International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 5, Issue 9 - 2018

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2018.05.09.009

Acute and Sub-Acute Toxicity Evaluation of Siddha Formulation Kalingathi Ennai In Accordance With OECD Guideline

S. Brunda*[1], K.Nithya[2], N. Anbu[3], K. Kanakavalli[4]

[*1&2] P.G.Scholar, Government Siddha Medical College, Arumbakkam, Chennai 600106, Tamil Nadu, India.
[3]HOD,PG Department of Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai 600106, Tamil Nadu, India.
[4]Principal, Government Siddha Medical College, Arumbakkam, Chennai 600106, Tamil Nadu, India.
Corresponding Author : **Dr.S.Brunda,** P.G.Scholar, Government Siddha Medical College, 6 Anna Arch Road, NSK Nagar, Arumbakkam, Chennai 600106, Tamil Nadu, India

Abstract

Siddha drugs and their formulations have been considered to be safe and effective due to their negligible side effects. This assumption may have influenced the indiscriminate use of these formulations to a large extent amongst the rural and urban population. These formulations are usually administered over a long period of time for many clinical indications. Since the formulation Kalingathi Ennai (KE) prescribed for chronic ailments, it is essential to validate the short and long term safety margin of the drug before clinical application. The main aim of the present investigation is to establish the safety profile of the test drug KE by acute and sub-acute oral toxicities in both male and female wistar rats in accordance with OECD guidelines. In the acute study, a single dose of 2000 mg/kg was orally administered and animals were monitored for 14 days. In the sub-acute study, repeated doses (20, 100 and 200 mg/kg/day) of the test drug KE were administered for 28 days and biochemical, hematological and histopathological parameters were evaluated. Results of the present investigation showed that there was no sign of toxicity and no mortality after single or repeated administration of *the test drug KE at varying doses in tested animals.* The mean body weight, food/ water intake, behavioral and most of the biochemical and hematological parameters showed normal levels in both male and female rats. No significant toxicity was observed in the histological examination of kidney, liver and spleen tissues at the highest dose of KE. Single and repeated oral administration of the Siddha drug KE may be safe and considered as relatively non-toxic at the varying doses of 20, 100 and 200 mg/kg/day dose level. These results further suggest that acute or sub-acute oral administration of the test drug KE is safe and doesn't causes any potential toxic effect in rats.

Keywords: Siddha drug, Kalingathi Ennai, Safety margin, Biochemical , Hematological parameters, Acute and sub-acute oral toxicity.

1. Introduction

For centuries, herbal medicines and their formulations have been considered to be safe and effective due to their negligible side effects. This assumption may have influenced the indiscriminate use of these formulations to a large extent amongst the rural populace. These formulations are usually administered over a long period of time without proper dosage monitoring by the experts and lack of awareness of the toxic effects that might result from such prolonged usage. Therefore, scientific knowledge towards oral toxicity is much needed, which will not only help identify doses that could be used subsequently, but also to reveal the possible clinical signs elicited by agents under investigation.

Most of medications used for treatment of different diseases may have various side effects; 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 antioxidants and other secondary metabolites, there is a great interest in the use of medicinal plants as an alternative to synthesized medications [1-3]. 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 [4].

A scientifically carried out screening is therefore important in order to ascertain safety and efficacy of traditional and herbal products and also to establish the active components in them [5] Toxicity, safety, and efficacy data for any herbal preparation in suitable animal models as per regulatory norms can greatly help in predicting toxicity and providing guidelines for selecting a safe dose in humans. Hence the present research work aimed at evaluating the short and long term safety of the Siddha formulation Kalingathi Ennai by acute and sub-acute toxicity studies in suitable rodent model.

2. Materials and Methods

2.1. Animal

Healthy adult Wistar albino rat were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air. A 12 light / dark cycle were maintained. Room temperature was maintained between 22 $\pm$ 2^0Cand 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 C.L.Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India with the IAEC approval number XLVIII/02/CLBMCP/2017

2.2. Acute toxicity Study

The animals were randomly divided into control group and treatment groups of 6 female wistar albino rats of 3 in each group .The animals were fasted overnight (12- 16 hrs) with free access to water. Group I served as control and the study was conducted with single oral administration of study drugKalingathi Ennai (KE) 2000mg/kg (p.o) to group II rats. 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 [6]. 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. Sub-Acute toxicity Study

The animals were randomly divided into control group and drug treated groups 48 wistar albino rats (24 male and 24 female) were selected and divided into 4 groups. Each group consist of 12 animals (6 Males and 6 Females). First group served as a control and other three group were treated with test drug KE (20, 100 and 200 mg/kg/day) for 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. The female rats were nulliparous and non-pregnant.

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 28^{th} day, the animals were fasted for overnight with free access to water. On 29^{th} day the animals were sacrificed with excess anesthesia. Blood samples were collected from aorta and stored in EDTA (ethylenediamine –tetra acetate) for Hematological analysis and for serum generation for biochemical analysis. The vital organs were harvested and carefully examined for gross lesions. The organs were preserved in 10% formalin for histopathological assessment and interpretation [7].

2.4. Hematological analysis

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

2.5. Biochemical analysis [8]

Serum samples were analyzed for Bilirubin, Aspartate Transaminase (AST), Alanine amino Transaminase (ALT) and Alkaline Phosphatase (ALP), High Density Lipoprotein (HDL), Low density Lipoprotein (LDL) , Very low density Lipoprotein (VLDL) , Triglycerides (TGL), Total Cholesterol ,Total protein, Urea, Blood urea nitrogen (BUN), Creatinine, Albumin, Total Protein, Glucose, Uric acid, using auto analyzer.

2.6. Histopathological evaluation [9]

Vital organs were harvested and the 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.

2.7. Statistical analysis[10]

The statistical analysis will be carried by one way ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean ± 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.

3. Results

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

The dose of KE used for acute toxicity study is 2000mg/kg 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.

Table 1:Effect of Kalingathi Ennai on clinical signs in Acute Oral Toxicity Study

SN	Group Control	Observation	SN	Group Test group	Observation
1	Body weight	Normal	1	Body weight	Normally increased
2	Assessments of posture	Normal	2	Assessments of posture	Normal
3	Signs of Convulsion Limb paralysis	Normal	3	Signs of Convulsion Limb paralysis	Absence of sign (-)
4	Body tone	Normal	4	Body tone	Normal
5	Lacrimation	Normal	5	Lacrimation	Absence
6	Salivation	Normal	6	Salivation	Absence
7	Change in skin color	No significant color change	7	Change in skin color	No significant color change
8	Piloerection	Normal	8	Piloerection	Normal
9	Defecation	Normal	9	Defecation	Normal
10	Sensitivity response	Normal	10	Sensitivity response	Normal
11	Locomotion	Normal	11	Locomotion	Normal
12	Muscle gripness	Normal	12	Muscle gripness	Normal
13	Rearing	Mild	13	Rearing	Mild
14	Urination	Normal	14	Urination	Normal

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

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

Table 2:Effect of Kalingathi Ennai on Body weight of rats in acute toxicity study

Dose	Days		
	1	7	14
Control	176.21± 3.22	177.2± 4.27	179.2 ± 4.82
KE 2000 mg/kg	172.5± 3.18	174.2± 3.26	175.4 ± 3.27
P value (p)*	NS	NS	NS

3.3.Effect of KE on Body weight rats in Sub-acute oral toxicity study.

No significant toxicity was observed in rats during the 28 consecutive days of treatment via oral route with KE at low, mid and high dose of 20, 100 and 200 mg/ kg b.w. The results were tabulated in Table 3.

Table 3:Effect of Kalingathi Ennai on Body weight of rats in Sub-acute toxicity study

Dose	Days				
	1	7	14	21	28
Control	165.6± 2.76	166.4 ± 3.42	167.7 ± 3.26	169.2 ± 3.73	170.7 ± 1.31
Low dose	162.2 ± 4.12	162.7 ± 2.64	163.9± 1.51	164.9 ± 1.66	164.42± 2.76
Mid dose	167.6± 1.24	167.9 ± 4.74	169.4 ± 8.92	169.1 ± 6.36	170.7 ± 9.12
High dose	174.4± 3.74	174.6 ± 6.32	175.6 ± 2.86	176.1± 8.82	175.32 ± 2.42
P value (p)*	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

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

No significant change was observed in body weight of both male and female rats treated with KE at low, mid and high dose of 20, 100 and 200 mg/ kg b.w.The results were tabulated in Table 4 and 5.

.

Table 4:Effect of Kalingathi Ennai on food intake of rats in Sub-acute toxicity study

Dose	Days				
	1	7	14	21	28
Control	37.12 ±5.37	38.5±3.22	39.5±3.37	38.5±3.37	37.12±3.12
Low dose	33.7±2.12	35.3±1.42	35.9±1.68	36.4±2.62	35.9±8.42
Mid dose	34.2±3.64	35.9±3.64	36.2±6.15	37.4±2.18	35.2±2.64
High dose	35.2±2.14	35.2±2.18	36.6±2.14	37.2±4.28	37.2±2.18
P value (p)*	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table 5:Effect of Kalingathi Ennai on water intake of rats in Sub-acute toxicity study

Dose	Days				
	1	7	14	21	28
Control	31.5 ± 8.95	32.0 ±6.23	28.5±6.23	29.12±8.19	31.5±3.96
Low dose	29.5±3.31	29.9±6.62	31.7±4.02	32.2±4.29	34.9±3.13
Mid dose	30.7±3.93	30.3±3.11	30.1±2.83	31.4±2.11	31.4±1.14
High dose	31.1±1.12	31.2±2.43	32.7±2.53	33.2±1.89	34.4±2.45
P value (p)*	NS	NS	NS	NS	NS

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.5.Effect of KE on Hematological parameters of rats in Sub-acute oral toxicity study

No statistically significant differences were recorded in hematological parameters of rats treated with KE at low, mid and high dose of 20, 100 and 200 mg/ kg b.w.. The results were tabulated in Table 6.

Table 6: Haematological parameters of rats exposed to Kalingathi Ennai

Category	Control	Low dose	Mid dose	High dose	P value (p)*
Haemoglobin(g/dl)	14.8±1.88	12.98±1.28	13.01±1.26	14.18±3.96	N.S
Total WBC (×10^3 l)	10.91±2.59	12.25±3.53	12.18±3.61	12.96±3.47	N.S
Neutrophils(%)	32.65±1.06	34.23±2.54	34.91±1.36	33.40±2.80	N.S
lymphocyte (%)	69.34±2.48	70.22±3.42	71.48±2.66	71.20±3.96	N.S
Monocyte (%)	0.78±0.17	0.81±0.12	0.84±0.11	0.95±0.16	N.S
Eosinohil(%)	0.64±0.09	0.19±0.12	0.78±0.06	0.42±0.04	N.S
Platelets cells10^3/µl	687.17±8.76	698.71±8.16	705.18±4.0	712.16±4.64	N.S
Total RBC 10^6/µl	7.99±0.12	6.82±1.87	6.92±0.59	6.18±0.72	N.S
PCV%	37.79±0.6	36.35±1.53	38.2±1.18	36.82±2.14	N.S
MCHC g/dL	33.6±2.23	34.19±1.19	35.18±1.92	34.13±1.94	N.S
MCV fL(µm^3)	49.17±3.64	48.20±1.24	49.28±1.24	49.99±1.84	N.S

N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.6.Effect of KE on Biochemical parameters of rats in Sub-acute oral toxicity study

No statistically significant differences were recorded in biochemical parameters of rats treated with KE at low, mid and high dose of 20, 100 and 200 mg/ kg b.w. The results were tabulated in Table 7 -9.

Table 7: Biochemical parameters of rats exposed to Kalingathi Ennai

Biochemical Parameters	Control	Low dose	Mid dose	High dose	P Value (p)*
Glucose (R) (mg/dl)	76.45±13.4	76.16±8.54	79.64±9.20	77.42±11.6	N.S
T.Cholosterol(mg/dl)	115.26±1.83	112.45±1.13	112.42±1.98	115.22±1.83	N.S
Trigly(mg/dl)	46.35±1.48	45.32±1.48	45.58±1.26	46.66±1.45	N.S
LDL	72.81±2.13	70.14±2.34	71.8±2.94	72.64±6.12	NS
VLDL	15.2±2.44	14.42±4.63	14.44±6.64	14.94±5.14	NS
HDL	26.66±6.88	27.96±2.34	27.88±5. 66	29.78±6.22	NS
Ratio 1(T.CHO/HDL)	4.42±2.44	4.36±1.44	4.84±2.44	4.86±1.92	NS
Ratio 2(LDL/HDL)	2.83±4.22	3.02±1.52	2.96±4.80	2.86±3.82	NS
Albumin(g/dL)	3.63±0.17	3.13±1.12	3.10±1.92	2.94±3.86	NS

NS- Not Significant,**(p > 0.01), * (p >0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table 8: Renal function test of rats group exposed to Kalingathi Ennai

Parameters	Control	Low dose	Mid dose	High dose	P Value (p)*
Urea (mg/dl)	13.35±0.99	12.91±1.86	13.16±1.98	13.18±3.92	N.S
Creatinine(mg/dl)	0.28±0.08	0.16±1.16	0.12±0.14	0.18±1.22	N.S
BUN(mg/dL)	15.02±0.10	14.80±1.20	14.66±0.44	15.10±2.32	NS
Uric acid(mg/dl)	5.17±0.35	5.25±1.43	5.02±1.35	5.18±1.08	NS

NS- Not Significant, **(p > 0.01), * (p >0.05) , n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table 9: Liver Function Test of rats exposed to Kalingathi Ennai

Parameters	Control	Low dose	Mid dose	High dose	P Value (p)*
T Bilirubin(mg/dl).	0.48±0.07	0.43±1.26	0.64±1.28	0.68±1.25	N.S
SGOT/AST(U/L)	79.95±1.39	77.15±1.31	78.71±1.83	80.35±3.03	N.S
SGPT/ALT(U/L)	31.23±1.28	31.81±3.52	30.14±3.18	31.9±1.88	N.S
ALP(U/L)	143.25±8.70	141.9±8.17	142.16±4.10	144.33±4.25	NS
T.Protein(g/dL)	5.32±0.38	5.28±0.34	5.21±1.33	5.13±1.06	N.S

NS- Not Significant, **(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

3.7. Effect of KE on Histopathological changes of rats in Sub-acute oral toxicity study

No abnormality were detected in the histopathological analysis of organs (Kidney, Liver and Spleen) retrieved from the rats treated with KE at high dose of 200 mg/ kg b.w. The results were illustrated in figure 1 and 2.

Figure 1: Histopathological representation organs of control group rats in Sub-acute oral toxicity study

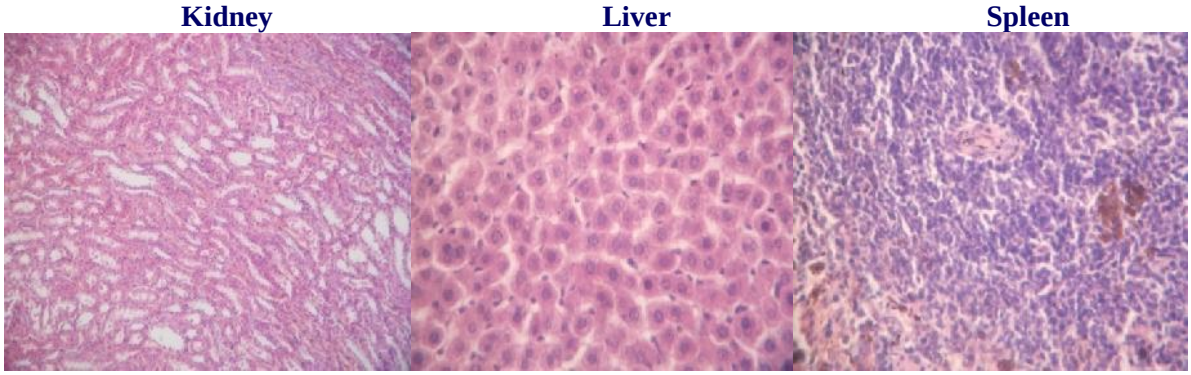
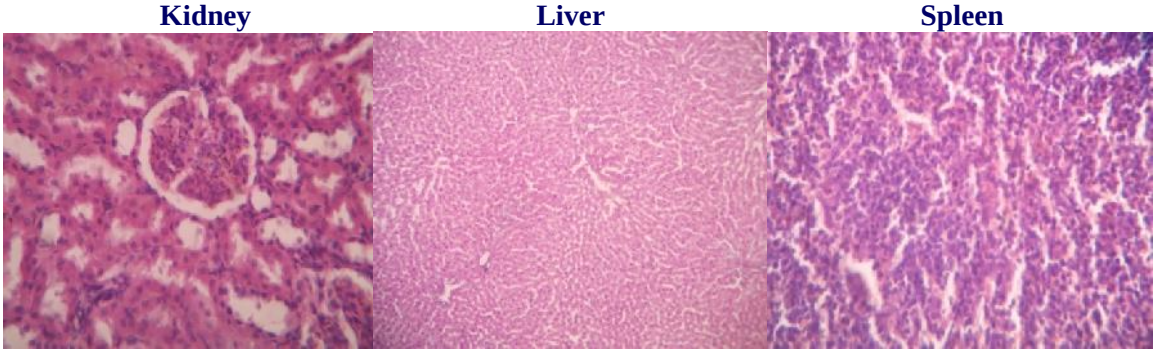


Figure 2 : Histopathological representation organs of KE treated rats in Sub-acute oral toxicity study



4. Discussion

Although siddha medicines used as the most common form of alternative medicine for treatment of various diseases all over the world, their toxicities and side effects are poorly known . The presence of many bioactive compounds with wider mechanisms of action in turn grabs the attention of the regulatory agencies to strongly impose on collection of toxicity related data's of siddha preparations before it application on human. The assessment of the lethal dose (LD_{50}) (the dose that kills 50% of test animals population) has now been used as a major parameter in measuring acute toxicity and also as an initial procedure for general screening of chemical and pharmacological agents for toxicity. Apart from mortality, other biological effects and the time of onset, duration and degree of recovery on survived animals, are also important in acute toxicity evaluation. Acute toxicity study solely gives information about LD_{50}, therapeutic index and the degree of safety of a pharmacological agent [11].

In acute toxicity study, there was no mortality up to a maximum dose of 2000 mg/kg body weight of KE after per oral administration. The changes in body weight have been used as an indicator of adverse effect of KE. Since no remarkable changes were observed in animal behavior, body weight and organ weight at dose in treated rats as compared to control group, it can be inferred that siddha formulation KE is nontoxic at the administered dose of 2000mg/kg.

Alteration in organ-to-body weight ratio may be as a result of organ damage. In sub-acute toxicity study it was observed that treatment with KE at 20, 100 and 200 mg/kg reveals no significant change in the body weight of the treated rats.

The haematological parameters can be used to determine the blood relating functions. The haemopoietic system is one of the most sensitive targets of toxic compounds and an important index of physiological and pathological status in both humans and animals. Treatment with KE at three dose level of low, mid and high dose posses no significant change in blood cell counts which suggested that the KE does not affect erythropoiesis, morphology, or osmotic fragility of blood cells [12].Transaminases (AST and ALT) and ALPs are generally used as indices for liver and kidney damage respectively [13,14]. No significant change was found in serum levels of AST, ALT, and ALP enzymes post KE drug administration. Assessment of liver and kidney function is a very vital index in evaluating the toxicity of drugs. Kidney function indices evaluated in this study were serum urea, creatinine and electrolyte concentrations [15]. Test drug KE therefore did not provokes any detrimental effect on liver and kidney. The nontoxicity of KE on specific organs was further confirmed by histopathological assessment. Histopathological examination of selected vital organs (liver, kidney and spleen) from both treated and control animals showed normal architecture, suggesting no microscopic changes and morphological disturbances were caused due to oral administration of KE at three dose level of 20, 100 and 200 mg/kg.Necrosis from hepatotoxic chemicals can occur within distinct zones in the liver, either distributed diffusely, or occur massively. Many chemicals produce zonal necrosis, *i.e*. necrosis confined to a specific zone of the hepatic acinus [16]. Histological observation of the vital organs of rats subjected to sub-acute toxicity study has revealed No significant changes suggesting that administration of the test drug KE at sub-acute has no systematic organ related toxicity. Microscopic observation of the kidney, liver and spleen of drug exposed rats shown normal histology of the organs with perfect morphological features. It showed that none of the organs were adversely affected, nor showed any signs of toxicity throughout the study.

5. Conclusion

From the evidence based data's obtained from the present research work it may conclude that siddha formulation Kalingathi Ennai is not toxic in all doses studied herein and did not produce any evident symptoms in the acute and sub-acute oral toxicity studies. The histology examination revealed no remarkable changes in the internal organs in both control and treated groups. Furthermore, the data of acute and sub-acute toxicity studies on this formulation were obtained in order to increase the confidence in its safety to humans for the use in the development of traditional pharmaceuticals.

Acknowledgments

I wish to acknowledge my thanks to The Noble research solutions, Chennai, Tamil Nadu, India for their technical assistance in publishing this research work.

References

1. Rakh MS, Chaudhari SR. Evaluation of CNS depressant activity of Momordica dioica Roxb willd fruit pulp. Int J Pharm Pharm Sci. 2010;2(4):124–6.
2. Gill NS, Bajwa J, Dhiman K, Sharma P, Sood S, Sharma PD. et al. Evaluation of therapeutic potential of traditionally consumed Cucumis melo seeds. Asian J Plant Sci. 2011;10(1):86–91.
3. Huang C, Zhou T, Chen Y, Zhang S, Chen G. (-)Epicatechin regulation of hydroxysteroid sulfotransferase sta (rsult2a1) expression in female rat steroidogenic tissues. J Pharmacol Toxicol. 2011;6(4):349–60.
4. Alam MB, Hossain MS, Chowdhury NS, Mazumder MEH, Haque ME, Islam A. In vitro and in vivo antioxidant and toxicity evaluation of different fractions of Oxalis Corniculata linn. J Pharmacol Toxicol. 2011;6(4):337–48.
5. Chakravarty B. Herbal medicines. Safety and efficacy guidelines. Regul Aff J. 1993;4:699–701.
6. OECD guideline for testing of chemicals. Guideline 423 ,17th December 2001.
7. OECD Guide lines 407 for testing of chemicals .Repeated dose 28-Day Oral Toxicity Study in Rodents. 2008:2- 8.
8. Jain N, Sharma P, Sharma N, Joshi S C. Haemato-biochemical profile following sub acute toxicity of malathion in male albino rats. Pharmacologyonline. 2009;2:500–506.
9. Suvarna SK, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. 7th edn, Churchill Livingstone, London.2013.
10. Visweswara Rao. Biostatistics., A manual of statistic methods for use in Health, Nutrition and Anthropology, Rajkamal Electrical press, Delhi, 2007.226-312.
11. Akhila JS, Deepa S, Alwar MC. Acute toxicity studies and determination of median lethal dose. Curr Sci. 2007;93:917–20.
12. Odeyemi O.O., Yakubu M.T., Masika P.J., Afolayan A.J. Toxicological evaluation of the essential oil from *Menthalongifolia* L. subsp. capensis leaves in rats. J. Med. Food. 2009;12:669–674.
13. Sheth SG, Gordon FD, Chopra S. Nonalcoholic steatohepatitis. Ann Intern Med. 1997;126:137–145.
14. James D, Tania SB, Sara ET. et al. Alanine Aminotransferase Levels and Fatty Liver in Childhood Obesity: Associations with Insulin Resistance, Adiponectin, and Visceral Fat. J Clin Endocrinol Metab. 2006;91:4287–4294.
15. S. Muhammad, L.G. Hassan, S.M. Dangoggo, S.W . Hassan, K.J. Umar, R.U. Aliyu**Acute** and subchronic toxicity studies of kernel extract of *Sclerocarya birrea* in rats**.** Sci World J, 6 (3) (2011), pp. 11-14.
16. Roberts, R.C. James, M.R. Franklin. Hepatotoxicity: toxic effects on the liver.P.L. Williams (Eds.), Principles of toxicology: environmental and industrial applications (2nd ed.), John Wiley & Sons, Inc., New York (2003), pp. 111-128.

Access this Article in Online	
	Website: www.ijarbs.com
Quick Response Code	Subject: Siddha Medicine
DOI:10.22192/ijarbs.2018.05.09.009	

How to cite this article:
S. Brunda, K.Nithya, N. Anbu, K. Kanakavalli. (2018). Acute and Sub-Acute Toxicity Evaluation of Siddha Formulation Kalingathi Ennai In Accordance With OECD Guideline. Int. J. Adv. Res. Biol. Sci. 5(9): 92-100.
DOI: http://dx.doi.org/10.22192/ijarbs.2018.05.09.009