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Toxicity study on Sanga Thiravagam for polycystic ovarian syndrome

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Abstract

Siddha medicine is an Indians oldest system of medicine for health and longevity. One of the community based prevalence study using the Rotterdam criteria found that about 18%of women had PCOS and that 70% of them were previously undiagnosed. The prevalence of pcos depends on the choice of diagnostic criteria. The world health organisation, estimates that it affects 116million women worldwide as of 2010(3.4%of the people).In this study,author have documented both Acute and sub acute toxicity study on Sangathiravagam for pcos.

Keywords: Siddha medicine, sanga thiravagam, pcos, toxicity study, soothagavayu.

Introduction

The classical siddha literature Aathmaratchamirthamennumvaithiyasangiragam quoted about soothagavaayu. The clinical features of soothagavaayu may be correlated with polycystic ovarian syndrome in modern aspects. Siddha literature pararasekaram cite that any imbalance in three humors may inhibit the release of ovum from the ovaries. This may be related to the pcos due to hormonal imbalance. Pcos is one of the leading cause of female subfertility and the most frequent endocrine problem in women of reproductive age.

Materials and Methods

Acute and sub acute toxicity study on Sanga Thiravagam in rodents

Animal Model.

Mice of either sex weighing 25-30g and rats weighing 110-140g were obtained from the animal house of K.k college of pharmacy . The animals were used with the approval of the Institute animal ethics committee and obtained from K.K.college of Pharmacy Chennai.

They were fed with a balanced standard pellet diet and maintained under standard laboratory conditions, providing 24-28° 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. The animals were acclimatized for one week under laboratory conditions.

Acute toxicity study-OECD 425 guidelines

Acute oral toxicity test for the SangaThiravagam was carried out as per OECD Guidelines 425. As with other sequential test designs, care was taken to ensure that animals are available in the appropriate size and age range for the entire study. The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation cannula. The fasted body weight of each animal is determined and the dose is calculated according to the body weight. 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 behaviour and other physiological activities. Single animals are dosed in sequence usually at 48 h intervals. However, the time interval between dosing is 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

In a 28-days sub acute toxicity study, twenty four either sex rats 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 Sanga Thiravagam (p.o) for 28 days at a dose of 2.5, 5.0 and 10.0 ml/kg respectively. The animals were then observed daily for gross behavioural changes and any other signs of subacutetoxicity. 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 28 days they were fasted overnight, each animal was anaesthetized with diethyl ether, 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˚ 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 hematologicl blood chemical analyses were taken from retro orbital vein. Heparinized blood samples were taken for determining complete blood count(white blood cell count, differential white cell blood cell count, plateletcount, red blood cell count, hematocrit and haemoglobin) by semiautomated hematology analyser. The serum from non heprinized blood was carefully collected for blood chemistry and enzyme analysis (glucose,creatinine,totalprotein,albumin,total and direct bilirubins, serum glutamate-oxaloacetate transaminase (SGOT) ,and serum glutamate pyruvate transaminase (SGPT),and alkaline phosphatase (ALP) were automatically determined using autoanalyzer.

Necropsy

All rats were sacrified after the blood collection. The positions, shapes ,sizes and colors of internal organs were evaluated. The spleen, testes, pancreas, lung, 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. 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) test using GraphpadInstat –V3 software $p<0.05$ were considered significant.

Results

1. All the animals from control and all the treated dose groups upto 10ml/kg survived throughout the dosing period of 28 days.
2. No signs of major or significant intoxication were observed in animals from lower to higher dose groups during the dosing period of 28 days,
3. Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days.
4. Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days.
5. Ophthalmoscopic examination conducted prior to and at the end of dosing period on animals from control and all the treated dose groups did not reveal any abnormality.
6. Haematological analysis conducted at the end of the dosing period on day 28 revealed no remarkable abnormalities attributable to the treatment.
7. Biochemical analysis conducted at the end of the dosing period on day 28 revealed no remarkable abnormalities attributable to the treatment.
8. Functional observation tests conducted at termination revealed on abnormalities
9. Urine analysis conducted at the end of the dosing period in week 4,revealed no abnormality attributable to the treatment
10. Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls.
11. Gross pathological examination did not reveal any abnormality.
12. Histopathological examination did not reveal any abnormality.

Table 1: Dose finding experiment and its behavioural Signs of toxicity

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

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 weight of albino rats exposed to- Sanga Thiravagam for 28 days

Dose ml/kg/day	Days				
	1	7	14	21	28
Control	112.24±2.01	114.65±4.22	115.88±5.21	117.43±1.08	119.64±4.22
2.5	113.22±0.21	114.65±9.23	116.20±0.04	118±2.22	120.88±3.67
5	116.23±4.12	116.89±4.26	118.90±5.89	119.21±3.22	119.98±0.21
10	114.65±5.02	114.88±5.27	116.47±0.26	117.80±4.21	118.66±0.86

Values are mean of 6 animals .E.M. nsP>0.05 Vs control

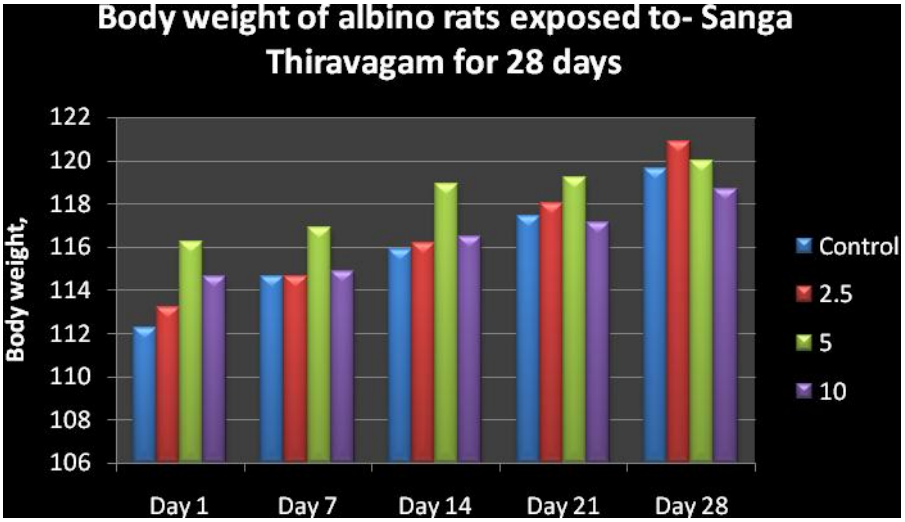

Table 3 water intake of albino rats exposed to Sanga Thiravagam for 28 days

Dose (ml/kg/day)	Days(ml/rat)				
	1	7	14	21	28
Control	60.12±2.12	58.26±2.12	56.24±2.46	58.42±2.68	56.24±2.80
2.5	58.24±4.16	56.28±3.86	54.80±3.24	52.80±2.24	54.24±4.68
5	54.28±6.42	55.46±2.86	56.20±4.22	56.80±4.86	58.20±2.24
10	52.65±8.22	53.40±6.46	54.20±4.20	56.20±2.40	58.20±2.66

Values are mean of 6 animals S.E.M. nsP>0.05 Vs control

Table 4 Food intake of albino rats exposed to SangaThiravagam for 28 days

Dose (ml/kg/day)	Days(ml/rats)				
	1	7	14	21	28
Control	40.12±2.12	42.48±2.46	44.68±2.80	46.44±2.42	44.26±2.56
2.5	42.46±2.12	43.28±2.43	44.28±2.48	44.36±3.09	45.60±2.87
5	41.68±2.14	42.80±2.65	43.44±3.10	44.68±2.49	46.42±2.15
10	42.28±2.14	40.28±2.15	43.27±2.90	42.03±3.16	44.46±2.60

Values are mean of 6 animals S.E.M. nsP>0.05 Vs control

Table 5.Hematological parameters after 28 days treatment with Sanga Thiravagam -in rats

Parameters	Control	2.5ml/kg	5ml/kg	10ml/kg
Red blood cell(mm3)	8.90±0.64	8.92±0.24	8.10±0.36	8.12±0.66
HB(%)	15.10±0.36	15.29±0.42	15.20±0.43	15.68±0.28
Leukocyte(x10 6 /ml)	10218±124.12	10298±110.46	10264±146.22	10264±119.24
Platelets/µl	1264±30.12	1197±31.22	1138±32.10	1198±32.14
MCV(gl)	54.22±5.12	53.29±5.86	54.20±5.22	54.28±5.02
Neutrophil	5.42±2.46	5.46±3.26	5.55±2.46	5.28±2.12
Lymphocytes	92.08±2.10	91.28±2.86	90.14±2.14	92.46±2.80
Monocytes	2.20±0.28	2.48±0.48	2.42±0.49	2.46±0.28
Eosinophil	1.00±0.01	1.00±0.12	1.00±0.24	1.00±0.46
Basophil	0	0	0	0
ESR(mm)	1±00	1±00	1±00	1±00
PCV	44.20±2.12	45.98±2.19	45.10±2.18	45.16±2.16

Values are mean of 6 animals .E.M. nsP>0.05 Vs control

Table 6: Effect of treatment with Sanga Thiravagam-biochemical parameters

Dose (ml/kg)	Control	2.5 ml/kg	5ml/kg	10ml/kg
Total BIlirubin(mg/dl)	0.221±0.14	0.242±0.12	0.226±0.02	0.226±0.25
BIlirubin direct (mg/dl)	0.1±0.04	0.1±0.04	0.1±0.04	0.1±0.06
BIlirubin indirect(mg/dl)	0.1±00	0.1±00	0.1±00	0.1±00
ALP(U/L)	380.68±12.64	386.22±10.26	368.14±10.48	364.48±10.39
SGOT(U/L)	155.24±5.18	156.56±5.28	158.34±5.28	159.20±5.26
SGPT(U/L)	44.28±2.30	44.68±2.48	44.88±2.68	44.69±2.14
Total protein(g/dl)	8.24±0.44	8.46±0.34	8.22±0.22	8.64±0.32*
Albumin(g/dl)	3.22±0.24	3.34±0.34	3.36±0.40*	3.43±0.26*
Globulin(g/dl)	5.08±0.12	5.60±0.16*	5.12±0.24*	5.23±0.24*

Values are mean of 6 animals S.E.M. *P<0.05; Vs Control

Table 7 RFT

Dose(ml/kg)	Control	2.5ml/kg	5ml/kg	10ml/kg
Urea(mg/dl)	56.24±2.26	55.30±2.46	55.45±2.46	55.86±2.52
Creatinine(mg/dl)	0.78±0.06	0.78±0.08	0.76±0.08	0.77±0.08
Uric acid(mg/dl)	1.6±0.12	1.6±0.13	1.6±0.12	1,6±0.12
Na m.mol	142.40±5.24	143.20±5.26	142.67±5.21	140.70±5.22
K m,mol	19.87±2.12	20.22±2.46	20.15±2.12	20.89±2.80
Clm.mol	100.18±5.12	101.24±5.29	101.46±5.22	100.26±5.26

Values are mean of 6 animals S.E.M. nsP>0.05 Vs control

Table 8 Lipid profile

Dose(ml/kg)	Control	2.5ml/kg	5ml/kg	10ml/kg
Total cholesterol(mg/dl)	42.34±3.01	42.43±3.02	42.86±3.06	42.90±3.26
HDL(mg/dl)	14.21±1.68	14.22±1.88	14.66±1.09	14.68±1.22
LDL(mg/dl)	42.06±2.25	42.98±2.46	43.01±2.86	43.20±2.10
VLDL(mg/dl)	15.20±2.26	15.32±2.28	15.28±2.10	15.68±2.88
Triglycerides(mg/dl)	87.02±2.22	88.12±2.09	88.20±2.99	88.27±2.12
TC/HDL ratio(g/dl)	3.85±0.21	3.21±0.25	3.58±0.86	3.76±0.88
Blood glucose(mg/dl)	128.14±2.24	128.18±2.87	129.12±2.10	128.17±2.67

Values are mean of 6 animals S.E.M. nsP>0.05 Vs control

Table 9 Urine Analysis

Parameters	Control	2.0ml/kg	5ml/kg	10ml/kg
Colour	Yellow	Yellow	Yellow	Yellow
Transparency	Clear	Slightly turbid	Slightly turbid	Slightly turbid
PH	>7.0	>7.8	>8.0	>8.9
Specific Gravity	1.010	1.010	1.010	1.010
Protein	Nil	Nil	2+	3+
Glucose	Nil	Nil	NIL	NIL
Bilirubin	_Ve	-Ve	-ve	-ve
Ketones	+ve	+ve	+ve	+ve
Blood	Absent	Absent	Absent	Absent
Pus Cells	0-cells/HPF	1-cell/HPF	2-cells/HPF	2-cells/HPF
RBC	Nil	Nil	Nil	1-cell/HPF
Epithelial cells	Nil	1-cell/HPF	Nil	Nil
Crystals	Nil	Nil	NIl	Nil
Casts	NIl	Nil	Nil	Nil
Uribilinogen	Normal	Abnormal	Abnormal	Abnormal

Table 10 Effect of oral administration of Sanga Thiravagam on organ weight

Dose(ml/kg)	Control	2.5ml/kg	5ml/kg	10ml/kg
Liver(g)	5.68±0.12	5.40±0.16	5.38±0.14	5.32±0.18
Heart(g)	0.64±0.02	0.64±0.02	0.62±0.02	0.62±0.02
Lung(g)	1.46±0.04	1.45±0.06	1.44±0.06	1.44±0.02
Spleen(g)	0.65±0.02	0.66±0.02	0.66±0.02	0.66±0.04
Ovary(g)	1.70±0.12	1.87±0.15	1.89±0.16	1.90±0.12
Testes(g)	1.46±0.12	1.48±0.14	1.42±0.12	1.42±0.12
Brain(g)	1,55±0.12	1.54±0.15	1.55±0.12	1.52±0.16
Kidney(g)	0.72±0.04	0.74±0.04	0.74±0.04	0.72±004
Stomach(g)	1.38±0.12	1.37±0.12	1.38±0.14	1.38±0.14

Values are mean of 6 animals S.E.M. nsP>0.05 Vs control

Conclusion

Based on these findings, no toxic effect was observed upto 10ml/kg of Sanga Thiravagam via oral route over a period of 28 days. So it can be concluded that the Sanga Thiravagam can be prescribed for therapeutic use in human with dosage recommendations of upto 10ml/kg body weight.

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