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ACUTE AND 28 DAY REPEATED ORAL TOXICITY STUDY OF *SATHAKUPPAI CHOORANAM*- A SIDDHA POLY HERBAL FORMULATION IN WISTAR ALBINO RATS

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ABSTRACT

Sathakuppai chooranam(SC) is the poly herbal formulation mentioned in ancient Siddha literature, *Agathiyar attavanai vagadam*. To evaluate it safety, acute and 28-day repeated oral toxicity studies were performed following OECD test guidelines 423 and 407, respectively. In acute study, SC was administered orally at 5, 50, 300, and 2000 mg/kg body weight. Animals were observed for toxic signs for 14 days. Gross pathology was performed at the end of the study. In 28 day repeated dose toxicity study, SC was administered at 200 and 400 mg/kg body weight daily, for 28 days. Animals were observed for mortality, behavioral changes, body weight, feed and water intake, hematology, biochemical parameters, gross pathology, relative organ weight and histopathological examination were performed. In acute toxicity study, no treatment related death or toxic signs were observed. It revealed that the LD_{50} cut-off value of SC is greater than 2000 mg/kg body weight. The 28 day repeated dose study did not show evidence of any treatment related changes in all observations up to the high dose level. There is no significant differences in organ weight, haematological and biochemical parameters of treated animals when compared with the control. Histopathological examination revealed no abnormalities. It revealed that the NOAEL > 400mg/kg/day in Wistar albino rats.

KEYWORDS: Siddha medicine, Sathakuppai chooranam, Poly herbal, Acute toxicity study.

INTRODUCTION

Medicinal plants play a key role in the human health care. About 80% of the world population rely on traditional medicine which is based on plants.[1] The use of medicinal plants for healing purposes has been increasingly popular as they are believed as beneficial, free of side effects[2], and their efficacy and cost effectiveness. These drugs are either single plant extracts or fractions or mixtures of extracts from different plants. These plant extracts are standardized for their safety and efficacy.[3] In Siddha system of medicine *Sathakuppai chooranam(SC),* a herbal formulation mentioned in ancient Siddha literature *Agathiyar attavanai vagadam*[4], has been prescribed for the management of Psychological and neurological disease like *Mathaazhivu (Depresion), pithavigaram (Hallucination), Thokamenmmai (Insomnia), Kudiverinoi (Alchololic dependence).* The World Health Organization estimates that about 140 million people throughout the world suffer from alcohol dependence. Alcohol dependence (AD) is the increased prevalence in India for the recent years there has been rapid proliferation of city bars and people are fast shedding their inhibitions about alcohol as a life style. In AD, Alcoholic liver disease and cirrhosis of liver is the common complication.[5] We will be more concern about alcohol de- addiction drugs. Severe acute alcoholic hepatitis has a poor outcome with standard supportive management. For example, the mortality rate of patients with severe alcoholic hepatitis in two prospective studies was 35% and 46%, respectively. The addition of acute renal failure worsens the prognosis further. *Sathakuppai chooranam* is traditionally widely used medicine in south India especially in Tamilnadu. However, till date, no safety profile of this formulation available. This study evaluate acute and sub-acute toxicity profile of poly-herbal siddha formulation of SC in laboratory animals which will provide effective documentation for its safety aspect in human usage of this drug.

MATERIALS AND METHODS

Preparation of test drug Sathakuppai chooranam

The ingredients of SC such as *Sathakuppai (Anethumgraveolens), Narseeragam (Cuminumcyminum), Peruseeragam (Pimpinellaanisum), Karuseeragam (Nigella sativa), Elam (Elettariacardamomum), Sannalavangapattai (Cinnamomumverum), Athimathuram (Glycyrrhizaglabra), Kothamalli (Coriandrumsativum), Lavangam (Syzygiumaromaticum)* each 35 gm (1palam) were procured from reputed raw drug shop, Broadway, Chennai and was authenticated (certificate no.NISMB2032015) by the botanist, National Institute of Siddha, Chennai-47. After the purification process of the ingredients of *Sathakuppai*

chooranam, which was prepared as mentioned in the Siddha literature, *Agathiyar attavanai vagadam* at Gunapadam laboratory in National Institute of Siddha, Chennai-47.

Experimental Animals

Healthy wistar albino rats in both sex (150-200g) were obtained from the animal house of King Institute of Preventive Medicine, Guindy, Chennai and maintained in the animal laboratory of K.K. College of Pharmacy, Gerugambakkam, Chennai. The animals were housed in polypropylene cages with bedding materials and kept under the well – ventilated and standard environmental condition of temperature 23± 2°, under 12/12 hrs light and dark cycle and 40-65% of relative humidity. The animals had standard pellet diet (by Godrej foods pvt. Ltd, Bangalore) for feeding and free access to RO water. The animals were kept in their cages for 7 days prior to dosing to allow for acclimatization to the laboratory conditions. The principles of laboratory animal care were followed. The toxicity studies were carried out at K.K. College of Pharmacy, Gerugambakkam, Chennai. The study was done after getting permission from the Institutional Animal Ethics Committee. (Ref: 4532/KKCP/2015).

Study design for toxicity studies

Acute toxicity study

The acute oral toxicity study was performed as per OECD guidelines 423 for testing chemicals (adopted- Dec,2001) with minor modification.[6] As per the guidelines female rats were divided into five groups, each group containing 3 rats (nulliparous and non-pregnant), first group treated as a control and other four groups treated with test drug SC at different doses 5mg, 50mg, 300mg, 2000mg per kg.b.wt respectively. All the animals were observed individually after dosing once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for furthere 14 days. Animals were observed for mortality and signs of tremors, convulsions, salivation, diarrhoea, and coma. Accessibility of food and water, Changes in skin and fur, eyes and mucous membranes, sleep pattern, locomotion were noted. Body weight of individual animals were noted weekly once. At the end of the study period (15th day), the overnight fasted animals(except water) were weighed and sacrificed under the excessive euthanaesia and subjected to gross pathological examination of major internal organs. Maximum tolerated dose was calculated in accordance with Globally harmonized system of classification and labelling of chemicals.[7]

28 day repeated dose oral toxicity study

Sub-acute toxicity studies were carried out according to OECD guideline-407 (revised-18 December 2007) with minor modification.[8] Based on the acute toxicity study 1/10(200mg/kg) and 1/5 (400mg/kg) of the acute non toxic dose was selected as the therapeutic dose. The therapeutic dose is fixed as such that it has two doses. High dose is upto the toxic level and the low dose is around the sub therapeutic dose. Both sex of the rats were divided into 3 groups, each group contains 10 animals (5 male and 5 female). Control animals (first group) treated with water (vehicle), the other two groups SC was administered at the dose of 200 & 400 mg/kg/day for 28 days. The toxic symptoms such as signs of toxicity, mortality and body weight changes(once a week) were monitored till the end of the study. Daily observation of food and water intake were calculated and expressed as 7 days cumulative value. On 29th day, the overnight fasted (except water) animals were anesthetized with excessive anesthesia and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of hematological parameters and another tube for biochemical parameters, without any anticoagulant which was centrifuged at 4000 rpm at 4°C for 10 minutes to obtain biochemical parameters such as glucose, total protein, albumin, triglycerides, cholesterol levels, bilirubin, urea, creatinine, Serum glutamate oxaloacetate transaminase/ Aspartate amino transferase (SGOT/AST), Serum glutamate pyruvate transaminase/ Alanine amino transferase (SGPT/ALT) and alkaline phosphatase (ALP) were estimated as per the calorimetric procedure.

The heparinized blood was used for haematological parameters such as haemoglobin content, total red blood corpuscles (RBC), total white blood corpuscles (WBC) count, hematocrit, platelets, lymphocytes and neutrophils were measured.

All the animals were sacrificed after the blood collection on 29th day. Necropsy of all animals was carried out for the gross pathological examination of major internal organs and the weights of the organs including liver, kidneys, spleen, brain, heart and lungs were recorded. The organs such as heart, kidneys, liver, spleen and stomach were removed from all animals and weighed on an electronic balance. The organs (3-5µm thick) were preserved and were fixed in 10% formalin and stained with hematoxylin and eosin. Histopathological examinations were performed on the preserved slides.

Statistical analysis

Data were presented as mean ± SEM (Standard error of mean). Findings such as body weight changes, relative organ weight, hematological and biochemical parameters were subjected to One-way ANOVA followed by Dunnett's test using a computer software program. (Graph Pad Prism 5.0). Significance between the mean value of test and control group.$P < 0.05$ was considered as significant.

RESULT

Acute toxicity study

There were no mortality and toxicity signs observed in both control and SC treated groups throughout the study period. No significant difference in toxicity signs, weight gain and no gross pathological changes in treated groups. The result of the Acute toxicity study as per OECD guideline- 423 indicates that the LD_{50} of SC is more than 2000mg/ kg and the test drug SC comes under the category- 5 according to the Globally harmonized system of classification and labelling of chemicals.

Sub acute toxicity study

There were no treatment related toxic signs and mortality observed in both male and female rats treated at mid (200 mg/kg) and high dose (400mg/kg) levels. There were no significant changes in body weight, food and water intake (Table 1,2&3) of both control and drug treated groups. The haematological (Table4) and biochemical parameters (Table5) are within the normal range of rats in experimental groups.[9] There were no abnormalities in the gross and histo-pathological (Figure 1) studies. It revealed that the NOAEL (No-Observed Adverse Effect Level) of SC is greater than the 400 mg/ kg/ day in rats, hence, it can be concluded that the oral administration of SC is safe.

Table 1: Effect of SC on body weight in Wistar albino rats- 28 day repeated oral toxicity study.

Dose mg/kg/day	1	7	14	21	28
Control	155.53 ± 5.88	159.33 ± 6.47	160.66 ± 7.02	162.83 ± 7.48	164.5 ± 7.91
Mid dose (200mg/kg)	157.01 ±10.37	159.51±10.52	161.66±10.85	164.86±11.22	167.01±13.74
High dose (400mg/kg)	161.33 ±13.05	164.83±13.44	166.33±13.68	170.16±13.78	173.16±14.17

Values were expressed as mean ± SEM. for n=10 rats in each group. One way ANOVA followed by Dunnett's test.

Table 2: Effect of SC on water intake in Wistar albino rats- 28 day repeated oral toxicity study.

Dose (mg/kg/day)	Days(gms/ rats)				
	1	7	14	21	28
Control	32 ± 0.92	33.36 ± 0.23	33.11 ± 0.23	33.19 ± 0.12	33.37 ± 0.32
Mid dose (200mg/kg)	33.09 ± 0.49	33.52 ± 1.52	34.33 ± 1.51	34 ± 1.1	34.64 ± 1.65
High dose (400mg/kg)	32.92 ± 1.51	33 ± 1.79	33.39 ± 1.51	34.19 ± 1.33	34.58 ± 1.52

Values were expressed as mean ± SEM. for n=10 rats in each group. One way ANOVA followed by Dunnett's test.

Table 3: Effect of SC on food consumption in Wistar albino rats- 28 day repeated oral toxicity study.

Dose (mg/kg/day)	Days(gms/ rats)				
	1	7	14	21	28
Control	25.5±0.04	25.16±1.12	24.09 ± 0.71	25.16 ±1.97	25.66 ± 1.37
Mid dose (200mg/kg)	26.5±0.82	26.16±0.75	25.06 ± 0.95	26.13 ±1.49	26.33 ± 1.12
High dose (400mg/kg)	24.31±2.02	24.8 ± 1.33	24.18 ± 1.10	25.33 ±1.15	25.83 ± 1.17

Values were expressed as mean ± SEM. for n=10 rats in each group. One way ANOVA followed by Dunnett's test.

Table 4: Effect of SC in haematological parameters in Wistar albino rats- 28 day repeated oral toxicity study.

Hematological parameter	Control	Trial drug	
		Mid dose (200mg/kg)	High dose (400mg/kg)
Total R.B.C. count ($\times 10^6$ mm^{-3}).	9.09±0.15	9.20±0.14	9.11±0.16
Total W.B.C. Count ($\times 10^3$ mm^{-3}).	12.67±0.22	12.15±0.17	12.23±0.28
Haemoglobin (Hb) (g/dl)	15.61±0.36	14.17±0.34	16.63±0.66
Hematocrit (%).	44.21±1.01	43.61± 1.72	42.4±1.36
Platelets ($\times 10^3$ mm^{-3}).	834.91±24.01	827.21±22.43	839.81±21.56
Lymphocytes(%).	84.7±3.32	81.8±7.33	82.8±4.43
Neutrophils (%).	20.6±0.65	21.6±0.52	20.2±0.91

Values were expressed as mean ± SEM. for n=10 rats in each group. One way ANOVA followed by Dunnett's test.

Table 5: Effect of SC on biochemical parameters in Wistar albino rats- 28 day repeated oral toxicity study.

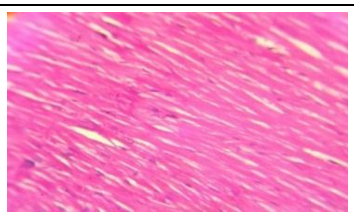
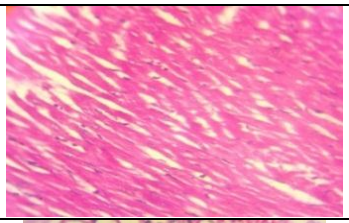
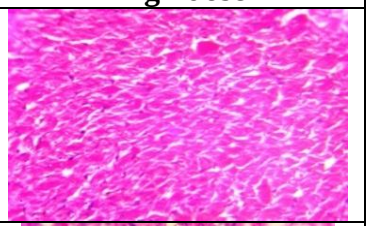
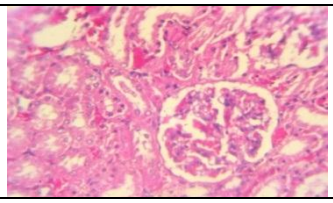
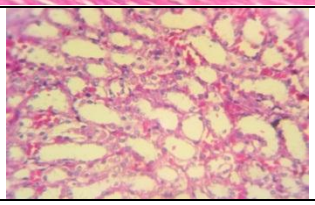
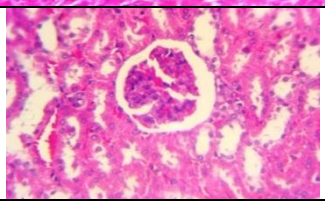
Biochemical parameter	Control	Trial drug	
		Mid dose (200mg/kg)	High dose (400mg/kg)
Creatinine (mg/dl)	0.5890±0.079	0.59±0.04	0.57±0.07
Urea (mg/dl)	15.30 ± 0.47	14.50±0.45	15.9±0.57
Glucose(F)(mg/dl)	85.8±12.6	84.9±12.4	85.2±12.5
Triglycerides (mg/dl)	52±1.13	51.90±1.633	52.45±2.01
Total Cholesterol (mg/dl)	46.60±1.21	46.92±1.08	47.12±1.02
Total protein (mg/dl)	4.29±0.26	4.20±0.65	4.26±0.864
Albumin (g/dl)	3.20±0.41	3.21±0.33	3.22±0.77
SGOT (IU/L)	121.41±2.68	121.56±1.33	119.61±3.123
SGPT (IU/L)	69.40±1.57	69.712±2.121	69.72±1.35
ALP (IU/L)	112.6±4.67	113.01±1.029	112.41±4.154
T. Bilirubin (mg/dl)	0.256±0.032	0.254±0.069	0.254±0.096

Values were expressed as mean ± SEM. for n=10 rats in each group. One way ANOVA followed by Dunnett's test.

Table 6: Effect of SC on relative organ weight in Wistar albino rats- 28 day repeated oral toxicity study.

Dose	Relative Organ Weight of rats					
	Liver	Kidney	Brain	Lungs	Heart	Spleen
Control	2.79± 0.1	0.66±0.02	0.38±0.02	0.29±0.01	0.30±0.01	0.15±0.01
Mid dose (200mg/kg)	2.88±0 .1	0.645± 0.02	0.39±0.01	0.30±0.02	0.30±0.01	0.167±0.01
High dose (400mg/kg)	2.81±0.1	0.661±0.03	0.39±0.01	0.30± 0.01	0.31±0.01	0.156±0.01

Values were expressed as mean ± SEM. for n=10 rats in each group. One way ANOVA followed by Dunnett's test.

S.no	Organ name	Control	Mid dose	High dose
1	Heart			
2	Kidney			

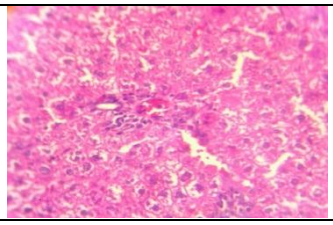
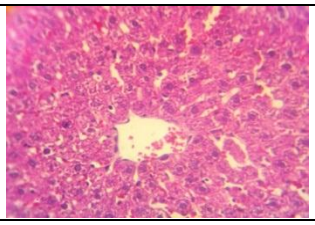
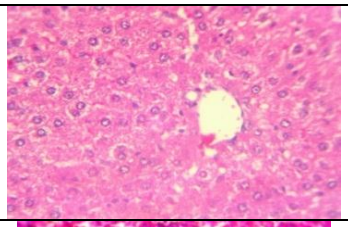
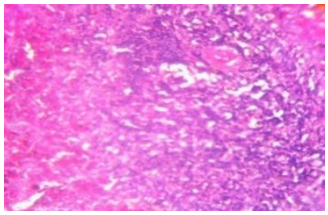
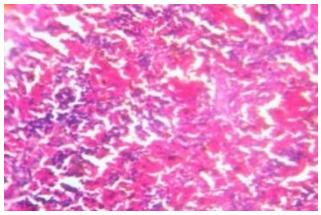
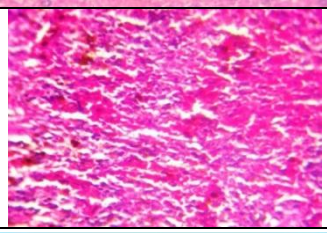
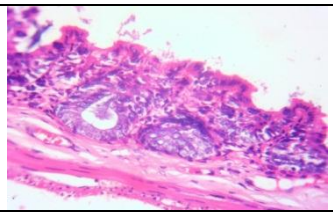
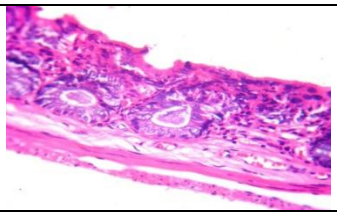
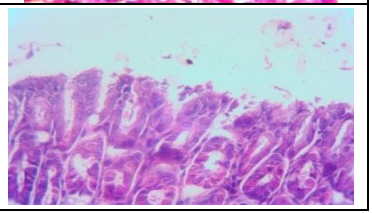
3	**Liver**			
4	**Spleen**			
5	**Stomach**			

Figure 1: Histopathological investigation of control and SC treated animals for 28 day repeated oral toxicity.

Histopathological examinations of the tissues revealed no abnormalities in control, mid (200 mg/kg/day) & high dose (400 mg/kg/day) of SC treated experimental animals.

DISCUSSION

Herbal medicines have attained greater importance as an alternative to conventional therapy. To optimize the safe use of a plant-based medicine, one should take into account their historical applications on humans and animals as well as toxicity evaluation of the medicinal herbs and their active components.[10] Many screening methods are employed to determine the safety and efficacy of these herbal medicines and also to establish the active component of the herbal products.[11] Toxicity is an expression of being poisonous, indicating the state of adverse effects led by the interaction between toxicants and cells.[12]

Siddha system of medicine is an ancient medical system of Dravidian origin which is prevalent mainly in Southern parts of India, especially in Tamil Nadu. In this present study, evaluate the toxicological and safety profile of *Sathakuppai chooranam*, a Siddha formulation which is used to treat liver disorder associated with alcoholism by acute and 28 day repeated toxicity studies as per OECD guidelines 423 and 407 respectively in Wistar rats and the results were analyzed.

In acute toxicity study of SC were administered in four different doses in step up fashion as per OECD 423 guidelines. There was no mortality and signs of toxicity were observed in SC treated animals throughout the study period, the LD_{50} of SC is more than 2000mg/ kg and it comes under the category- 5 according to the globally harmonized system of drugs. Therefore it can be concluded that the single administration of SC is non-toxic and safe for oral administration.

Examination of clinical signs plays a important role in toxicological studies.[13] mortality and morbidity were recorded throughout the study period. In repeated dose 28 days study as per OECD guidelines 407 revealed there were no mortality and morbidity in two different dose levels (mid dose (200 mg/kg/day) & high dose (400 mg/kg/day)) of SC.

There was a normal water intake (Table2) and food consumption (Table3) and there was an obvious weight gain (Table1) in the SC treated groups when compared with control, it revealed that the test drug did not adversely affect the basic metabolic functions of the experimental animal.

Clinical biochemistry and haematological parameters plays an important role to identify the toxicity induced by drugs.[14] Hematopoietic system is one of the targets for toxic compounds and is an important index of physiological and pathological states.[15] Analysis of haematological parameters (Table4) showed no significant changes in SC treated groups compared with control, it indicates it is the safe formulation and no pathological changes in haemopoietic system.

SGOT and SGPT are good indicators of liver function and biomarkers to predict the possible toxicity of drugs.[16] Any elevation pertaining to these enzymes indicate their outflow into the blood stream due to damage in liver parenchymal cells. Elevated bilirubin levels are an indication of altered liver functions and a small elevation is an important indicator of liver damage in laboratory animals or could be a sign of biliary duct obstruction. In order to assess the synthetic capacity of the liver, determination of plasma proteins like albumin is required and decrease in plasma proteins therefore tend to reflect chronic damage.[17] There were no significant differences in the levels of SGOT, SGPT, bilirubin and total protein (Table5) between the control and treated groups. These indicate that SC did not cause any damage to the liver.

The estimation of urea and creatinine are sensitive indicators of nephrotoxicity.[18] The normal values of kidney parameters such as blood urea and creatinine (Table5) suggest that SC did not have nephrotoxicity. In the present study, there were no treatment related abnormalities in renal function and other biochemical parameters suggesting that SC is non-toxic.

There were no significant changes in organ weight (Table6) of drug treated animals when compared with control. The evaluation of histopathological changes in organs remains a cornerstone in safety assessment of medicines.[19] In gross and histopathological examination (Figure 1), no abnormality was recorded in test groups.

From the above results, it can be indicated that the No Observed Adverse Effect Level of *Sathakuppai chooranam* was greater than 400mg/kg/day.

CONCLUSION

In-vivo toxicity study of SC revealed that there was no mortality and signs of toxicity up to 2000mg/kg in acute toxicity study, it comes under the category- 5 according to the Globally harmonized system of drugs and labeling of chemicals. In 28 day repeated oral administration of the test drug showed the No Observed Adverse Effect Level of SC greater than 400mg/kg/day. In this toxicity study reveals that SC is fairly non-toxic, it is safe for human consumption. Further, sub-chronic toxicity study of the test drug SC are recommended and clinical trials for treating liver disease associated with alcoholism.

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REFERENCE

1. *A.Subramonium, P. Pushpangadan: Development of phytomedicines for liver diseases, Indian journal of pharmacology, 1999; 31: 166-175.*
2. *da Costa Lopes L, Albano F, Augusto TravassosLaranjaG,MarquesAlves L, Fernando Martins e Silva L, Poubel de Souza G,etal.Toxicological evaluation by in vitro and in vivo assays of an aqueous extract prepared from Echinodorusmacrophyllus leaves. Toxicol Lett., 2000; 116: 189-98.*

3. *Sharma A: Antihepatotoxic activity of some plants used in herbal formulations. Fitoterapia, 1991; 62: 131-138.*
4. *Arangarajan.S, Agathiyar attavanai vagadam, Thanjavur (Tamil nadu): Saraswathymazhallibrarary, Mar 2007, 2nd Edition, P.No: 82.*
5. *Balammal.G, Alcoholism – A Review. Journal of Pharmaceutical Biology., 2013; 3(1): 1-13.*
6. *OECD: Guidelines for the Testing of Chemicals/Section 4: Health Effects Test No. 423: Acute Oral toxicity - Acute Toxic Class Method. 2002, Paris, France: Organization for Economic Cooperation and Development.*
7. *OECD Forum for the Future., Organisation for Economic Co-operation and Development: Advisory Unit on Multi-Disciplinary Issues. Expo 2000/OECD Forum for the Future: 21st Century Technologies: balancing economic, social and environmental goals: main issues and summary of the discussions of a Conference held on 7th and 8th December at SchlossKrickenbeck, Germany. 1998, Paris: OECD.*
8. *Organization for Economic Cooperation and Development; Guidelines for the Testing of Chemicals/Draft Updated Test Guideline 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents. 2008.*
9. *Petterino C, Argentino-Storino A: Clinical chemistry and haematology historical data in control Sprague–Dawley rats from pre-clinical toxicity studies. Exp Toxicol Pathol., 2006; 57: 213-219. 10.1016/j.etp.2005.10.002.*
10. *Mukinda JT, Syce JA: Acute and chronic toxicity of the aqueous extract of Artemisiaafra in rodents. J Ethnopharmacol, 2007; 112: 138-144. 10.1016/j.jep.2007.02.011.*
11. *SimKT, Sri Nurestri AM, Sinniah SK, Kim KH, Norhanom AW: Acute oral toxicity of Pereskiableo and Pereskiagrandiofolia in mice. Pharmacogn Mag., 2010; 6: 67-70. 10.4103/0973-1296.59969.*
12. *Das N, Goshwami D, Hasan S, RaihanSZ. Evaluation of acute andsubacute toxicity induced by methanol extract of Terminalia citrineleaves in Sprague Dawley rats. J Acute Dis., 2015; 4(4): 316-21.*
13. *Stevens KR, Mylecraine L: Issues in chronic toxicology. Principles and Methods of Toxicology. Edited by: Hayes AW. 1994, New York: Raven Press, 673-third.*
14. *Petterino C, Argentino-Storino A: Clinical chemistry and haematology historical data in control Sprague–Dawley rats from pre-clinical toxicity studies. Exp Toxicol Pathol., 2006; 57: 213-219. 10.1016/j.etp.2005.10.002.*

15. *Tatke PA. Nidhiya IS, Deshpande SG: Safety profile of a polyherbal formulation (Gynocare capsules) in female rats by subchronic oral toxicity study. Toxicol Int., 2012 May; 19(2): 106-11.*
16. *Hilaly JE, IsrailiZH, Lyouss B: Acute and chronic toxicological studies of Ajuva Iva in experimental animals. J Ethnopharmacol, 2004; 91: 43-50. 10.1016/j.jep.2003.11.009.*
17. *RasekhHR, Nazari P, Kamli-Nejad M, Hosseinzadeh L. Acute and subchronic oral toxicity of Galegaofficinalis in rats. Journal of Ethnopharmacology, 2008; 116: 21–26.*
18. *William M. kluwe Renal function tests as indicators of kidney injury in subacute toxicity studies, Toxicology and Applied Pharmacology, 15 march 1981; 57(3): 414-424.*
19. *P. Greaves, Histopathology of Preclinical Toxicity Studies: Interpretation and Relevance in Drug Safety Evaluation, Academic Press, New York, NY, USA, 3rd edition, 2007.*