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Research article

Evaluation of acute and sub-acute toxicity of *sivanar amirtham* in albino mice and wistar albino rats

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ABSTRACT

The objective of present study is to evaluate the acute and sub-acute toxicity of Sivanar Amirtham in Swiss albino mice and Wistar albino rats. A single dose of extract (2000 mg/kg) was given orally to the animals in the acute study, and they were observed for 14 days. In the sub-acute investigation, biochemical, haematological, and histological parameters were assessed after successive doses of Sivanar Amirtham (100, 200, and 400 mg/kg/day) were given for 28 days. After a single or repeated dosage of Sivanar Amirtham, there was no evidence of toxicity or mortality. Sivanar Amirtham median lethal dose (LD50) was determined to be greater than 2000 mg/kg. When comparing the weights of the treatment and control groups, the Sivanar Amirtham caused no significant differences in body weights or organ weights. Most biochemical and haematological markers were within normal limits. Only the Sivanar Amirtham doses of 200 mg/kg and 400 mg/kg caused significant increases in MCV levels in both male and female rats. Significant increases in SGPT levels were seen in both male and female rats at dosages of 200 mg/kg and also increase in the activities of SGPT in female rats at a dosage of 400 mg/kg Sivanar Amirtham ($p<0.05$ and $p<0.01$, respectively). Every organ's histological evaluations were normal, except a tiny change in the liver and kidney. Oral administration of the Sivanar Amirtham is considered relatively non-toxic.

Keywords: Sivanar Amirtham, Acute Toxicity, Sub-acute Toxicity, Hematology, Serum biochemistry, Histopathology.

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INTRODUCTION

Now a day's the traditional system of medicine became significantly more popular all over the globe because of less toxic, fewer side effects, and curative properties. India is known for its traditional medicinal systems like Ayurveda, Siddha, and Unani. Medical systems were found mentioned even in the ancient Vedas and other scriptures [1]. Siddha system of medicine, one of the oldest Asian traditional medical systems, derives drugs from plants, animal products, minerals, and metals which are originating in ancient Tamilakam in South India [2]. Sivanar Amirtham is a popular herbo-mineral formula utilized in traditional ayurvedic to deal with a variety of illnesses. Sivanar Amirtham is a combination of a total of nine ingredients like Dryopteris filix-max, Elemental Mercury (purified), Aconitum napellus, Elemental sulfur (purified), Zingiber officinale, Piper nigrum, Piper longum, Arsenic disulfide (purified), Borax (purified).

Traditionally Sivanar Amirtham is used to promote the strength of bones and joints by anti-inflammatory and analgesic activity. It is also used to treat stiffness of muscles, coccyx pain, back spasm, and back injury. Partially it is also useful in hypothyroidism, ankylosing spondylitis, sensory neural hearing loss, and avascular necrosis [3].

The great authorities known as Siddhars devised and founded this method around 2500 years ago, hence the name Siddha Medicine. The medicines were created as a result of the Siddhars' extensive research on herbs, minerals, and animals. This dimension of medicine was opened up by Agastya Muni [4]. When compared to other ancient medical systems, Siddha has a relatively broad scope. Thousands of raw medications are employed in the Siddha system. Before the preparation of medications, Siddha physicians placed a high priority on the purification of raw substances. Herbal items are used to make more than 80% of Siddha medications. However, herbal remedies are ineffective in some serious conditions. Siddhars mentioned some herbo-metal and herbo-mineral formulations in that situation. Standard purification and preparation processes are used to make these medicines. The end product is then extremely small, appearing to be nano microns in size or even smaller. As a result, it is

quickly incorporated into our system and never causes toxicity [5].

18 prime Siddhars, who are followers of the primordial Guru, have also offered their unique knowledge and experience in this discipline. According to legend, Adiyogi himself practiced it, and Agastya was the one who brought it to south India.

He came up with a highly effective combination, as well as some amazing material utilization [6].

Siddha medicine aims to make the body perfect, indestructible, and long-lasting. Siddhars have emphasized daily and seasonal routines, as well as dietary, practices, as well as a code of ethics for living a healthy life [7]. Most of the Siddha preparations in clinical practice currently are prescribed only with the support of ancient literature.

For many rural populations in underdeveloped nations, traditional medicine may give new chemicals that help counteract the high cost and hazardous effects of existing drugs. Because no research has been done on Sivanar Amirtham intake, a holistic approach to evaluating their efficacy and safety profile is required. As a result, the current study used swiss albino mice and wistar albino rats to test the safety of Sivanar Amirtham in acute and sub-acute toxicity studies.

MATERIALS AND METHOD

Collection of drug

The Sivanar Amirtham, a Siddha Sasthriya Medicine was collected from SKM Siddha and Ayurveda Company (India) Private Limited, an ISO 9001: 2008 & GMP certified company, Saminathapuram, Modakkurichi, Erode, Tamilnadu, India- 638104.

Experimental animals

Female Swiss albino mice (20–30 g) and both male and female wistar albino rats (170–200 g, 6 weeks old) were obtained from MSRUAS's animal house and used in acute and sub-acute toxicity experiments. The animals were kept in polypropylene cages with free access to water and a standard pellet diet under conventional laboratory settings with 12 hr/ 12 hr light/dark cycles at roughly 22 °C and 12 hr/ 12 hr light/dark cycles. The study was conducted at the MSRUAS, Bangalore after obtaining Institutional Animals Ethical Committee clearance bearing the number, IAEC no: XVIII/MSRFPH/M-05/08.02.2017.

Acute toxicity study

The oral acute toxicity of Sivanar Amirtham was tested on Swiss albino mice (25-30gm) using the OECD guideline 423 and a limit test dose of 2000 mg/kg. All of the animals were fasted overnight before receiving a dose of free extra water. The animals were split into five groups, each with three animals. The control group received 0.3 ml sterile distilled water after an overnight fast, while the treatment group received 300, 1000, and 2000 mg/kg b.wt. given orally. The body weight of each animal was calculated according to the bodyweight before dose administration. All mice were monitored for mortality or any delayed signs of toxicity at 1, 2, and 4 hours following administration with the test medication, as well as regularly during the first 24 hours and then daily until 14 days. [8] During the test period, any changes in skin, hair, eyes, and mucus membranes, food and water intake, body weight, respiration rate, and behavioural, neurological, and autonomic profiles were observed [9].

Sub-acute oral toxicity study

For 28 days, forty female and male rats (five each) were given a daily oral dose of Sivanar Amirtham (100, 200, and 400 mg/kg) and/or an equal volume of distilled water in a sub-acute toxicity study. Before treatment, all rats were weighed, as well as once a week throughout the test period.

Hematological and biochemical examination

On the 29th day, all the animals were sacrificed by anaesthesia (chloroform) after overnight fasting (8 h). The blood sample was collected into a test tube with and without EDTA as an anticoagulant respectively for biochemical and hematological parameters. The blood without EDTA was evaluated for biochemical analysis after centrifugation at 2500 r/min for 15 min to obtain serum and stored at −20 °C until assayed for biochemical estimation. After collecting the blood, all the vital organs such as the brain, lungs, liver, kidney, heart, stomach, small intestine, pancreas, ovary, and testes were separated, weighed each organ on an electronic balance and relative organ body weight of both control and treated groups were determined and compared [10].

Effect of Sivanar Amirtham on hematological parameters

Using an automatic hematology analyser, the control and drug-treated groups' red blood cell count, hematocrit, mean cell volume, haemoglobin, white blood cell count, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume, monocyte, neutrophil, lymphocyte, and platelet count were determined and compared to the control group (Mindy- bc 500).

Effect of Sivanar Amirtham on serum biochemical parameters

Biochemical parameters such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, high-density lipoprotein, total bilirubin (T-BIL), bilirubin direct, bilirubin indirect, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, gamma-glutamyl. The autoanalyzer was used to examine all parameters (Olympus au 400).

Statistical analysis

Statistical analysis was performed as mean ± SEM (n = 5). Differences between groups were analysed followed by Dunnett's multiple comparisons (ANOVA) test. A probability level of $P < 0.001$ and $P<0.005$ was accepted statistically.

RESULTS

According to the World Health Organization, traditional medicine is used by 80 percent of the people in rural areas, and

medicinal plants have been used as medicine by humans for over 60000 years. Siddha Medicine is a traditional medical system. Traditional medicine is gaining popularity in the poor world, and it is growing by the day. [11, 12] Consumers continue to doubt the efficacy and safety of traditional herbal treatments, even though many are available but only a few have been proven in research trials [13].

Acute toxicity study

During the 14-day observation period, mice given a fixed dose of Sivanar Amirtham (2000 mg/kg) showed no clinical symptoms of toxicity. In female mice, there were no aberrant gross results. There were no substantial changes in body weight growth. All of the animals lived to the end of the experiment, showing that the median lethal dose (LD50) of Sivanar Amirtham for female mice is greater than 2000 mg/kg [14].

Sub-acute toxicity Studies

Effect of Sivanar Amirtham on the general behaviour of rats

The rats' general behaviour did not change significantly after receiving Sivanar Amirtham by mouth. In comparison to the control group, the rats' body weight and food intake did not vary significantly. Sivanar Amirtham (100, 200, and 400 mg/kg) was given to rats for 28 days and no mortality was observed. In comparison to control groups, there was no evidence of toxicity over the experimental period. According to data presented in Table 1.

Table 1: Effects of Sivanar Amirtham on body weight of treated rats

DOSE (mg/kg/day)	DAYS (g/rat)				
	1	7	14	21	28
CONTROL					
Male	241.4±1.41	252.2±1.95	265.8±1.36	272.8±1.47	278.1±1.06
Female	139.6±1.11	230.2±1.08	234±1.76	238±1.55	242.2±1.15
SIVANAR AMIRTHAM 100					
Male	241.4±1.30	220.2±1.62	230.6±1.34	236.6±1.23	240.4±1.77
Female	167±1.31	149.2±1.95	151.6±1.53	154±1.67	158.3±1.23
SIVANAR AMIRTHAM 200					
Male	220.4±1.63	239.2±1.73	248±1.39	259.8±1.704	266.6±1.56
Female	221.2±1.48	170.2±1.89	173.8±1.54	177±1.93	180.6±1.09
SIVANAR AMIRTHAM 400					
Male	232.4±1.07	248.6±1.83	254.8±1.57	266.4±1.13	271±1.09
Female	227±1.58	211.6±1.63	215±1.10	218±1.94	220.6±1.40

Effect of Sivanar Amirtham on hematological parameters of rats

Hematological analysis showed no significant change in HB, Leukocyte, Platelets, Neutrophil, Lymphocyte, Monocyte, Eosinophil, Basophil, ESR, PCV, MCHC in rats treated with Sivanar Amirtham (100 mg/kg, 200 mg/kg and 400 mg/kg) did not differ significantly from those of control rats. However, the administration of the Sivanar Amirtham to rats at 200 mg/kg and 400 mg/kg resulted in a statistically decrease in RBC. At the medium and high dose (200 mg/kg, 400 mg/kg), the Sivanar Amirtham caused a significant increase in MCV. The results of the haematological parameters were shown in (Table 2).

Table 2: Effect of Sivanar Amirtham on hematological parameters of treated rats

Parameters	Control		SAS 100 (mg/kg)		SAS 200 (mg/kg)		SAS 400 (mg/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female
RBC ($X10^6/mm^3$)	7.024±0.43	6.354±0.15	6.574±0.24	6.3±0.16	5.182±0.01**	5.732±0.21*	5.178±0.009**	5.268±0.13**
HB (g/dL)	15.72±0.30	14.88±0.22	15.3±1.04	14.34±0.22	15.62±0.26	15.12±0.34	13.76±0.70	14.46±0.97
Leukocyte ($X10^3/mm^3$)	9.158±0.80	9.598±0.57	7.94±0.10	9.06±0.56	9.36±0.46	8.74±0.45	9.2±0.27	8.38±0.32
Platelets ($10^5/mm^3$)	5.376±0.211	5.348±0.32	5.574±0.22	6.012±0.11	5.502±0.27	6.102±0.02	5.762±0.24	5.51±0.23
MCV (gl)	72.24±1.59	64.02±1.195	72.92±1.87	74.54±1.39	95.42±1.08**	84.88±1.33**	91.94±1.51**	87.7±1.27**
Neutrophil (%)	68.2±1.15	68.4±0.87	69.8±1.24	74.8±1.15	76.6±1.06	76.0±1.70	62.8±1.22	71.8±1.44
Lymphocyte (%)	23±1.14	17.8±1.15	24.8±0.73	18.4±0.87	39±1.44	22.4±1.33	26.4±1.93	21.8±1.74
Monocyte (%)	3.0±0.54	2.2±0.37	3.0±0.44	2.0±0.54	1.6±0.90	2.4±0.67	2.4±1.93	2.4±0.67
Eosinophil (%)	3.2±0.58	3.2±0.37	4.4±0.67	3.8±0.58	4.0±0.77	4.0±0.70	3.2±0.58	2.0±0.54
Basophil (%)	00±00	00±00	00±00	00±00	00±00	00±00	00±00	00±00
ESR (mm)	1.0±00	1.0±00	1.0±00	1.0±00	1.0±00	1.0±00	1.0±00	1.0±00
PCV (%)	49.844±0.51	44.68±1.11	51.43±0.15	46.9±1.88	49.46±0.41	48.4±0.09	47.64±0.78	46.1±0.31
MCHC (g/dl)	31.55±0.70	33.42±0.84	29.72±1.96	30.83±1.66	31.58±0.6207	31.23±0.71	28.92±1.602	31.41±1.26

RBC- Red blood cells, WBC- White blood cells, PCV- Packed cell volume, MCV- Mean corpuscular volume, MCH- Mean corpuscular haemoglobin, MCHC- Mean corpuscular haemoglobin concentration, Comparisons were made between Control and Three treated groups (100mg/kg, 200mg/kg and 400mg/kg). Values are expressed as mean ± SEM. *P<0.05; **P<0.01 compared to control.

Effects of Sivanar Amirtham on biochemical parameters

Sivanar Amirtham caused no significant changes in blood

glucose, total bilirubin, ALP, SGOT, total protein, albumin, globulin, GGT, urea, creatinine, uric acid, Na, K, Cl whilst there was a limited increase in the activities of SGPT both male and female rats at dosages of 200 mg/kg and also increase in the activities of SGPT in female rats at a dosage of 400 mg/kg of Sivanar Amirtham (Table3&4).

Table 3: Effect of Sivanar Amirtham on biochemical parameters (LFT)

Parameters	Control		SAS 100 (mg/kg)		SAS 200 (mg/kg)		SAS 400 (mg/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female
Total Bilirubin (mg/dL)	0.852±0.01	0.718±0.04	0.88±0.02	0.746±0.04	0.882±0.02	0.618±0.02	0.644±0.03	0.036±0.01
Bilirubin direct (mg/dL)	0.044±0.006	0.052±0.007	0.040±0.184	0.064±0.008	0.082±0.112	0.068±0.025	0.097±0.010	0.071±0.022*
Bilirubin indirect (mg/dL)	0.172±0.012	0.22±0.01	0.176±0.01	0.184±0.01	0.528±0.11**	0.278±0.02	0.24±0.02	0.282±0.03
ALP (U/L)	118.4±1.50	145.2±1.08	132.12±1.84	149.52±1.02	135.04±1.06	137.16±1.43	127.18±1.07	145.48±1.96
SGOT (U/L)	85.4±1.18	122.4±1.01	86.8±1.59	161.72±1.06*	112.92±1.81	132.48±1.78	106±1.26	143±1.91
SGPT (U/L)	49.62±0.92	71.4±0.87	52.52±0.43	74.36±1.18	75.2±1.35**	49.46±1.44**	50.88±1.60	52.42±1.19**
Total Protein (g/dL)	6.34±0.41	7.34±0.17	6.60±0.40	8.18±0.22	7.2±0.22	6.92±0.35	7.11±0.37	7.32±0.29
Albumin (g/dL)	5.034±0.51	4.92±0.17	5.272±0.49	5.17±0.18	5.9±0.23	5.14±0.21	4.426±0.22	5.954±0.25
Globulin (g/dL)	3.792±0.34	3.834±0.25	3.94±0.36	3.938±0.30	3.82±0.08	3.36±0.23	3.322±0.25	3.44±0.18
GGT (U/L)	10.38±1.47	13.8±0.80	12.4±1.06	14±1.84	16.6±1.80	17.8±1.35	16.94±1.50	16.16±1.55

ALP- Alkaline phosphate, SGOT- Serum glutamic-oxaloacetic transferase, Serum glutamic pyruvic transaminase, GGT-Gamma-glutamyl transferase, Values are expressed as mean ± SEM. *P<0.05; **P<0.01 compared to control.

Table 4: Effect of Sivanar Amirtham on biochemical parameters (RFT)

Parameters	Control		SAS (100 mg/kg)		SAS (200 mg/kg)		SAS (400 mg/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female
Urea (mg/dL)	53.04±1.42	39.74±1.27	57.26±0.40	40.58±1.83	50.98±1.88	43.3±1.72	56.76±1.28	48.12±1.34
Creatinine (mg/dL)	0.638±0.080	0.692±0.048	0.664±0.093	0.724±0.06	0.85±0.140	0.756±0.041	0.768±0.098	0.796±0.03
Uric acid (mg/dL)	2.052±0.18	1.724±0.200	2.178±0.02	1.548±0.231	2.52±0.144	2.104±0.029	1.576±0.272	1.584±0.258
Na (m.mol)	67.406±1.79	67.926±1.99	68.659±1.17	69.759±1.58	70.62±1.70	68.71±1.12	65.288±1.28	66.788±1.79
K (m.mol)	3.16±0.39	3.187±0.41	3.399±0.41	3.323±0.40	3.4±0.30	3.57±0.36	3.006±0.49	3.089±0.053
Cl (m.mol)	48.486±1.53	48.026±1.37	49.899±1.97	47.22±1.11	52.42±1.63	51.91±1.46	51.08±1.53	50.78±1.47

Na- Sodium, K- Potassium, and Cl- Chloride, Values are expressed as mean ± SEM. **P<0.01 compared to control.

Table 5: Lipid profile- after 28 days treatment with Sivanar Amirtham in Wistar albino rats

Parameters	Control		SAS (100 mg/kg)		SAS (200 mg/kg)		SAS (400 mg/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female
Total cholesterol (mg/dL)	94.53±1.90	91.44±1.31	95.3±1.64	93.8±1.62	97.3±1.92	96.4±1.37	101.4±1.75	99.8±1.46
HDL (mg/dL)	61.56±1.059	52.46±1.69	61±1.22	48.4±1.60	64.4±1.31	58±1.42	51.8±1.88	46.8±1.26
LDL (mg/dL)	39.88±1.41	50.17±1.55	42.2±1.241	35.6±1.24	39.8±1.88	54.4±1.84	45.6±1.44	40.00±1.70
VLDL (mg/dL)	20.6±1.55	20.32±1.42	16.2±1.20	19.6±1.31	24±1.36	18.6±1.56	24.4±1.13	23.2±1.24
Triglycerides (mg/dL)	115.94±1.39	111.98±1.26	118.4±1.43	117.4±1.66	140.6±1.17	105.4±1.16	114.4±1.816	121.2±1.41
Blood glucose (mg/dL)	99.6 ± 1.92	90.6±1.34	97.2 ± 1.02	94.8±1.99	101.8 ± 1.92	95.6±1.74	97.6 ±1.481	101.8 ±1.44

Lipid profile analysis showed no significant change in total cholesterol, HDL, LDL, VLDL, Triglycerides, Blood glucose. The results of the lipid profile are shown in (Table 5).

HDL- High-density lipoprotein, LDL- Low-density lipoprotein, VLDL- very low-density lipoprotein, Values are expressed as mean ± SEM. *P<0.05; **P<0.01 compared to control. Values are expressed as mean ± SEM. Results are not statistically significant compared to control.

Urine analysis showed no significant change in color, transparency, Specific gravity, specific, pH, protein, glucose, bilirubin, ketones, blood, urobilinogen, pus cells, RBCs, epithelial cells, crystals. The results of the urine analysis are shown in (Table 7)

Table 6: Effect of Sivanar Amirtham on organ weights

Isolated Organs	Control	Sivanar Amirtham (100 Mg/Kg)	Sivanar Amirtham (200 Mg/Kg)	Sivanar Amirtham (400 Mg/Kg)
Brain (g)	1.41±0.06	1.65±0.064	1.67±0.052	1.74±0.097
Lungs (g)	1.13±0.089	1.448±0.0864	1.452±0.043	1.522±0.05
Heart (g)	0.948±0.129	0.762±0.0096	0.84±0.036	0.92±0.04
Liver (g)	7.2±0.25	6.678±0.26	7.198±0.39	7.534±0.24
Spleen (g)	0.7±0.07	0.62±0.02	0.748±0.07	1.016±0.046
Stomach (g)	1.25±0.08	1.34±0.07	1.44±0.08	1.374±0.03
Kidney (g)	0.76±0.06	0.786±0.02	0.778±0.01	0.824±0.01
Ovary (g)	0.046±0.002	0.046±0.004	0.044±0.002	0.046±0.004
Testes (g)	1.276±0.10	1.166±0.02	1.13±0.02	1.392±0.07
Pancreas (g)	0.894±0.02	0.898±0.07	1.006±0.03	0.882±0.01

Table 7: Urine Analysis - after 28 days treatment with SAS in Wistar albino rats

Parameters	Control		SAS (100 mg/kg)		SAS (200 mg/kg)		SAS (400 mg/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female
Colour	Straw Yellow	Straw Yellow	Straw Yellow	Straw Yellow	Straw Yellow	Straw Yellow	Straw Yellow	Straw Yellow
Transparency	Clear	Clear	Clear	Clear	Clear	Clear	Slightly turbid	Clear
Specific gravity	1.010	1.010	1.010	1.010	1.010	1.010	1.010	1.010
pH	7.0	7.0	6.0	7.0	7.0	7.0	7.0	7.0
Protein	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Glucose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Bilirubin	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Ketones	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve
Blood	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Urobilinogen	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Pus cells	0-cells/HPF	0-cells/HPF	1-cell/HPF	1-cell/HPF	2-cells/HPF	1-cells/HPF	1-cell/HPF	2-cells/HPF
RBCs	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Epithelial cells	Nil	Nil	Nil	Nil	1-cell/HPF	Nil	2-cell/HPF	1-cell/HPF
Crystals	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Histopathological studies

Histological assessment of liver, kidneys indicates a moderate version in rats treated sub acutely with Sivanar Amirtham on the doses 200 and 400 mg/kg. Histopathology of all vital organs was shown in (Figure 1-12).

Figure 1: Histopathology of Bone of rat treated with SAS: - (1.1) Control- shows normal connective tissue with fibroblasts; (1.2) SAS 100 mg/kg - shows connective tissue with normal fibroblasts; (1.3) SAS 200 mg/kg - shows mild infiltrations with fibroblasts; (1.4) SAS 400 mg/kg - shows edematous connective tissue with fibroblasts.

Bone

(a) Control (b) Low dose (100 mg/kg) (c) Medium dose (200 mg/kg) (d) High dose (400 mg/kg)

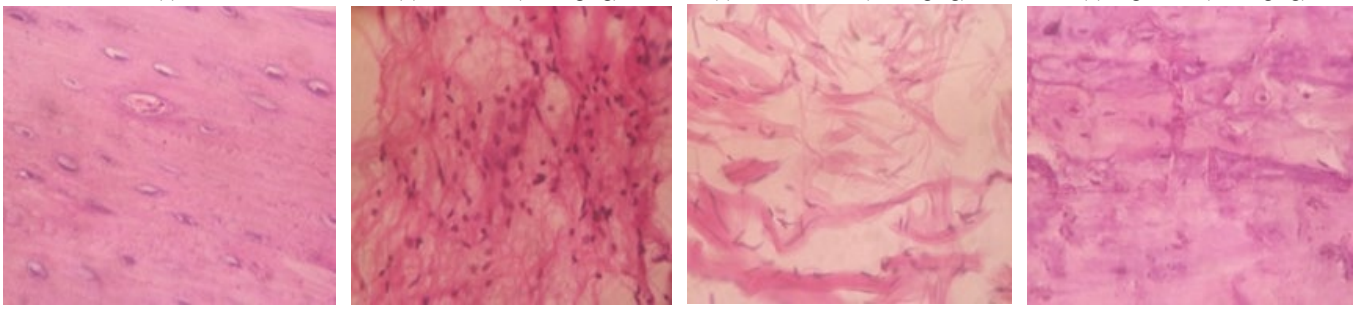


Figure 2: Histopathology of Brain of rat treated with SAS: (2.1) Control- shows normal nerve fibers and astrocytes; (2.2) SAS 100 mg/kg - with normal astrocytes and nerve fibers; (2.3) SAS 200 mg/kg- shows normal nerve fibers and astrocytes; (2.4) SAS 400 mg/kg) - shows normal nerve fibers and astrocytes.

Brain

(a) Control (b) Low dose (100 mg/kg) (c) Medium dose (200 mg/kg) (d) High dose (400 mg/kg)

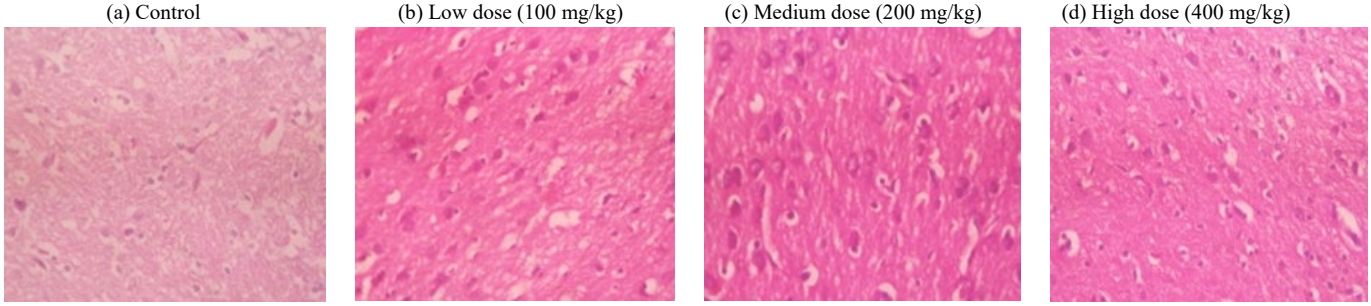


Figure 3: Histopathology of Heart of rat treated with SAS: (3.1) Control- normal myocytes and cardiac muscle bundles; (3.2) SAS 100 mg/kg- with normal myocytes and muscle fibers; (3.3) SAS 200 mg/kg- cardiac muscle fibers and myocytes appear normal and no hemorrhage seen in the muscle bundles; (3.4) SAS 400 mg/kg - myocytes and cardiac muscle bundles appear normal.

Heart

(a) Control (b) Low dose (100 mg/kg) (c) Medium dose (200 mg/kg) (d) High dose (400 mg/kg)

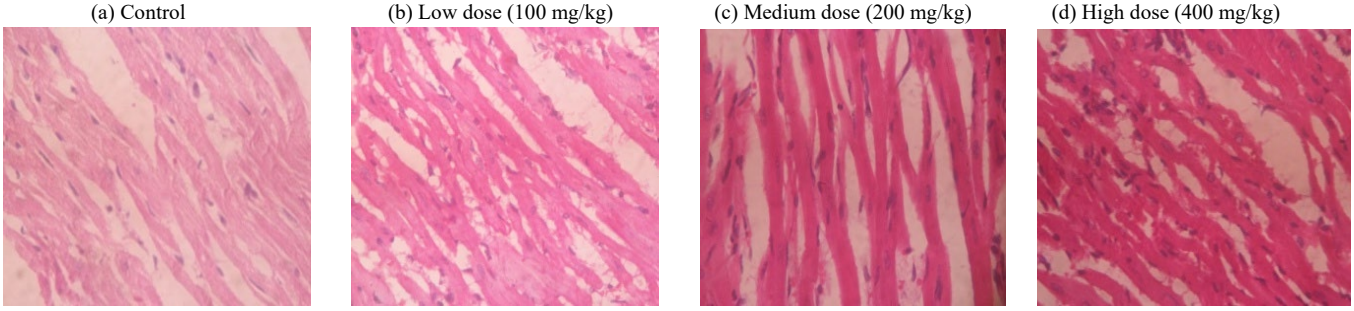


Figure 4: Histopathology of Intestine of rat treated with SAS: (4.1) Control- shows prominent columnar epithelial cells and mucus secretion; (4.2) SAS 100 mg/kg - with prominent columnar epithelial cells, mucus secretion, and mild infiltrations; (4.3) SAS 200 mg/kg - shows prominent mucus secretion; (4.4) SAS 400 mg/kg- shows prominent columnar epithelial cells and mucus secretion.

Intestine

(a) Control (b) Low dose (100 mg/kg) (c) Medium dose (200 mg/kg) (d) High dose (400 mg/kg)

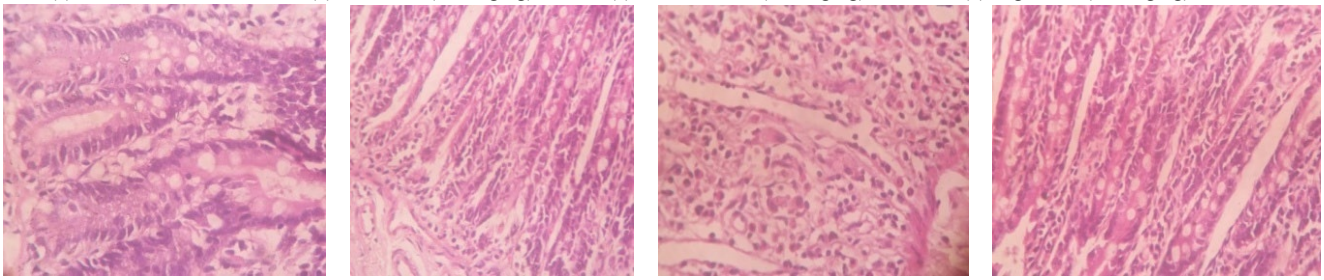


Figure 5: Histopathology of Kidney of rat treated with SAS: (5.1) Control- shows normal renal tissue with glomeruli and tubule; (5.2) SAS 100 mg/kg- shows normal glomeruli and renal tubules and mild infiltration of cells; (5.3) SAS 200 mg/kg- shows renal tissue with tubular damage and Glomeruli show mild shrinkage; (5.4) SAS 400 mg/kg - shows renal tissue with tubular epithelial damage and normal glomeruli.

Kidney

(a) Control (b) Low dose (100 mg/kg) (c) Medium dose (200 mg/kg) (d) High dose (400 mg/kg)

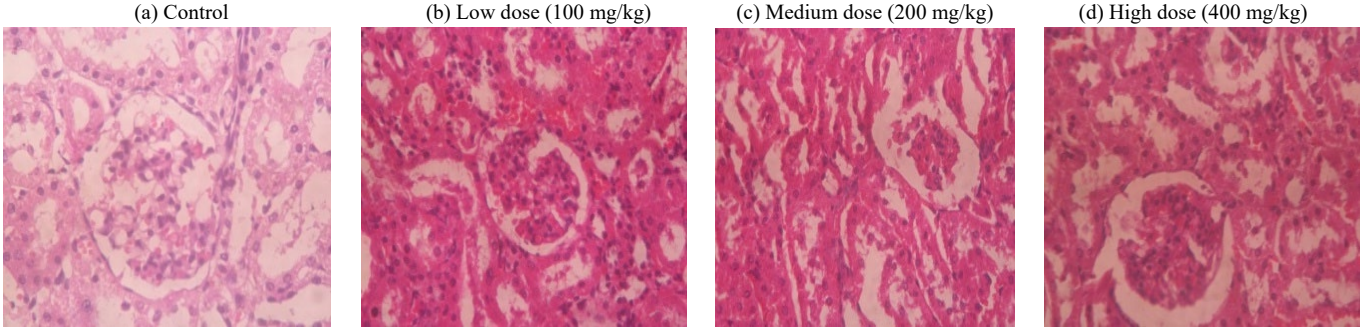


Figure 6: Histopathology of Liver of rat treated with SAS: (6.1) Control- shows normal hepatocytes and infiltration of mononuclear cells; (6.2) SAS 100 mg/kg - with mild congestion and infiltration of mononuclear cells; (6.3) SAS 200 mg/kg - mild edema, congestion of blood vessels; (6.4) SAS 400 mg/kg - dilatation of sinusoids and infiltration of mononuclear cells

Liver

(a) Control (b) Low dose (100 mg/kg) (c) Medium dose (200 mg/kg) (d) High dose (400 mg/kg)

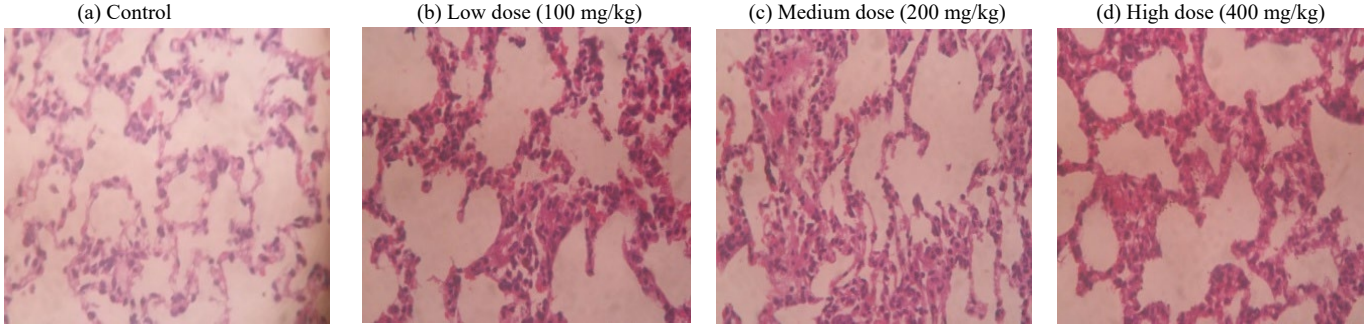


Figure 7: Histopathology of Lungs of rat treated with SAS: (7.1) Control- shows normal alveoli; (7.2) SAS 100 mg/kg - mild congestion of alveolar tissue; (7.3) SAS 200 mg/kg- Congested alveolar wall is seen; (7.4) SAS 400 mg/kg- mild congestion of alveolar tissue with mild alveolar septal edema.

Lungs

(a) Control (b) Low dose (100 mg/kg) (c) Medium dose (200 mg/kg) (d) High dose (400 mg/kg)

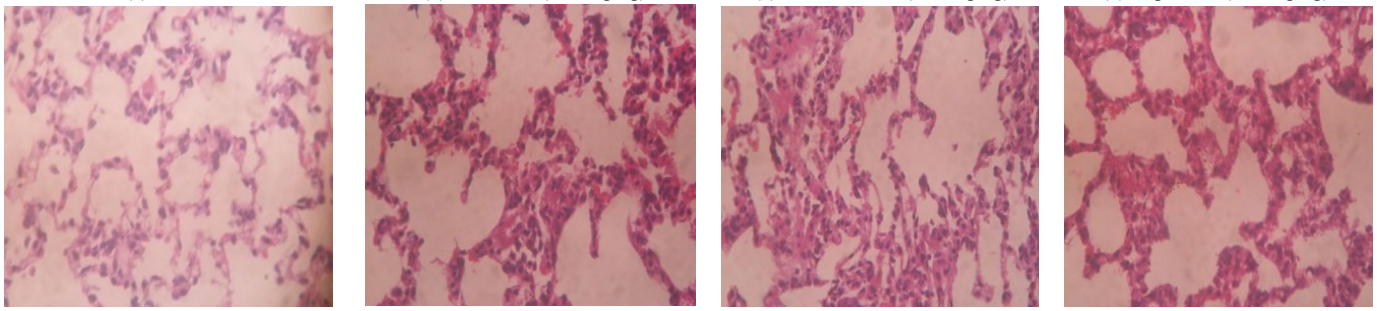


Figure 8: Histopathology of Ovary of rat treated with SAS: (8.1) Control- shows ovarian stroma with follicles and Corpus luteum; (8.2) SAS 100 mg/kg - shows normal ovarian follicles and Corpus luteum; (8.3) SAS 200 mg/kg- shows normal ovarian follicles and Corpus luteum; (8.4) SAS 400 mg/kg- shows normal ovarian follicles and Corpus luteum.

Ovary

(a) Control (b) Low dose (100 mg/kg) (c) Medium dose (200 mg/kg) (d) High dose (400 mg/kg)

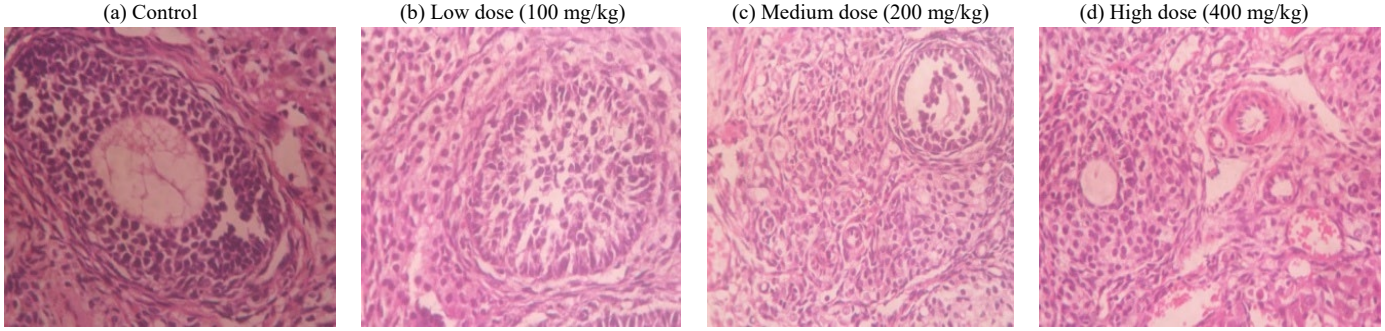


Figure 9: Histopathology of Pancreas of rat treated with SAS: (9.1) Control- shows pancreas with normal acini and islet cells; (9.2) SAS 100 mg/kg - shows pancreas with normal acini and islet cells; (9.3) SAS 200 mg/kg - shows pancreas with normal acini and islet cells; (9.4) SAS 400 mg/kg - shows pancreas with normal acini and islet cells.

Pancreas

(a) Control (b) Low dose (100 mg/kg) (c) Medium dose (200 mg/kg) (d) High dose (400 mg/kg)

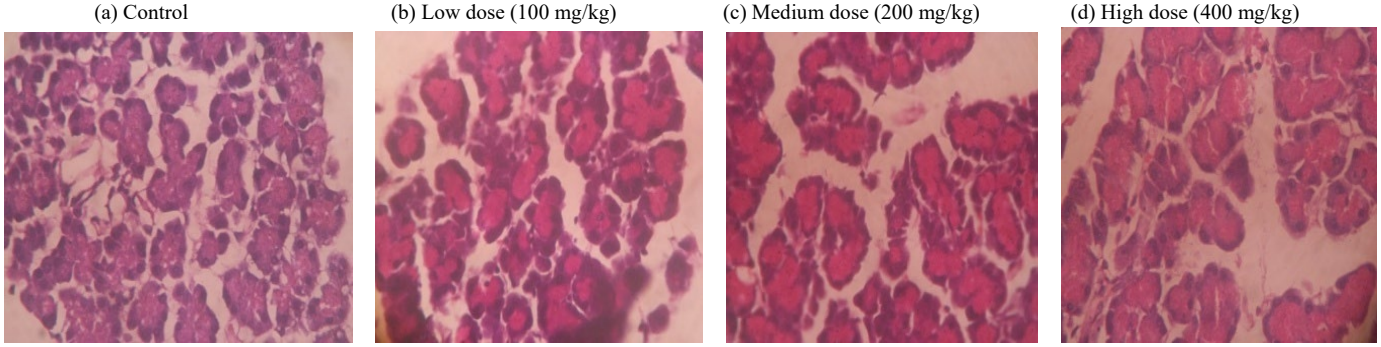


Figure 10: Histopathology of Spleen of rat treated with SAS: (10.1) Control- shows normal spleen with lymphoid aggregation; (10.2) SAS 100 mg/kg - shows lymphoid hyperplasia; (10.3) SAS 200 mg/kg - shows lymphoid hyperplasia;10.4) SAS 400 mg/kg- shows congestion with lymphoid hyperplasia.

Spleen

(a) Control (b) Low dose (100 mg/kg) (c) Medium dose (200 mg/kg) (d) High dose (400 mg/kg)

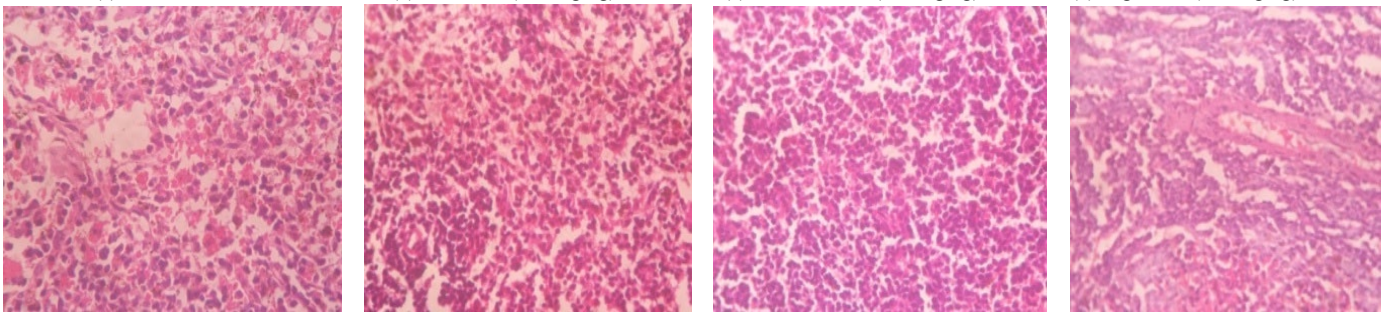


Figure 11: Histopathology of Stomach of rat treated with SAS: (11.1) Control- shows normal mucosal glands with columnar epithelial cells; (11.2) SAS 100 mg/kg- shows near-normal mucosal gland; (11.3) SAS 200 mg/kg - shows normal mucosal glands with columnar epithelial cells; (11.4) SAS 400 mg/kg - shows hyperplasic mucosal glands, congestion, and superficial erosion

Stomach

(a) Control (b) Low dose (100 mg/kg) (c) Medium dose (200 mg/kg) (d) High dose (400 mg/kg)

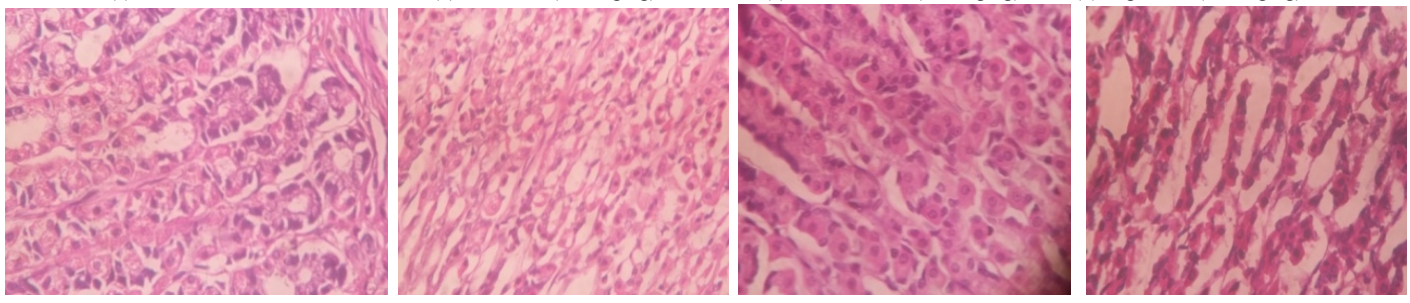
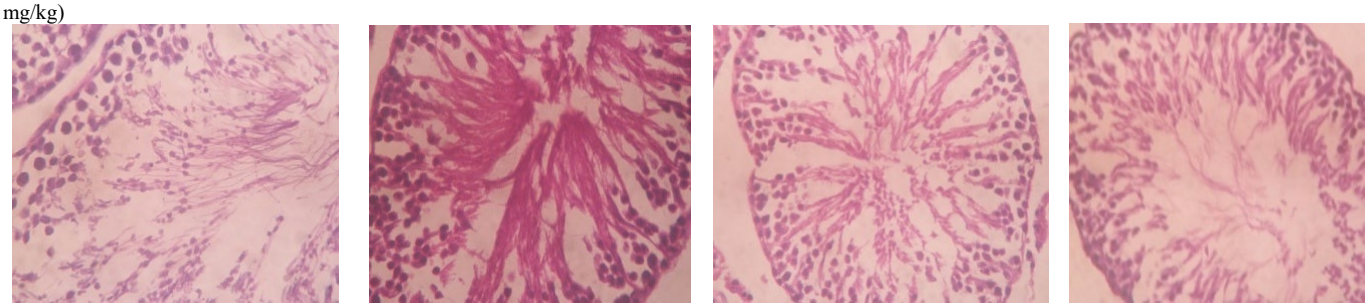


Figure 12: Histopathology of Testes of rat treated with SAS: (12.1) Control- shows normal seminiferous tubules with spermatogenesis; (12.2) SAS 100 mg/kg - seminiferous tubules with normal spermatogenesis; (12.3) SAS 200 mg/kg - seminiferous tubules with normal spermatogenesis; (12.4) SAS 400 mg/kg - seminiferous tubules with normal spermatogenesis

Testes

(a) Control (b) Low dose (100 mg/kg) (c) Medium dose (200 mg/kg) (d) High dose (400 mg/kg)



DISCUSSION

For masses of years, natural medicine had been the basis for the remedy of numerous illnesses. In screening herbal products for pharmacological activities, assessment, and evaluation of the toxic traits of a natural compound are normally preliminary steps. Regardless of the pharmacological beneficial effects of Sivanar Amirtham, designated knowledge about the toxic effect of this Siddha medicine is lacking [15-17]. Hence, the modern-day observation changed into undertaken to evaluate and cognizance on the intense and sub-acute toxicity of Sivanar Amirtham in mice and rats. The acute toxicity takes a look at is utilized to check the harmful outcomes of an agent to the organism given as a single or short-term exposure [18].

The repeated dose toxicity tests provide information on poisonous outcomes, identification of target organs, effects on animal physiology, hematology, the biochemical profile, and histopathology. These checks are required utilizing regulatory corporations to symbolize the toxicological potential of any substance [19-21]. In this study, throughout sub-acute exposure, all animals have been active and answered undoubtedly to stimuli. No deaths and no medical signs and symptoms of nearby or systemic poisonous outcomes had been found. The behaviour of the animals became recorded day by day (popular health and scientific symptoms of toxicity) and no modifications have been determined [22].

In trendy, an increase or decrease inside the frame weight of an animal has been used as a hallmark of an unfavourable impact of medication and chemicals. In the present study, the frame weight and the relative organ weights of all treated rats did no longer fluctuate drastically from those of the control groups. It indicates that the extract did not affect appetite or unfavourable outcomes on the increase of the animals [23].

In this observe hematological, liver function and renal feature checks have been performed. Lipid profiles had been additionally measured. In the hematological take a look at Hb and MCV level of the groups 200 and 400 mg/kg/bw have been significant modifications while compared to the control. Serum ranges of three enzymes (ALP, SGOT, and SGPT) are normally used as clinical biochemistry markers associated with liver damage. Among these enzymes, serum ranges of SGPT increase both male and female rats at dosages of 200 and also increase in the activities of SGPT in female rats at a dosage of 400 mg/kg of Sivanar Amirtham had been important changes while as compared to the control. Besides that, all of the values discovered are in the everyday range for the species.

Histological assessment of liver, kidneys indicates a moderate version in rats treated subacutely with Sivanar Amirtham on the doses 200 and 400 mg/kg. In light of this discrepancy biochemical and histological result of the liver it is able to be said that Sivanar Amirtham does not present liver toxicity sufficient to have an alteration of the features of the liver. This can be because of the short period of treatment (28 days), and consequently, a chronic have a look at is needed for the complete information of the liver of this Siddha medicinal drug.

CONCLUSION

In experimental animals given the medication orally at a high dose of 400 mg/kg, no death was observed. The drug's toxicity investigation in rats revealed the drug's benign nature on the hepatic, renal, and hematological systems, even at a high dose level of daily administration for 28 days. It is obvious from the foregoing findings that Sivanar Amirtham had no harmful effect on rats.

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