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ACUTE ORAL TOXICITY STUDY OF CHANDRAKANTHI CHOORANAM

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ABSTRACT
Chandrakanthi Chooranam (*CKC*) is a classical preparation which consists of twenty five ingredients and is indicated for the treatment of oligozoospermia, vaginal diseases, veneral diseases, polyuria and in all biliousness. The present study was aimed to conduct acute oral toxicity study in wistar albino rats and to establish the safety of the *CKC* and this article presents the results of 14 days acute oral toxicity study. Acute oral toxicity study was carried out following the World Health Organization (WHO) guidelines 2000. The therapeutic dose of *CKC* is 12 gm/day. For acute oral toxicity study for *CKC* the higher dose selected was 10 times the therapeutic dose (10.8 gm/kg b.wt of rat; as single dose) to the rats. On necropsy, no gross pathological abnormalities were observed in the vital organs of the rats.

KEYWORDS: Chandrakanthi chooranam, WHO guidelines, acute toxicity, oligozoospermia, sastric preparation.

INTRODUCTION
Siddha herbo mineral formulation *Chandrakanthi Chooranam* is a sastric preparation with the reference Chikicha rathna deepam-Part 2, which comes under drugs and cosmetic act 1940.[1] *Chandrakanthi Chooranam (CKC)* consists of twenty five ingredients and is indicated for the treatment of oligozoospermia, vaginal diseases, veneral diseases, polyuria and in all biliousness.[2] Analytical studies such as physicochemical standards, preliminary phytochemical analysis, TLC/HPTLC finger printing profiles, safety evaluation such as microbial contamination, heavy metal determination, pesticide residues, mycotoxins, TGA analysis, ICP-OES analysis was evaluated in *CKC.* The results showed the presence of amino acids, steroids, triterpenes,flavonoids, phenols, tannins, anthraquinones and saponins; ICP-OES analysis for heavy metals were found to be below detection level and showed the presence of nutritional elements such as calcium, magnesium, iron, zinc and copper; pesticide residues and aflatoxins were absent and the formulation was free of microbial contamination.[3] Safety and the efficacy study depend on the methods adopted in the drug preparation and variation from the traditional knowledge (method) may not give the desired outcome.[4] Acute toxicity studies (initial assessment in toxic studies) present fast, significant information and may specify whether additional toxicity studies must be conducted. It gives information on the health risk that is possible to occur from the short-term experience to a drugs and is performed in all compounds.[5] Even though *CKC* is used in Siddha system of medicine and it is a classical prepartion, in view to toxicity there is no documents and published reports.

The present study was aimed to conduct acute toxicity study in wistar albino rats and to establish the safety of the *CKC*. This article presents the results of 14 days oral acute toxicity study to ensure the safety of the drug.

MATERIALS AND METHODS

Table 1: The ingredients, anatomical parts used and their quantities in CKC.

S.No	Ingredients	Part used	Quantity
1	Nerunjil (*Tribulus terrestris* Linn)	Fruit	35gms
2	Nilapanai (*Curculigo orchioide* Gaertn)	Rhizome	35gms
3	Murungai (*Moringa oleifera* Lam)	Seed	35gms
4	Poonaikaali (*Mucuna prurita* Hook)	Seed	35gms
5	Iluppai poo (*Madhuca longifolia* Linn)	Flower	35gms
6	Bhumi chakkarai (*Maerua arenaria* Hook)	Root tuber	35gms
7	Seerakam (*Cuminum cyminum* Linn)	Fruit	35gms
8	Lavangabathiri (*Cinnamomum tamala* Nees)	Leaf	35gms
9	Lavangapattai (*Cinnamomum verum* Presl)	Stem Bark	35gms
10	Kirambu (*Syzygiumaromaticum* Linn)	Flower bud	35gms
11	Elavampisin (*Bombax ceiba* Linn)	Gum	35gms
12	Drakshai (*Vitis vinifera* Linn)	Fruit	35gms
13	Koshtam (*Costus speciosus* Koen)	Root	35gms
14	Athimathuram (*Glycyrrhiza glabra* Linn)	Root	35gms
15	Sirunagappo (*Mesua ferrea* Linn)	Flower	35gms
16	Perichankai (*Phoenix dactilifera* Linn)	Unripe fruit	35gms
17	Moongil uppu (*Bambusaaurundinaceae* Willd)	Salt	35gms
18	Jaathikkai (*Myristica fragrans* Houtt)	Seed	35gms
19	Korai kizhangu (*Cyperus rotundus* Linn)	Rhizome	35gms
20	Takkolam (*Ilicium verum* Hook)	Flower	35gms
21	Maramanjal (*Coscinium fenestratum* Gaertn)	Stem bark	17.5gms
22	Aadaathoda (*Adhatoda vasica* Nees)	Seed	35gms
23	Maruthani (*Lawsonia inermis* Linn)	Seed	35gms
24	Ponnakani (*Alternanthera sessilis* Linn)	Seed	35gms
25	Gomutra silasathu (*Asphaltum punjabinum)*	Fine-ash	35gms

Identification and Authentication of Raw Drugs

Adhatoda vasica seeds were procured from the Research Institute for Indian System of Medicine, Joginder Nagar, Mandi, Himachal Pradesh, India. *Alternanthera sessilis* seeds were collected from the herbal garden, National Institute of Siddha, Chennai, India. Other herbal drugs were procured from Govindhasamy chetty store, Chennai, India. Gomutra silasathu (mineral drug) was procured from SKM, Tamil Nadu, India. Drugs were identified, authenticated and voucher specimen (NIS/MB/59/2012) was deposited in the Department of Medicinal Botany, National Institute of Siddha, Chennai.

Purification process of the herbal and mineral ingredients[1, 6, 7, 8]

Primarily all the drugs were purified as per the procedures mentioned in Siddha literature. *Glycyrrhiza glabra, Coscinium fenestratum, Cyperus rotundus and Maerua arenaria* were washed in water, outer skin were peeled off and then dried in the sunlight. Seeds present in *Phoenix dactilifera and Myristica fragrans* were removed, outer portion were dried in sunlight and used. *Curculigo orchioides* was dried, powdered and then par boiled in milk for 1 samam [3hours], then dried under sunlight and then powdered. Impurities of *Costus speciosus, Cuminum cyminum, Mesua ferrea, Bombax ceiba, Cinnamomum verum, Cinnamomum tamala, Ilicium verum, Syzygium aromaticum, Bambusa aurundinaceae, Madhuca longifolia, Tribulus terrestris, Vitis vinifera, Moringa oleifera, Adhatoda vasica, Mucuna prurita, Lawsonia inermis* and *Alternanthera sessilis* were removed and dried in sunlight. *Gomutra silasathu* was mixed with the cow's urine and then filtered with a thick cloth. And then dried in the sunlight. Layer was formed on the filtrate which was then removed and dried up. This method was repeated until no more layer was formed (7 times).[9]

Preparation of *Gomutra Silasathu parpam*[1]

Gomutra Silasathu parpam (one among the 25 ingredients of CKC) was prepared as per the method demonstrated in the Siddha literature¨ 35gms [1 palam] of the silasathu (purified) was soaked in pulitha arisi kazhuviya neer (fermented rice water) for three days. Fresh fermented rice water was used for each days. On the 4th day, the drug was put on the mortar to triturate with the pulitha kazhuneer for twelve hours (4 samam). Pellets were prepared and dried. Following drying, the pellets were placed in between two earthen pots, positioned one above the other. The earthen pots were sealed with cloth (cotton) smeared by fuller's earth and dried in the sun light. Pellets were then put in to oxidation process (Pudam) with twenty five cow-dung cakes. After ignition, it was permitted to quench itself. The finishing products were taken out, pulverized and then stored in air tight containers.

Preparation of the study drug *Chandrakanthi Chooranam*[1]

All the purified herbal ingredients and *silasathu parpam* was together powdered and shifted in to a 100 size mesh. Chooranam was par boiled with milk (final purification process) then finally dried and stored.

Acute toxicity study

Acute toxicity study was carried out following the World Health Organization (WHO) guidelines [2000].[10]

Drug and Dose

The therapeutic dose for the study drug (CKC) for acute toxicity study was calculated by extrapolating the human-clinical dose (12 gm/day) to rat dose (216mg / 200gm b.wt; 1.08 gm /kg b.wt) which was based on the ratio of the body surface.[11] Drug was made in to suspension by adding with its vehicle milk [2ml] in mortar-pestle. The drug was administered to rats with respect to their individual weights.

Dose calculation[11]

Table 2: Dose calculation for rats in toxicity study.

Dose for a rat weighing 200gm = Human absolute dose X conversion factor (Human to clinical)
Dose for a rat weighing 200gm = 12 X 0.018 = 0.216gm/200gm b.wt
Dose for a rat weighing 1kg = 1.08 gm/kg b.wt

For acute toxicity study the higher dose selected was 10 TD (10.8 gm/kg b.wt; single dose) to the rats.

Route of administration

Oral route was selected for acute toxicity study as it is the clinical route of administration.

Procurement and rearing of experimental animals

Adult male wistar rats weighing 130-220 gms were used for the acute toxicity study. The animals were procured from National Centre for Laboratory Animal Sciences (NCLAS), NIN, Hyderabad. They were housed three per cage under standard laboratory conditions at a room temperature at 20±2^{0}C. Ventilated by air conditioning with 100% fresh air and humidity was maintained between 50-70%. The animals were subjected under standard photo-periodic condition of 12:12 hr light dark cycle. The animals were fed with standard rodent pellet procured from M/s. Provimi Animal Nutrition India Pvt Ltd, Bengaluru and purified RO water (Kent RO water filter cum purifier) ad libitum. Animals were acclimatized to laboratory conditions one week prior to the initiation of the experiments. The protocol for experimentation was approved by Institutional Animal Ethics Committee (Ref.no: NIS/IAEC/I/2011/2(A)) of National Institute of Siddha, Chennai, Tamilnadu, India.

Acute toxicity study

Experimental design

Table 3: Experimental design in acute toxicity study.

Sample Size	18 wistar rats
Sex	Male
Route of Administration	Oral
Experiment Duration	14 days
Drug	*Chandrakanthi chooranam*
Dose	10.8 gm/kg/p.o (10 times the dose equivalent to human therapeutic dosage was selected to ascertain its safety potential)

Animal grouping and interventions

The animals were randomly divided into three groups (I, II and III) of six rats (n=6) each. Individual identification of the animal was made by marking. Group I animals served as control and received 10ml/kg b.wt of distilled water. Group II received once with 10ml/kg bwt of milk and served as vehicle control. Group III served as the treated groups and received 10 times the dose equivalent to human therapeutic dose [10.8gm/kg/p.o.] of *CKC.*

Table 4: Animal grouping and intervention in acute toxicity study.

Groups	Intervention	No of Rats
Normal Control- Group I	Distilled water	6
Vehicle control - Group II	Milk	6
10 x TD - Group III	*CKC* (10.8g / kg b.wt)	6

In-life observation

Doses were administered to the wistar rats which were overnight fasted with water *ad libitum.* All the rats were observed for general conditions, signs of toxic symptoms and mortality for every hour during the first day with particular concentration given during the first 4 h and thereafter every day for 14 days. Parameters such as mortality, allergic reactions, skin colour changes, response to handling, secretions, pilo-erection, posture, gait, diarrhoea, tremors, sleep, convulsion signs, circling, depression, sedation, excitement and cyanosis were observed and then recorded.

Physiological parameters

Feed and water consumptions were recorded daily. Individual body weight of the wistar rats were recorded previous to the dosing, on the 7th day and prior to the sacrifice on the 14th day.

Gross necropsy

After the observation period of 14 days, all surviving rats were sacrificed and were subjected to the complete gross necropsy on 15th day to examine any signs of systemic-toxicity. External surface of the body, cranial, orifices, thoracic and the abdominal cavities and its contents were examined. Lastly, the vital organs like heart, lungs, liver, kidneys, spleen, brain and testis was grossly examined.

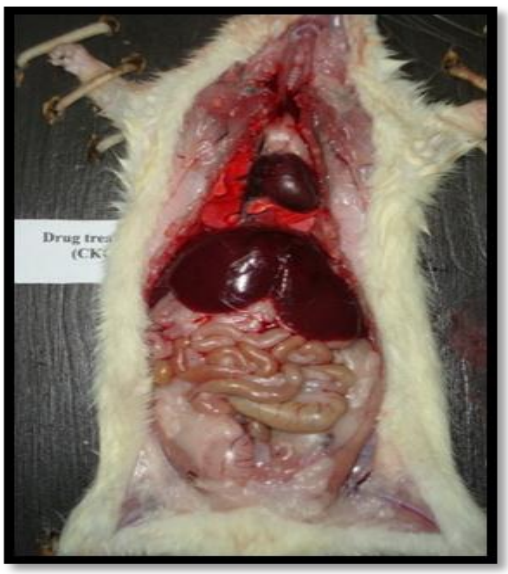
RESULTS AND DISCUSSION

Effect on feed, water intake and survival of wistar rats

No abnormal changes was observed in the feed and water intake of wistar rats between the control and treated groups in acute toxicity study. No mortality were observed and the survival was hundred percent.

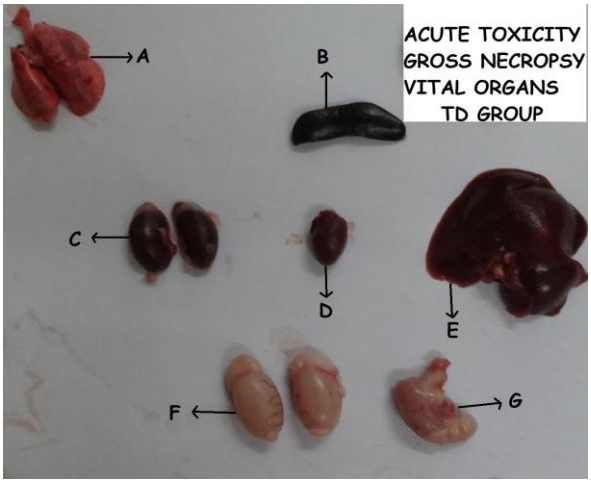
Acute Toxicity study – Gross Necropsy

The study drug CKC treated rats at 10 TD dose level.did not show any death, behavioural changes and toxic signs immediately after dosing, during 14 days and at the end of the trial. On necropsy, no gross pathological abnormalities were observed in the vital organs (fig 1, fig 2 and fig 3) and hence the toxicity of the drug at 10 TD dose level can be ruled out.



10 TD Group

Fig 1: Gross necropsy of the vital organs in acute toxicity study.



A-Lungs; B- Spleen; C- Kidney; D- Heart; E- Liver; F- Testis; G- Caecum

Fig 2: Gross Necropsy of the vital organs in acute toxicity study (10TD group).

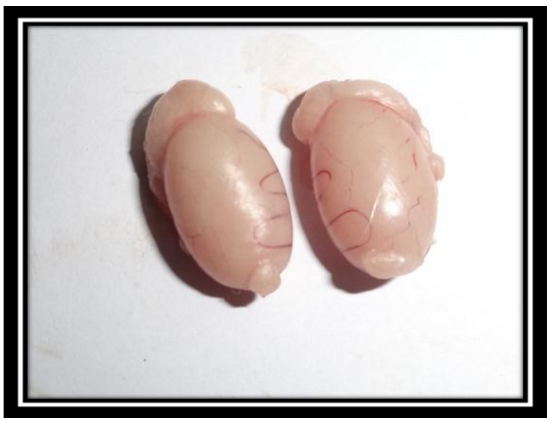


Fig 3: Gross Necropsy of testis the target organ.

Body weight gain

Weight gain (body) of vehicle control group showed significant (P<0.05) increase and 10 TD group showed non significant increase when compared to that of the control group. (Table 5).

Table 5: Final body weight gain of each group in acute toxicity study.

Groups	Body weight change in (g)
Normal control	37 ± 2.0490
Vehicle control	44.83 ± 1.7400*
10 TD CKC	48.5 ± 9.4080
Values are expressed as mean ±S.E.M; *P<0.05	

CKC was orally given at dose of 10 times the therapeutic dose (10 TD). Rats were observed for general conditions, signs of toxic symptoms and mortality for every hour during the first day with particular concentration given during the first 4 h and thereafter every day for 14 days. 10 TD dose level did not show any death, behavioural changes, toxic signs during 14 days and showed non significant changes in body weight when compared to that of the control group. On necropsy, no gross

pathological abnormalities were observed in the vital organs and hence the acute toxicity study indicates that the drug is well tolerated up to 10 times [10.8gm/kg b.wt] the therapeutic dose in tested wistar rats.

CONCLUSION

Acute oral toxicity study of the drug *Chandrakanthi chooranam* revealed that it didn't produce any signs of toxicity sign and is well tolerated up to 10 times [10.8gm/kg b.wt] the therapeutic dose in wistar rats.

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