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Evaluation of Acute and Subacute toxicity of Siddha herbal formulation *Pungampoo chooranam*

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Abstract: *The Siddha system of medicine was not a discovery but a gradual evolution during successive periods of history by sages of South India called as Siddhars. Even though the materials used as drugs by Siddhars could be classified into herbal, inorganic substances and animal products great emphasis was given to herbs. Pungampoo Chooranam is one such herbal formulation consisting of flowers of single herb Pongamia pinnata. Although this herbal formulation has been indicated for the treatment of Diabetes mellitus in the Siddha classical literature Boga Munivar Vaithiyam – 700, its scientific data on its safety profile is still deficient. So the present study was performed to evaluate the toxicological potential of Pungampoo Chooranam. In acute oral toxicity study, Pungampoo Chooranam was administered at 2000mg/kg orally and animals were observed for toxic signs at 30 min, 1, 2 and 4 hours and thereafter once a day for the next 14 days. In repeated dose-28 day toxicity study, the animals were divided into three groups of 6 animals each consist of 3 male and 3 female rats. Group-1 animals received saline 5 ml/kg b.w (p.o). Group II Animals received low dose of test drug Pungampoo Chooranam 200 mg/kg (p.o). Group III animals received high dose of test drug 400 mg/kg (p.o) once daily for 28 days respectively. The study results showed that neither the acute toxicity study of Pungampoo Chooranam at the dose level of 2000mg/kg nor the repeated dose study did not produce any toxic sign or mortality during study. In repeated dose toxicity study, no significant changes were observed in the haematological and biochemical parameters, relative organ weight, gross necropsy and histopathological examination with Pungampoo Chooranam treatment. The Results of the present study suggest that LD50 of Pungampoo Chooranam >2000mg/kg.*

Keywords*:* Siddha, *Pungampoo Chooranam*, Acute oral toxicity, Sub acute toxicity, *Pongamia pinnata*

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I. Introduction

Diabetes mellitus (DM) is one among the major metabolic disorders presently connected with several long term complications, morbidity and mortality in the affected individuals. In India more than 40 million people are affected which represent nearly 20% of total diabetes population worldwide[1]. Traditional systems of medicine such as *Siddha* system continues to be extensively practiced due to increase in population rise, inadequate supply of drugs, unaffordable high cost of treatments, side effects of some synthetic drugs and development of resistance to currently used drugs. *Pungampoo Chooranam* is one such herbal formulation which has been indicated in *Siddha* literature for the treatment of Diabetes mellitus. Though herbal medicines are time–tested and are considered to be safe, most of them are not scientifically validated for their safety profile. Hence the present study was performed to evaluate the acute and sub acute toxicity of the *Siddha* herbal formulation *Pungampoo Chooranam* in experimental rodents. Through this study the safety of this herbal drug can be established for the clinical use of this traditional formulation among the diabetic patients.

II. Materials And Methods

2.1. Study Drug

The hertbal ingredients thatwere used for the preparation of trial drug Pungampoo chooranam consist of Pungampoo (Pongamia glabra flowers).The Herbal drugs were procured from traditional reputed shops of Chennai and authenticated by botanist. The above drugs were then prepared as pills as per Siddha text.

2.2. Preparation of trial drug

The shade dried flowers of Pungam tree (Pongamia glabra flowers) were roasted slowly by adding little bit of cow's ghee, and then powdered and sieved using cloth.

2.3. Experimental animals husbandry

Healthy adult Wistar albino rat weighing between 170-200 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit (AHU). A 12 light / dark cycle were maintained .Room temperature was maintained between 22 $\pm$ 2^0 Cand relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama University, Chennai, Tamil Nadu, India (SU/CLATR/IAEC/IV/023/2016).)

2.4. Acute toxicity study

Acute toxicity study was carried out in accordance with guidelines of Organization for Economic Co-operation and Development (OECD-423) for testing of chemicals with minor modifications[1]. One group consist of 6 female rats (nulliparous and non pregnant) were used for this study. The dose utilized for evaluation of acute toxicity study is about 2000 mg/kg (p.o) higher than that of the therapeutic dose. The animals were fasted overnight (12- 16 hrs) with free access to water. The study was conducted with single oral administration of study drug *Pungampoo Chooranam* 2000mg/kg (p.o). The animals were 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 . Body weight was recorded periodically. At the end of the experiment all animals were subjected for gross necropsy and observed for pathological changes[2].

2.5. Repeated dose 28-day oral toxicity study

Sub-acute (Repeated dose 28-day oral toxicity study)was carried out as per OECD guidelines Guideline-407 [2].Healthy adult Wistar albino rats of both sex were used for the study. The Animals were divided into three groups of 6 animals each consist of 3 male and 3 female rats. Group-1 animals received saline 5 ml/kg b.w (p.o). Group II Animals received low dose of test drug *Pungampoo Chooranam* 200 mg/kg (p.o). Group III animals received high dose of test drug 400 mg/kg (p.o) The animals were randomly divided into control group and drug treated groups for two different doses viz. low dose (200 mg/kg b.w) and high dose (400 mg/kg b.w). The animals were administrated with the study drug once daily for 28 days. All the experimental animals were observed for clinical signs of mortality and morbidity once a day, preferably at the same time each day, till the completion of treatment[3].

2.5.1Observations

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 .At the end of the stipulated treatment period (end of 28^{th} day), the overnight fasted (water allowed) animals were anaesthetized, blood samples were collected by retro-orbital puncture. On 29^{th} day the animals were sacrificed with excess anesthesia. Blood samples were collected from aorta and stored in EDTA (ethylenediamine –tetra actate) for Hematological analysis using established procedures and automated Bayer Hematology analyzer for Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White blood cell count (WBC), Platelet Count, Hemoglobin (Hb), Mean cell Haemoglobin Concentration (MCHC), Mean Red Cell

Volume (MCV), Mean Cell Hemoglobin (MCH), Mean platelet volume (MPV), Neutrophils, Eosinophil's, Basophils, Lymphocytes and Monocytes[2]. Serum samples were analyzed for High Density Lipoprotein (HDL), Low density Lipoprotein (LDL) , Very low density Lipoprotein (VLDL) , Triglycerides (TGL), Total Cholesterol , Blood urea nitrogen (BUN), Creatinine, Albumin, Total Protein, Glucose, Uric acid, Aspartate Transaminase (AST), Alanine amino Transaminase (ALT) and Alkaline Phosphatase (ALP) using Mind ray auto analyzer model BS 120[3]. The vital organs including heart, brain, lungs, spleen, kidneys, liver, stomach, testes, and ovary were harvested and carefully examined for gross lesions. The organs were preserved in 10% formalin for histopathological assessment and interpretation[4,5].

2.6. Statistical analysis

The statistical analysis was carried by one way ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean ± standard error .A statistical comparison was carried out using the Dunnet's test for the control and treatment group.

III. Results And Discussion

3.1. Acute toxicity study

Observation included the change in skin, fur, eyes and mucus membrane. Appearance of toxicity related to central nervous system, Cardiovascular system and Autonomic nervous system such as tremors, convulsions, sedation, stereotypic behavior, respiratory distress, cardiovascular collapse, response to sensory stimuli, salivation, diarrhea, , pilo erection, Muscular co ordination, Muscular grip, posture, gait, limb paralysis, lethargy, sleep, coma and mortality were observed with special attention. The results revealed no treatment related death or signs of toxicity in the treated animals throughout the study. Body weight gain was also observed (Fig-1) when compared with before and after treatment of the observed groups. Further, there were no gross pathological abnormalities which prove the LD50 value was found to be greater than 2000mg/kg b.wt. Hence according to the Globally Harmonised System of Classification and Labelling of chemicals, *Pungampoo Chgooranam* can be classified as Category−5 and provides direct relevance for protecting human and animal health.

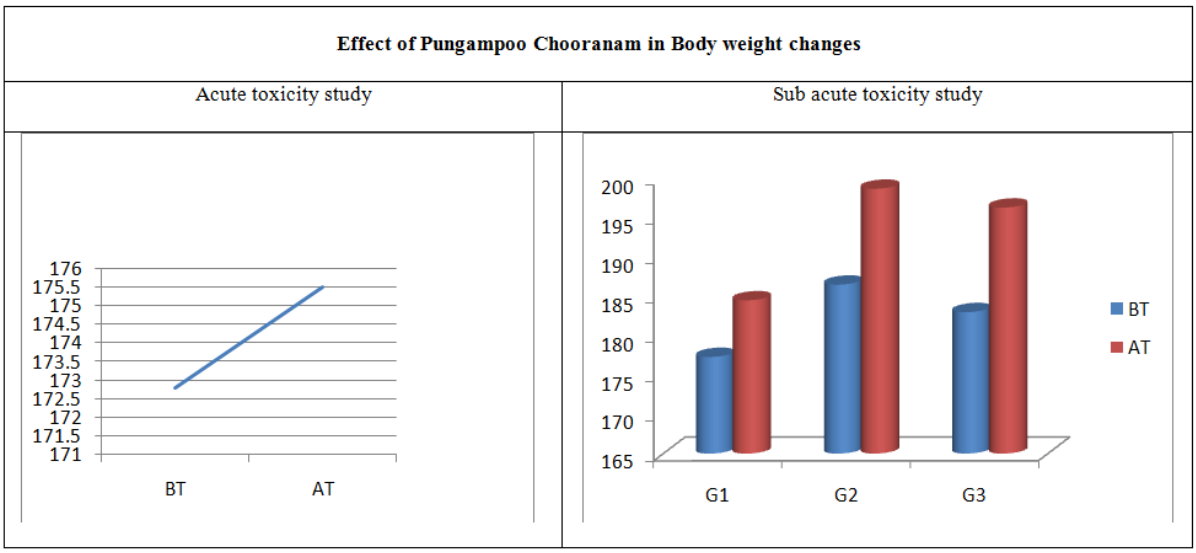

Figure-1.Changes in the body weight of rats treated with *Pungampoo Chooranam (Acute toxicity study.*

The above graph reveals that there was a significant increase in body weight after treatment with *Pungampoo Chooranam*.

3.2. Repeated oral toxicity study

Table-1. Quantitative data on the food and water intake of rats treated with *Pungampoo Chooranam* for 28 days in Sub-acute toxicity study

Stat. Measures	**Group I** (control)		**Group II** (200 mg/kg)		**Group III** (400 mg/kg)	
	Food intake	**Water intake**	**Food intake**	**Water intake**	**Food intake**	**Water intake**
Mean	17.75	29.92	17.75	29.92	20.08	34.42
Std. Deviation	0.5693	0.9574	3.775	1.101	3.957	2.267
Std. Error	0.2846	0.4787	1.887	0.5507	1.978	1.133

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test

There were no treatment-related toxicity signs and mortality observed in both sexes of rats treated at 200mg and 400 mg/kg orally for a period of 28 days and in the satellite group of rats. Bodyweight gain was observed between control and treated groups during the study (Fig-1). Food and water consumption of treated groups were found to be insignificant in both the sexes when compared to the control groups. Since there is no significance decrease in mean body weight and there is considerable increase in mean body weight of control and treatment groups, it can draw an inference towards the drug Pungampoo Chooranam has no inclination to produce drastic tissue destruction nor does it seem to interfere with absorption of the nutrients[6].

Figure-1. Administration of trial drug *Pungampoo Chooranam* to experimental animals

Table-2. Effect of *Pungampoo Chooranam* on Haematology profile of rats in sub-acute toxicity study

Groups	**Stat. Measures**	**WBC count ($\times 10^3$ µl)**	**RBC ($\times 10^6$ µl)**	**PLT ($\times 10^3$ µl)**	**MCV (fl)**	**MCH (pg)**	**MCHC (g/dl)**	**HGB (g/dl)**
Group I (control)	Mean	12.23	5.817	918.7	60.92	19.82	31.42	11.07
	Std. Deviation	2.719	0.9683	71.46	2.062	2.04	1.292	1.507
	Std. Error	1.11	0.3953	29.17	0.842	0.8328	0.5275	0.6152
Group II (200mg/kg)	Mean	11.12	7.783	710	58.97	20.22	32.48	11.17
	Std. Deviation	1.046	1.055	279.5	3.893	3.268	1.393	1.508
	Std. Error	0.4269	0.4308	114.1	1.589	1.334	0.5689	0.6157
	Mean	11.73	6.15	725.2	59.7	19.3	31.15	12.57

Group III (400 mg/kg)	Std. Deviation	2.858	0.7064	299.3	3.3	2.467	1.924	1.94
	Std. Error	1.167	0.2884	122.2	1.347	1.007	0.7856	0.7919

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table-3. Effect of *Pungampoo Chooranam* on Haematology profile of rats in sub-acute toxicity study.

Groups	**Stat. Measures**	**Lymph (%)**	**Mon (%)**	**Neutrophils (X 10^3/mm^3)**	**Eosinophils (%)**	**Basophils (%)**	**MPV (fl)**
Group I (control)	Mean	69.98	2.633	2.083	1.283	0.3333	6.483
	Std. Deviation	3.602	1.102	0.9453	0.2639	0.5164	1.003
	Std. Error	1.47	0.4499	0.3859	0.1078	0.2108	0.4094
Group II (200mg/kg)	Mean	78.53	2.15	2.233	1.333	0.5	6.1
	Std. Deviation	7.821	0.9874	0.7394	0.2582	0.5477	1.468
	Std. Error	3.193	0.4031	0.3018	0.1054	0.2236	0.5994
Group III (400mg/kg)	Mean	77.45	3.65	1.883	1.417	0.5	6.117
	Std. Deviation	8.302	1.093	0.736	0.2041	0.5477	1.373
	Std. Error	3.389	0.4463	0.3005	0.08333	0.2236	0.5606

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Table-4. Effect on biochemical parameters-Sub acute toxicity study

Biochemical parameters	**Group I** (control)			**Group II** (200mg/kg)			**Group III (**400 mg/kg)		
	Mean	S.D	S.E	Mean	S.D	S.E	Mean	S.D	S.E
Blood sugar ® (mg/dl)	82.33	13.75	5.613	82.5	12.69	5.182	79.33	11.59	4.731
BUN (mg/dl)	19.67	2.338	0.9545	13.33	2.875	1.174	17.5	2.074	0.8466
Creatinine(mg/dl)	0.7167	0.2927	0.1195	0.5667	0.2251	0.09189	0.7667	0.2422	0.09888
Total cholesterol (mg/dl)	122.7	6.088	2.486	111	16.6	6.777	115.3	19.24	7.856
Triglycerides (mg/dl)	74.5	10.41	4.249	87	10.26	4.187	66.83	4.916	2.007
HDL (mg/dl)	59.17	15.88	6.483	61	13.81	5.639	71.33	13.85	5.655
LDL l (mg/dl	55	7.849	3.204	31	17.66	7.211	41.5	8.432	3.442
VLDL (mg/dl)	14.43	3.189	1.302	17.73	3.333	1.361	14.85	1.577	0.6438
total protein (g/dl)	5.483	1.08	0.4408	4.967	1.172	0.4787	5.433	0.6713	0.2741
albumin (g/dl)	2.75	0.5648	0.2306	3.783	0.7679	0.3135	2.45	0.6834	0.279

(AST) (IU/ml)	101.3	20.53	8.381	88.17	19.96	8.15	109.7	13.6	5.554
ALT) (IU/L)	20.5	2.881	1.176	39.33	6.121	2.499	24.83	5.419	2.212
(ALP) (IU/L)	139.2	58.25	23.78	163.3	27.72	11.32	154	63.17	25.79

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Hematological profile such as Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White blood cell count (WBC), Platelet Count, Hemoglobin (Hb), Mean cell Haemoglobin Concentration (MCHC), Mean Red Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean platelet volume (MPV), Neutrophils, Eosinophil's, Basophils, Lymphocytes and Monocytes were found to be within the normal physiological limits for rodents and no significant change has been observed in treatment groups when compared with the control groups(Table-2&3). Hence there is no serious toxicological implications such as destruction of Erythrocytes[7]. Lipid profiles such as HDL, LDL , VLDL , TGL, Total Cholesterol did not show any significant changes. The main product of protein metabolism is urea and an increased level of urea in the blood is an indicator of renal impairment. The present study showed no significant changes pertaining to renal parameters. Aspartate Transaminase (AST), Alanine amino Transaminase (ALT) which are the indicators of hepatocellular injury also did not show any significant alterations in the *Pongampoo chooranam* treated groups and control groups(Table-3&4). The histopathological studies revealed no significant weight changes and normal architectural changes in the vital organs such as heart, brain, lungs, spleen, kidneys, liver, stomach, testes, and ovary suggesting that the preparationis devoid of serious organ degenerative potential both dose levels.

IV. Conclusion

The present Acute and sub acute toxicity results suggest that LD50 of *Pungampoo Chooranam* >2000mg/kg. Further studies on long term toxicity and clinical trials may be may be rational to substantiate the study results.

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