INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES
(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com
DOI:10.22192/ijcrcps Coden: IJCROO(USA) Volume 4, Issue 2 - 2017

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2017.04.02.004

Acute and sub acute toxicity studies on Sarva Noi Linga Chenduram in rodents.

Dr A.Punitha[1*], Dr S.Visweswaran[2], Dr A .Rajendra kumar[3], Dr K.Manickavasakam[4].

[1]Department of Gunapadam
[2]Lecturer, Department of Gunapadam, National Institute of Siddha, Chennai -47.
E-mail: svisu11@gmail.com
[3]Research officer, SRRI, Puducherry.
[4]Former Director, National Institute of Siddha, Chennai -47
*Corresponding Author: punidranbu@gmail.com

Abstract

The aim and objective of the study was to prepare and evaluate the safety of "Sarva Noi Linga Chenduram" in animal model. Sarva Noi Linga Chenduram was prepared by standard operative procedure mentined in the siddha text.To evaluate its safety, acute and 28-day repeated oral toxicity studies were performed following OECD test guidelines 425 and 407, respectively.

In acute toxicity study, the animals were treated with Sarva Noi Linga Chenduram 1000mg/kg were showed tolerance with negligible toxic signs. Hence the one tenth of the dose was selected as median therapeutic dose for the further study. Gross pathology was performed at the end of the study. In repeated dose toxicity study Sarva Noi Linga Chenduram, was administered at 50,100,200 mg/kg body weight daily for 28 days. The results of haematological investigations, revealed mild changes (P>0.05) when compared with those of respective controls. The results obtained from the study showed it was safe and need further clinical studies.

Keywords: Sarva Noi Linga Chenduram, Siddha, acute ,sub –acute toxicity .

Introduction

Siddha System is one of the traditional and pioneer systems of medicine. Siddha medicines are broadly classified in to internal medicines and external medicines each constitutes of 32 types. They are prepared from herbs, metals, minerals and biological resources. A total number of 4,448 diseases is mentioned in Siddha text as well as with line of treatment. In India, about 80 % of population depends on traditional medicine, out of which, nearly 70-75 % depend on the traditional medicine[1]. Sarva Noi Linga Chenduram is one of the medicines mentioned in the siddha text[2]. The ingredients of Sarva Noi Linga Chenduram are lingam(Mercuric sulphide) and Venkaram (Borax). Sarva Noi Linga Chenduram is indicated for the therapaeutic management of Kalladaippu(renal calculi). Chenduram are sulphide form of metals and minerals. There is a controversy regarding the risk of toxic metals and minerals in Siddha herbo-mineral preparations, hence toxicological parameters were investigated.

Materials and Methods

Drugs, Chemicals and Reagents:

For the toxicological study Sarva Noi Linga Chenduram was prepared in Gunapadam lab ,NIS Chennai. All other

reagents, assay kits and chemicals used in this work were purchased from Sigma Chemical Co. St Louis, MO, USA.

Collection and authentication of raw drug:

The raw drugs were procured from raw drug store in Chennai and authenticated by competent authority of Department of Gunapadam.

Preparation of the medicine:

Ingredients:

Purified Lingam (Cinnabar)	-	35g
Purified Venkaram (Borax)	-	140g

Purification methods:

Purification of Lingam:[3]

It was kept on a mud vessel and heated in low fire. The juices of citrus lemon, Acalypa indica, cow's milk were mixed in equal proportions. The mixed liquid was poured drop by drop on lingam while heating**.**

Purification of Venkaram:[4]

Venkaram ground by the citrus lemon juice and then dried it .

Method of medicine preparation:

Lingam ground into tiny particles.Venkaram got placed in a mud vessel and heated in a low fire. When venkaram started melting purified lingam was sprinkled little by little. It had to be mixed well.Before melting of venkaram all quantity of lingam was sprinkled. After that the medicine was taken away from the heat. By the time, it got completely condensed. Then it was well ground in the kalvam and stored in an air tight container.

Animals:

Mice of either sex weighing 25-30g and rats weighing 210-240g were obtained from the animal house of Vels University. The animals were used with the approval of the Institute animal ethics committee and obtained from Vels University, Chennai. They were fed with a balanced standard pellet diet and maintained under standard laboratory conditions, providing 24-28^{0}C temperature, standard light cycle (12 h light, 12 h dark) and water ad libitum. Animals were kept in cages with raised floors of wide mesh to prevent coprophagy. Animal welfare guidelines were observed during the maintenance period and experimentation. The rats were randomly assigned to control and different treatment groups, six animals per group. (Approval number: XIII/VELS/PCOL/36/2000/CPCSEA/IAEC/08.08.2012).

The animals were acclimatized for one week under laboratory conditions.

Preparation of drug and stock solution[5]:

The suspension of siddha drug Sarva noi linga chendooram in 2% (w/v) CMC was prepared for oral administration by gastric intubation method.

Acute toxicity study-oecd 425 guidelines

Acute oral toxicity test for the Sarva Noi Linga Chenduram was carried out as per OECD Guidelines 425.[5] As with other sequential test designs, care was taken to ensure that animals were available in the appropriate size and age range for the entire study.

Administration of the drug:

The test substance was administered in a single dose by gavage using a stomach tube or a suitable intubation cannula. The fasted body weight of each animal was determined and the dose was calculated according to the body weight. After the substance has been administered, food was with held for a further 2 hours in mice. The animals were observed continuously for the first 4 h and then each hour for the next 24 h and at 6 hourly intervals for the following 48 h after administering of the test drug, to observe any death or changes in general behavior and other physiological activities. Single animals were dosed in sequence usually at 48 h intervals. However, the time interval between dosing was determined by the onset, duration, and severity of toxic signs. Treatment of an animal at the next dose was delayed until one is confident of survival of the previously dosed animal.

Observation of toxicity signs:

General behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes, change in skin and fur, mortality and the body weight changes were monitored daily. The time of onset, intensity, and duration of these signs, if any, was recorded.

Sub-acute toxicity:

Sub acute toxicity study [7] was carried out according to OECD GUIDELINES 407. In a 28-days sub acute toxicity study, twenty four rats of either sex were divided into four groups of 6 rats each. Group I that served as normal control was administered with distilled water (p.o.) while groups II, III and IV were administered daily with the Sarva Noi Linga Chenduram (p.o.) for 28 days at a dose of 50,100,200 mg/kg respectively. The animals were then observed daily for gross behavioral changes and any other signs of subacute toxicity. The weight of each rat was recorded on day 0 and weekly throughout the course of the study, food and water consumption per rat was calculated. At the end of the 28 days they were fasted overnight, each animal was anaesthetized with diethylether [8] following which they were then dissected and blood samples were obtained by cardiac puncture

into heparinised tubes. The blood sample collected from each rat was centrifuged with 3000 X g at 4^{o}C for 10 min to separate the serum and used for the biochemical assays.

Hematological and blood biochemical analyses:

At the end of the study, all animals were kept fasted for 16-18 h and then anesthetized with anesthetic ether on the 28th day. Blood samples for hematological and blood chemical analyses were taken from retro orbital vein[9.] Heparinized blood samples were taken for determining complete blood count (white blood cell count, differential white blood cell count, platelet count, red blood cell count, haematocrit, and hemoglobin) by semiautomated hematology analyzer. The serum from non-heparinized blood was carefully collected for blood chemistry and enzyme analysis (glucose, blood urea nitrogen (BUN), creatinine, total protein, albumin, total and direct bilirubins, serum glutamate-oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) were automatically determined using autoanalyzer.

Necropsy:

All rats were sacrificed after the blood collection. The position, shapes, sizes and colors of internal organs were evaluated. The Spleen, Testes, Pancreas, Lungs, Liver, Brain, Heart, Stomach, Intestine, Bone, Ovary, and Kidney tissues were excised from all rats to visually detect gross lesions, and weighed to determine relative organs weights and preserved in 10% neutral formalin for histopathological assessment[10]. The tissues were embedded in paraffin, and then sectioned, stained with haematoxylin and eosin and were examined microscopically.

Statistical analysis

Values were represented as mean ± SEM. Data were analysed using one-way analysis of variance (ANOVA) [11] and group means were compared using the Tukey-Kramer Multiple Comparisons Test using GraphPad Instat-V3 software. P values < 0.05 were considered significant. **(**Table 1-10).

Results and Discussion

In acute toxicity study, the animals treated with 1000mg/kg were showed tolerance with negligible toxic signs. Hence the one tenth of the dose was selected as median therapeutic dose for the further study. In sub acute toxicity study, animals were shown significant toxic clinical signs during the dosing period of 28 days. Results of body weight determination of animals of control and different dose groups exhibited reduction in body weight (P>0.05) after one week of the dosing period.

During dosing period, the quantity of food consumed by animals from different dose groups was found to be comparable (P>0.05) and normal with that of control animals. Ophthalmoscopic examination of animals in control and Sarva Noi Linga Chenduram treated group revealed abnormality as liver damage. Urine analysis data of control group and Sarva Noi Linga Chenduram treated group of animals determined revealed abnormalities like increase in urine volume and colour was reddish brown. Gross pathological examination of animals in control as well as the Sarva Noi Linga Chenduram treated group revealed abnormalities like liver damage at higher dose treated animals and also and microanatomical changes in bone and spleen tissue.

Table 1: Dose finding experiment and its behavioral Signs of Toxicity

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	500	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	+	-
2	1000	+	+	-	+	+	+	-	+	+	+	-	-	-	+	-	-	-	+	+	-
3	2000	+	+	-	+	+	+	-	+	+	+	-	-	-	+	+	+	-	+	+	+

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15.Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20. Mortality

Table 2. Body wt (g) of rats exposed to *Sarva Noi Linga Chendooram* for 28 days.

Dose (mg/kg/day)	*Days*				
	1	7	14	21	28
Control	110.52±4.85	114.21±5.46	118.10±5.05	121.18±5.12	126.31±4.84
50	118.25±4.45	112.10±5.12	101.12±5.12	100.02±4.22*	108.20±5.01
100	115.33±5.14	110.22±5.00	104.02±5.13	108.17±4.22	110.10±5.11
200	116.46±5.28	109.10±4.72	110.12±5.00	110.48±5.10	110.10±4.10

Values are mean ± S.E.M. (Dunnet 't' test). *P>0.05; N=6.

Table 3. Food (g/day) intake of rats exposed to *Sarva Noi Linga Chendooram* for 28 days.

Dose (mg/kg/day)	Days (gms/rats)				
	1	7	14	21	28
Control	40.00±2.25	43.16±2.12	45.10±2.46	44.64±2.58	45.15±2.15
50	45.30±2.41	44.10±2.18	44.20±2.30	45.14±2.29	46.00±4.02
100	43.35±2.10	45.42±2.63	45.15±2.34	44.25±2.18	45.14±3.00
200	44.15±2.22	45.24±2.55	45.34±2.52	45.43±2.60	45.40±2.48

Values are mean ± S.E.M. (Dunnet 't' test). ^{ns}P>0.05. N=6.

Table 4. Water (ml/day) intake of rats exposed to *Sarva Noi Linga Chendooram* for 28 days.

Dose (mg/kg/day)	Days(ml/rat)				
	1	7	14	21	28
Control	48.10±2.45	50.18±3.30	52.48±3.14	52.04±3.13	51.46±3.81
50	50.12±2.42	50.42±3.12	51.12±4.10	50.00±3.52	49.42±2.42
100	49.00±2.20	44.48±3.01	46.67±3.28	48.02±2.48	49.40±3.20
200	50.00±2.18	50.23±2.04	50.15±3.10	51.32±2.74	50.17±3.00

Values are mean ± S.E.M. (Dunnet 't' test). ^{ns}P>0.05. N=6.

Table 5. Hematological parameters after 28 days treatment with *Sarva Noi Linga Chendooram*.

Parameter	Control	50 mg/kg	100 mg/kg	200 mg/kg
Red blood cell (mm^3)	5.21±0.44	5.26±0.32	5.18±0.40	5.12±0.41
HB (%)	14.65±0.38	15.12±0.40	15.52±0.45	16.00±0.32
Leukocyte ($x10^3$/Cu.mm)	8.72±1.1	8.58±0.34	8.40±1.00	8.32±1.12
Platelets(K/µl)	448±25.00	456±30.41	476±22.14	481±23.04
MCV (gl)	52.21±4.26	51.34±4.26	52.10±4.71	53.12±4.20
N	15.14±1.42	15.42±1.19	14.22±0.50	14.87±1.14
L	84.12±2.54	83.92±2.48	82.00±2.75	83.21±2.92
M	1.50±0.38	1.42±0.40	1.41±0.32	1.42±0.36
E	1.01±0.10	1.00±0.21	1.00±0.18	1.00±0.14
B	0±0.00	0±0.00	0±0.00	0±0.00
ESR(mm)	1±00	1±00	1±00	1±00
PCV	46.30±2.45	46.42±2.81	45.75±3.00	45.66±2.38

Values are mean ± S.E.M. (Dunnet 't' test). *P<0.05; **P<0.01. N=6.

Table 6. Effect of treatment with *Sarva Noi Linga Chendooram on* biochemical parameters.

Dose (mg/kg)	Control	50 mg/kg	100 mg/kg	200 mg/kg
Total Bilirubin (mg/dL)	0.28±0.04	0.28±0.04	0.27±0.05	0.28±0.05
Bilirubin direct (mg/dL)	0.20±0.05	0.24±0.04	0.22±0.05	0.23±0.05
ALP (U/L)	102.10±10.00	104.42±9.20	110.18±10.05	112.32±10.27
SGOT (U/L)	114.05±4.14	112.10±4.00	110.42±4.44	112.10±5.85
SGPT(U/L)	34.10±2.66	35.14±3.15	35.04±2.18	35.62±2.14
Total Protein(g/dl)	6.34±1.42	6.70±0.14	7.11±0.29	7.10±0.27
Albumin(g/dl)	2.41±0.28	2.71±0.25	2.82±0.38	2.90±0.22
Globulin(g/dl)	4.18±0.20	5.30±0.24**	4.77±0.24	4.70±0.24

Values are mean ± S.E.M. (Dunnet 't' test). **P<0.01 Vs control group N=6.

Table-7 RFT

Dose (mg/kg)	Control	50 mg/kg	100 mg/kg	200 mg/kg
Urea (mg/dL)	4.56±1.88	5.44±1.25	5.78±1.22	5.74±1.70
Creatinine (mg/dL)	0.73±0.04	0.72±0.04	0.73±0.05	0.82±0.04
Uric acid (mg/dL)	3.52±0.15	4.12±0.18*	4.10±0.18	4.21±0.14*
Na m.mol	115.46±5.04	115.51±5.10	115.10±4.22	114.10±4.10
K m.mol	5.25±2.46	5.45±1.67	5.44±1.50	4.80±2.31
Cl m.mol	102.24±4.30	101.00±4.45	98.44±4.24	100.77±4.04

Values are mean ± S.E.M. (Dunnet 't' test). *P<0.05; Vs control group N=6.

Table-8. Lipid Profile

Dose (mg/kg)	Control	50 mg/kg	100 mg/kg	200 mg/kg
Total cholestrol(mg/dL)	80.11±2.80	79.14±2.37	78.78±3.02	76.99±2.90
HDL(mg/dL)	123.25±2.50	122.20±2.27	123.10±3.00	121.25±2.24
LDL(mg/dL)	42.12±2.55	42.24±2.80	43.00±2.88	42.62±2.04
VLDL(mg/dl)	26.10±2.40	27.38±2.14	26.10±2.48	25.40±2.25
Triglycerides (mg/dl)	27.24±2.62	26.28±2.28	27.20±3.00	28.08±2.55
Blood glucose (mg/dl)	92.40±4.50	94.24±3.15	94.22±3.00	95.28±2.42

Values are mean ± S.E.M. (Dunnet 't' test). ^{ns}P>0.05. Vs control group N=6.

Table-9 Urine Analysis

Parameters	Control	50 mg/kg	100 mg/kg	200 mg/kg
Colour	Yellow	Reddish Yellow	Reddish Brown	Reddish Brown
Transparency	Clear	Slightly turbid	Turbid	Turbid
Specific gravity	1.010	1.010	1.010	1.010
PH	>7.2	>7.4	>7.2	>8.4
Protein	Nil	1+	1+	2+
Glucose	Nil	Nil	Nil	Trace
Bilirubin	-ve	-ve	-ve	-ve
Ketones	-ve	-ve	-ve	+ve
Blood	Absent	Absent	Absent	Absent
Urobilinogen	Normal	Normal	Normal	Normal
Pus cells	0-cells/HPF	1-cell/HPF	2-cells/HPF	1-cell/HPF
RBCs	Nil	Nil	0-1cells/HPF	Nil
Epithelial cells	Nil	1-cell/HPF	Nil	1-cell/HPF
Crystals	Nil	Nil	Nil	Nil
Casts	Nil	Nil	Nil	Nil
Others	Bacteria seen	Bacteria seen	Bacteria seen	Bacteria seen

Table 10. Effect of oral administration of *Sarva Noi Linga Chendooram* on organ weight

Dose (mg/kg)	Control	50 mg/kg	100 mg/kg	200 mg/kg
Liver (g)	3.10±0.10	3.14±0.12	3.12±0.10	3.08±0.12
Heart (g)	0.32±0.04	0.31±0.05	0.30±0.04	0.32±0.04
Lung (g)	0.44±0.12	0.45±0.12	0.43±0.10	0.42±0.10
Spleen (g)	0.45±0.05	0.46±0.05	0.46±0.05	0.46±0.05
Ovary (g)	1.43±0.12	1.44±0.12	1.45±0.14	1.46±0.12
Testes (g)	2.12±0.12	2.10±0.17	2.12±0.12	2.20±0.14
Brain (g)	2.11±0.10	2.15±0.12	2.16±0.14	2.12±0.15
Kidney (g)	0.80±0.05	0.78±0.05	0.81±0.05	0.82±0.05
Stomach (g)	1.14±0.12	1.15±0.11	1.12±0.13	1.14±0.12

Values are mean ± S.E.M. (Dunnet 't' test). ^{ns}P>0.05. Vs control group N=6.

The results of haematological investigations, revealed mild changes (P>0.05) when compared with those of respective controls. Results of Biochemical investigations conducted on days 28 revealed the significant changes in the values of different parameters when compared with those of respective controls. Globulin showed increased levels in animals in 50mg/kg dose group (P<0.01), Total Protein level is elevated in animals of 100 and 200mg/kg dose group but it is statistically not significant. Uric acid level was elevated in animals of 100 and 200mg/kg group (P<0.05). Other all biochemical and Haematological parameters were found to be within normal limit as compared to control group values.

Conclusion

Toxic effect was observed at 200mg/kg of Sarva Noi Linga Chenduram treated via oral route over a period of 28 days. This study explored that Sarva Noi Linga Chenduram can be prescribed for therapeutic use in human with the dosage recommendations of upto maximum of 100mg/kg body weight p.o. for long term administrations the 20-30% of dose reduction is very essential to avoid organ damage.

References

1. Singh R, Gautam N, Mishra A, Gupta R. Heavy Metals and Living Systems: An Overview. Indian J Pharmacol 2011;43:246-253.
2. ..Abdhulla sahib Anupoga vaidhya navaneetham part 4 P.NO 52, 53
3. Dr R.Thiyagarajan,gunapadam thathu seeva vaguppu,4th edition 2004 p.no 272
4. Dr R.Thiyagarajan,gunapadam thathu seeva vaguppu,4th edition 2004 p.no 272
5. N. Kabilan, S. Tamil Selvi and N. Senthamarai Selvi.Anti-hyperglycemic activity of the herbo-mineral siddha preparation in alloxan induced diabetic rats. J. Nat. Prod. Plant Resour., 2013, 3 (2):42-47
6. Organization for the Economic Cooperation and Development (OECD) *Guidelines for Testing of Chemicals*. Paris, France: OECD; 2005. (Guidenace, no. 425. Up and Down Procedure).
7. O. A. Salawu , B. A. Chindo , A. Y. Tijani *, I. C. Obidike , T. A. Salawu A. James Akingbasote African Journal of Pharmacy and Pharmacology Vol. 3(12). pp. 621-626, December, 2009
8. Draft OECD Guidance Document on Histopathology for inhalation toxicity studies, Supporting TG 412 (Subacute Inhalation Toxicity: 28-Day) and TG 413 (Subchronic Inhalation Toxicity: 90-Day (Version 15 June 2009).
9. Janet Hoff, LVT, RLATG . Methods of Blood Collection in the Mouse. November 2000 Lab Animal Volume 29, No. 10.
10. Vogel G, Vogel W. *Drug Discovery and Evaluation: Pharmacological Assays*. Berlin, Germany: Springer; 1997.
11. Michael F. W. Festing. Design and Statistical Methods in Studies Using Animal Models of Development

How to cite this article:

A.Punitha, S.Visweswaran, A. Rajendra kumar, K.Manickavasakam.. (2017). Acute and sub acute toxicity studies on Sarva Noi Linga Chenduram in rodents. Int. J. Curr. Res. Chem. Pharm. Sci. 4(2): 13-18.

DOI: http://dx.doi.org/10.22192/ijcrcps.2017.04.02.004

Access this Article in Online

Website: www.ijcrcps.com

Subject: Siddha Medicine

Quick Response Code

DOI: 10.22192/ijcrcps.2017.04.02.004