IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
October 2015 Vol.:4, Issue:3
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Acute and Sub Acute Toxicity Study on *Elathi kuligai* in Wistar Rats

IJPPR
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ISSN 2349-7203
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Submission: 1 October 2015
Accepted: 9 October 2015
Published: 25 October 2015

HUMAN JOURNALS

www.ijppr.humanjournals.com

Keywords: *Elathi kuligai*, Toxicity, Bronchitis, Oral route

ABSTRACT

Siddha system is the one of the oldest and most ancient traditional prevailing medicinal systems in India especially in our south India. In our system first preference is given to plant-based medicine. Plant-derived drugs have been a part of the evolution of human health care for thousands of years in India. *Elathi kuligai* is prepared as per classical siddha text book for respiratory problem in paediatric age group [1]. It is a poly herbal drug preparation. Before conducting clinical trial, preclinical study should be undergone as per WHO guidelines. The present preclinical study aimed to carry out safety and toxicity of *Elathi kuligai*. Male and female Wistar rats of age 6 – 8 weeks old and weighing 220–240 gm were used for this study. Acute and Sub-acute toxicity studies 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 dose level of 200 mg/kg and 400 mg/kg of body weight of *Elathi kuligai* to Wistar rats, there was no characteristic clinical sign of toxicity or mortality or severe toxicological effects on selected body organs, biochemical indices and hematological and histopathological markers of rats during the sub-acute periods of study was observed.

INTRODUCTION

Bronchitis is characterized by the development of cough or small sensation in the back of the throat with or without production of sputum that is expectorant or coughed up from the respiratory tract. Bronchitis refers to non specific bronchial inflammation and is associated with a number of childhood condition, acute bronchitis is a syndrome, usually viral in origin, with cough as a prominent feature [2]. Bronchitis is a major health issue in many developing and developed countries. Bronchitis, both acute and chronic is prevalent throughout the world and is one of the top five reasons for childhood physician visits in countries. Bronchitis occurs most commonly in children younger than 2 years with another peak seen in children aged 9-15 years [3]. Bronchitis is inflammation of the bronchial airway. In children, bronchitis caused by virus (90%) like rhinovirus, corona virus, influenza, a & b para influenza, adenovirus, or less often by a mycoplasmo pneumonia, Chlamydia, streptococci and bacterial infection (10%). Non infectious inflammation of the bronchi caused by physical and chemical irritants such as inhale dust, pollen grain and organic substance etc.[4] [9].

In the Siddha text book,[8] it is said that dry or productive cough with expectoration wheeze, malaise, fever, constipation, URTI like nasopharyngitis, rhinitis and sore throat are the symptoms of *Kabasuram*. *Kabasuram* shows symptoms similar to bronchitis are specifically taken for the dissertation, which is a respiratory disease encountered by a large population of children today and limit their daily activities.

Apart from lifestyle recommendations which will balance *Kabakuttram*. Several herbs are useful for controlling the symptoms of bronchitis. Prior to the initiation of human trial, the safety of the drug is to be proved [5]. A preclinical study is important to determine a safety dose for human trial [6]. The present preclinical study aimed to evaluate the acute and sub-acute toxicity of *Elathi kuligai*. This study provides vital information about efficacy and safety of *Elathi kuligai*.

MATERIALS AND METHODS

SOP OF *ELATHI KULIGAI*

Elathi kuligai [1] is a Herbal Siddha formulation comprising of sixteen different types of herbs like Elam *(Elettaria cardamomum*), Ilavangam (*Syzygium aromaticum*), Chukku (*Zingiber*

officinale), Vellilolthiram *(Symplocos racemosa*) Santhanam *(Santalum album*), Kadugurogini (*Picrorhiza scrophulariiflora*), Elupaipoo *(Madhuca longifolia*), Nannari Root (*Hemidesmus indicus*), Vettiver *(Vetiveria zizanioides)* Athimathuram *(Glycyrrhiza glabra*), Koraikilangu *(Cyperus rotundus),* Koththamalli vithai (*Coriandrum sativum*) Thiratchai *(Vitis vinifera*) Paerichai (*Phoenix dactylifera*) sarkkarai and Sugarcane juice *(Saccharum officinarum*) [7] [10][11][12][9].

The purified raw drugs are made into fine powder, then it is grinded in *Kalvam* with sugarcane juice and makes its 370 mg pills and dry. The trial drug *Elathi kuligai* is stored in clean dry air tight container and it is dispensed to the patients in packets.

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 and obtained from Qualigen Fine Chemicals Pvt. Ltd. Wistar rats of either sex weighing about 220-250 gm were obtained from the animal house of King Institute of Preventive Medicine, Guindy, Alanthur Road, SIDCO Industrial estate, Chennai-600 032, Tamil Nadu. The animals were acclimated to standard laboratory condition (temperature between 22± 2° and humidity 60- 70%) and illumination cycle changed on 12 hr light/ dark cycle. The animals were housed in polypropylene cages and were housed in groups of three animals of similar sex; feed with Standard pellet feed was provided. Potable water passed through *ad libitum* in rat feeding bottles with stainless steel sipper tubes. The present study was approved by Institutional Animal Ethical Committee (IAEC), C.L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai-97.

Acute toxicity study in rats

Acute toxicity study was carried out in three female nulliparous and non- pregnant Wistar rats. They were used for acute oral toxicity study according to Organization for Economic Cooperation Development 423 (13). Healthy female rats weighing 220–240 gm were used for this study. Study carried out on female rats divided into two groups of 3 animals each under fasting condition (16 hrs prior to test animals deprived for food not for water). *Elathi kuligai* was administered orally 2000 mg/kg body weight to different groups of rats and observed for

toxicological signs of toxicity. They were observed for every one hour for first 24 hours and every day for about 14 days from the beginning of the study. All animals were observed for change in skin colour, body weight, mucous membrane, nasal, autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation), and central nervous system (drowsiness, posture, tremors and convulsions) changes respectively in Table: 1

Table1: 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	Absence
Change in skin color	No significant color change
Piloerection	Not observed
Defecation	Regular Solid consistency
Sensitivity response	Normal
Locomotion	Normal
Muscle gripness	Normal
Rearing	Normal
Urination/Color	Normal

Effect of *Elathi kuligai* on Mortality rate of the study animals on Acute toxicity study

Treatment	Mortality observed for the duration of 1- 14 days
GROUP I - CONTROL	NIL
GROUP II- TREATMENT	NIL

Sub-Acute Oral Toxicity

This study was carried out as per Organization for Economic Co-operation and Development, Guideline-407(14). Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Three rats of same sex were housed per cage. Eighteen rats (09 male and 09 female healthy animals) were randomly divided into three groups of 6 animals. Treatment groups were dosed daily for the period of 28 days. Control group animal left untreated. Animal belongs to group II treated with low dose of the test drug 200 mg/kg and Group III treated with high dose of the test drug *Elathi kuligai* 400 mg/kg oral by gastric intubation technique. All animals were observed daily for clinical signs of toxicity. The drug was administered daily for 28 days on same time and observed at least twice for morbidity and mortality. Body weights and food consumption of the animals were evaluated weekly (Table: 2) & (Table: 1).

Table 2: Food Intake & Body Weight of Rats Treated with *Elathi kuligai* for 28 Days

GROUPING		**Food (g/day/rat)**	**Body weight (g)**
CONTROL	Mean	24	234.5
	Std. Deviation	2.449	3.209
	Std. Error	1	1.31
LOW DOSE	Mean	22.67	233.5
	Std. Deviation	2.658	2.51
	Std. Error	1.085	1.025
HIGH DOSE	Mean	25	234.7
	Std. Deviation	2.098	2.805
	Std. Error	0.8563	1.145

Effect of *Elathi kuligai* on Mortality rate of the animals on Sub-Acute toxicity study

Treatment	**Mortality observed for the duration of 1- 28 days**
GROUP I - CONTROL	NIL
GROUP II- LOW DOSE	NIL
GROUP III- HIGH DOSE	NIL

Haematological and Biochemical Investigations

Blood was collected through retro-orbital sinus from all the animals of different groups on 29th day. The blood was collected in tubes containing Heparin/EDTA as an anticoagulant. Animals were fasted overnight prior to the blood collection. Haematological and biochemical parameters were determined using Auto analyzer using standard kits and the data are provided.

Table 3: Effect of test drug on Hematological and Biochemical analysis

GROUPING		Total red cells count ($\times 10^6$ µl)	Total WBC count ($\times 10^3$ µl)	Platelet count ($\times 10^3$ µl)	Packed cell volume (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Blood sugar® (mg/dl)	BUN (mg/dl)
CONTROL	Mean	7.333	9.667	529.5	49.33	59	26.67	43	80.67	17.67
	Std. Deviation	1.366	1.366	34.43	4.033	4.94	3.327	5.797	9.026	3.67
	Std. Error	0.5578	0.5578	14.05	1.647	2.017	1.358	2.366	3.685	1.498
LOW DOSE	Mean	7.167	8.667	550.3	55	59.83	33.33	43.33	81.67	18
	Std. Deviation	1.169	1.633	45.52	4.517	6.113	3.983	6.439	8.262	4.243
	Std. Error	0.4773	0.6667	18.58	1.844	2.496	1.626	2.629	3.373	1.732
HIGH DOSE	Mean	5.667	8.667	536.3	52.67	58.83	31.5	47.67	84.83	20.83
	Std. Deviation	1.211	1.506	38.27	9.873	6.047	2.258	4.033	7.679	3.189
	Std. Error	0.4944	0.6146	15.62	4.03	2.469	0.922	1.647	3.135	1.302

Table 4: Effect of test drug on Serum creatinine and lipid profile

GROUPING		Serum creatinine (mg/dl)	Serum total cholesterol (mg/dl)	Serum tri glyceride level (mg/dl)	Serum HDL cholesterol (mg/dl)	Serum LDL cholesterol (mg/dl)	Serum VLDL cholesterol (mg/dl)	Serum total protein (g/dl)
CONTROL	Mean	1.067	102.8	53	22.83	52.33	35.83	6.317
	Std. Deviation	0.4082	4.956	6.229	3.251	4.179	2.317	1.733
	Std. Error	0.1667	2.023	2.543	1.327	1.706	0.9458	0.7073
LOW DOSE	Mean	0.9833	100.7	48.5	24.33	54.5	40	6.467
	Std. Deviation	0.343	6.802	5.718	3.266	2.51	1.265	2.597
	Std. Error	0.14	2.777	2.335	1.333	1.025	0.5164	1.06
HIGH DOSE	Mean	0.8833	101	46.83	23.33	53	36.5	5.733
	Std. Deviation	0.1722	6.481	4.07	1.751	3.033	5.282	1.999
	Std. Error	0.07032	2.646	1.662	0.7149	1.238	2.156	0.816

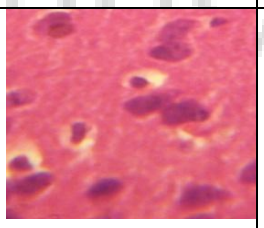
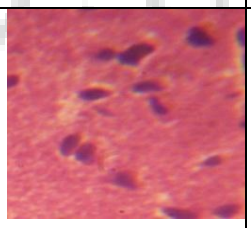
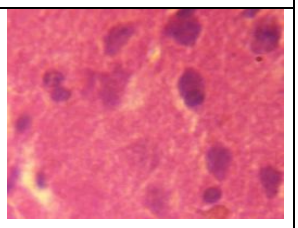
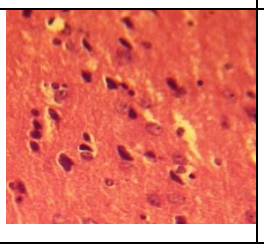
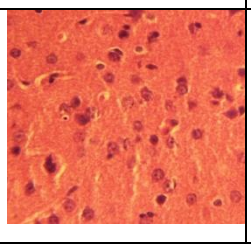
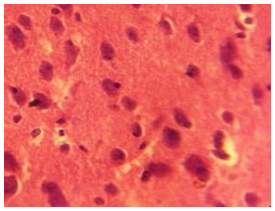
Table 5: Effect of test drug on Albumin and Liver enzymes analysis

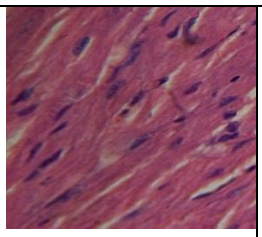
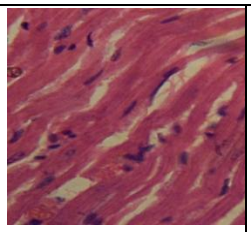
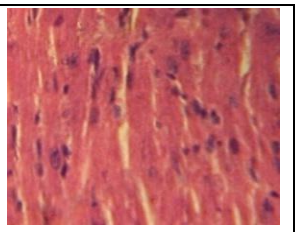
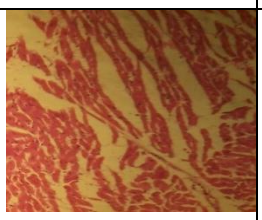
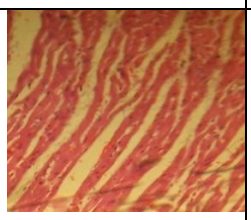
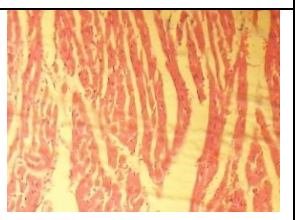
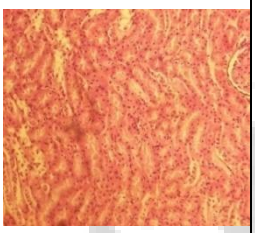
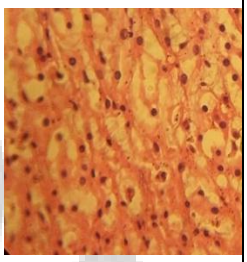
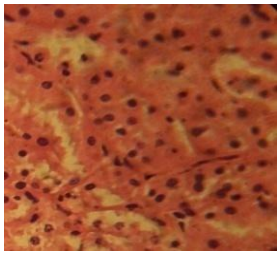
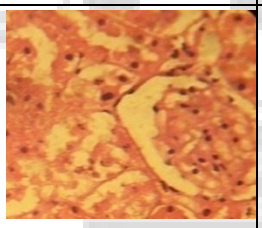
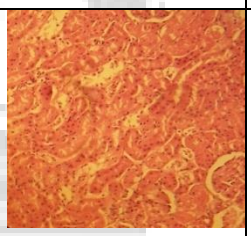
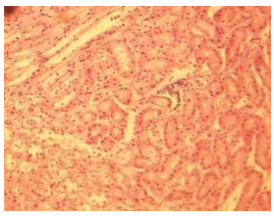
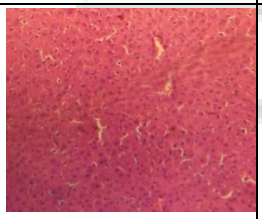
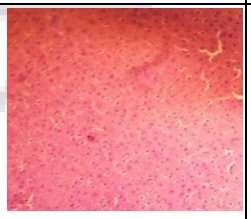
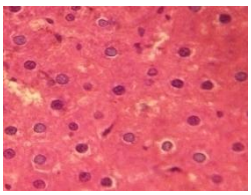
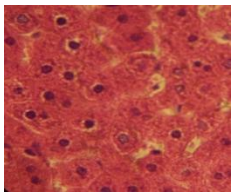
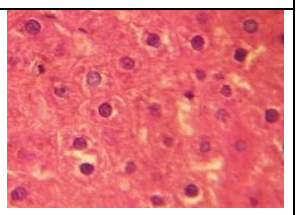
GROUPING		Serum albumin (g/dl)	SGOT (AST) (IU/ml)	SGPT (ALT) (IU/L)
CONTROL	Mean	4.833	123	73.17
	Std. Deviation	1.291	14.99	3.764
	Std. Error	0.527	6.121	1.537
LOW DOSE	Mean	4.433	128.3	67.5
	Std. Deviation	1.937	14.38	6.189
	Std. Error	0.7906	5.869	2.527
HIGH DOSE	Mean	4.383	125.3	67.33
	Std. Deviation	1.146	7.891	4.32
	Std. Error	0.4679	3.221	1.764

Table 6: Effect of test drug on Blood cell count

GROUPING		HB (g/dl)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)	Basophils (%)
CONTROL	Mean	16.67	75.67	35	1.367	0.85	0
	Std. Deviation	1.506	1.966	4.733	0.2875	0.2074	0
	Std. Error	0.6146	0.8028	1.932	0.1174	0.08466	0
LOW DOSE	Mean	16.17	73.5	32.67	1.55	0.7	0.6667
	Std. Deviation	1.472	2.881	0.8165	0.295	0.3033	0.5164
	Std. Error	0.6009	1.176	0.3333	0.1204	0.1238	0.2108
HIGH DOSE	Mean	15.83	75.67	37.17	1.767	0.9833	0.5
	Std. Deviation	1.329	2.422	2.927	0.383	0.5742	0.5477
	Std. Error	0.5426	0.9888	1.195	0.1563	0.2344	0.2236

HISTOPATHOLOGIGAL STUDY REPORT

GROUPING SAMPLE	CONTROL	LOW DOSE	HIGH DOSE
BRAIN (Magnification 45x)			
BRAIN (Magnification 10x)			

HEART (Magnification 45x)			
HEART (Magnification 10x)			
KIDNEY (Magnification 45x)			
KIDNEY (Magnification 10x)			
LIVER (Magnification 45x)			
LIVER (Magnification 10x)			

Effect of Test drug on organ morphology

Grouping	Kidney	Liver	Heart	Lungs	Spleen	Pancreas	Brain	Ovaries	Testes
Group I - Control	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Group II- Low Dose	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Group III - High Dose	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Necropsy

All animals were sacrificed by cervical dislocation on 29th day. Necropsy of all animals were carried out and the weights of the vital organs were recorded (heart, liver, kidneys and brain).

Histopathology

During necropsy the target organs viz., heart, liver, kidneys and brain were collected and preserved in 10 % neutral formalin buffer for the histopathological evaluation. The organs from control and treated animals were preserved in 10 % neutral formalin buffer for histopathological examination.

DISCUSSION

Histopathological examination, Kidneys show normal in all the three groups, renal cortex and medulla appears normal, arrangement of Nephrotic bundles appears regular and highly intact. In Heart Cardiac myocyte appears larger with regular fiber length; myocardial cells appear intact and prominent nuclei with no major signs of abnormalities in all the three groups. Liver shows normal Hepatic Parenchymal lining, hepatic veins appear normal, no signs of Necrosis or Cirrhosis. No signs of inflammation in all the three groups. Brain shows no signs of hemorrhage and apoptosis, edema and Inter neuronal distance appears regular with no signs of degeneration and no ischemic brain tissue damage in all three groups.

No signs of behavioral changes. Hematological and biochemical abnormalities were observed.

Food consumption and body weight gain were found to be comparable throughout the dosing period of 28 days.

CONCLUSION

The current toxicity study proves that recommended dose of the Siddha drug *Elathi kuligai*, does not produce any pathological symptoms throughout the dosing period of 28 days. So the safety drug dose of *Elathi kuligai* is 400 mg/kg /body weight.

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