World Journal of Pharmaceutical ReseaRch
SJIF Impact Factor 5.045
Volume 3, Issue 8, 778-787. Research Article ISSN 2277 – 7105

ACUTE AND SUBACUTE TOXICITY STUDY ON AMIRTHA SANJEEVI KULIGAI

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Article Received on
08 August 2014,

Revised on 30 August 2014,
Accepted on 24 Sept 2014

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ABSTRACT

Single and polyherbal preparations are being traditionally used in Indian system of medicine especially in siddha medicine. Mostly herbal preparations have less side effects so it is suitable for paediatric use. Amirtha Sanjeevi Kuligai(ASK) is prepared as per classical text book of Balavagadam for respiratory problem in paediatric age group. Before conducting clinical trial, preclinical study should be undergone as per WHO guidelines. The clinical trial has been approved by IEC (IEC.NO - GSMC-CH/1/2013/016). The present preclinical study aim is to carry out safety and toxicity of ASK (IAEC. NO: XXXIX/13/CLBMCP/2013/ dated 29.6.2013). Adult both sex of swiss albino rats weighing 220- 250 gm were used. Acute and Sub-acute toxicity were carried out as per OECD guidelines 423 and 407. Hematological parameters, biochemical parameters, histo-pathological study were performed for all animals. The study concludes that on oral administration of 400 mg/kg of body weight of ASK to swiss albino rats, there was no characteristic clinical sign of toxicity or mortality observed.

KEYWORDS: Amirtha Sanjeevi Kuligai, Toxicity, Respiratory disease, and oral.

INTRODUCTION

Asthma is a chronic disorder of the bronchial tree, characterized by completely or partially reversible airway obstruction, which may improve spontaneously or may subside only after specific therapy. Airway hyper responsiveness is defined as the narrowing of the airways as response to a variety of stimuli, such as allergens and nonspecific triggers and infections.

Asthma is a chronic disorder of both children and adults, with 300 million individuals afflicted worldwide (Global Initiative for Asthma (GINA) guidelines) [1]. Although the prevalence of asthma has increased over the last decades, especially so in children, there is still no sound explanation for this increase [2].Asthma symptoms include recurrent wheezing, coughing, chest tightness, and dyspnoea, with nightly and early morning symptoms being more prevalent, whereby quality of life is often reduced [3]. Approximately 60–75% of school-aged children with asthma have an allergy [4]. Childhood Bronchial Asthma varies widely from country to country. At the age of six to seven years, the prevalence ranges from 4 to 32 [5]. It has also increased the number of preventable hospital emergency visits and admissions. Apart from being the leading cause of hospitalization for children, it is one of the most important chronic conditions causing elementary school absenteeism [6], [7]. Childhood Bronchial Asthma has multifactor causation. Geographical location, environmental, racial, as well as factors related to behaviors and life-styles are associated with the disease [8],[9], [10]. Child hood bronchial asthma closely correlates with the description of the disease "SOOLI KANAM" recorded thousands years ago by the ancient Siddhar's. Sooli Kanam having the clinical features like wheezing, cough, decreased physical activity, poor diet intake etc described by Siddhar's [11]. In siddha literary various herbal drugs either single or compound drugs are prescribed for the management of many respiratory problems .The goal of the therapy is not only to treat the condition but also to enhance the immune system of the child. One such medicine is *Amirtha Sanjeevi Kuligai*a poly herbal formulation which is indicated for Sooli Kanam (Childhood bronchial asthma) in siddha literature Balavagadam[12]. *AmirthaSanjeeviKuligaihave the ingredients like Ferulla asafoedita,Costus specious root, Electariacardamomumfruit, Syzygiumaromaticumflower bud, Santalum album wood, Picrrorhoeakurora, Madhuca longifolia flower, Hemidusmus indica root, Plectranthus vettiveriodes root, Vettiveria zizanoides root, Piper longum fruit, Glycyrrhiza glabra, Cyperus rotundus root tuber, Vitis viniferadry fruit, Phonex dactylifera fruit and Saccharum officinarum juice. Literature review of the drug ingrediants Ferulla asafoedita[13], Electaria cardamomum[13],Piper longum[13],Picrrorhoea kurora[14], and Hemidusmus indica[15] possess Anti-asthmatic activity.* A preclinical toxicity study is mandatory in determining a safety dose for human trial [16]. Prior to the initiation of human trial the safety of the drug is to be proved[17]. The present preclinical study aimed at evaluating the acute and sub-acute toxicity of ASK. This study provides vital information about efficacy and safety of ASK.

MATERIALS AND METHODS

The ingredients of the drugs *Amirtha Sanjeevi Kuligai* were procured from TAMPCOL drug shop. The raw drugs were identified and authenticated in Post Graduate Department of Gunapadam, Govt.Siddha Medical College, Arumbakkam, Chennai. All the ingredients were taken in equal quantity then it was powdered finely and required quantity of sugar cane juice was added little by little and it was grinded in a mortar for 12 hours, then it was made into 370 mg pills, dried and preserved in an air tight container. This pills was labelled as ASK and used for the present study.

Amirtha Sanjeevi Kuligai

Adjuvant: Hot Water

Chemicals, Reagents and Animals

All chemicals and reagents were obtained from sigma chemicals Ltd, USA. All other reagents used in the study were of analytical grade were obtained from Qualigen fine chemicals Pvt. Ltd. Swiss albino rats of either sex weighing about 220-250 gm were obtained from the animal house of king institute of preventive medicine, Guindy, Chennai. The animals were acclimated to standard laboratory condition (temperature – 24 to 28^{o}C and humidity 60- 70%) and maintained on 12 hr light/ dark cycle. The animals were housed in polypropylene cages and fed with standard rodent pellet obtained and water ad libitum. The present study was approved by the Institutional Animal Ethical Committee (IAEC), C.L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai-97, with the approval number: IAEC/XXXIX/13/CLBMCP/2013/ dated 29.6.2013.

Acute Toxicity Study: Three female nulliparous and non- pregnant rats were used for acute oral toxicity study according to Organization for Economic Cooperation Development

(OECD) guideline 423[18]. ASK was administered orally 2000 mg/kg body weight of different groups of rats and absorbed for toxicological study. The animals were observed individually after dosing the first 30 mins, periodically during the first 24 h, with special attention given during the first 4h, and daily thereafter, for 14 days. Observations included changes in skin, fur, eyes, mucous membrane (nasal), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation), and central nervous system (drowsiness, gait, tremors and convulsions) changes respectively **(Table: 1)**. Mortality, if any was determined over a period of 2 weeks.

Table: 1 Dose Finding Experiment and Behavioral Signs of Toxicity.

Group	**Day**
Body weight	Normal
Assessments of posture	Normal
Signs of Convulsion Limb paralysis	Absence of sign (-)
Body tone	Normal
Lacrimation	Absence
Salivation	Mild
Change in skin colour	No significant colour change
Piloerection	Not observed
Defecation	Loose stools observed occasionally
Sensitivity response	Normal
Locomotion	Normal
Muscle grip ness	Normal
Rearing	Normal
Urination	Normal

Sub -Acute Oral Toxicity

In this study, the animals were divided into three groups of each 6 animals (3 males and 3 females) and treated with low (200 mg/ kg of body weight) and high dose 400mg /kg of body weight) levels to administered for 28 days . Group 1 received 0.025% CMC in water and served as control, Groups 2 and 3 received 200 mg /kg and 400 mg/kg ASK(suspended in 0.02% CMC solution) body weight orally, respectively. The drug was administered daily for 28 days at the same time and observed at least twice for morbidity and mortality. Body weights and food consumption of the animals were evaluated weekly **(Table:2).**Whereas this sub- chronic oral toxicity study was carried out according to OECD guideline 407[19, 20].

Table 2: Food Intake &Body Weight of Rats Treatment with Ask For 28 Days

Grouping		Food (g/day/rat)	Body weight (g)
Control			
	MEAN	23.67	235
	SD	5.241	5.865
	SE	2.14	2.394
LOW DOSE			
	MEAN	23	229.7
	SD	4.517	3.615
	SE	1.844	1.476
HIGH DOSE			
	MEAN	26	230.5
	SD	3.795	8.643
	SE	1.549	3.528

Values are mean of 6 animal's ± S.E.M. (Dunnets test) $^{ns}p< 0.05$

Hematological and Blood Biochemical Analysis

On the 29 th day, of the sub – chronic oral toxicity, over a period of fasting the rats were anesthetized with ether and blood sample for haematological and biochemical analysis were collected by cardiac puncture method into tubes with and without Ethylene diamine tetra acetate (EDTA), respectively. Haematological parameter observed and recorded (Table 3 andTable 4) Biochemical parameter such as serum cholesterol, LDL, HDL, Total protein, SGOT and SGPT also recorded (Table 5 and Table 6)

Table 3: Hematologicalparameters after 28 Days Treatment with Ask In Rats.

Grouping	Total red cells count (×10 6 µl)	Total WBC count (×10 3 µl)	Platelet count (×10 3 µl)	Packed cell volume (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Blood sugar ® (mg/dl)	BUN (mg/dl)
CONTROL									
MEAN	8.167	8.5	569.5	45.67	59.83	25.67	44.67	75.33	16.67
SD	1.835	3.209	6.716	4.457	5.981	3.83	4.719	5.354	2.582
SE	0.7491	1.31	2.742	1.82	2.442	1.563	1.926	2.186	1.054
LOW DOSE									
MEAN	8.433	8.65	567.7	44.83	58	23.5	45.83	74.17	14.33
SD	0.784	2.716	6.563	4.355	5.477	3.937	3.656	4.834	2.251
SE	0.3201	1.109	2.679	1.778	2.236	1.607	1.493	1.973	0.9189
HIGH DOSE									
MEAN	8.833	9.183	568.3	48.17	58	24.83	43.33	74.33	14
SD	0.709	1.459	4.412	2.137	4.733	5.419	3.67	3.724	2
SE	0.2894	0.5958	1.801	0.8724	1.932	2.212	1.498	1.52	0.8165

Values are mean of 6 animals ± S.E.M. (Dunnets test)$^{ns}p<0.05$

Table 4: Hematological Parameters after 28 Days Treatment with Ask In Rats.

Grouping		HB (g/dl)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)	Basophils (%)
CONTROL	M	31.83	50.67	52.5	12.47	1.183	0.65
	SD	20.72	27.11	20.04	16.87	0.6524	0.505
	SE	8.459	11.07	8.18	6.888	0.2664	0.2062
LOW DOSE	M	15.67	66.67	39.17	1.417	0.6167	0.1667
	SD	2.658	4.457	6.706	0.2639	0.2317	0.4082
	SE	1.085	1.82	2.738	0.1078	0.09458	0.1667
HIGH DOSE	M	14	66.33	35	1.517	0.6	0.3333
	SD	2.098	4.082	6.033	0.2229	0.2366	0.5164
	SE	0.8563	1.667	2.463	0.09098	0.09661	0.2108

Values are mean of 6 animals ± S.E.M. (Dunnets test)$^{ns}p<0.05$

Table 5: Biochemical Parameters after 28 Days Treatment with Ask In Rats.

Grouping		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)
CONTROL	M	0.8833	99.17	46.17	29	48	36.5
	SD	0.3312	2.483	5.981	3.899	2.191	4.087
	SE	0.1352	1.014	2.442	1.592	0.8944	1.668
LOW DOSE	M	0.7	100.8	42.5	29.83	44	34
	SD	0.228	3.312	2.739	5.076	3.847	3.742
	SE	0.09309	1.352	1.118	2.072	1.571	1.528
HIGH DOSE	M	0.7833	106.8	48	29.33	44.33	34.67
	SD	0.2401	3.43	3.098	5.317	4.32	3.615
	SE	0.09804	1.4	1.265	2.171	1.764	1.476

Values are mean of 6 animals ± S.E.M. (Dunnets test)$^{ns}p<0.05$

Table 6: Biochemical Parameters after 28 Days Treatment with Ask In Rats

Grouping		Serum total protein (g/dl)	Serum albumin (g/dl)	SGOT (AST) (IU/ml)	SGPT (ALT) (IU/L)
CONTROL	M	5.117	2.9	226.7	48
	SD	0.7195	0.4775	18.02	36.84
	SE	0.2937	0.1949	7.356	15.04
LOW DOSE	M	4.517	2.467	214.8	72.5
	SD	0.3656	0.3266	3.656	2.429
	SE	0.1493	0.1333	1.493	0.9916

HIGH DOSE	M	4.317	2.617	214.2	69
	SD	0.3545	0.4355	2.714	5.797
	SE	0.1447	0.1778	1.108	2.366

Histo-Pathological Study

Animals in the study were also subjected to a full, detailed gross necropsy. The positions, shapes, size and colour of the internal organs (heart, kidney, brain, liver) were also recorded. The liver, heart, kidney, brain samples from each group were preserved in 10% buffered formalin and processed for routine paraffin block preparation. Sections of thickness of about 5 µm were cut and stained with hematoxylin and eosin for histopathological investigation [21].

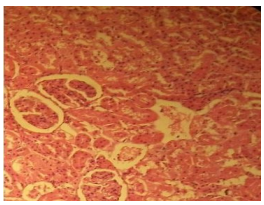
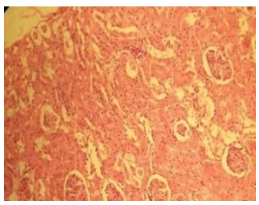
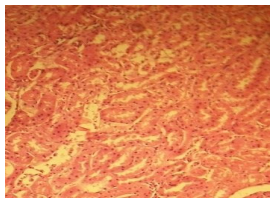
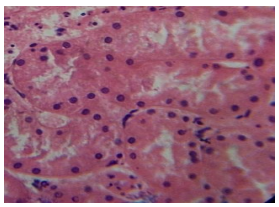
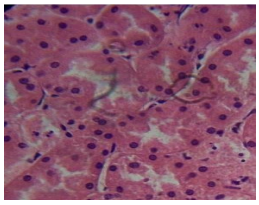
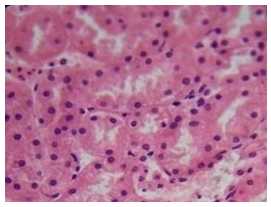
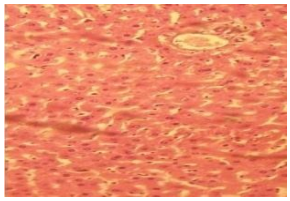
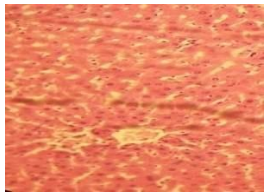
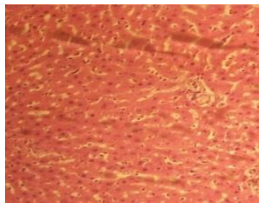
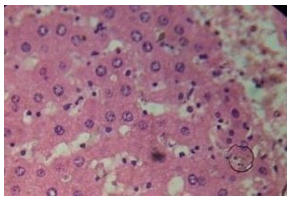
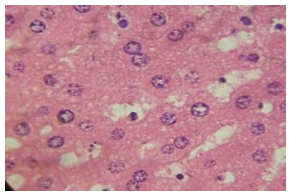
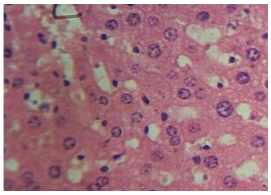
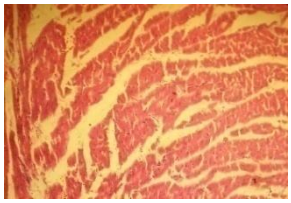
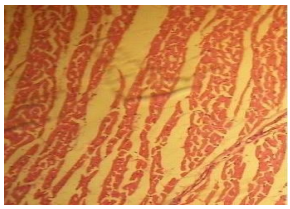
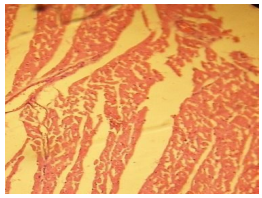
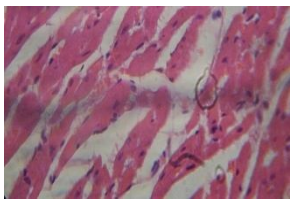
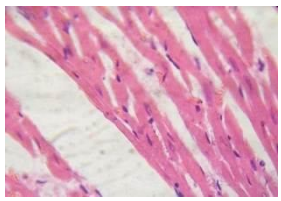
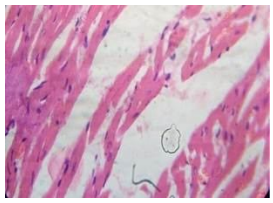
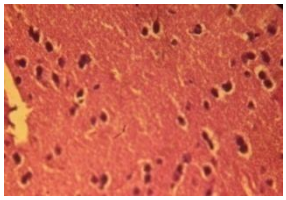
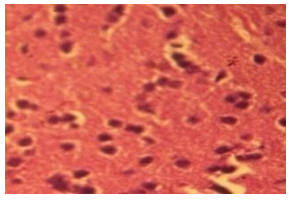
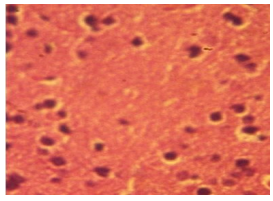
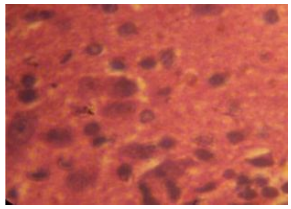
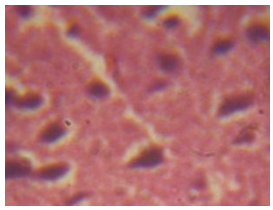
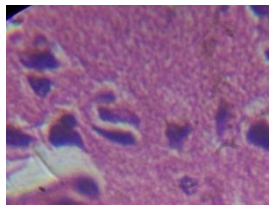
Statistical Analysis

All of the data were expressed as mean ± SEM. Statistical significance between more than two groups were using one way ANOVA followed by Bonferroni multiple comparision test. Calculations were using GraphPad prism software. The significance level was set at p value ≤ 0.05 for all tests.

DISCUSSION

1. All the animals both control and dose treated group upto 400mg/kg survived throughout the period of 28 days.
2. No signs of major or significant intoxication were observed in animals from lower to higher dose groups during the dosing period of 28 days.
3. No signs of Behavioral changes, Hematological and Biochemical abnormalities were observed.
4. Food consumption and body weight gain were found to be comparable throughout the dosing period of 28 days.
5. Opthalmoscopic examination, conducted prior to and at the end of dosing periods on animals from control and all the treated dose groups did not reveal any abnormality.
6. Gross pathological examination did not reveal any abnormality.
7. Histopathological examination, liver shows lumen of hepatic veins appear normal, No signs of necrosis. Kidney shows normal arrangements of nephrotic bundle in all the three groups. Heart shows no signs of lesion or infracts was observed in all the three sroups, Brain shows normal histology with regular neuronal alignment further there was no considerable observation of signs of oedema or degeneration.

Histo Pathological Analysis of Sub-Acute Toxicity Study.

GROUP SAMPLE	CONTROL	LOW DOSE	HIGH DOSE
KIDNEY (Magnification 10x)			
K IDNEY (Magnification 45x)			
LIVER (Magnification 10X)			
LIVER (Magnification45X)			
HEART (Magnification 10X)			
HEART (Magnification 45X)			
BRAIN (Magnification10X)			
BRAIN (Magnification45X)			

CONCLUSIONS

Based on above finding, no toxic effect was observed upto 400mg/kg of body weight of AmirthaSanjeeviKuligai treated via oral route over a period of 28 days. So this study concluded that the AmirthaSanjeeviKuligai is suitable with the dosage recommendations of upto 400mg/kg body weight p.o.

ACKNOWLEDGEMENTS

The grateful author, the principal, Govt. Siddha Medical College, Chennai, Tamil Nadu.

REFERENCES

1. "GINA report," Global Strategy for Asthma Management and Prevention, 2011, http://www.ginasthma.org/.
2. W. Eder, M. J. Ege, and E. Von Mutius, "The asthma epidemic," New England Journal of Medicine, 2006; 355(21): 2226–2235.
3. W. M. C. Van Aalderen, G. G. Meijer, Y. Oosterhoff, and A. O. Bron, "Epidemiology and the concept of underlying mechanisms of nocturnal asthma," Respiratory Medicine, 1993; l(87): 37–39.
4. S. Illi, E. von Mutius, S. Lau, B. Niggemann, C. Grüber, and U. Wahn, "Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study," Lancet, 2006; 368(9537): 763–770.
5. International study of Bronchial Asthma and allergies in childhood (ISAAC). Worldwide variations in the prevalence of Bronchial Asthma symptoms. Euro Respir J. 1998; 12:315-35.
6. Reid J, Marciniuk DD, Cockcroft DW. Bronchial Asthma management in the emergency department. Can Respir J. 2000; 7:255-60.
7. Górkan F, Ece A, Haspolat K, Derman O, Bosnak M. Predictors for multiple hospital admissions in children with Bronchial Asthma. Can Respir J. 2000; 7:163-6.
8. World Health Organization. Bronchial Asthma: scope of the problem. Available from: http://www.who.int. [last cited on 2005 Aug 23].
9. Ahmad OB, Lopez AD, Inoue M. The decline in child mortality: a reappraisal. Bull World Health Organ. 2000; 78:1175-91.
10. Gakidou E, Oza S, Fuertes CV, Lee DK, Sousa A, Hogan MC, et al. Improving child survival through environmental and nutritional interventions: the importance of targeting interventions toward the poor. JAMA, 2007; 298:1876-87.

11. K.S. Murugesamudhaliyar,Kuzhanthaimaruthuvam (Balavagadam), Dept. of Indian Medicine and Homeopathy, 294.
12. K.S. Murugesamudhaliyar,Kuzhanthaimaruthuvam(Balavaagadam), Dept.of Indian Medicine and homeopathy, 331.
13. C.P. Khare (2007) Indian Medicinal Plants .Springer, NY 10013, USA.
14. SiddhenduBhattacharjee, IJPRN ISSN: 2277-8713; IJPRBS, 2013; 2(4): 1-16.
15. Bhujbal et al, Pharmacology online, 2009; 1: 209-216.
16. Anoop A, Jagadeesan A, Subramaniam S, Toxicological study on LingachenduramI,a siddha drug, IJPS; 2002; 64(1): 53.
17. Thillaivanan et al, Acute and sub-acute toxicity study of Isappukolchooranam IJPSR, 2013; 4(11): 4448- 4456
18. Paris: Organization for Economic Co-operation and Development. Acute oral toxicity, acute oral toxic class method guideline-423. (2002)
19. Paris: Organization for Economic Co-operation and Development. Repeated dose 28-day oral toxicity test method guideline-407. (1995)
20. Chandra P, Sachan N, Ghosh AK, Kishore K. Acute and Sub-chronic Oral Toxicity Studies of a Mineralo-herbal Drug Amlena on Experimental Rats. Int J Pharm Res Innov, 2010; 1: 15–8.
21. Phoolchantral et al, Acute, sub-chronic oral toxicity studies and evaluation of antiulcer activity of Sooktyn in experimental animals JAV Pharm, Technol Res, 2012; 3(2): 117-123.