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Toxicity Profiling of Siddha Formulation Sarakondrai Chooranam by Acute and Repeated Oral dose Toxicity Studies in Wistar Rats

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Abstract

Toxicity Studies become an integral part of drug discovery and development process which involves different models, ranging from test tube experiments to cell cultures, animals, healthy human subjects. Although herbal medicine as the most common form of alternative medicine are widely used for treatment of various diseases all over the world now as per the regulatory guidelines it become mandatory to evaluate the short term and long term toxicities studies in rodents to establish the safety before utilizing the same for the clinical aspect. In Siddha system of traditional medicine it was clearly mentioned in detail about the toxic effects of various herbs and mineral components and also detailed in depth about the procedures for purification and detoxification of the same. But still now there is very limited documentary literatures available on the toxicity profiling of most of the Siddha preparation, hence the main aim of the present investigation is to carry out the systematic acute and repeated oral dose toxicity studies of the Siddha formulation Sarakondrai Chooranam (SKC) on rodents and to establish the safety of the drug. In short term acute toxicity study the drug SKC was administered in single dose of 2000 / kg b.w (p.o).Potential drug toxicity related to C.N.S, A.N.S and C.V.S were observed up to 14 days. In sub-acute repeated oral dose toxicity study the formulation SKC was administered at two dose level such as low and high dose of 200 and 400 mg / kg b.w (p.o) for four weeks. Results obtained from the acute and sub-acute study reveals that the Siddha formulation SKC doesn't reveal any significant change in body weight, behavior and mortality in treated rats and no sign of toxicity was registered throughout the study. Further it was observed that SKC at both the dose level did not modify the weight index, food and water intake in treated animals. There is no significant change in haematological, bio-chemical and histopathological observation of animals treated with SKC at both the dose level of 200 and 400 mg / kg b.w (p.o) when compare to control group animals. From the evidence based results of the present investigation it was concluded that the Siddha preparation SKC has wide safety margin in tested rodents and further long term usage of this formulation will be considerably safe for the ailment of various disease in humans.

Keywords: Siddha, Sarakondrai Chooranam, Toxicity Studies, OECD, Acute study, Sub-acute.

1. Introduction

Toxicity testing of new compounds is essential for drug development process. The pre-clinical toxicity testing on various biological systems reveals the species, organ and dose- specific toxic effects of an investigational product. The toxicity of substances can be observed by (a) studying the accidental exposures to a substance (b) *in vitro* studies using cells/ cell lines (c) *in vivo* exposure on experimental animals. The Organization for Economic Co-operation and Development (OECD) Guidelines for the testing of chemicals are a collection of the most relevant internationally agreed testing methods used by government, industry and independent laboratories to characterize potential hazards of new and existing chemical substances, chemical preparation and mixtures. They cover tests for the physico-chemical properties of chemicals, human health effects, environmental effects, degradation and accumulation in the environment [1].

Rodents are the most widely used animals for the evaluation of pre-clinical drug testing with respect to the toxicity studies of Siddha formulations, because of the genomic resemblance with that of the human. It is estimated, the global annual usage of non-human vertebrates ranging from 115.3 to 126.9 million in various levels of pre-clinical testing [2].

Short-term toxicity studies with rodents are generally conducted for 14 or 28 days. Results of these studies [3]can help to predict appropriate doses of the test substance for future sub-chronic or chronic toxicity studies [4]⁻ It can be used to determine NOELs (No observable effect level) for some toxicology endpoints.

Cassia fistula Linn is a Semi-Wild Indian Labernum also known as the Golden Shower, is distributed in various countries including Asia, Mauritius, South-Africa, Mexico, China, West Indies, East Africa, and Brazil as an ornamental tree for its beautiful branches of yellow flowers. Recognize by the British pharmacopoeia [5]. It is widely used for its Medicinal properties, its main property being that of a mild laxative suitable for children and pregnant women. It is also a purgative due to the wax aloin and a tonic [6]. It has been reported to treat many other Intestinal disorders like healing ulcers [7,8]. The plant has a high Therapeutic value and it exerts an anti-pyretic and analgesic effect [9,10]. The main aim of the present investigation is to evaluate the safety of the Siddha formulation Sarakondrai Chooranamin rodents at fixed dose level by acute and sub-acute repeated oral toxicity studies in accordance with OECD guidelines.

2. Materials and Methods

2.1. Collection of plant materials

The fresh leaf and flower of *C. fistula* (Sarakondrai) were collected from Southern Zone of Tamil Nadu, India. Plant specimen were identified and authenticated by the Pharmacognosist, SCRI, Chennai, Tamil Nadu, India.

2.2. Formulation of Sarakondrai Chooranam (SKC) [11].

Leaves and flowers of *Cassia fistula* commonly known by its Tamil name Sarakondrai. Fresh samples are collected and they are dried in shade. After that they are finely powdered and formulated as per the procedure described by Athmaratchamirtham Ennum Vaithya Sara Sangraham.

2.3. Toxicological Profiling of Sarakondrai Chooranam

2.3.1. Animal

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 $22 + 2^{\circ}$ C and 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/08/2016.

2.3.2. Acute toxicity Study

The animals were fasted overnight (12- 16 hrs) with free access to water. The study was conducted with single oral administration of study drug *Sarakondrai Chooranam* (SKC) 2000mg/kg (p.o). The animals were observed continuously for first 72 h and then 14 days for emerging signs of behavioral changes, body weight changes and for mortality.

Occurrence of toxicity in animals were observed continuously for the first 4 to 24 h and observed periodically for the next 14 days. Observation includes the change in skin, fur, eyes and mucus membrane. Appearance of C.N.S,C.V.S and A.N.S related toxicity such as tremors, convulsions, sedation, steric behavior, respiratory distress, cardiovascular collapse, response to sensory stimuli, salivation, diarrhea, lethargy, sleep, coma and mortality were observed with special attention [12]. Body weight was recorded periodically. At the end of the experiment all animals were subjected for gross necropsy and observed for pathological changes.

2.3.3.Sub-Acute toxicity Study

The animals were randomly divided into control group and trial drug SKC 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. The animals in group I (control group) received normal saline 5 ml/kg b.w. The animals in group II received low dose of *SarakondraiChooranam*200 mg/kg b.w(p.o) and group III received high dose of *Sarakondrai Chooranam* 400 mg/kg b.w(p.o).

The rats were weighed periodically and observed for signs of toxicity pertains to C.N.S, C.V.S, A.N.S including behavioral changes, food - water intake and morphological changes. At the end of 28th day, the animals were fasted for overnight with free access to water. On 29th day the animals were sacrificed with excess anesthesia. Blood samples were collected from aorta and stored in EDTA (ethylenediamine-tetra actate) for Hematological analysis and for serum generation for biochemical analysis. 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 [13].

2.3.4. Haematological analysis

Blood samples were analyzed using established procedures and automated Bayer Haematology analyzer. Parameters evaluated include Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White blood cell count (WBC), Platelet Count, Haemoglobin (Hb), Mean cell Haemoglobin Concentration (MCHC), Mean Red Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean platelet volume (MPV), Neutrophils, Eosinophil's, Basophils, Lymphocytes and Monocytes.

2.3.5.Bio-chemical analysis [14]

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.

2.3.6.Histopathological evaluation [15]

Organs included of heart, brain, lungs, spleen, kidneys, liver, stomach, testes and ovary. Histological slides of organs were made and observed under the microscope. The pathological observations of cross-section of these organs were performed on gross and microscopic bases. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological changes.

2.3.7. Statistical analysis [16]

The statistical analysis will be carried by one way ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean \pm standard error. A statistical comparison was carried out using the Dunnet's test for the control and treatment group. P-values less than 0.05 were set as the level of significance.

Results

3.1. Effect of SKC on clinical signs of Female rats in Acute Oral Toxicity Study

The dose of SKC used for acute toxicity study is 2000mg/kg which is higher than the normal therapeutic dose. No mortality observed at this dose level, further no significant change with respect to clinical signs on acute toxicity observed for (24-48 h) and a long period (14 days). The results were tabulated in Table 1.

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Parameter	Observation
Clinical Signs Parameters for the duration of 14 days	Test Drug 2000mg/ Kg
Number of animals observed	6 Female
Lacrimation	Absence
Salivation	Absence
Animal appearance	Normal
Tonic Movement	Absence
Clonic Movement	Absence
Laxative action	Absence
Touch Response	Normal
Response to Sound	Normal Response
Response to Light	Normal Response
Mobility	Normal Response
Respiratory Distress	Nil
Skin Colour	Normal
Stereotype behaviour	Absence
Piloerection	Absence
Limb Paralysis	Absence
Posture	Normal
Open field behaviour	Normal
Gait Balancing	Normal
Freezing Behaviour	Absent
Sings of Stress and Anxiety	None Observed
Muscular coordination	Normal
Muscle grip	Normal
Sedation	Absence
Social Behavior	Normal
Urine Analysis	No Abnormality
Urine Colour	Yellowish
Urine pH	7
Urine -Glucose	Absence
Urine -Ketones	Absence
Urine- Bilirubin	Absence
Urine-Blood Cells	Negative
Urine - Pus cells	Negative
Mortality	Nil

Table 1: Effect of Sarakondrai Chooranamon clinical signs of Female rats in Acute Oral Toxicity Study

3.2. Effect of SKC on Body weight of Female rats in acute toxicity study

No significant change was observed in body weight of female rats treated with SKC at the dose of 2000mg/ kg. The results were tabulated in Table 2.

Table 2: Effect of Sarakondrai Chooranam on Body weight of Female rats in acute toxicity study

Group	Before Treatment Weight in Gms	After Treatment Weight in Gms
Test Drug SKC 2000mg/ Kg	182.5 ± 6.56	185.8 ± 6.43

Values are mean \pm S.D (n = 6 per group). Statistically using one way ANOVA followed by Dunnett's test.

3.3. Effect of SKC on clinical signs of male and female rats in Sub-acute oral toxicity study.

SKC at low and high dose of 200 and 400 mg/ kg b.w. The results were tabulated in Table 3.

No significant toxicity was observed in rats during the 28 consecutive days of treatment via oral route with

Table 3: Effect of Sarakondrai Chooranam on clinical signs of male and female rats in
Sub-acute oral toxicity study.

Parameter	Group I	Group II	Group III
Clinical Signs Parameters for the			
duration of 28 days	Control	SKC 200mg/ Kg	SKC 400mg/ Kg
	3 Male and 3	3 Male and 3	_
Number of animals observed	Female	Female	3 Male and 3 Female
Lacrimation	Absence	Absence	Absence
Salivation	Absence	Absence	Absence
Animal appearance	Normal	Normal	Normal
Tonic Movement	Absence	Absence	Absence
Clonic Movement	Absence	Absence	Absence
Laxative action	Absence	Absence	Moderate
Touch Response	Normal	Normal	Normal
	Normal		
Response to Sound	Response	Normal Response	Normal Response
	Normal		
Response to Light	Response	Normal Response	Normal Response
Mobility	Normal	Normal	Normal
Respiratory Distress	Nil	Nil	Nil
Skin Color	Normal	Normal	Normal
Stereotype behavior	Absence	Absence	Absence
Piloerection	Absence	Absence	Absence
Limb Paralysis	Absence	Absence	Absence
Posture	Normal	Normal	Normal
Open field behavior	Normal	Normal	Normal

Gait Balancing	Normal	Normal	Normal
Freezing Behaviour	Absent	Absent	Absent
Sings of Stress and Anxiety	None Observed	None Observed	None Observed
Muscular coordination	Normal	Normal	Normal
Muscle grip	Normal	Normal	Normal
Sedation	Absence	Absence	Absence
Social Behavior	Normal	Normal	Normal
Urine Analysis	No Abnormality	No Abnormality	No Abnormality
Urine Colour	Yellowish	Yellowish	Yellowish
Urine pH	7	6	6
Urine -Glucose	Absence	Absence	Absence
Urine -Ketones	Absence	Absence	Absence
Urine- Bilirubin	Absence	Absence	Absence
Urine-Blood Cells	Negative	Negative	Negative
Urine - Pus cells	Negative	Negative	Negative
Mortality	Nil	Nil	Nil

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3.4. Effect of SKC on Body weight rats in Subacute oral toxicity study.

SKC at low and high dose of 200 and 400 mg/ kg b.w. The results were tabulated in Table 4.

No significant toxicity was observed in rats during the 28 consecutive days of treatment via oral route with

Table 4: Effect of Sarakondrai Chooranam on Body weight of rats in Sub-acute oral toxicity study

Group	Before Treatment Weight in Gms	After Treatment Weight in Gms
Group I	187.3 ± 5.64	198.7 ± 6.40
Group II	185.3 ± 3.93	197.3 ± 4.08
Group III	180.3 ± 6.37	192.7 ± 6.80

Values are mean \pm 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.

3.5. Effect of SKC on food and water intake of rats in Sub-acute oral toxicity study.

dose of 200 and 400 mg / kg b.w. The results were tabulated in Table 5.

No significant change was observed in body weight of both male and female rats treated with SKC at the

Table 5:Quantitative data on the food and water intake of rats treated with Sarakondrai Chooranam for 28 days in Sub-acute toxicity study

Group	Food intake	Water intake		
Group I	19.25 ± 3.07	41.25 ± 1.37		
Group II	18.25 ± 2.84	27.75 ± 1.31		
Group III	16.83 ± 3.49	39.25 ± 1.34		

Values are mean \pm 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.

3.6. Effect of SKC on Hematological and Biochemical parameters of male and female rats in Sub-acute oral toxicity study

treated with SKC at the dose of 200 and 400mg / kg b.w. The results were tabulated in Table 6-9.

No statistically significant differences were recorded in haematological and bio-chemical parameters of rats

Table 6: Effect of Sarakondrai Chooranam on Haematology profile of rats in sub-acute toxicity study.

Group	WBC count (×10 ³ µl)	RBC (×10 ⁶ μl)	PLT (×10 ³ μl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	HGB (g/dl)
Group I	13.5±1.53	7.217±1.55	684.3±90.86	63.22±4.26	19.37±3.83	31.47±2.08	11.88±2.10
Group II	9.983±1.49	6.417±1.29	764.3±137.4	59.12±6.00	20.37±1.28	32.65±1.52	11.1±1.23
Group III	11.08±2.22	7.033±1.55	511.8±98.96	61.12±5.25	23.03±3.16	32.12±1.55	11.65±2.59

Values are mean \pm 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 7: Effect of Sarakondrai Chooranam on Haematology profile of rats in sub-acute toxicity study.

Group	Lymph (%)	Mon (%)	Neutrophils (X 10 ³ /mm ³)	Eosinophils (%)	Basophils (%)	MPV (fl)
Group I	70.74±6.62	2.1±0.78	2.53±1.00	1.3±0.28	0.5±0.54	6.26±1.28
Group II	70.92±5.51	3.9±1.34	1.96±0.75	1.63±0.34	0.16±0.40	6.98±0.84
Group III	72.65±8.05	2.55±1.34	2.38±1.09	1.343±0.34	0.33±0.51	6.65±1.01

Values are mean \pm 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 8:Effect of Sarakondrai Chooranam on Serum Bio-chemistry profile of rats in sub-acute toxicity study

Group	Blood sugar ® (mg/dl)	BUN (mg/dl)	Serum creatinine (mg/dl)	Serum total cholesterol (mg/dl)	Serum triglycerides level (mg/dl)	Serum HDL cholesterol (mg/dl)	Serum LDL cholesterol (mg/dl)	Serum VLDL cholesterol (mg/dl)
Group I	86.17±8.931	11.17±3.251	0.6167±0.2994	117.2±16.98	80.67±11.93	68.67±12.45	29.67±17.75	16.8±21.707
Group II	94.17±6.432	12.5±3.017	0.75±0.2074	105.5±17.83	81.83±11.51	69.17±9.496	26±5.404	13.6±2.589
Group III	76.67±14.17	16.5±4.764	0.75±0.2168	119.3±18.38	70.83±8.75	47.67±4.412	53.67±7.789	19.05±4.723

Values are mean \pm 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.

Group	Group Serum total protein (g/dl)		(AST) (IU/ml)	(ALT) (IU/L)	(ALP) (IU/L)	
Group I	4.91±2.04	3.71±1.08	99.5±22.51	32.83±10.57	247±20.91	
Group II	5.5±0.76	2.98±0.62	107.8±19.96	21.33±6.77	107.2±18.13	
Group III	4.91±1.35	2.26±0.48	134.5±5.82	39.83±7.30	158.8±72.28	

Fable 9 : Effect of Sarakondrai Chooranam on Serur	n Bio-chemistry profile of rats in sub-acute	toxicity study
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Values are mean \pm 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.

3.7. Effect of SKC on gross organ weight of male and female rat in Sub-acute oral toxicity study

There was no significant change in the gross organ weight of the male and female rats treated with the test drug SKC. The results were tabulated in Table 10.

Table 10 : Quantitative data on absolute organ weight of rats treated with SarakondraiChooranamfor 28 days in Sub-acute toxicity study.

Group	HEART (gms)	LIVER (gms)	KIDNEYS (gms)	SPLEEN (gms)	BRAIN (gms)	LUNG (gms)	STOMACH (gms)	TESTES (gms)	UTERUS & OVARY (gms)
Group	$0.54 \pm$	$6.62 \pm$	$1.50 \pm$	$0.4 \pm$	$1.66 \pm$	$1.63 \pm$	1.19 ± 0.26	$2.53 \pm$	1.13 ±
Ι	0.05	0.78	0.19	0.12	0.17	0.16	1.18 ± 0.26	0.90	0.40
Group	$0.69 \pm$	$5.20 \pm$	$1.50 \pm$	$0.63 \pm$	$1.75 \pm$	1.7 ±	1.25 + 0.29	$3.03 \pm$	1.36 ±
II	0.10	0.66	0.16	0.16	0.17	0.15	1.25 ± 0.28	0.58	0.20
Group	$0.68 \pm$	$7.17 \pm$	$1.32 \pm$	$0.61 \pm$	$1.65 \pm$	1.6 ±	1.41 ± 0.41	$2.63 \pm$	1.2 ± 0.1
III	0.14	0.54	0.19	0.11	0.10	0.32	1.41 ± 0.41	0.83	1.2 ± 0.1

Values are mean \pm S.D (n = 6 per group of which 3 males and 3 females) for Heart, Liver, Kidney, Brain, Spleen, Lung, Stomach. Values are mean \pm S.D (n = 3 per group per sex) for testes , ovary and uterus for Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

3.8. Effect of SKC on Histopathological changes of male and female rat in Sub-acute oral toxicity study

No abnormality were detected in the histopathological analysis of organs (Kidney, Heart, Liver, Brain, Lung, Spleen and Stomach) retrieved from the rats treated with low and high dose of SKC. Appearance of inter neuronal space and count appears normal with regular morphology of brain parenchymal cells. No signs of degeneration and haemorrhage were observed in sample belongs to group I,II and III as shown in figure 01 and 02.

Nucleus appears prominent with regular arrangement of fibres. No evidence of atherosclerosis and

thrombosis. No evidence of collagen deposition in myocardium were observed in samples belongs to group I, II and III as shown in figure 03 and 04. No signs of airway secretion and bronchial secretion. Bronchial blood vessels and connective tissue appears normal with no signs of pulmonary oedema were observed in both control and treated rats as shown in figure 05 and 06.

Appearance of proximal and distal convolutes tubules was normal with no evidence of atrophy. No evidence of interstitial inflammation and lymphocyte accumulation in sample belongs to group I,II and III. As shown in figure 07 and 08. Marginal infiltration of inflammatory cells were observed in sample belongs to 3HM on periportal region of liver section belongs to group I, II and III. As shown in figure 09 and 10. Lymphoid follicles appears normal . Marginal sinus (MS) of the rat and its sinus lining cells appears normal. Erythropoietic cells (EP) are scattered throughout the red pulp of both the samples. Appearance of central artery and marginal sinus are normal in sample belongs to group I, II and III as shown in figure 11 and 12.

Microscopic analysis of stomach sample reveals normal anatomy of muscular stomach with epithelial layer keratinized stratified squamous epithelium, Lamina propria and Sub-mucosa were observed in sample belongs to group I, II and III. As shown in figure 13 and 14. Appearance of endometrium, myometrium and uterine glands was normal. Arrangement of stratum basale, functionale and surface epithelium seems normal in samples belongs to group I,II and III as shown in figure 15. Histopathological analysis of ovary showing normal corpus luteum(CL) and Primordial follicles with few mature ovarian follicles with no signs of abnormality. Appearance of antral follicle, primary oocyte and secondary follicles are normal in sample belong to group I,II and III as shown in figure 16.

Histo cytology of testicular tissue shows well differentiated germ cells with respect of spermatogonia includes spermatid and sperm were observed in sample belongs to group I,II and III as shown in figure 17.

Figure 01: Histopathology of Brain (Female Rat) in Sub-acute toxicity Study Low Power Magnification 10X



GROUP I

GROUP II

GROUP III

Figure 02: Histopathology of Brain (Male Rat) in Sub-acute toxicity Study Low Power Magnification 10X









GROUP I

GROUP II

GROUP III





High Power Magnification 40X



Figure 06: Histopathology of Lung (Male Rat) in Sub-acute toxicity Study



Figure 08: Histopathology of Kidney (Male Rat) in Sub-acute toxicity Study



Figure 10: Histopathology of Liver (Male Rat) in Sub-acute toxicity Study



Figure 11: Histopathology of Spleen (Female Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



Figure 12: Histopathology of Spleen (Male Rat) in Sub-acute toxicity Study

Low Power Magnification 10X







Int. J. Adv. Res. Biol. Sci. (2017). 4(12): 40-59 Figure 14: Histopathology of Stomach (Male Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



High Power Magnification 40X



Figure 15: Histopathology of Uterus (Female Rat) in Sub-acute toxicity Study



Int. J. Adv. Res. Biol. Sci. (2017). 4(12): 40-59 Figure 16: Histopathology of Ovary (Female Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



GROUP I

GROUP II

GROUP III

High Power Magnification 40X



Figure 17: Histopathology of Testes (Male Rat) in Sub-acute toxicity Study



4. Discussion

Herbal medicines are the most popular form of therapy for most of the world's population. A large number of population in the developing countries still rely on herbal medicine practitioners to meet their primary healthcare needs. The major reasons behind utilization of herbal medicines are that they are affordable, easily accessible, patient oriented and closely relates to patient's belief. This practice being natural and safe, perceived non-toxic by the general as population.Nearly 80% of India's one billion populations use at least one or the other product belongs to Indian system of traditional medicines. Use of traditional medicines has been conventionally increased globally.

Most of medications used for treatment of different diseases may have various side effect. Therefore, alternative approaches with more effectiveness, efficacy and safety are needed. There are only 35% of natural compounds available in current drugs. As plants, in general, are considered to have potential bioactive substances such as anti-oxidants and other secondary metabolites, there is a great interest in the use of medicinal plants as an alternative to synthesized medications[17-19]. According to several studies, medicinal plants and plant-originated products are suggested to be safer and less harmful for human body compared with modern synthetic drugs [20]. However, Plants and plant originated medicines like synthetic drugs have adverse effects. Medicinal plants have also been historically used for a long time in traditional treatment of diseases all over the world. As a result, different aspects of medicinal plants have been on the focus of intense scientific researches throughout of the world. Hence the World health organization (WHO) and US Food and Drug Administration (FDA) strongly insisting towards the slogan of safety rather than efficacy. As per the regulations the drugs irrespective of herbal or synthetic have to be proven its safety systematic toxicity studies before through administering the same in humans.

Toxicity study is an experimental screening method used to confirm safety of traditional preparations and herbal drugs in animal model trials. Investigation of acute toxicity is usually used as the first step in the toxicological analysis and aimed to understand the biological activity of a chemical and its mechanism of action. The information about acute systemic toxicity acquired in this test is used in hazard identification and risk management regarding with production, handling and use of chemicals [21]. Acute toxicity testing is carried out to determine the effect of a single dose on a particular animal species. In general, it is recommended that acute toxicity testing be carried out with two different animal species (one rodent and one non-rodent). In acute toxicological testing, the investigational product is administered at different dose levels and the effect is observed for 14 days. All mortalities caused by the investigational product during the experimental period are recorded and morphological, biochemical, pathological and histological changes in the dead animals are investigated. The results of the acute toxicity profiling of the drug SKC reveals no significant change in body weight, behavioral and mortality at the dose of 2000mg/kg.

Repeated dose toxicity testing is carried out for a minimum of 28 days. The test substance is administered daily for a certain period through the oral route. The test substance is administered regularly at a specific time. Usually, a rodent of any gender and age 5–6 weeks is used for repeated dose toxicity testing. In sub-acute toxicity study treatment with SKC at the dose level of 200 and 400mg/kg did not reveal any significant change in body weight, food and water intake in both male and female rats. Further there is no alteration in AST, ALT and ALP level of treated animals which denotes the hepato- protective nature of the drug.

Clinical bio-chemistry and haematological data hold significant role in determining the toxicity induced by drugs. Blood parameters analysis is relevant to risk evaluation as the haematological system has a higher predictive value for toxicity in humans (91%) when assays involve rodents and non-rodents [22]. Blood forms the main medium of transport for many drugs and xenobiotics in the body and for that matter components of the blood such as red blood cells, white blood cells, haemoglobin and platelets are at least initially exposed to significant concentrations of toxic compounds. Damage to and destruction of the blood cells are inimical to normal functioning of the body [23]. Results of haematological and serological profiling of SKC at the dose of 200 and 400mg/kg did not shown any signs of haematopoietic toxicity and there is no change in the blood and serum parameters of the treated animals.

The evaluation of histopathological changes in organs remains a cornerstone in safety assessment of medicines [24]. Absolute terminal organ weight and percent relative organ weight indicative of test compound caused changes in functioning of target organs, changes in phospholipid metabolism, over or of enzymes undersecretion and hormones. hypo/hyperplasia and possible tissue necrosis [25]. In the present investigation there was no abnormality were detected in the histopathological analysis of organs (Kidney, Heart, Liver, Brain, Lung, Spleen, Stomach, Testes and Ovary) retrieved from the rats treated with low and high dose of SKC.

5. Conclusion

Toxicology is a branch of science that deals with toxins and poisons and their effects and treatment. Toxicological screening is very important for the development of new drugs and for the extension of the therapeutic potential of existing molecules. From the results of the present toxicology study of the trial drug SKC it was concluded that the drug is relatively nontoxic, causes no apparent organ damage or mortality in both the acute and sub-acute repeated oral toxicity study in treated animals. Hence from the results, it was concluded that the drug SKC was safe and reveals no signs of significant toxicity for long term treatment for the chronic disease condition.

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