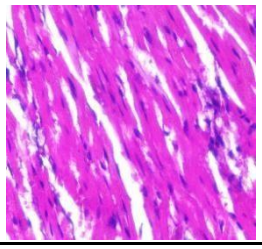
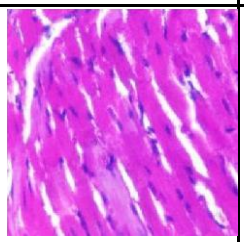
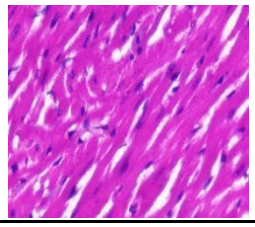
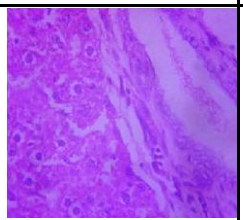
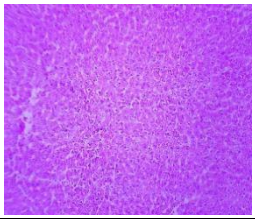
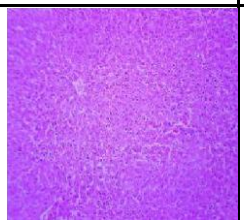
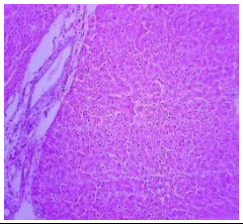
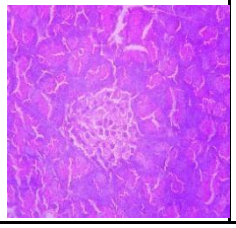
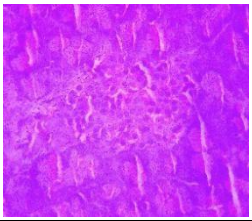
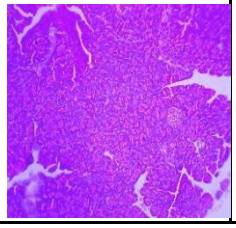
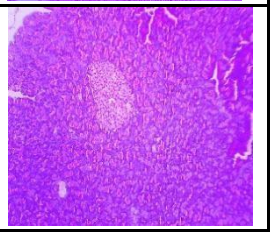
Organs	**Normal control**	**SVU (200mg/kg)**	**SVU (400mg/kg)**	**SVU (600mg/kg)**
BRAIN				
HEART				
LIVER				
PANCREAS				

Figure-2. Effect of SVU on Histopathology of vital organs in Sub acute toxicity study

Histopathological examinations were performed on the liver, kidney,brain,pancreas and heart, to assess whether or not organs or tissues had been damaged. We found that none of the organs of the rats given daily Siddha formulation Soodha vallathi urundai at a dose of (200,400 and 600 mg/kg) showed any morphological alterations or abnormalities under the light microscope

Discussion

The present study focused on acute toxicity evaluation of Siddha Formulation *Soodha Vallathi Urundai*. The 14-day acute toxicity study of the said formulation did not cause any mortality or behavioral, motor-neuronal abnormalities in rats. The monitoring of skin and fur, eyes, behavioural pattern such as gait and posture and autonomic and central nervous system activities of treatment rats remained unchanged with the treatment of Siddha formulation namely *Soodha vallathi Urundai* when compared with those of control group. This showed that the oral LD50 of Siddha formulation namely *Soodha vallathi Urundai* was greater than 2000mg/Kg body weight.

The monitoring of body weight and food/water consumption of the experimental animals is important while studying the toxicity and safety of a natural product since it denotes the physiological and metabolic status of the animals and its nutrition. In the present study, food and water intake of the rats from the time of acclimatization till the end of the experiment followed a common and steady pattern. None of the experimental groups suffered loss in weight or gained overweight which suggested that the Siddha formulation *Soodha Vallathi Urundai* did not induce significant changes in the appetite and did not exert any deleterious effects on the general health status and metabolic growth of the rats. It was also noted that the The pattern of body weight and feed consumption was not altered significantly ($P>0.05$).Therefore the Siddha Formulation *Soodha Vallathi Urundai* which suggested that the formulations did not induce any deleterious effects on growth and development of the rats[12].

In Subacute toxicity study, after 28 days of treatment, all the animals exhibited a steady increase in body weight from 186.20±4.20gm of normal control group rats to 194.50±4.50 gm. It indicates that the daily intake of the Siddha formulation *Soodha vallathi Urundai* at a dose of (200,400 and 600 mg/kg) did not alter food intake. Furthermore, it possibly shows that weight gain and appetite stability were not impeded by the Siddha formulation *Soodha vallathi Urundai* at a dose of (200,400 and 600 mg/kg) during the exposure period.

The relative organ weight index is used as another basic indicator to assess the deleterious effects of the formulation's active metabolites. The relative organ weight gives a preliminary insight to the swelling or damage caused by any harmful agent[11] The toxic effect of ingested herbal remedies in the body is most likely to be felt by important organs such as the spleen, heart, liver and kidneys because of the vital roles that they play in the body[13] The liver and kidneys are major targets of xenobiotic action, with the liver being the main organ for xenobiotic biotransformation, while the kidney serves as excretory organ of xenobiotics [14] In this study, the relative organ weight of liver, kidneys, Heart, stomach, spleen, testis, or ovary were determined and there was no significant changes ($P> 0.05$) in relative organ weight as compared with their respective control groups. An increase in organ weight suggests the occurrence of hypertrophy while a decrease suggests necrosis in the target organ[15]

In subacute toxicity study, our findings revealed that there was no significant increase in organ weight (Table 2), suggesting that the Siddha formulation *Soodha Vallathi Urundai* at a dose of (200,400 and 600 mg/kg) was not toxic to the animals at the tested doses.The assessment on the hematological parameters is important as it can point directly to the systemic effects caused by the administered formulations. Haematopoiesis is the process of blood cell formation. Changes in the haematopoietic

system have a higher predictive value for human toxicity when data are translated from animal studies[16] From the results of acute toxicity study, the hematological profile of all the treated rats showed no significant difference (P> 0.05) in comparison with the control group.In sub acute toxicity study, administration of Siddha formulation Soodha vallathi urundai at a dose of (200,400 and 600 mg/kg) in rats for a period of 28 days produced no significant change in all blood parameters except an increase in lymphocytes and mean platelets volume[14].

Several important biochemical parameters were also included in this toxicity study. The primary organs prone to the toxic effects of medicines are the kidney and liver.The kidney function parameters such as serum creatinine, urea, and total protein were determined, while the level of AST and ALT was determined to assess the liver function.The results of the acute toxicty study suggested that the kidney and liver functions were not altered for Siddha Formulation *Soodha Vallathi Urundai* treated rats.

There were no statistically significant differences in creatinine, urea, total protein, AST, and ALT levels between controls and treated animals ($P > 0.05$). Hence, these findings suggest that Siddha Formulation *Soodha Vallathi Urundai* did cause any deleterious effects on kidney and liver of the rats.The role of liver and kidney functions that are important for survival of animals can be measured by serum biochemical analysis, which are crucial in the toxicological evaluation[17].

The liver enzymes (aminotransferases; ALT and AST) describe its cellular integrity, while albumin and total protein levels describe its functionality[18] AST and ALT are principally produced by the liver cells and any assault to the liver may lead to an increase in the serum level of these enzymes. High levels of liver enzymes are signs of hepatocellular toxicity[19], whereas a decrease may indicate enzyme inhibition[20].ALT found in abundance in kidneys, testes, cardiac and skeletal muscles[21] is the most sensitive marker of liver damage or toxicity. The functionality of the liver was assessed by the serum total protein, bilirubin and albumin.A high serum level of urea indicates that the kidneys may not be working properly, or that the animal is dehydrated whereas, low urea levels are seen in acute liver failure or overhydration[22]. Creatinine clearance, an indicator of glomerular filtration rate is used for assessing kidney function. A reduction in serum levels of total proteins, bilirubin and albumin depicts reduced synthetic function, which is evident in liver damage or diseases. Our study showed a non-significant change in total protein and albumin serum levels from the control group ($P > 0.05$). In Sub acute toxicity, the Siddha formulation *Soodha vallathi Urundai* at a dose of (200,400 and 600 mg/kg) did not cause any significant change in the creatinine levels when compared with the control suggesting that the Siddha formulation *Soodha vallathi Urundai* at a dose of (200,400 and 600 mg/kg) may not be toxic to the kidney.

Histopathological studies were conducted on kidney and liver of all the rats. In acute toxicity study, the gross examination of the organs did not show any signs of necropsy and abnormal morphological changes. The microscopic examination of the hematoxylin eosin stained tissue sections also recorded insignificant changes as compared with the control rats' tissues.Histology of the kidneys in sub acute toxicity study the rats did not produce any toxic changes confirming the safety of the Siddha formulation Soodha vallathi urundai at a dose of (200,400 and 600 mg/kg) in the vital organs[23][24]. The results from this acute oral toxicity study suggested that the Siddha Formulation Soodha Vallathi Urundai are relatively nontoxic and the no-observed-adverse-effect level (NOAEL) of Siddha Formulation *Soodha Vallathi Urundai* was determined as 2000mg/Kg body weight/day. In the subacute toxicity study, no deaths were recorded after oral administration of 200, 400 and 600 mg/kg for 28 d. The animals did not present any behavioral changes when subjected to Hippocratic screening for this experimental period (28 d). There were no significant changes in food or water consumption among treated rats throughout the 28-day study, which is an indicator that the diet and water were well tolerated by the animals..

Conclusion

The results of the acute toxicity study indicate that the Siddha formulation Soodha vallathi urundai both at a dose of (200,400 and 600 mg/kg) administered through oral route to rats, using the up and

down method of acute toxicity testing did not produce any sign of toxicity and death in the animals. According to OECD criteria under its Globally Harmonised Classification System (GHS) for chemical substances and mixtures, substances with LD50>2000 to 5000mg/kg are categorised as unclassified or category 5 . The Sub acute toxicity showed that the administration of the Siddha formulation Soodha vallathi urundai at a dose of (200,400 and 600 mg/kg) to Wistar rats was not toxic in any of the tested doses and no evident histopathological damage in the vital rat organs, regardless of gender. Furthermore, the results obtained from both acute and subacute toxicity studies of Siddha formulation Soodha vallathi urundai *could* thus give insight to its safety in humans.

REFERENCES

1. Subbarayappa BV. Siddha medicine: an overview. The Lancet. 1997 Dec 20;350(9094):1841-4.
2. Fowler MW. Plants, medicines and man. Journal of the Science of Food and Agriculture. 2006 Sep;86(12):1797-804.
3. Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular aspects of Medicine. 2006 Feb 1;27(1):1-93.
4. Norrman J, Söderqvist T, Volchko Y, Back PE, Bohgard D, Ringshagen E, Svensson H, Englöv P, Rosén L. Enriching social and economic aspects in sustainability assessments of remediation strategies–Methods and implementation. Science of the Total Environment. 2020 Mar 10;707:136021.
5. Mench M, Lepp N, Bert V, Schwitzguébel JP, Gawronski SW, Schröder P, Vangronsveld J. Successes and limitations of phytotechnologies at field scale: outcomes, assessment and outlook from COST Action 859. Journal of Soils and Sediments. 2010 Sep;10:1039-70.
6. Sharada AC, Solomon FE, Devi PU. Toxicity of Withania somnifera root extract in rats and mice. International journal of pharmacognosy. 1993 Jan 1;31(3):205-12.
7. Brunda S, Nithya K, Anbu N, Kanakavalli K. Acute and Sub-Acute Toxicity Evaluation of Siddha Formulation Kalingathi Ennai In ACCordance With OECD Guideline. Int. J. Adv. Res. Biol. Sci. 2018;5(9):92-100.
8. Brunda S, Nithya K, Anbu N, Kanakavalli K. Acute and Sub-Acute Toxicity Evaluation of Siddha Formulation Kalingathi Ennai In ACCordance With OECD Guideline. Int. J. Adv. Res. Biol. Sci. 2018;5(9):92-100
9. Ibrahim MB, Sowemimo AA, Sofidiya MO, Badmos KB, Fageyinbo MS, Abdulkareem FB, Odukoya OA. Sub-acute and chronic toxicity profiles of Markhamia tomentosa ethanolic leaf extract in rats. Journal of ethnopharmacology. 2016 Dec 4;193:68-75.
10. Sellers RS, Mortan D, Michael B, Roome N, Johnson JK, Yano BL, Perry R, Schafer K. Society of Toxicologic Pathology position paper: organ weight recommendations for toxicology studies. Toxicologic pathology. 2007 Aug;35(5):751-5.
11. Yam MF, Lim CP, Fung Ang L, Por LY, Wong ST, Asmawi MZ, Basir R, Ahmad M. Antioxidant and toxicity studies of 50% methanolic extract of Orthosiphon stamineus Benth. BioMed research international. 2013;2013(1):351602.
12. Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, Johnson JK, Schafer K. Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. Toxicologic pathology. 2007 Aug;35(5):742-50.
13. Ezeja MI, Anaga AO, Asuzu IU. Acute and sub-chronic toxicity profile of methanol leaf extract of Gouania longipetala in rats. Journal of ethnopharmacology. 2014 Feb 12;151(3):1155-64.
14. Hoff-Brand AV, Pettit JE. Hematological parameters in essentials of haematology. 4th ed. New Jersey: Blackwell Science; 2000.
15. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90-day oral gavage toxicity study of d-methylphenidate and d, l-methylphenidate in Sprague–Dawley rats. Toxicology. 2002 Oct 15;179(3):183-96.

16. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M. Concordance of the toxicity of pharmaceuticals in humans and in animals. Regulatory toxicology and pharmacology. 2000 Aug 1;32(1):56-67.
17. Bariweni MW, Yibala OI, Ozolua RI. Toxicological studies on the aqueous leaf extract of Pavetta crassipes (K. Schum) in rodents. Journal of Pharmacy & Pharmacognosy Research. 2018;6(1):1-6.
18. Adeoye BA, Oyedapo OO. Toxicity of erythrophleum guineense. African Journal of Traditional, Complementary and Alternative Medicines. 2004;1(1):45-54.
19. Brautbar N, Williams II J. Industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms. International journal of hygiene and environmental health. 2002 Jan 1;205(6):47991.
20. Kazeem M. Hypolipidemic and toxicological potential of aqueous extract of Rauvolfia vomitoria Afzel root in wistar rats. J Med Sci. 2013 May 15;13:253-60.
21. Friedman LS, Martin P, Munoz SJ. Liver function tests and the objective evaluation of the patient with liver disease. Hepatology: a textbook of liver disease. 1996;1:791-833.
22. Lum G, Leal-Khouri S. Significance of low serum urea nitrogen concentrations. Clinical chemistry. 1989 Apr 1;35(4):639-40.
23. El Hilaly J, Israili ZH, Lyoussi B. Acute and chronic toxicological studies of Ajuga iva in experimental animals. Journal of ethnopharmacology. 2004 Mar 1;91(1):43-50.
24. Raina P, Chandrasekaran CV, Deepak M, Agarwal A, Ruchika KG. Evaluation of subacute toxicity of methanolic/aqueous preparation of aerial parts of O. sanctum in Wistar rats: Clinical, haematological, biochemical and histopathological studies. Journal of Ethnopharmacology. 2015 Dec 4;175:509-17.