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ACUTE ORAL TOXICITY STUDY OF THE SIDDHA MEDICINE GOWTHAMAR CHOORANAM IN WISTAR ALBINO RATS

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ABSTRACT
Gowthamarchooranam is one of the poly herbal medicine in siddha system in the treatment of jaundice and respiratory diseases. The preparation was taken from *The Pharmacopoiea of siddha research medicines.*[1] The study was conducted as per the guidelines of Organization for Economic Cooperation and Development. The experimental protocol was approved by the institutional ethical committee (IAEC) under CPCSEA (approval no: KKCP/2014/020/CPCSEA). It is the principle of the test that based on a series of 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 given orally to a group of experimental animals at one of the defined doses. No mortality was observed in Gowthamar Chooranam during this acute oral toxicity study at the dose level of 5mg, 50mg, 300mg and 2000mg. Hence we can conclude that the drug is safe for clinical use.

KEYWORDS: Acute oral toxicity, Gowthamarchooranam, OECD, Toxicity, Herbal.

INTRODUCTION
Gowthamarchooranam is one of the poly herbal medicine in siddha system in the treatment of Jaundice and respiratory diseases. The preparation was taken from *The Pharmacopoiea of siddha research medicines*by Dr.Shanmugavelu L.I.M.H.P.I.M, Dr.G.T.Naidu. The ingredients of the chooranam are Sitrarathai, Kadukkaithol, Arisithippili, Jaathikkai, Vaalmilagu and Sarkarai. All the ingredients of the chooranam have many pharmacological activity like Hepato protective, Anti diabetic, anti oxidant, anti hypertensive, Anti Inflammatory, Anti Microbial. Here the purpose of the study was to test the acute oral toxicity. Plants or drugs must be ensured to be safe before they could be used as medicines. Oral toxicity study is the principle of the test that based on a series of 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 given orally to a group of experimental animals at one of the defined doses. The substance is tested using a series of procedure, each step using three animals of a single sex. The result of mortality of the animals dosed at one step will determine the next procedure, i.e.; − no further procedure is needed − additional animals with be the same dose − dosing of animals at the next higher or lower doses. The method will enable a conclussion with respect to classifying the test substance to one of a series of toxicity classes.

METHODOLOGY
The siddha drug *Gowthamarchooranam* was prepared as per the siddha literature The Pharmacopoiea Of Siddha Research Medicines.

Selection of Animals
- The animal models used in this study were Wistar albino rats.
- Healthy Female Wistar albino rats weighing 150-250gm were obtained from the animal house of Kings Institute, Guindy, Chennai.
- Females should be nulliparous and non-pregnant.
- Each animal must be around 8 and 12 weeks old at the time of dosing.

The studies were conducted in the animal house of KK College of pharmacy.

Housing and feeding conditions

- Animals were housed under standard laboratory conditions.
- They were maintained in a ventilated room. The temperature in the room should be $22^0(\pm3^0)$.
- The relative humidity should be at least 30% and not exceed 70% (50%-60%).
- Lighting should be artificial; it is maintained as 12h light/dark cycle.
- Animals were kept in a clean polypropylene cage.
- Rats were fed with standard pellet diet (SaiMeera Foods, Bangalore) and water *ad libitum*.

Preparation of animals

All the animals were randomly selected and marked on its fur for its individual identification. They were acclimatized to the laboratory conditions at least one week prior to the commencement of the study.

EXPERIMENT PROCEDURE

Administration of doses

Gowthamar Chooranam prepared as per the classical Siddha literature was suspended in 2% CMC with uniform mixing and was administered to the groups of Wistar albino rats. It is given in a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance 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 recorded systematically and continuously observed 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. They were deprived of food, but not water 16–18 h prior to the administration of the test suspension. Finally, the number of survivors was noted after 24 h and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.[2]

Number of animals and dose levels

Since this test drug has been under practice for long time and likely to be non-toxic, a limit test at one dose level of 2000 mg/kg body weight will be carried out with 6 animals (3 animals per step).[3]

Duration of Study : 48 hrs
Evaluation : 14 Days

Limit test

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.[4]

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.

OBSERVATIONS

- The animals were observed individually after dosing at least once during the first 30mins and periodically during the first 24 hrs.
- Special attention: First 1-4 hrs after administration of drug, and
- It is observed daily thereafter for a total of 14 days, except when they needed to be removed from the study and killed humanely for animal welfare reasons or are found dead.

a. Mortality

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hour following drug administration on day 1 of the experiment and daily twice thereafter for 14 days.[5]

b. Body weight

Body weights will be recorded at day: -1, day 1, 2, 7 and 14 of the study.[6]

RESULTS

Table 1: Behavioral Signs of acute oral Toxicity.

SL	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

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.	**2000mg**	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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.

DOSE	DAYS		
	1	7	14
CONTROL	214.6±31.474	214.2 ± 14.162	215.2 ± 24.22
HIGH DOSE	213.5± 22.25	213.4 ± 2.12	213.4 ± 2.62
P value (p)*	NS	NS	NS

DISCUSSION

- The acute oral toxicity potentials of *GC*in Wistar albino rats were studied.
- In the sighting study, the test substance was administered in sequential manner to one animal each at 2000 mg kg-1 body weight followed by two animals at 2000 mg kg-1 body weight.
- According to OECD guidelines, for acute oral toxicity LD_{50} dose of 2000mg/kg of the drug is found to be safe.
- The treated animals were observed for mortality, untoward clinical/toxic signs, alterations in body weight gain and necropsy findings during the study.
- The treated animals survived throughout the study period and did not reveal any treatment related major abnormal clinical signs at the test dose levels.
- Morphological characters like changes in skin, eyes, fur, nose appeared normal.
- The rats did not reveal any observable signs of central nervous system.
- The rats showed signs of alertness, pile erection, grooming and touch response at the dose level of 2000mg/kg of body weight.
- The overall percentage of body weight gain in rats treated with the drug every weekly was found to be normal indicating that the test animals were in a healthy condition during the days of observation period.

CONCLUSION

Normally herbal drugs are always considered as safe for human use. Though it is safe there is a need for global acceptance, even herbal medicine also have to be scientifically proved for its non toxic effects. From this toxicity analysis it was observed that the test drug Gowthamarchooranam didn't possessed any mortality. Finally we can conclude that the drug Gowthamarchooranam possess no toxicity and safe for consumption. Further research studies have to be followedregarding the therapeutic efficacy of this polyherbal siddha formulation *Gowthamarchooranam* in preclinical and clinical aspects. This may lead to deliver this wonderful drug to people to get cure from hepatic and respiratory diseases.

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