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LONG TERM TOXICITY STUDY OF CHANDRAKANTHI CHOORANAM

Clinical Research

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ABSTRACT

WHO guidelines intends to specify the standard system in toxicological studies (non-clinical) associated in assessing the herbal medicine safety.In Siddha science, 96 thathuvam (principles) deals with the basic components of human body which include intellectual, physiological, physical and psychological components of the individual. Siddha herbo mineral formulation, *Chandrakanthi Chooranam* (*CKC*) is a sastric preparation indicated for oligozoospermia; poly urea; vaginal disease; veneral disease and biliousness.Acute oral toxicity study of *CKC* revealed that it is well tolerated up to 10.8gm/kg b.wt [10 times the therapeutic dose(1.08 gms/day)] in wistar rats. The present long term toxicity study is the further extension of the acute toxicity study and to validate the safety profile of *CKC* in wistar rats and was carried out following the World Health Organization (WHO) guidelines 2000. Three dose levels of 1.08gm/kg b.wt (therapeutic dose); 3.24g/kg b.wt (3 x Therapeutic dose) and 5.4gm/kg b.wt. (5 x Therapeutic dose) were administered to rats for duration of 90 days. Long term toxicity study was assessed by estimating physiological, biochemical, hematological and histopathological effects of *CKC* on rats.

KEYWORDS

Chandrakanthi chooranam, WHO guidelines, Siddha, long term toxicity, oligozoospermia

INTRODUCTION

In Siddha science, 96 thathuvam (principles) deals with the basic components of human body which include intellectual, physiological, physical and psychological components of the individual.Siddha system had already gained an insight in individualistic management through thega ilakkanam (biotype - characterization of an individuals) which reduces the possibility of misdiagnosis/incorrect treatments, which now contemporary research is testing to achieve through pharmacogenetics and pharmacogenomics.[1] Siddha herbo mineral formulation *Chandrakanthi Chooranam (CKC)* is a sastric preparation consists of twenty five ingredients, indicated for oligozoospermia; poly urea; vaginal disease; veneral disease and biliousness.[2] Standardization is important in evaluating the quality/safety of the polyherbal formulation and the data obtained from the previous study ensured that CKC is of standard the quality.[3]

Phytochemical analysis of *CKC* revealed the presence of amino acids; steroids; triterpenes; flavonoids; phenols; tannins; anthraquinones and saponins. The physico-chemical analysis showed 8.458% on loss on drying (at 105°C),13.043% -total ash, 3.392%-water soluble ash,5.611% - acid insoluble ash, 4.04% - acid soluble ash, 19.25% - water soluble extractive, 16.85% - alcohol soluble extractive. Heavy metals (ICP-OES analysis) were found to be below detection level; nutritional elements (ICP –OES analysis) were found to be:calcium (6482.9 ppm), magnesium (1870 ppm), iron (988.6 ppm), zinc (21.98 ppm) and copper (8.09 ppm) respectively.Microbial contamination (bacterial count and fungal count) were found to be within the limits. Specific pathogens (E.coli;Salmonella spp;Staphylococcus aureus;Pseudomonas aeruginosa) were found to be absent.Aflatoxins (B1; B2; G1; G2) were found to be BDL (below detection limit). Pesticide residues (organochlorine;organophosphorus) were not detected.[3]

Thin Layer Chromatography (TLC) of *CKC* at UV 254 nm showed 5 green spots (at Rf value of 0.19; 0.14; 0.24; 0.43 and 0.75), under UV 366 nm showed 8 spots (at Rf value of 0.09-pale blue; 0.23-greenish blue; 0.32-greenish blue; 0.41-greenish blue; 0.48 -greenish blue; 0.54-pale blue; 0.59-blue and 0.65-greenish blue) and after derivatization with vanillin sulphuric acid showed 7 spots (at Rf value of 0.05-purple; 0.17-purple; 0.38-purple; 0.44-purple; 0.58-purple; 0.69-bluish purple; 0.96-purple).The high performance thin layer chromatography (HPTLC) of choloroform extract of *CKC* at UV 254 showed 7 peaks (at Rf value of 0.09; 0.14; 0.24; 0.31; 0.43; 0.54 and 0.75), at UV 366 showed 9 peaks (at Rf value of 0.09; 0.12; 0.23; 0.32; 0.41; 0.48; 0.54; 0.59 and 0.65), at 540 nm after derivatization showed 11 peaks (at Rf value of 0.15; 0.36; 0.44; 0.54; 0.67; 0.72; 0.76; 0.78; 0.81; 0.87 and 0.90). [3]

The toxic effects of herbal preparations on target organs of animals and humans are enormous hence have elicited tremendous medical concerns. The potential toxic substances in plants such as antinutritional factors, heavy metals and phytochemicals, have been the subject of many investigations.[4] Acute oral toxicity study of *CKC* revealed that it is well tolerated up to 10.8gm/kg b.wt [10 times the therapeutic dose (12gms/day)] in wistar rats.[5] The present long term toxicity study of CKC in wistar rats is the further extension of the acute toxicity study and to validate the safety profile of CKC.

MATERIALS AND METHODS

Ingredients of Chandrakanthi Chooranam

Nerunjil (*Tribulus terrestris* Linn) fruit; Nilapanai (*Curculigo orchioide* Gaertn) rhizome; Murungai (*Moringa oleifera* Lam) seed; poonaikaali (*Mucuna prurita* Hook) seed; iluppai poo (*Madhuca longifolia* Linn) flower; Bhumi chakkarai (*Maerua arenaria* Hook) root tuber; Seerakam (*Cuminum cyminum* Linn) fruit; Lavangabathiri (*Cinnamomum tamala* Nees) leaf; Lavangapattai (*Cinnamomum verum* Presl) stem bark; Kirambu (*Syzygiumaromaticum* Linn) flower bud; Elavampisin (*Bombax ceiba* Linn) gum; Drakshai (*Vitis vinifera* Linn) fruit; Koshtam (*Costus speciosus* Koen) root; Athimathuram (*Glycyrrhiza glabra* Linn) root; Sirunagappo (*Mesua ferrea* Linn) flower; Perichankai (*Phoenix dactilifera* Linn) unripe fruit; Moongil uppu (*Bambusa aurundinaceae* Willd) salt; Jaathikkai (*Myristica fragrans* Houtt) seed; Korai kizhangu (*Cyperus rotundus* Linn) rhizome; Takkolam (*Ilicium verum* Hook) flower; Maramanjal (*Coscinium fenestratum* Gaertn) stem bark; Aadaathoda (*Adhatoda vasica* Nees) seed; Maruthani (*Lawsonia inermis* Linn) seed; Ponnakani (*Alternanthera sessilis* Linn) seed; Gomutra silasathu (*Asphaltum punjabinum) parpam* - Fine-ash [2]

Raw Drugs: Procurement, Identification and Authentication

Adhatoda vasica seeds (Research Institute for Indian System of Medicine, Joginder Nagar, Mandi, Himachal Pradesh); *Alternanthera sessilis* seeds (National Institute of Siddha – Herbal garden, Chennai); other herbal drugs (Govindhasamy chetty store, Chennai); Gomutra silasathu (SKM, Tamil Nadu) were procured respectively.Herbal drugs were identified and authenticated by Assistant Professor of Botany, National Institute of Siddha, Chennai. Mineral drug was identified and authenticated by Prof. V. Ram Mohan (Department of Geology) University of Madras, Chennai. Voucher specimen: NIS/MB/59/2012 was deposited in the Medicinal botany department, of National Institute of Siddha, Chennai.

Preparation of Chandrakanthi Chooranam:
Raw drugs were purified as per standard siddha literature and preparation of CKC were carried out according to the methods specified in standard siddha text. [2, 3, 6, 7, 8, 9]

Long term toxicity study
Long term toxicity study was carried out following the World Health Organization guidelines [WHO; 2000] [10]

Dose calculation for rats in toxicity study
Dose for a rat weighing 200gm = Human absolute dose X conversion factor (Human to clinical) [12 X 0.018 = 0.216gm/200gm b.wt]**.**Dose for a rat weighing 1kg = 1.08 gm/kg b.wt**.** [11] For long term toxicity study three doses were selected.

Table 1: Three dose levels in long term toxicity study

1 x Therapeutic dose = 1.08 gm/kg b.wt (1TD)
3 x Therapeutic dose = 3.24 gm/kg b.wt (3 TD)
5x Therapeutic dose = 5.4 gm/kg b.wt (5 TD)

Route of administration:
Oral route was selected for long term toxicity study.

Procurement and rearing of experimental animals:
Male wistar rats of weight 130-220 gms were used for long term toxicity study. Rats were procured from the National Centre for Laboratory Animal Sciences [NCLAS], NIN; Hyderabad. Rats were housed three per cage. Standard laboratory state of room temperature 20 ± 2^0 C; ventilation by air conditioning with fresh air (100%) and humidity between 50-70% were maintained. Standard photo-periodic state of 12:12 hr, light dark cycle were maintained. Rats were fed with rodent pellet (standard) obtained from M/s. Provimi Animal Nutrition India Pvt Ltd; Bengaluru and with purified water [Kent RO ,water filter/purifier) ad libitum. Rats were acclimatized to the laboratory state one week prior to the initiation of the study. The protocol for the study was approved by the Institutional Animal Ethics Committee [Ref.no-NIS/IAEC/I/2011/2(A)] of National Institute of Siddha; Chennai; Tamilnadu.

Experimental design

Table 2: Experimental design in long term toxicity study

Sample Size	**30 wistar rats**
Sex	Male
Route of Administration	Oral
Experiment Duration	As a rule of WHO-guidelines for the clinical administration of drug, between 1-6 months, the toxicity study administration period is from 3- 6 months and as a result 3 months [90 days] was selected as the treatment schedule.
Drug	*Chandrakanthi chooranam*
Dose	Three dose levels were studied to produce the range of toxic effects and mortality rates in wistar rats Therapeutic dose: (1x TD) 1.08gm/kg b.w Average dose: (3 x TD) 3.24g/k g b.w Higher dose: (5 x TD) 5.4 g / kg b.w

Animal grouping and interventions
The wistar rats were randomly divided into five groups (I, II, III, IV and V) of six rats (n=6) each. The groups of animals were transferred in to different cages and were marked for their identifications. Group I animals served as control and received 10ml/kg b.wt of distilled water. Group II received 10ml/kg b.wt of milk and served as vehicle control. Group-III rats received *CKC* at a dose equivalent to human therapeutic dose (1.08gm/kg/p.o), Group-IV rats received 3 times the dose equivalent to human therapeutic dose (3.24g/k g/ p.o.) of *CKC*. Group V rats received 5 times the dose equivalent to human therapeutic dose (5.4gms/kg b.wt) of *CKC*.

Table 3: Animal grouping and interventions in long term toxicity study

Groups	Intervention	No of Rats
Normal Control - Group I	Distilled water	6
Vehicle control - Group II	Milk	6
1x TD - Group III	*CKC* (1.08 g / kg b.wt)	6
3 Xtd - Group IV	*CKC* (3.24g / kg b.wt)	6
5 x TD - Group V	*CKC* (5.4 g / kg b.wt)	6

Sampling, Sacrifice and Surgical procedure:
Twenty-four (24) hours after the 90th day of treatment, following over-night fasting (12 h) blood was collected from the rats through retro orbital- plexus into 2 tubes, one contains EDTA for the analysis of haematological parameters and the other in to a sterile plain bottle without additives wich was centrifuged at 4000 rpm at 4◦C for 10 mts to get the serum for biochemical estimations. The resulting samples was obtained and stored at −20 °C prior to the biochemical estimation. After this all the wistar rats were sacrificed with the excess anaesthesia. The abdominal cavity was opened via a midline incision.The organs like heart, lungs, spleen, kidneys, liver, testis, brain, stomach and thymus were excised and examined for gross lesions and weighed. Representative tissue samples were preserved in 10% formalin solution for histopathological evaluation.

Pathological Evaluation
Final body weight, organ weight, blood picture, blood biochemical markers and histological examination of internal organs were determined.

Physiological parameters
Daily consumption was measured by calculating difference between the amounts of food and water given and their remnants on the next day. The body weights of all rats were recorded [gm] before and after the session [90days] of each respective drug administration (before sacrifice).

Organ weights
Organs like heart, lungs, spleen, kidneys, liver, testis, brain, stomach and thymus were dissected out, freed from the surrounding fats and connective tissues. Mopped with the tissue paper and was weighed (absolute). Organs were weighed on the digital balance soon after the dissection to avoid drying [wet weight were recorded].

Blood chemistry- panel
Hematological Assays
Variables were analyzed using automated bayer- hematology analyzer which includes total white blood cell count (WBC), lymphocytes(LYMP), Monocytes (MONO), Granulocytes(GRAN), Total red blood cell count (RBC), platelet count (PLT), platelet crit (PCT); mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH),mean corpuscular haemoglobin concentration (MCHC), haemoglobin (Hb), Hematocrit (HCT) or packed cell volume (PCV), red blood cells width (RDW), Platelet distribution width (PDW), mean platelet volume (MPV)

Biochemical Assays
Biochemical variables like glucose, urea, creatinine, total cholesterol, triglycerides (TGL), total Protein (TPN), albumin, globulin, total bilirubin, Aspartate amino transferase (AST) and Alanine amino transferase (ALT) were determined using chemical analyzer RA 50. Electrolyes were analyzed by Transasia electrolyte analyzer-EC lyte transasia.

Histopathological study
The tissues examined were: Heart, lungs, spleen, intestine, kidneys, liver, testis, brain and thymus. Tissue samples were preserved in formalin (10%) for histopathological evaluation. The tissue samples were usually embedded into paraffin; 2-m thick sections were stained with H & E (hematoxylin and eosin). All the sample slides were examined microscopically for any pathological observations.

RESULTS
Body Weight Gain
Body weight gain of vehicle control, 3 TD ($P < 0.05$) and 5TD ($P<0.001$) group showed significant increase than the control group. TD group showed non significant changes ($P > 0.05$) in the body weight gain. (Table 4)

Table 4: Final body weight gain of each group in long term toxicity study

`Groups	Body weight change in (g)
Normal control	122 ± 5.9910
Vehicle control	148 ± 7.8730*

1 TD CKC	158 ± 18.2890
3 TD CKC	166 ± 12.9630*
5 TD CKC	171 ± 9.4960***
Values are expressed as mean ±S.E.M; *P<0.05; ***P<0.001	

Hematology

Values of Hb; RBC; WBC; differential counts; MCH; MCHC; Platelets; MPV; PLT; PCT; HCT of the male wistar rats treated with the study drug of all doses levels (TD, 3TD, 5TD) showed non-significant changes when compared to the control group. MCV value of the vehicle control group -showed significant increase ($p < 0.05$) (within normal laboratory limits).The results do not indicate serious pathological conditions. (Table 5)

Clinical Chemistry

The values of trigycerides; globulin; bilirubin, sodium and blood sugar of wistar rats treated with the three dose levels (TD, 3TD and 5TD) showed non significant changes (P >0.05) when compared with that of the control groups. Blood urea was found to be significantly decreased (P< 0.05) in TD group; showed non significant change in vehicle, 3TD and 5TD group. Serum creatinine was found to be significantly increased ($P < 0.05$) in vehicle and TD group (within normal limits); non significant changes (P >0.05) in 3TD and 5 TD groups. Total cholesterol showed non significant changes (P >0.05) in 5TD group; significant increase in vehicle (P< 0.05), 3TD group (P< 0.05) and TD (P< 0.01) group (within normal limits)

Total protein was increased significantly (P< 0.05) in 5TD group and showed non significant changes (P >0.05) in vehicle, TD and 3TD group. Albumin was increased significantly (P< 0.05) in 5TD and showed non-significant changes (P >0.05) in vehicle, TD and 3 TD groups (within normal range).AST was increased significantly in 3TD (P< 0.05) and vehicle group (P< 0.01); non significant changes (P >0.05) in TD and 5TD group. ALT showed significant decrease (P< 0.05) in 3TD group and non significant changes (P >0.05) in vehicle, TD and 5 TD groups. Potassium showed significant decrease (P< 0.01) in 5TD group and non significant changes (P >0.05) in vehicle, TD and 3 TD groups. Chloride showed significant decrease in (P< 0.01) vehicle, (P< 0.05)

Table 5: Effect of CKC on hematological parameters in wistar rats

Parameters	Normal control	Vehicle control	1 TD CKC	3 TD CKC	5TD CKC
Hb	9.1 ± .7358	12.3±1.0327	10.9±.4879	10.4± 1.4652	10.1±.7055
RBC	5.7± .4317	7.8 ± .6669	7.0 ± .3363	6.6 ± .9262	6.4 ± .4446
WBC	7.7± 1.1870	9.083 ± 1.0167	6.2±.4757	7.0±1.2028	7.3±1.0920
LYM	56.31 ± 2.1911	56.85± 1.3076	56.68± .9816	52.33± 1.6368	58.31±1.1083
MON	3.7±.1962	3.9± .3981	4.0 ±.1310	3.8 ± .3842	4.0 ± .1803
GRAN	39.9± 2.0241	39.2 ± 1.4098	39.2± .9290	43.7 ± 1.3686	37.8 ±1.1545
MCV	46.13± .4455	47.93±.3989*	46.16±.3148	46.21±.5023	46.71± .2960
MCH	15.78±.2182	15.68 ±.1759	15.30±.1915	15.46±.0919	15.98 ±.1249
MCHC	34.383± .3458	32.85±.4794	33.30±.2769	33.58± .3563	34.2 ±.2887
PLT	230.50±16.707	277± 28.931	233.67 ±15.549	323.33± 63.566	257.33 ±15.794
MPV	5.683 ±.0401	5.750 ± .0671	5.700 ±.0683	5.967 ±.0919	5.700 ±.0365
PCT	0.1305 ±0.009	0.1593 ±0.018	0.1325 ± 0.007	0.1933±0.0395	0.1475±0.008
HCT	26.367 ±2.0089	37.583 ±3.2963	32.700 ±1.5360	31.000 ±4.5195	29.717 ±2.2198
RDW	12.033 ±.4310	11.517 ±.3311	11.650 ±.1688	12.350 ±.2110	11.750 ±.1432
PDW	14.583± .0833	14.733±. .0615	14.767 ±..0760	14.867 ±..0989	14.667 ±..0333
Values are expressed as mean ±S.E.M; *P<0.05					

3TD and (P< 0.05) 5TD group and non significant changes (P >0.05) in TD group. All

Table 6: Effect of CKC on biochemical parameters in wistar rats

Parameters	Normal control	Vehiclecontrol	1 TD CKC	3 TD CKC	5TD CKC
Glucose	96.17 ± 7.472	110.83±16.660	80.50±4.660	79.67±5.942	90.33±3.739
Urea	35 ± 1.317	29.17 ± 4.183	28.83 ± 1.558*	30.17 ±3.124	35 ± 4.575
Creatinine	0.6±.0365	0.7 ±.0428*	0.8±.0365 *	0.5±.0885	0.7±.1014
Cholesterol	63.33 ± 6.200	84.33± 4.645*	95.17±9.958**	83.17±5.388*	76.11±16.105
Triglycerides	96.50±14.843	123.50± 6.206	135.33±13.583	103.33 ±9.032	132.67±16.760
Protein	7.6± .2887	7.0 ±.1926	7.0 ±.3983	7.8 ±.6019	10.35 1.1147*
Albumin	3.2±.2692	3.2±.1116	3.2±.1470	3.3±.2500	4.3±.2257 *
Globulin	3.7±.1478	3.7 ±.2044	3.7±.4301	4.5±.5426	5.9 ±.9807
Bilirubin	0.3± .0333	0.5±.0931	0.4±.0703	0.5± .1195	0.4 ±.1057
AST	136.6±3.774	154± 7.317**	138.50 6.412	173.83±14.481*	160.67±17.416
ALT	63.50± .992	74 ±9.274	60.17± 2.798	53.33± 3.593*	57.17 ±3.114
Sodium	134.33± 1.085	133.33± .803	133.50±.619	132.83±.654	133.83 ±.477
Potassium	7.0±.000	7.6± .3117	7.4 ±.1910	6.6± .2473	7.3 ±.0957**
chloride	106.83±.792	104.33±.494**	105.17± .477	104.33± .494*	104.67± .333*
Values are expressed as mean ±S.E.M; *P<0.05 ; ** P< 0.01					

were within normal limits. (Table 6)

Organ Weights

Absolute weights of brain, liver, stomach, kidney and thymus were found to be comparable with those of the control group rats. Increase in absolute weight of testis ($p < 0.05$) was noted in animals of 3 TD group. Absolute weight of heart was found to be significantly decreased ($p < 0.05$) in 5TD group and absolute weight of lungs was found to be significantly decreased ($p < 0.05$) in 5TD and vehicle group. (Table 7)

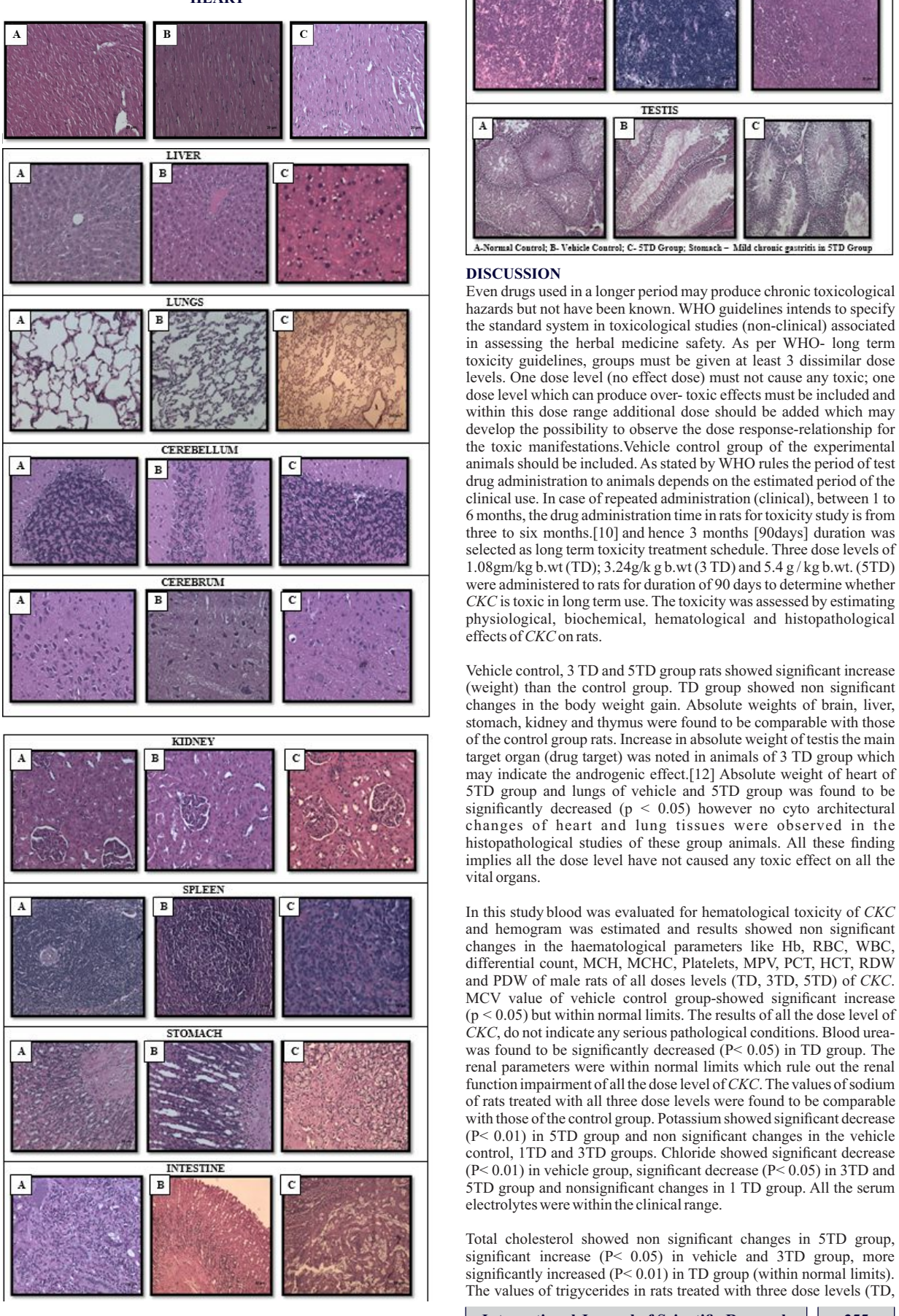
Histopathology

Vital organs like brain, heart, liver, kidney, spleen, lungs, stomach, intestine, thymus and testis from both treated (1TD, 3TD, 5TD) and control (normal and vehicle) group animals showed normal architecture. Mild focal chronic gastritis was observed in glandular part of the stomach in one animal of 5TD group (1/6). [Figure 1]

Table 7: Effect of different dose levels of CKC on organ weight [mg] in wistar rats

	Normal control	Vehicle control	1 TD CKC	3 TD CKC	5TD CKC
Brain	1.60 ± .0719	1.76 ± .0585	1.52± .0650	1.56± .0290	1.62 ± .0785
Heart	1.01± .0369	0.97± .03337	0.94 ± .0682	0.91± 0.0463	0.9 2± .0428*
Lungs	1.77±. 0.1173	1.34 ±. 0.0683	1.46 ±0.0440	1.57± 0.1183	1.39± 0.0726*
Liver	10.13 ± 0.3979	8.47 ± .3597	8.91± 0.5843	8.68 ± 0.7400	9.68± .3103
Stomach	1.41± 0.1244	1.44 ± 0.1064	1.45 ± .0263	1.46 ± 0.0444	1.48 ± .0292
Thymus	0.43 ± 0.0445	0.38 ± 0.0230	0.37 ± 0.0111	0.32 ± 0.0329	0.33 ± 0.0403
Testis	2.75 ± 0.0839	2.83 0.0612	2.84 ±0.1359	2.86± 0.0469*	2.89 ± 0.0549
Kidney	1.31± 0.0852	1.19 ±0.0486	1.2 1± 0.0930	1.10 ± 0.0535	1.12 ± 0.0441
Values are expressed as mean ±S.E.M; *P<0.05					

Figure 1: Histopathological photomicrographs of rats in longterm toxicity study



DISCUSSION

Even drugs used in a longer period may produce chronic toxicological hazards but not have been known. WHO guidelines intends to specify the standard system in toxicological studies (non-clinical) associated in assessing the herbal medicine safety. As per WHO- long term toxicity guidelines, groups must be given at least 3 dissimilar dose levels. One dose level (no effect dose) must not cause any toxic; one dose level which can produce over- toxic effects must be included and within this dose range additional dose should be added which may develop the possibility to observe the dose response-relationship for the toxic manifestations.Vehicle control group of the experimental animals should be included. As stated by WHO rules the period of test drug administration to animals depends on the estimated period of the clinical use. In case of repeated administration (clinical), between 1 to 6 months, the drug administration time in rats for toxicity study is from three to six months.[10] and hence 3 months [90days] duration was selected as long term toxicity treatment schedule. Three dose levels of 1.08gm/kg b.wt (TD); 3.24g/k g b.wt (3 TD) and 5.4 g / kg b.wt. (5TD) were administered to rats for duration of 90 days to determine whether *CKC* is toxic in long term use. The toxicity was assessed by estimating physiological, biochemical, hematological and histopathological effects of *CKC* on rats.

Vehicle control, 3 TD and 5TD group rats showed significant increase (weight) than the control group. TD group showed non significant changes in the body weight gain. Absolute weights of brain, liver, stomach, kidney and thymus were found to be comparable with those of the control group rats. Increase in absolute weight of testis the main target organ (drug target) was noted in animals of 3 TD group which may indicate the androgenic effect.[12] Absolute weight of heart of 5TD group and lungs of vehicle and 5TD group was found to be significantly decreased ($p < 0.05$) however no cyto architectural changes of heart and lung tissues were observed in the histopathological studies of these group animals. All these finding implies all the dose level have not caused any toxic effect on all the vital organs.

In this study blood was evaluated for hematological toxicity of *CKC* and hemogram was estimated and results showed non significant changes in the haematological parameters like Hb, RBC, WBC, differential count, MCH, MCHC, Platelets, MPV, PCT, HCT, RDW and PDW of male rats of all doses levels (TD, 3TD, 5TD) of *CKC*. MCV value of vehicle control group-showed significant increase ($p < 0.05$) but within normal limits. The results of all the dose level of *CKC*, do not indicate any serious pathological conditions. Blood urea-was found to be significantly decreased ($P< 0.05$) in TD group. The renal parameters were within normal limits which rule out the renal function impairment of all the dose level of *CKC*. The values of sodium of rats treated with all three dose levels were found to be comparable with those of the control group. Potassium showed significant decrease ($P< 0.01$) in 5TD group and non significant changes in the vehicle control, 1TD and 3TD groups. Chloride showed significant decrease ($P< 0.01$) in vehicle group, significant decrease ($P< 0.05$) in 3TD and 5TD group and nonsignificant changes in 1 TD group. All the serum electrolytes were within the clinical range.

Total cholesterol showed non significant changes in 5TD group, significant increase ($P< 0.05$) in vehicle and 3TD group, more significantly increased ($P< 0.01$) in TD group (within normal limits). The values of trigycerides in rats treated with three dose levels (TD,

3TD and 5TD) were found to be comparable with those of the control group. The normal limits of cholesterol and trigycerides rule out the cardio vascular disorder and coronary artery diseases. The present study showed non significant changes in blood sugar level of vehicle and in all three dose level groups.

AST- [Aspartate amino transferase] was increased significantly (P< 0.05) in 3TD and (P< 0.01) in vehicle group and showed non significat changes in 1 TD and 5TD groups. ALT [Alanine amino transferase] showed significant decrease (P< 0.05) in 3TD group and non significant changes in vehicle, 1TD and 5TD group. Albumin was increased significantly (P< 0.05) in 5TD and showed nonsignificant changes in rest of the dose levels. Globulin showed non significant changes in all the dose level groups. (Within normal limits) The values of bilirubin of rats treated with three dose levels (TD, 3TD and 5TD) and vehicle control were found to be comparable with those of the control group.Total protein showed non significant changes in vehicle, 1 TD and 3TD groups and was increased significantly in (P< 0.05) 5TD group. The protein level in 5TD group can be substantiated by the results of the histopathological study of the liver and by further corroboration with abnormalities found in the organ weight of liver which revealed the non toxicological significance when compared to that of the control group. The cause for increase in total protein level is not known with assurance but might be due to the increase in hepatic protein synthesis/ decreased degradation.

Histopathological examination of vital organs like brain, heart, liver, kidney, spleen, lungs, stomach, intestine, thymus and testis from both treated (1TD, 3TD, 5TD) and control (normal and vehicle) group animals showed normal architecture. Mild chronic gastritis was found in glandular part of the stomach in one animal of 5TD group (1/6). Psychological stress induces modifications of motility, secretion, visceral sensitivity, and local inflammatory responses in the GI tract. [22] The pathological change observed in one wistar rat in the higher dose group may be due to the psychological stress and it may be due to an individual variation, since such pathological changes was not found in other animals of that group, in which higher dose was given. Thus histopathological studies also confirmed the safety data, along with other physiological, biochemical and heamatological parameters after *CKC* treatment.

CONCLUSION

In conclusion the long term toxicity study of *CKC* of therapeutic dose (1.08gm/kg b.w) for 90 days in wistar rats demonstrated that it is safe with respect to animal survival, haemotological, biochemical and histopathological findings and is considered to be used in clinical application.

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