



ACUTE AND SUB ACUTE TOXICITY STUDIES OF A SIDDHA SASTRIC FORMULATION *LINGA CHENDHURAM* IN WISTAR RATS

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ABSTRACT

Linga Chendhuram (LC) is a herbo-mineral formulation cited in Siddha literature *Siddha Vaithiya Thirattu* comprises of purified *Lingam* (Cinnabar) processed in herbal juice – *Citrullus colocynthis* and used in the management of various types of fever, arthritis and anaemia at 65 mg/dose. Acute and Sub-acute oral toxicity of *Linga Chendhuram* was carried out in Wistar rats under OECD guidelines 423 and 407. In acute study, LC was administered at 2000mg/kg orally and animals were observed for toxic signs for 14 days. In sub-acute toxicity study, test groups were treated with LC at 18, 90 and 180 mg/kg/day along with 2 ml of diluted honey and control group with distilled water 2 ml/day for 28 days daily. LC at 2000mg/kg produced no treatment related toxic signs or mortality during the study. Haematological and biochemical parameters were analyzed using auto analyzer with standard kits and one way ANOVA followed by Dunnett test was performed for significant analyses. Gross necropsy and histopathology studies using H&E stain were done on major organs. LD50 was found more than 2 g/kg. No-Observed-Adverse-Effect level of LC was seen at 180 mg/kg in 28 days of treatment. No abnormal findings were noted in high dose group organs. Administration of LC at its human therapeutic dose of 195 mg/kg in rat (180 mg/kg) is safe.

KEYWORDS: *Siddha*, *Linga Chendhuram*, Acute oral toxicity, Sub-acute toxicity.

INTRODUCTION

Siddha system is one among the Indian systems of Medicine has been practicing in Tamil speaking part of world. Initially, it is practiced in a Guru-Shisya Parampara, now has been institutionalized. This system was founded by 'Siddhars' who are known for their versatile abilities. They were the masters in natural chemistry and formulated medicinal preparations utilizing various natural resources such as plants, minerals, metals (forms of metals and metalloids derived from the elemental form) and animals. Purified form of Mercury and Sulphur plays a vital role in the formulation of Siddha drugs used for the treatment of chronic and auto immune diseases. *Lingam* is a mercuric compound, chemically red sulphide of mercury having specific gravity 8.1. This sulphide occurs in nature as a fine grained, dark red, very heavy mineral ore called cinnabar. This compound is entirely different from *Kajjali*. *Kajjali* is a black sulphide compound prepared artificially by grinding equal parts of mercury and sulphur till the globules disappear. But *Lingam* is a naturally occurring red sulphide compound and having different properties besides *Kajjali*. This is the most stable form than black form. Cinnabar contains 96% HgS, and Hg is a highly toxic heavy metal. The different chemical forms (organic and inorganic) of mercurial compounds exhibit different degrees of toxicity with effects including hearing loss, vision disturbance, motor deficits, and retarded or abnormal walking ability [1,2]. Despite its toxicity, cinnabar has traditionally been used in Indian system of medicine. Drugs having these heavy metals more than their

allowable limits are banned in many countries. JAMA published an article reporting that the presence of heavy metals such as Lead, Cadmium, Arsenic and Mercury over the permissible limit of WHO in Ayurvedic drugs [3]. Based on that, the western countries bans the traditional drugs in the market, after that Department of AYUSH, Govt. of India made valuable effort in overcoming this issue and strongly incised to do characterization and toxicological studies in experimental animals for proving the safety of the traditional drugs. *Lingam* based preparation is effective in the treatment of diarrhea, pyrexia, delirium, urticaria, diuresis, tuberculosis, scabies, unknown insect bites, syphilis, leprosy, eczema, skin diseases, throbbing pain (Soolai) and *Vatha* diseases. *Lingam* involves as an ingredients in various preparation such as *Linga chendhuram*, *Padigalinga thuvar*, *Jathi Jambheera kuzhambu*, *Brammanandha bairavam*, *Vasantha kusumagaram*, etc [4]. Arun Sudha *et al.*, (2009) has been reported that *Linga chendhuram* has the presence of nano particles in the range of 40 -50 nm [5]. EDAX, XRD and TGA showed the presence of HgS as the main constituent under the permissible limit. ICP-OES analysis has shown the presence of Ca, Cd, Cr, Cu, Fe and Mg in below 1% of *Linga chendhuram*. The toxicological level of *Linga chendhuram* is not found yet. In view of this issue, we validated the safety of *Linga chendhuram* in animal model.

MATERIALS AND METHODS

Preparation of *Linga Chendhuram*

Linga Chendhuram (LC) was prepared by the method described in *Siddha Vaithiya Thirattu*^[6]. The raw materials used in the preparation were *Lingam* (Cinnabar) and entire herb *Atruthumatti* (*Citrullus colocynthis*). The raw *lingam* was procured from registered Siddha Medical practitioner Dr. Murugesan MD *Siddha*, Orathanadu, Tamilnadu. The *Lingam* got authentication from Dr. M. Suresh Gandhi, Assistant professor, Department of Geology, Madras University. The plant material *Atruthumatti* was collected from herbal garden of National Institute of Siddha, Chennai and got authentication from the botanist (Specimen Voucher No. NIS/MB/97/2013) of National Institute of Siddha, Chennai. Before the actual preparation process, the *lingam* was purified by *Surukku* method done as per *Gunapaadam Thathu & Jeeva Vaguppu*^[4]. LC was prepared in *Gunapadam* (*Siddha* pharmacology) lab of National Institute of Siddha, Chennai, India, following standard procedures mentioned in the literature. The purified *Lingam* is placed in a new open mud pan and subject to heat. Then, the juice of *Atruthumatti samulam* is added drop by drop over the *Lingam* to fuse until addition of entire juice. After this process, *Lingam* is taken to 'Kalvam' (a traditional grinding tool made of black stone) and ground it into fine powder. This finished red colour formulation will be stored in an appropriate vessel.

Experiment animal's husbandry

The study protocol has got approval from Institutional Animal Ethical Committee of National Institute of *Siddha*, Chennai (1248/ac/09/CPCSEA/5-12/2011). Male and female Wistar Albino rats (8-12 weeks and males; 120-160g; females: 120-160g) were obtained from the animal house of King Institute of Preventive Medicine, Guindy, Chennai and maintained in the animal house of National Institute *Siddha*, Chennai. Animals were housed in individually in polypropylene cages in a ventilated room (air cycles: 15/min; 70:30 exchange ratio) under an ambient temperature of 22±2°C and 40-65% relative humidity, with a 12-h light/dark artificial photoperiod. The animals received RO water *ad libitum* and fed with Amruth Rodent pellet.

Acute oral toxicity study

This study was carried out by following the procedure with the starting dose of 2000 mg/kg body weight of test drug mentioned in OECD 423 guideline^[7, 8]. Six female rats were randomly selected and acclimatized prior to the study. Each selected animal was kept in separate poly propylene cage and marked with picric acid on the fur for identification. The rats were fasted overnight before the administering of test drug. After the administration of test drug, the rats were deprived of feed for 16 h and water was not allowed for initial 3 h. Initially, the starting dose 2000 mg/kg of LC was chosen and administered to three rats and observed for mortality and clinical signs of toxicity (General behaviour, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) at 30 min, 1, 2 and 4 hours and thereafter once a day for the next 14 days. Since there was no mortality and abnormal signs, further three animals were administered with LC at the same dosage of

2000 mg/kg and observed for mortality and clinical signs of toxicity. LD50 cut-off value of LC was determined in accordance with Globally Harmonized System of Classification and Labeling of chemicals.

Sub-acute oral toxicity study

This study was carried out by following OECD guidelines^[9]; adopted for the testing of chemicals - 407 (3rd October, 2008) and was modified according to the experimental need. In the literature *Siddha Vaithiya Thirattu*, the human intended dosage for LC was recommended as 65 mg three times a day (195 mg/day). On the basis of body surface area conversion against human dose, 18 mg/kg/day dosage of LC was calculated for rat. In the present study, three doses of LC of 18 mg/kg/day (Low dose), 90 mg/kg/day (Intermittent dose) and 180 mg/kg/day (High dose) were selected for administration.

The both sexes of Wistar Albino rats were randomized into four groups of ten animals each (5 males, 5 females). Group I received a vehicle (Honey 1ml + distilled water 1ml) and served as control group. Group II, III and IV served as low, intermittent and high doses of LC respectively. All the test substances were administered once daily via oral route through gastric lavage for 28 days.

All the experimental animals were observed for mortality and morbidity twice a day, till the completion of treatment. Clinical observations were made once daily to detect signs of toxicity, preferably at the same time(s) in each day (1h after vehicle or LC administration). The focus of the observations was the same as described above for the acute toxicity study. Body weights of the animals were recorded once in a week. The amounts of food and water given and their remnants on the next day were measured to calculate the difference, which was regarded as daily consumption and the data were expressed as 7 days cumulative value. At the end of 28th day treatment, live rats were fasted over night and on 29th day under light chloroform anaesthesia blood were drawn from the retro orbital sinus in a potassium tube EDTA and a tube without anti coagulant.

Haematological and biochemical parameters

The haematological parameter tests such as Haemoglobin (Hb), Red Blood Cell count (RBC), White Blood Cell count (WBC), WBC Differential count - Lymphocyte, Monocyte and Granulocyte, Red Cell Distribution Width (RDW), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet (Plt), Platelet Crit (PCT), Platelet Distribution Width (PDW) and Mean Platelet Volume (MPV) were done in the EDTA mixed blood samples using Erba Mannheim® haematology analyser. The blood samples without anticoagulant were used for estimating biochemical parameters such as Glucose, Cholesterol, Triglyceride (TG), Protein, Urea, Creatinine, Bilirubin, Serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT) and Alkaline Phosphatase (ALP) using Erba system Pack kits in Fully Automated Biochemistry analyzer. Sodium, Potassium and Chloride content were estimated by using electrolyte analyser from Roche®.

Necropsy and Histo-pathological study

After withdrawal of blood, all the rats were sacrificed for gross necropsy and histopathological study. Organs including brain, trachea, lungs, heart, liver, kidney, stomach, spleen, intestine, testis, seminal vesicle, bladder, uterus and ovaries were studied for gross necropsy and weighed for calculating relative organ weight. Histopathological studies on liver, kidney, lungs, stomach, heart, spleen, brain, and femorotibial joints were carried out for control and high dose group. The tissues of collected organs were fixed in 10% Neutral buffered formalin for 24 h. The tissues were trimmed, embedded in molten paraffin wax and sectioned (4-5 microns thickness) using rotary microtome. The sections were floated in hot water and placed in the glass slide. The slides were stained with Haematoxylin and Eosin (H&E), mounted in DPX and examined under light microscope.

Statistical analysis

All data were expressed as mean \pm standard deviation (SD). The test groups were compared with control for testing significance by one way ANOVA followed by Dunnet test using GRAPH PAD INSTAT version 3 software programmes. Values of $p < 0.05$ were considered significant.

RESULTS**Table 1: Effect of *Linga chendhuram* on body weight gained in male wistar albino rats - 28 day repeated oral toxicity study**

Treatment	Initial day (g)	On7th day (g)	On14th day(g)	On 21th day (g)	On 28th day (g)
Control (Honey2ml)	146.28 \pm 4.32	152.36 \pm 8.68	168.46 \pm 10.46	180.74 \pm 12.14	184.42 \pm 08.14
18 mg/kg	148.46 \pm 9.24	156.4 \pm 8.28	170.2 \pm 11.67	181.4 \pm 15.2	190.8 \pm 10.46
90 mg/kg	136.46 \pm 11.22	143.4 \pm 14.32	161.8 \pm 16.2	168.6 \pm 16.24	176.24 \pm 14.38
180 mg/kg	139.16 \pm 8.67	144.02 \pm 10.84	162.46 \pm 11.26	178.64 \pm 16.43	184.2 \pm 12.46

Values are expressed as mean \pm S.D. (n = 5)

Table 2: Effect of *Linga chendhuram* on body weight gained in female wistar albino rats - 28 day repeated oral toxicity study

Treatment	Initial day (g)	On7th day (g)	On14thday (g)	On21th day (g)	On28th day(g)
Control (Honey2ml)	148.46 \pm 11.32	153.6 \pm 12.38	158.6 \pm 10.12	164.6 \pm 8.64	168.8 \pm 8.32
18 mg/kg	144.24 \pm 14.14	152 \pm 6.3	158.4 \pm 11.62	162 \pm 5.64	169.6 \pm 6.32
90 mg/kg	136.46 \pm 2.14	142.6 \pm 8.24	150.6 \pm 11.58	158.64 \pm 10.26	164.6 \pm 16.42
180 mg/kg	142.46 \pm 4.92	146.6 \pm 6.24	151.7 \pm 8.42	159.4 \pm 11.64	163.72 \pm 8.96

Values are expressed as mean \pm S.D. (n = 5)

Table 3: Effect of *Linga chendhuram* on relative organ weight in male wistar albino rats - 28 day repeated oral toxicity study

Organ	Control (Honey2ml)	<i>Linga chendhuram</i>		
		18 mg/kg	90 mg/kg	180 mg/kg
Brain	0.97 \pm 0.13	0.95 \pm 0.06	1.04 \pm 0.09	0.96 \pm 0.04
Heart	0.39 \pm 0.05	0.41 \pm 0.01	0.39 \pm 0.03	0.36 \pm 0.04
Liver	3.46 \pm 0.24	3.70 \pm 0.14	3.84 \pm 0.42	3.59 \pm 0.32
Spleen	0.45 \pm 0.03	0.51 \pm 0.07	0.41 \pm 0.06	0.45 \pm 0.03
Kidney	0.86 \pm 0.04	0.91 \pm 0.05	0.92 \pm 0.12	0.84 \pm 0.04
Testis	1.04 \pm 0.09	1.10 \pm 0.11	1.08 \pm 0.08	1.06 \pm 0.12

Values are expressed as mean \pm S.D. (n = 5)

Acute oral toxicity study

The acute toxicity study showed no mortality of rats up to the dosage of 2000mg/kg. No behavioural changes or abnormal clinical signs of toxicity were observed up to the above dosage throughout the end of 14 day study period. No gross pathological abnormality in the organs was found even at this high dose. LD50 value was found to be more than 2000 mg/kg body weight and therefore this test drug *Linga chendhuram* falls under (Unclassified) category V with reference to Globally Harmonized classification System (GHS) (OECD, 2000).

Sub-acute oral toxicity study

For a period of 28 consecutive days of oral treatment of LC at 18, 90 & 180mg/kg/day in both sexes of rat, no treatment related toxicity signs or mortality were observed. Feed and water consumption of treated groups were found not to be significantly affected or changed in both sexes compared to the vehicle treated rats. But no significant change in the body weight gained or lost in the treated test groups were observed compared with control group during the study (Table I & II). The absolute and relative organs weight was also not altered by LC treatments (Table III & IV). If LC were a toxic substance, there would have been a minimal reduction in body weight gain and internal organs weight.

Table 4: Effect of *linga chendhuram* on relative organ weight in female wistar albino rats - 28 day repeated oral toxicity study

Organ	Control (Honey2ml)	<i>Linga chendhuram</i>		
		18 mg/kg	90 mg/kg	180 mg/kg
Brain	1.03±0.02	1.05±0.05	1.06±0.04	1.08±0.02
Heart	0.42±0.03	0.42±0.02	0.40±0.03	0.40±0.01
Liver	4.01±0.32	4.10±0.19	4.25±0.02	4.08±0.16
Spleen	0.46±0.03	0.44±0.03	0.43±0.03	0.42±0.04
Kidney	0.76±0.08	0.80±0.02	0.72±0.02	0.82±0.02
Testis	0.08±0.02	0.07±0.02	0.07±0.02	0.06±0.01

Values are expressed as mean ± S.D. (n = 5)

Among haematological parameters analyzed in male rats, MCH (pg) and MCHC (g/dl) level was significantly reduced in test groups at three dose levels on compared with control group. Among hematological parameters analyzed in female rats, Monocyte ($10^9/L$) was significantly reduced in 90mg/kg LC treated group, Haemoglobin (g/dL), Total RBC ($10^{12}/L$), RDW (%), Hematocrit (%) and Platelet crit (%) values were significantly increased in 18mg/kg of LC on compared with control group. Among biochemical parameters analyzed in male rats, Urea (mg/dl), Creatinine (mg/dl) and ALP (U/L) was significantly reduced and Bilirubin (mg/dl) was significantly increased in 18mg/kg of LC on compared with control group. The haematological and biochemical parameters show no significant different between the test groups on compared with control group.

Table 5: Effect of *Linga chendhuram* on hematological parameters in male Wistar albino rats - 28 day repeated oral toxicity study

Parameter	Control (Honey 2ml)	<i>Linga chendhuram</i>		
		18 mg/kg	90 mg/kg	180 mg/kg
Total WBC ($10^9/L$)	9.6±2.01	11.24±2.93	10.24±3.58	8.74±1.46
Lymphocyte ($10^9/L$)	5.06±0.79	7.2±2.59	5.6±2.43	5.68±1.76
Monocyte ($10^9/L$)	0.3±0.12	0.34±0.09	0.24±0.05	0.26±0.05
Granulocyte ($10^9/L$)	3.62±0.80	3.68±0.61	4.34±2.89	3.78±1.08
Hemoglobin (g/dL)	17.5±2.79	18.32±1.99	13.04±2.29	13.72±3.76
Total RBC ($10^{12}/L$)	9.89±1.51	10.57±0.77	7.71±1.32	8.3±2.12
RDW (%)	11.4±1.24	11.4±0.83	10.94±0.53	11.1±0.33
Hematocrit (%)	51.7±8.50	47.16±18.50	39.94±6.93	42.4±11.70
MCV (fL)	52.36±0.89	51±1.10	51.84±0.507	50.94±1.23
MCH (pg)	17.6±0.46	16.6±0.58**	16.84±0.28*	16.3±0.26**
MCHC (g/dL)	33.8±0.60	32.62±0.64*	32.58±0.56*	32.12±0.47**
Platelet ($10^9/L$)	96.4±33.5	136.4±60.69	151.8±65.50	153.6±65.05
Platelet crit (%)	0.05±0.02	0.08±0.35	0.08±0.37	0.09±0.03*
PDW (%)	15.3±0.19	15.2±0.25	15.28±0.37	15.16±0.15
MPV(fL)	6.16±0.19	6.0±0.15	5.92±0.21	6.06±0.11

WBC: White blood count; RBC: Red blood cell; RDW: Red cell distribution width; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PDW: Platelet distribution width; MPV: Mean platelet volume

Values are expressed as mean ± S.D. (n = 5). P value calculated using one way ANOVA followed by Dunnett test. Significance is indicated as *p < 0.05 vs control group.

Table 6: Effect of *Linga chendhuram* on hematological parameters in female wistar albino rats - 28 day repeated oral toxicity study

Parameter	Control (Honey 2ml)	<i>Linga chendhuram</i>		
		18 mg/kg	90 mg/kg	180 mg/kg
Total WBC ($10^9/L$)	7.34±2.94	9.8±1.69	8.18±0.5	7.2±1.8
Lymphocyte ($10^9/L$)	4.08±1.56	4.78±0.84	4.28±0.16	3.98±1.59
Monocyte ($10^9/L$)	0.28±0.14	0.24±0.05	0.14±0.05*	0.22±0.04
Granulocyte ($10^9/L$)	3.6±2.2	3.28±0.56	2.26±0.47	2.36±0.78
Hemoglobin (g/dL)	13.9±4.13	19.8±0.89**	17.9±2.54	17.42±1.04
Total RBC ($10^{12}/L$)	7.99±2.18	11.81±0.52**	10.8±1.4*	10.42±0.79*
RDW (%)	10.02±0.8	11.4±0.83*	10.94±0.53	11.1±0.33*

Hematocrit (%)	42.5±13.7	60.34±2.14**	51.38±7.18	45.1±3.24
MCV (fL)	52.6±3.28	51.16±1.04	53.68±0.62	52.8±0.95
MCH (pg)	17.3±0.62	16.6±0.58	16.84±0.28	16.3±0.26**
MCHC (g/dL)	33.08±1.2	32.62±0.64	32.52±0.76	33.5±0.66
Platelet (10 ⁹ /L)	83.4±18.6	157.2±30.43**	173.0±46.92**	134.0±30.43
Platelet crit (%)	0.05±0.0	0.1±0.02**	0.11±0.03**	0.08±0.02
PDW (%)	15.42±0.43	15.26±0.18	15.34±0.32	15.24±0.15
MPV (fL)	6.22±0.36	6.42±0.22	6.08±0.23	6.04±0.25

WBC: White blood count; RBC: Red blood cell; RDW: Red cell distribution width; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PDW: Platelet distribution width; MPV: Mean platelet volume

Values are expressed as mean ± S.D. (n = 5). P value calculated using one way ANOVA followed by Dunnett test. Significance is indicated as *p < 0.05 vs control group

Table 7: Effect of *Linga chendhuram* on biochemical parameters in male wistar albino rats - 28 day repeated oral toxicity study

Parameter	Control (Honey 2ml)	<i>Linga chendhuram</i>		
		18 mg/kg	90 mg/kg	180 mg/kg
Glucose (mg/dl)	72.6±11.71	77.2±9.5	83.2±11.7	72.6±12.2
Cholesterol (mg/dl)	70.0±9.4	83.8±6.04	77.2±6.97	81.4±10.18
Triglyceride (U/L)	82.6±16.31	91.2±9.4	84.2±5.82	95.8±5.94
Protein (g/dL)	6.8±0.08	7.2±0.56	7.18±0.71	7.2±0.81
Urea (mg/dl)	42.8±1.92	32.2±4.86*	35.2±5.8	42.6±7.43
Creatinine (mg/dl)	1.18±0.13	0.9±0.2*	0.92±0.14*	1.02±0.13
Bilirubin (mg/dl)	0.38±0.08	0.62±0.16*	0.48±0.16	0.48±0.08
SGOT (U/L)	201.4±43.52	177.2±17.93	184±37.23	174.2±35.94
SGPT (U/L)	86.4±20.0	75.4±13.63	72.0±13.78	55.0±9.24*
ALP (U/L)	429.8±36.8	293.8±35.92*	277.0±83.13**	375.2±83.67
Sodium (mmol/L)	144.0±5.43	140.2±7.69	137.3±16.21	142.3±10.01
Potassium (mmol/L)	4.6±1.3	4.81±2.32	4.01±1.21	4.9±1.20
Chloride (mmol/L)	104.6±4.22	112.4±10.9	107.7±8.01	112.1±10.42

SGOT: Serum Glutamic Oxaloacetic Transaminase; SGPT: Serum Glutamic Pyruvic transaminase; ALP: Alkaline Phosphatase

Values are expressed as mean ± S.D. (n = 5). P value calculated using one way ANOVA followed by Dunnett test. Significance is indicated as *p < 0.05 vs control group

Table 8: Effect of *Linga chendhuram* on biochemical parameters in female wistar albino rats - 28 day repeated oral toxicity study

Parameter	Control (Honey 2ml)	<i>Linga chendhuram</i>		
		18 mg/kg	90 mg/kg	180 mg/kg
Glucose (mg/dl)	79.6±7.43	83.8±11.4	94.4±17.7	97.0±17.6
Urea (mg/dl)	35.0±12.64	39.6±4.97	40.0±5.14	40.6±7.63
Creatinine (mg/dl)	0.86±0.11	0.72±0.08	0.8±0.0	0.84±0.13
Cholesterol (mg/dl)	70.0±9.4	76.0±14.31	65.0±8.27	70.8±25.8
Triglyceride (U/L)	177.6±32.3	179.8±54.96	215.8±42.28	188.4±76.33
SGOT (U/L)	129.4±34.93	155.0±31.21	135.6±26.81	151.8±30.54
SGPT (U/L)	50.8±17.76	47.8±8.58	54.8±13.06	52.0±10.14
ALP (U/L)	310.6±52.07	285.0±59.66	304.8±39.01	307.2±40.59
Bilirubin (mg/dl)	0.76±0.09	0.62±0.16	0.6±0.07	0.7±0.10
Sodium (mmol/L)	145.0±6.31	140.2±8.1	137.3±16.78	143.8±11.54
Potassium (mmol/L)	4.4±1.2	5.38±2.68	4.26±1.34	4.8±1.02
Chloride (mmol/L)	105.9±4.86	113.4±11.89	106.8±7.55	111.6±10.49

SGOT: Serum Glutamic Oxaloacetic Transaminase; SGPT: Serum Glutamic Pyruvic transaminase; ALP: Alkaline Phosphatase

Values are expressed as mean \pm S.D. (n = 5). P value calculated using one way ANOVA followed by Dunnett test. Significance is indicated as *p < 0.05 vs control group

The gross necropsy study on the organs revealed no abnormal pathological morphology. H&E stained kidney slices of female rats of high dose group showed changes in their architecture by the presence of minimal infiltration of mononuclear cells indicated slight inflammation of glomeruli (Figure 1C, 1D). But in the kidney of male rats, no evidence of such lesions was observed (Figure 1B). No abnormal changes were observed in other H&E stained slices of organs and they were not shown in the figure.

DISCUSSION

Linga Chendhuram (LC) is a *Sastric Siddha* formulation prepared using purified *Lingam* (Cinnabar) by triturating with the juice of *Citrullus colocynthis*. LC was used for long period in the therapeutic management of fevers, arthritis and anemia. Cinnabar (Mercury sulphide) did not produce much toxicity rather than other mercurial compounds since its poor absorption in GI tract [10]; However many studies reported that on chronic administration of Cinnabar produce severe toxic effects. While using toxic substance in the preparation of *Siddha* Formulation, it is mandatory to do the special purification process. On using *Lingam*, if it is not properly purified and the process of *Lingam* based formulation is incomplete, it will result in toxicity such as inflammation of alimentary canal, urinary disorders, neurological effects, burning sensation of all over the body, etc. This study tested the acute and sub acute toxicity of LC to substantiate its safety for human use. LC was orally administered at higher dose 2 gm/kg to the Wistar Albino rats in acute toxicity study and for 28 days of repeated (sub acute) toxicity study daily doses of 18, 90 & 180 mg/kg of body weight to the Wistar Albino rats. The median lethal dose for LC in rat was estimated more than 2000 mg/kg and this dose was 100 times more than that of the human therapeutic dose. It was clearly proved that the human therapeutic dose was absolutely free from acute toxicity. After completing sub acute oral toxicity study on LC at three dose levels 18, 90 & 180mg/kg/day, no fatal and abnormal signs happened in the rats. If any toxic substance is consumed for the above period, there will be change in the body weight due to altered metabolism. During the period, no significant reduction in the body and organ weight was observed revealed that LC does not much interfere in the metabolism.

From the results of present investigation on haematological and biochemical parameters, we observed some variations among the male and female rats. In male rats treated with test drug observed that MCH, MCHC, Urea, Creatinine and ALP levels were significantly reduced but bilirubin was significantly increased. But in female rats, no significant difference was observed on these levels. In female rats, Hb, RBC, RDW, Hct, and Platelet crit levels were significantly increased but monocyte level was significantly reduced. These changes were not noted in male rats. All the haematological and biochemical parameters were within the normal limits in both male

and female rats treated with LC at different dosages. The variations seen in the above said parameters were not much critical in establishing pathological lesions since differences observed were statistically on compared with control groups. If the drug is toxic, there will be reduction in Hb content, RBC count and Hct value and indicate the shortfall in Iron metabolism leading to disturbance in Erythropoiesis. Such condition seen in Anaemic condition. Here, LC does not interfere in the haematological parameters. Liver and Kidney plays a significant role in eliminating toxins or foreign materials. If these organs are damaged, it will result increased excretion of hepatic enzymes and protein, urea, and creatinine in the blood. All biochemical parameters investigated were within the normal limits in the test group's favors LC did not cause damage to the heart, liver, kidney and other organs. If there is any damage occurs, it will reflect in the gross and histological appearance of the sliced organs. No observable pathological findings were found in the organs of LC high dosed group confirmed that it is a safer drug.

CONCLUSION

Linga Chendhuram is a *Siddha Sastric* formulation prepared by processing purified Cinnabar with herbal juice of *Citrullus colocynthis* and having therapeutic value against fever with arthralgia at 65 mg/dose. To establish the safety profile of this formulation, acute and sub acute toxicity studies in Wistar albino rats were done following OECD guideline 423 and 407 respectively. The median lethal dose for *Linga Chendhuram* was found more than 2 g/kg. No-Observed-Adverse-Effect level of LC was seen at 180 mg/kg in 28 days of treatment. No abnormal findings were noted in high dose group organs. Under this study, it is concluded that *Linga Chendhuram* at its human therapeutic dose 195 mg/kg is safer in rat model.

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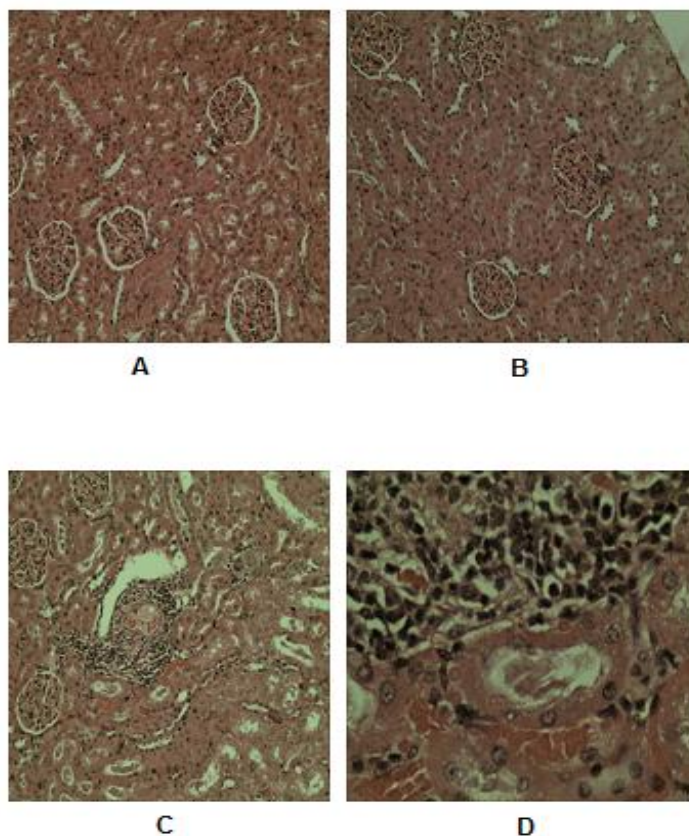


Fig.1: A - H&E sliced kidney of control group showing normal architecture (10X)

B - H&E sliced kidney of test group of male rat treated with LC at 180 mg/kg showing normal architecture (10X)

C - H&E sliced kidney of test group of female rat treated with LC at 180 mg/kg showing infiltration of mononuclear cells (10X)

D - H&E sliced kidney of test group of female rat treated with LC at 180 mg/kg showing infiltration of mononuclear cells (40X)