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ACUTE TOXICITY STUDY ON SIDDHA POLY HERBAL FORMULATION – SHAYATHIRKU ENNAI

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ABSTRACT

The Poly herbal formulation Shayathirku Ennai has been used in the treatment of Sura Peenisam in children (Sinusitis) and also other paediatric respiratory diseases. Shayathirku Ennai is mentioned in the Siddha text book "Agathiyar Vaithiya Vallathi – 600". Safety of the medicine is more important than the efficacy of the medicine. Hence evaluation of Toxicity study on Shayathirku Ennai is mandatory. According to OECD guidelines, acute Toxicity single doses administred and monitored for 14 days. The result obtained is significant and the P value is. Hence Shayathirku Ennai was non Toxic on acute Toxicity study.

KEYWORDS: Siddha Medicine, Polyherbal Formulation, Shayathirku Ennai, Acute Toxicity study.

INTRODUCTION

Siddha System of medicine has been used widely over the thousands of year in Tamil Nadu. There are three groups of drugs in Siddha System of medicine such as Plant products (Mula Vargam), Inorganic substances (Thathu Vargam), Animal products (Jeeva Vargam).

Shayathirku Ennai isone of the Polyherbal Formulation which consist of six major herbs Poovanthi Pattai, Kadukkai, Kasthuri Manjal, Vanniver, Karunjeeragam , Eranda Ennai. Toxicity Study of such medicine is essential factor to asses safety of the drug. It is used in the treatment of Sura Peenisam (Sinusitis in children).

Sura Peenisam is affecting more than 14 percentage of School going children leads to their poor day to day performance or activities.

The present study was carried out to rule out Safety of Shayathirku Ennai in the treatment of Sura Peenism (Sinusitis in Children).According to OECD Guideline – 423 Acute Oral Toxicity Study Of Shayathirku Ennai is evaluated. The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance. This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods.

The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment. In principle, the method is not intended to allow the calculation of a precise LD50, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test.

The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%. The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

MATERIALS AND METHODS

Source of Raw Drugs

- Poovanthi Pattai is collected from Thiruvannamali District.

- Vanni Ver is collected from Vadivudai Amman Kovil ,Thiruvettriur
- The other raw drugs is procured from a well reputed Indigenous drug shop from Parrys corner, Chennai.
- All raw drugs were authenticated by the Pharmacognosist, SCRI Chennai.

Purification of the Raw Drugs
Raw drugs are purified as mentioned in Sikicharathna Deepam Sarakku Suthi Muraigal.

Ingredients

S. No.	Name of the drug	Part used	Botanical name	Quantity
1	Poovanthi Pattai	Stem Bark	*Sapindus trifoliatus*	50 palam (1750) gms)
2	Eranda Ennai	Seed Oil	*Ricinus communis*	1 Padi (1.3 ltr)
3	Kadukkai	Fruit	*Terminalia chebula*	1 Palam (35 gms)
4	Kasthuri Manjal	Rhizome	*Curcurma aromatic*	1 Palam (35 gms)
5	Vanni Ver	Root	*Prosopis spicigera*	½ Palam (17.5 gms)
6	Karunjeeragam	Seeds	*Nigella sataiva*	½ Palam (17.5 gms)

Method of preparation
Poovanthi Pattai decoction is made by taking poovanthi pattai in a mud pot, 1.4 liters of water is added and boiled till the decoction is reduced upto 1.3 liters. To this decoction equal quantity of Eranda Ennai is added. Kasthuri Manjal, Kadukkai, Vanniver are made into a paste in stone morter and the paste is mixed into the above mentioned decoction and Ennai. This mixture is boiled till the mezhugu patham (Waxy Consistency) is obtained and Karunjeeraga powder is added at the end of this Ennai.

Therapeutic Dosage – 5ml for 8 to 12 years twice a day.

Principle of the Test
It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.

- No further testing is needed
- Dosing of three additional animals, with the same dose
- Dosing of three additional animals at the next higher or the next lower dose level.
- The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

Methodology
Selection of Animal Species
The preferred rodent species is the wistar albino rat, although other rodent species may be used. Healthy young adult animals are commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 6 to 8 weeks old and the weight (150-200gm) should fall in an interval within±20 % of the mean weight of any previously dosed animals.

Housing and Feeding Conditions
The temperature in the experimental animal room should be 22ºC $\pm$ 3ºC. Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals
The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions

Test Animals and Test Conditions
Sexually mature Female Wistar albino rats (150-200gm) were obtained from TANUVAS, Madhavaram, and Chennai. All the animals were kept under standard environmental condition (22±3 ° C). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore).

Preparation of animals
The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions

Preparation for Acute Toxicity Studies
Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the, **Shayathirku Ennai**
The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design

IAEC No: XLVIII/08/CLBMCP/2017

Test Substance : **Shayathirku Ennai**
Animal Source : TANUVAS, Chennai.
Animals : Wister Albino Rats (Female-3+3)
Age : >6 weeks
Body Weight on Day 0 :150-180 gm.
Acclimatization : Seven days prior to dosing.
Veterinary examination : Prior and at the end of the acclimatization period.
Identification of animals : By cage number, animal number and individual marking by using Picric acid.
Number of animals : 3 Female/group,
Route of administration : Oral
Diet : Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water : Aqua guard portable water in polypropylene bottles.
Housing & Environment : The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature : between 22ºC ± 3ºC.
Relative humidity : between 30% and 70%,
Air changes : 10 to 15 per hour and
Dark and light cycle : 12:12 hours.
Duration of the study : 14 Days

Administration of Doses

Shayathirku Ennai was administered to the groups of wistar albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three Female animals are used for each group. The dose level of 2000 mg/kg body weight was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously as per the guideline after drug administration. The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. Finally, the number of survivors was noted after 24 hrs and these animals were then monitered for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

OBSERVATIONS

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somato-motor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. The principles and criteria summarized in the Humane Endpoints Guidance Document will be taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanly killed. When animals are killed for humanly reasons or found dead, the time of death was recorded.

Acute Oral Toxicity Study of Shayathirku Ennai

Table 1: Dose finding experiment and its behavioral Signs of acute oral Toxicity Observation done.

S.L.	Group Control	Observation	SL	Group Test Group	Observation
1	Body weight	Normal	1	Body weight	Normally increased
2	Assessments of posture	Normal	2	Assessments of posture	Normal
3	Signs of Convulsion Limb paralysis	Normal	3	Signs of Convulsion, Limb paralysis	Absence of sign (-)
4	Body tone	Normal	4	Body tone	Normal
5	Lacrimation	Normal	5	Lacrimation	Absence
6	Salivation	Normal	6	Salivation	Absence
7	Change in skin color	No significant color change	7	Change in skin color	No significant color change

8	Piloerection	Normal	8	Piloerection	Normal
9	Defecation	Normal	9	Defecation	Normal
10	Sensitivity response	Normal	10	Sensitivity response	Normal
11	Locomotion	Normal	11	Locomotion	Normal
12	Muscle gripness	Normal	12	Muscle gripness	Normal
13	Rearing	Mild	13	Rearing	Mild
14	Urination	Normal	14	Urination	Normal

Behaviour

The animals will be observed closely for behaviour in the first four hours which includes abnormal gait, aggressiveness, exophthalmos, ptosis, akinesia, catalepsy, convolusion, excitation, head twitches, lacrimation, loss of corneal reflex, loss of traction, piloerection reactivity of touch, salivation, scratching, sedation, chewing, head movements, sniffing, straub, tremor and writhes, diarrhea, leathery, sleep and coma.

Body Weight

Individual weight of animals was determined before the test substance was administered and weights will be recorded at day 1, 7, and 14 of the study. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and humanly killed.

Food and water Consumption

Food and water consumed per animal was calculated for control and the treated dose groups.

Mortality

Animals were observed for mortality throughout the entire period.

RESULTS

All data were summarized in tabular form, (Table-1-5) showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test, description of toxic symptoms, weight changes, food and water intake.

No of animals in each group:3

Table 2: (Observational study Results).

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.	**Control**	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	**2000 mg/kg**	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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. Diarrhea 18. Writhing 19. Respiration 20. Mortality.
(+ Present, - Absent)

Table 3: (Body weight Observation).

	Days		
Dose	1	7	14
Control	220.11± 1.23	222.2± 1.42	224.2 ± 1.24
2000 mg/kg	202.1± 1.18	204.2± 1.26	206.4 ± 1.20
P value (p)*	NS	NS	NS

Table 4: (Water intake (ml/day) of Wistar albino rats group exposed to (Shayathirku Ennai*)*.

Dose	Days		
	1	6	14
Control	33.2 ± 1.34	33.9± 2.23	34.4± 2.13
2000 mg/kg	34.2±1.14	34.4±1.11	36. 6± 1.19
P value (p)*	NS	NS	NS

N.S- Not Significant,**($p > 0.01$), *($p > 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table 5: Food intake (gm/day) of Wistar albino rats group exposed to SHAYATHIRKKU ENNAI.

Dose	Days		
	1	7	14
Control	31.43±4.16	32.12±3.16	32.52±2.26
2000 mg/kg	35.12±4.14	35.21±1.12	36.22±1.14
P value (p)*	NS	NS	NS

CONCLUSION

The Acute Oral Toxicity study demonstrated that the Siddha formulation Shayathirku Ennai shows no acute toxicity .Higher dose is 2000mg per kg. So it can be concluded that the Shayathirku Ennai was prescribed for Therapeutic use. The study provides valuable data on Acute Toxicity profile of Shayathirku Ennai.

REFERENCES

1. Mohana Raj. T. Mathalai Noi Thoguthi, (1): 371.
2. Suraj gupte. The Short Textbook of Paediatrics.11nd ed. jaypee brothers(p)ltd;2 publication, 2009; 684.
3. An on JB (April 2010 "URI" Am.J.Med123 (4Suppl): s 16-25.doi:10.10 16j.Amjmed, 2010.02.003.PM D 2 35 632/
4. Halmilos DL (OCT 2011 "Chronic rhinosinusitis: epidemiology and medical management".The journal of allergy and clinical immunology 128(4:693-707;quiz 708 -9.doi :10,10 16/ j.jac .2 011.8.004 PMID 21890184.
5. Sunali s.Khanna,A.Gharpure.Correlation of incresed sinusitis and urban air polltion ind.J.sci.Res and Tech, 2012; 1(1): 14-17.
6. ISRN otolaryngology (SukhbirK.Shahid), 2012,10. 5402 / 2012/851831.
7. Agasthiyar Vaithiya Vallathi 600(Moolamum Uraium) 2nd ed.New Delhi Publication: Central Council For Research In Ayurvedha And Siddha, 2005; 166.
8. Murgesa mudaliyar KS Siddha Materia medica (Medicinal plant division) .2nd ed.Indian medicine and Homeopathy publication, 2008; 724, 38, 798,122,296,201.
9. www.oecd.org.
10. KannuSami Pillai C. Sikicharathna Deepam Sarakku Suthi Muraigal.1sted. Thirumagal Vilasa Achagam, 2011; 27,28,29,30,31,32.
11. OP Ghai, Ghai essential paediatrics 7th edition, CBS Publishers 7th Edition, 2010; 335.