Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 4, 2013

Research Article

ACUTE AND28-DAY SUBCHRONIC ORAL TOXICITY STUDY OFKADUKKAI MAATHIRAI, AN IRON BASED SIDDHA HERBAL FORMULATION IN WISTAR ALBINO RATS

DR.VELAYUDAM[1], DR.ILAVARASAN[2], DR.ARUL AMUTHAN[3]

[1]National Institute of Siddha, Chennai, India. [2]Captain Sriniwasa Murthy Research Institute for Ayurveda and Siddha Drug Development, Chennai, India. [3]Dept. of Pharmacology, Melaka Manipal Medical College, Manipal University, India. Email: dramuthanmd@yahoo.co.in,

Received: 28 Feb 2013, Revised and Accepted: 20 Jun 2013

ABSTRACT

Background: *Kadukkai maathirai*(KM),an iron based polyherbal formulation widely used in India for the treatment of anaemia and liver disease by Siddha physicians. The toxicological profile of the drug was not reported. The present study was carried out to determine toxicity profile of KM by acute and subchronic oral toxicity studies in rats.

Methods: Adult wistar albino rats of either sex weighing 150 – 200 g were used for the present study. In acute toxicity study, single oral dose of KM was administered and the animals were observed for 14 days. In 28-day sub chronic oral toxicity study, three doses (36, 180, 360mg/kg) of KM was administered orally for 28 days. Body weight, food intake and water intake were recorded at weekly interval. Hematological parameters like total RBC, total WBC count, WBC differential count and hemoglobin (Hb) were determined in whole blood. Biochemical parameters like, glucose, total protein, total bilirubin, total cholesterol, blood urea nitrogen (BUN), creatinine, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) were analyzed in serum. Histological changes were assessed in liver, kidney, lungs, heart, brain, ovary, testis and spleen.

Results: There was no significant alteration in body weight, food intake, water intake, urine parameters, hematology parameters, liver function enzymes and kidney function parameters. Histopathological studies of stomach, heart and brain showed normal architecture. There were mild inflammatory changes seen in histology of spleen, liver, kidney and lung of KM treated groups.

Conclusion: *Kadukkai maathirai* even at 10 times higher the therapeutic dose could be considered safe, thus having wide margin of safety.

Keywords: Siddha, Ayurveda, Complementary, Sub chronic toxicity, Traditional medicine, Iron

INTRODUCTION

Traditional medicines have been recognized by worldwide due to the claim of less adverse effects compared to modern medicine. In developing countries, these formulations are popular for the management of chronic ailments. Unlike single molecule, the polyherbal formulation might have multi-targeted mechanism of actions lead to synergistic effect. Siddha Medicine is one of the ancient traditional medical systems practiced in India, Srilanka, Malaysia, Singapore and by other Tamil speaking communities worldwide. It believes herbomineral preparations as powerful and emphasizes to administer while herbals alone could not provide relief [1]. Scientific validation of traditional system of medicines including Siddha Medicine is increasing and became need of the hour to explore innovative approaches like revere pharmacology and systems biology [2].

Kadukkai maathirai(KM),an iron basedherbomineral formulation commonly used by Siddha physicians to treat liver disorders and anaemia. It is administered daily as 400mg orally in two divided doses. The ingredients of KM include annabedhi (*Ferrous sulphate*), kadukkai (*Terminalia chebula*), milagu (*Piper nigrum*), karisaalai (*Eclipta alba*) and *Citrus limon*. The physicochemical analysis revealed that KM contains 18% iron and the presence of heavy metals was below the permissible limits[3]. Our earlier study proved the dose dependent hepatoprotective activity of KM in CCl_4 induced liver injury in rats[4].Literature survey revealed that the toxicity profile of this drug was not studied scientifically; hence this study was carried out to assess the toxicity profile of KM in Wistar rats.

MATERIAL AND METHODS

Kadukkai maathirai

KMwas prepared in our lab following procedures mentioned in the Siddha Hospital Pharmacopoeia, government of Tamil Nadu, India[5]. For the toxicity study, the powdered drug was made as suspension with 1% sodium carboxymethyl cellulose.

Experimental animal

The study protocol was approved by Institutional Animal Ethics Committee, AJ College of Pharmacy, Chennai, India (Approval no: AJ/IAEC/11/42). Healthy adult Wistar albino rats of either sex weighing between 150 and 200g were used. Animals were housed in standard environmental condition (temperature 28-30^{o}C, photoperiod: approximately 12 h natural light per day; relative humidity: 50-55%) and fed with diets and water *ad libitum*.

Acute toxicity study

Acute oral toxicity study was performed as per Organization for Economic Cooperation Development (OECD) guidelines[6].The limit test dose of 2000mg/kg was used as stipulated in OECD guidelines. Three overnight fasted female rats, each sequentially dosed at intervals of 48 h, were used for the test. All three animals were observed individually for acute toxic signs and behavioural changes 1 h post-dosing, and at least once daily for 14 days.

Sub-chronic toxicity test

28 days repeat-dose oral toxicity study was carriedout according to OECD guideline 407[7].The animals were divided into four groups of six animals in each (3 males and 3 females).To extrapolate the human toxicity profile in rats, three doses of KM were chosenfor this animal toxicity study from clinically used human dose (400 mg per day in human) by using body surface area dosing table[8].They were; therapeutic dose (36mg/kg), 5 times the therapeutic dose (180mg/kg) and 10 times therapeutic dose (360mg/kg). Group 1, 2 and 3 received three doses of KM 36, 180 and 360mg/kg body weight respectively. Group 4 received 10 ml/kg body weight of1% sodium carboxymethyl celluloseand served as control. All the groups were administered orally by respective treatment daily for 28 days daily at same time. Body weight, food intake and water intake were recorded at weekly intervals with simultaneous observation for toxic manifestation and mortality.

After 28 days of treatment, urine was collected by maintaining animals in metabolic cage for the analysis of pH, specific gravity, presence of blood, leucocytes, protein, bilirubin, urobilinogen, glucose, ketones and nitrate. The rats were anaesthetized and blood sample was collected through retro-orbital puncture into tubes with and without EDTA for hematological and biochemical analysis respectively. Total leukocyte and total erythrocyte were counted by

hemocytometer. Differential WBC count was done under compound microscope after staining with leishmans' stain. Hemoglobin (Hb) was estimated by Sahli's hemoglobinometer method.

Amount of glucose was estimated by ortgotoluidine-boric acid heat method. Total protein, albumin, cholesterol, triglyceride, total bilirubin, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were estimated. After euthanasia, vital organs such as liver, kidney, lung, heart, spleen, stomach and brain were dissected out and kept in 10 % formalin for histopathological studies.

Statistical analysis

Statistical analysis was done by using SPSS software version 11.5. Values were expressed as mean ± standard deviation (SD). The statistical evaluation was carried out by One-Way ANOVA followed by Tukey's test. Level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Acute toxicity study

Kadukkai maathirai did not produce any toxic symptoms or mortality at the dose level of 2000 mg/kg/po for short period (48 h) and long period (14 days).

Sub-chronic toxicity test

No behavioural abnormality or death was observed during the whole treatment period. No significant difference in body weight was observed between treatment and control groups (Table1). All three drug treated groups showed a slight reduction in food intake while comparing its baseline intake (Table 2).

Urine parameters

Table 3 shows urine parameters of rats after 28 day toxicity study. There was no statistically significant difference between treatment groups and control group in urine pH, specific gravity and urobilinogen. Blood, bilirubin, glucose and ketone bodies were absent in urine of all groups. Although presence of leucocytes, protein and nitrate were seen in all groups, there was no alteration in the drug treated groups compared to control (Table 3).

Blood parameters

Hematological parameters are tabulated in table 4. There was a slight reduction in total WBC count and mild increase in monocytes count in the KM treated groups when compared to control group. But, none of the alterations were statistically significant (Table 4).

Table 1: Mean body weight of rats during 28day administration of *Kadukkai maathirai*

Treatment (Oral)	Body weight in g				
	Day 0	Day 7	Day 14	Day 21	Day 28
KM (36 mg/kg)	152±5.80	168.83±6.79	174±5.93	174.66±8.75	172.83±6.11
KM (180 mg/kg)	151±5.49	165.25 ±10.82	169.33±12.51	174.5±10.80	172.33±7.31
KM (360mg/kg)	153±6.32	169.16±9.19	169.66±9.87	173±9.31	169±8.46
1% SCMC (10ml/kg)	151±6.38	160.66±6.91	160.77±9.65	161.33±8.73	168.8±5.40

Values are mean ±SD

KM - *Kadukkai maathirai;* SCMC - sodium carboxymethyl cellulose

Table 2: Food consumption by rats during 28day administration of *Kadukkai maathirai*

Treatment (Oral)	Food consumption in g/kg body weight/day				
	Day 0	Day 7	Day 14	Day 21	Day 28
KM (36 mg/kg)	60.21	58.73	56.60	56.58	56.89
KM (180 mg/kg)	61.11	58.49	51.18	43.93	46.42
KM (360mg/kg)	59.45	47.12	47.49	46.31	48.21
1% SCMC (10ml/kg)	61.21	64.15	60.00	63.03	66.29

KM - *Kadukkai maathirai;* SCMC - sodium carboxymethyl cellulose

Table 3: Urine analysis of rats after 28 days treatment with*Kadukkai maathirai*

Treatment (Oral)	*pH	*Specific gravity	Presence of blood	Leucocytes	Protein	Bilirubin	*Urobilinogen	Glucose	Ketones	Nitrite
KM (36 mg/kg)	9± 0.00	1.000± 0.01	nil	Moderate	Trace	Nil	1± 0.00	nil	nil	+
KM (180 mg/kg)	9± 0.00	1.005± 0.02	nil	Moderate	Trace	Nil	1± 0.00	nil	nil	+
KM (360mg/kg)	9± 0.00	1.000± 0.01	nil	Moderate	Trace	Nil	1± 0.00	nil	nil	+
1% SCMC (10ml/kg)	9± 0.00	1.000± 0.01	nil	Moderate	Trace	Nil	1± 0.00	nil	nil	+

*Values are mean ±SD

Table 4: Hematological parameters of rats after 28 days treatment with*Kadukkai maathirai*

Treatment (Oral)	Total WBC (10^3/mm^3)	Lymphocytes (%)	Monocytes (%)	Granulocyte / Neutrophil (%)	Total RBC (10^6/mm^3)	Hb (g/dl)
KM (36 mg/kg)	27.56± 4.11	64.78± 5.90	6.74± 0.90	25.36± 3.07	7.06± 0.65	15.33± 1.06
KM (180 mg/kg)	24.33± 4.77	60.97± 10.34	6.80± 1.55	26.03± 6.44	7.37 ±0.59	14.95± 1.15
KM (360mg/kg)	24.76±5.34	65.39±2.85	6.34±1.64	24.51±4.59	7.01±0.52	14.55±1.20
1% SCMC (10ml/kg)	33.18± 9.60	67.58± 3.33	4.70± 1.09	26.16± 4.87	7.40± 0.62	15.41± 0.80

Values are mean ±SD

All the biochemical parameters were similar to control group except the slight raise in triglyceride and alanine phosphatase level in the drug treated groups. However, there was no statistically significant difference was observed between drug treated and control groups (Table 5).

Table 5: Biochemical parameters of ratsafter 28 days of treatment with*Kadukkai maathirai*

Parameters	Treatment (oral)			
	KM (36 mg/kg)	KM (180 mg/kg)	KM (360mg/kg)	1% SCMC (10ml/kg)
Glucose (mg/dl)	79.71 ±13.88	87.45 ±12.17	87.40 ±8.21	90.79 ±11.37
Total Protein (g/dl)	6.32 ±0.29	6.25 ±0.41	6.47 ±0.25	6.23 ±0.28
Albumin (g/dl)	4.76 ±0.24	4.58 ±0.37	4.63 ±0.44	5.44±1.2
Cholesterol (mg/dl)	42.17 ±5.41	41.55 ±6.77	44.42 ±5.62	42.80 ±6.41
Triglycerides (mg/dl)	96.46 ±21.24	99.10 ±31.56	73.58 ±16.12	65.30 ±22.07
Bilirubin (mg/dl)	0.32 ±0.06	0.32 ±0.08	0.29 ±0.19	0.26 ±0.15
Urea (mg/dl)	27.04 ±3.12	26.53 ±2.74	34.83 ±3.17	29.38 ±3.79
Creatinine (mg/dl)	0.65 ±0.03	0.67 ±0.04	0.65 ±0.04	0.65 ±0.03
AST (U/L)	96.80 ±13.64	106.81 ±17.08	107.40 ±17.74	109.19 ±16.42
ALT (U/L)	39.06 ±9.60	41.65 ±8.79	43.04 ±5.64	39.17 ±7.82
ALP (U/L)	337.50 ±102.81	408.20 ±118.38	369.91 ±75.68	325.60 ±101.09

Values are mean ±SD

AST - aspartate aminotransferase; ALT - alanine aminotransferase; ALP - alkaline phosphatase

Histopathological Evaluation

Histopathological studies of stomach, heart and brain of KMtreated animals showed normal architecture **(Fig. 1-3**). Spleen has shown mild congestion**(Fig. 4).** There were mild inflammatory changes seen in liver, kidney and lung of KM treated groups (**Fig. 5-7).**

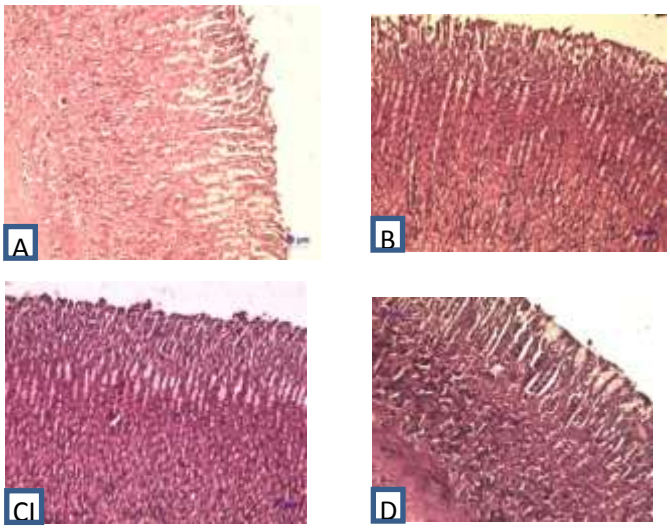

Fig. 1: Histology of stomach taken from *Kadukkai maathirai* (KM)treated and control group

[A - KM 36 mg/kg, B - KM 180 mg/kg, C - KM 360 mg/kg, D - 1% SCMC 10ml/kg]

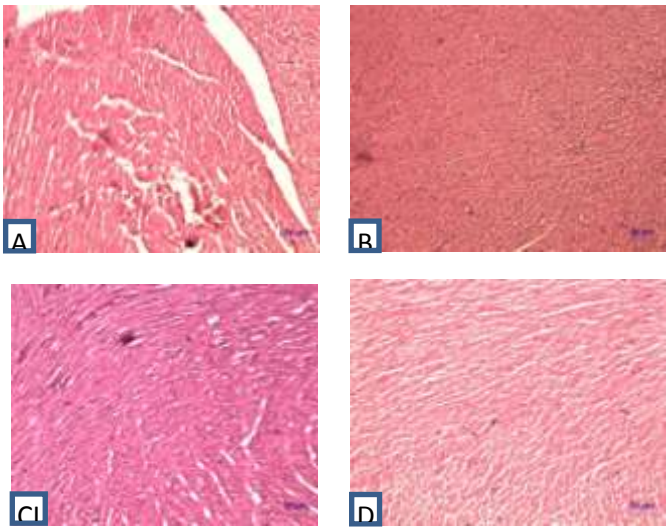

Fig. 2: Histology of heart taken from *Kadukkai maathirai* (KM)treated and control group

[A - KM 36 mg/kg, B - KM 180 mg/kg, C - KM 360 mg/kg, D - 1% SCMC 10ml/kg]

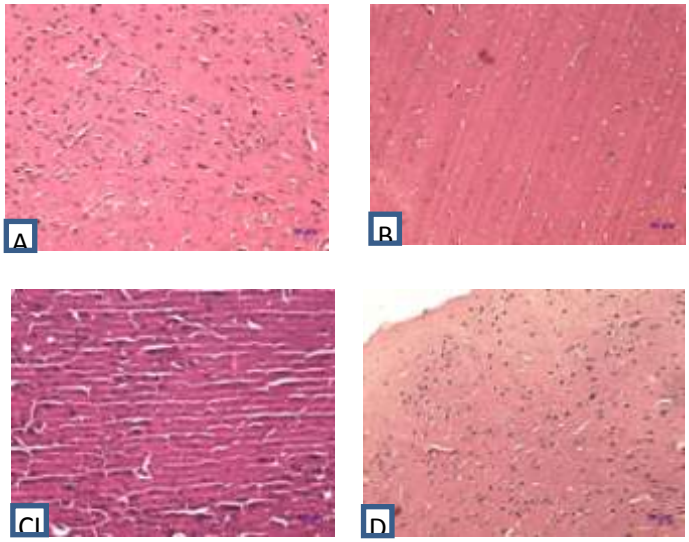

Fig. 3: Histology of brain taken from *Kadukkai maathirai* (KM)treated and control group

[A - KM 36 mg/kg, B - KM 180 mg/kg, C - KM 360 mg/kg, D - 1% SCMC 10ml/kg]

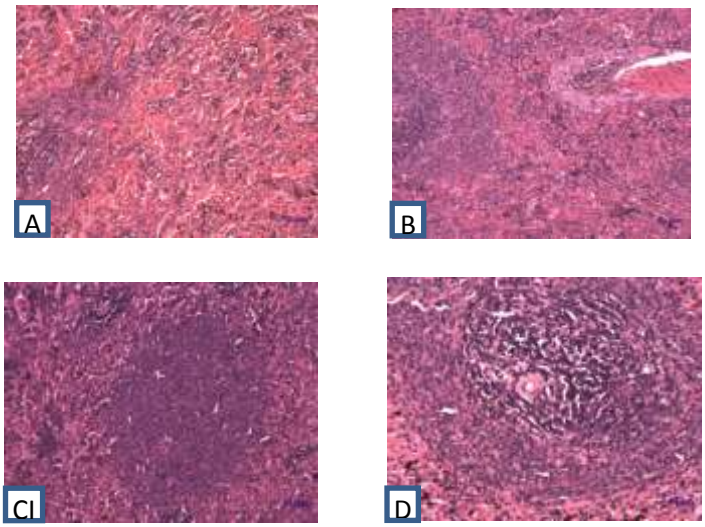

Fig. 4: Histology of spleen taken from *Kadukkai maathirai* (KM)treated and control group

[A - KM 36 mg/kg, B - KM 180 mg/kg, C - KM 360 mg/kg, D - 1% SCMC 10ml/kg]

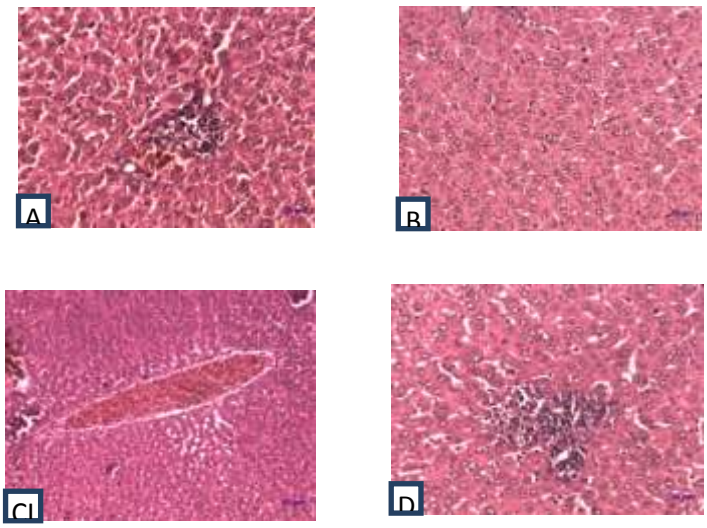

Fig. 5: Histology of liver taken from *Kadukkai maathirai* (KM)treated and control group

[A - KM 36 mg/kg, B - KM 180 mg/kg, C - KM 360 mg/kg, D - 1% SCMC 10ml/kg]

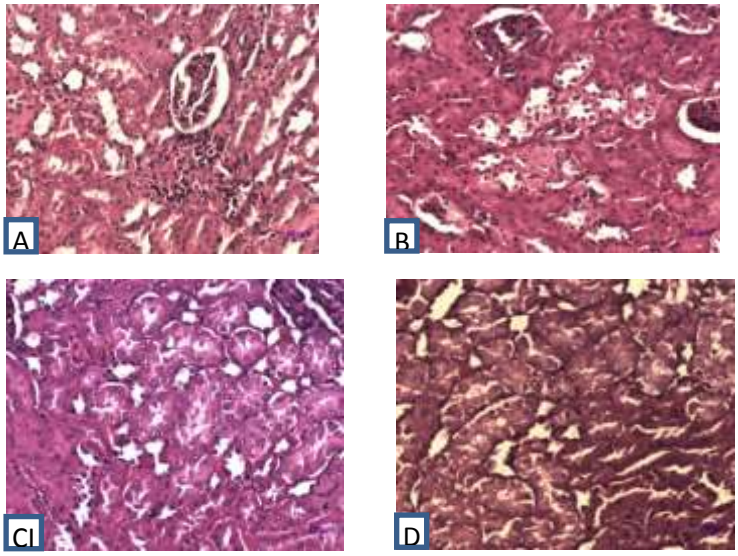

Fig.6: Histology of kidney taken from *Kadukkai maathirai* (KM)treated and control group

[A - KM 36 mg/kg, B - KM 180 mg/kg, C - KM 360 mg/kg, D - 1% SCMC 10ml/kg]

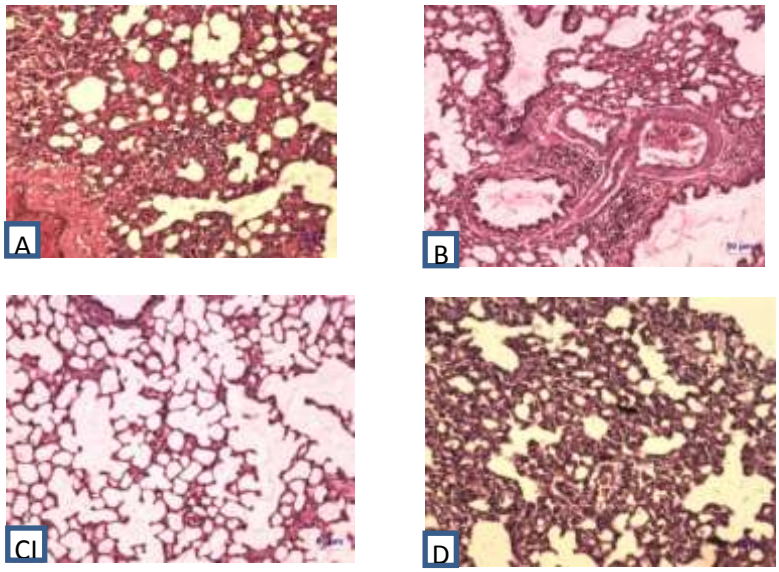

Fig. 7: Histology of lung taken from *Kadukkai maathirai* (KM)treated and control group

[A - KM 36 mg/kg, B - KM 180 mg/kg, C - KM 360 mg/kg, D - 1% SCMC 10ml/kg]

DISCUSSION

According to acute toxic class method (OECD – 423 guidelines), the LD 50 dose of 2000 mg/kg and above is categorized as (X 'unclassified). Thus, KM could possibly be considered as nontoxic drug. There were no significant changes in any of the hematological parameters in *Kadukkai maathirai* treated group compared with the control groups. The blood glucose level, which remained constant in all the groups of animals, shows the normoglycemic activity of the *Kadukkai maathirai*. Determination of BUN and serum creatinine shows that the *Kadukkai maathirai* did not produce any renal dysfunction.

AST, ALT, ALP and total bilirubin are good indices of liver function which indicate that there were no significant changes in the enzyme levels, when compared with the control animals. Hence the *Kadukkai maathirai* did not induce any toxicity to the liver and kidney. Determination of kidney function tests shows that the Kadukkai*maathirai*did not produce any renal dysfunction.

Normal architecture of stomach, heart and brain suggesting no detrimental changes and morphological disturbances were caused by the daily oral administration of the *Kadukkai maathirai* at the dose level of 36 mg/kg, 180 mg/kg and 360 mg/kg/po, for 28 days. Mild inflammatory changes in liver, kidney and lung suggested that the organs might be the target of KM on long term administration.

CONCLUSION

In acute and subchronic toxicity studies, we did not observe mortality or signs of toxicity because of administration of *Kadukkai maathirai.* No weight loss, no change in food/water intake, no statistically significant changes in blood, urine parameters and histopathology suggest the safety profile of KM on long term administration. We did not find any lowest observed adverse effect level (LOAEL) for KM. Thus, *Kadukkai maathirai* even at 10 times higher the therapeutic dose could be considered safe, thus having wide margin of safety. Since the toxicity in human cannot always be entirely extrapolated from animal studies, clinical evaluation should be performed to assess the safe dose range to be followed in humans.

REFERENCE

1. Sathish R, Madhavan R, VasanthiHR, Amuthan A. In-vitro alpha-glucosidase inhibitory activity of *abraga chendooram*, a siddha drug. Int J Pharmacol and ClinSci 2012;1:74-6.

2. Chitra B, Ramaswamy RS. Pharmacological analysis of a Siddha formulation *Karuncheeragachurnam*.Int J Pharm PharmSci 2012; 4: supple 2: 104-7.
3. Velayudam, Ilavarasan, Amuthan A. Physico-chemical evaluation of *kadukkai maathirai*and its tablet formulation, a Siddha iron preparation used in anaemia. Int J Pharmacol and ClinSci 2012;1:3-8.
4. Velayudam, Amuthan A, Ilavarasan. Hepatoprotective activity of *kadukkai maathirai*(A Siddha polyherbal formulation) against carbon tetrachloride induced liver damage in rat. Research Journal of Pharmaceutical Sciences 2012;1:17-21.
5. Narayanaswamy V. Pharmacopoeia of Hospital of Indian Medicine. Chennai: Tamilnadu Siddha Medical Board; 1995.p.34.
6. OECD. Guidelines for the Testing of Chemicals /Section 4: Health Effects Test No. 423: Acute Oral toxicity - Acute Toxic Class Method.Organization for Economic Cooperation and Development, Paris, France; 2002.
7. OECD. Repeated dose oral toxicity test method. In:OECD Guidelines for testing of chemicals, N°407. Organization for Economic Cooperation and Development, Paris, France; 2008.
8. Bhardwaj S, Gupta D. Study of acute, subactue and chronic toxicity test. International Journal of Advanced Research in Pharmaceutical& Bio Sciences 2012 1:103-29.