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EVALUATION OF ACUTE AND SUB ACUTE TOXICIY STUDY OF *KANDANGKATHIRI KIRUTHAM* (GHEE OF SOLANUM XANTHOCARPUM) IN ANIMAL MODEL.

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Abstract: *Kandangkathiri Kirutham* (ghee of Solanum xanthocarpum) is a siddha herbal formulation containing leaf juice of *Kandangkathiri* (Solanum xanthocarpum),cow's ghee, the paste of raw drugs namely *Sittrarathai* (Alpinia officinarum), *Sittramutti* (Sida cordifolia) , *Nerunjil ver* (Tribulus terrestris), *Chukku* (Zingiber officinale), *Milagu*(Piper nigrum), *Thippili* (Piper longum) mentioned for *Ullnaakku Azharchi*(Tonsillitis). **Aim**: To assess Acute and Sub acute toxicity of *Kandangkathiri Kirutham* in animal model. **Materials and Methods:** In acute toxicity study Wistar Albino rats were administered orally *Kandangkathiri Kirutham* in single dose of 200 mg/kg for 14 days and observed for behavioural changes and mortaliy, if any. In Sub acute toxicity study wistar Albino rats were administered orally 2 doses of *Kandangkathiri Kirutham* i.e.,low dose 200mg/kg and high dose 400mg/kg for 28 days. During 28 days of treatment,rats were observed for any changes in body weight, daily food & water intake. After 28 days,rats were sacrificed for hematological, biochemical & histopathological study. **Result:** There was no mortality or abnormal behavior observed in acute toxicity study and in Sub acute toxicity there was no changes in body weight, daily food & water intake when compared to control group rats on administering *Kandangkathiri Kirutham*. Further, hematological & biochemical parameters were also found normal. Histopathological study revealed normal architecture of kidney, liver, heart and brain of *Kandangkathiri Kirutham* treated rats. **Conclusion:** *Kandangkathiri Kirutham* is safe in rats.

Keywords: Acute toxicity, Sub acute toxicity, *Kandangkathiri Kirutham*)

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INTRODUCTION

Herbal medicines are generally complex mixtures of many bio active compounds. Safety is a fundamental principle in the provision of herbal medicines and herbal products for health care, and a critical component of quality control. Preclinical studies of herbal drugs provide scientific justification of traditional use and prove that they are safe and efficacious. Organization for Economic Co-operation and Development (OECD) sets guidelines regarding toxicity study. *Kandangkathiri Kirutham* is a herbal formulation consists of *Kandangkathiri* leaf juice-1.3 litre (Solanum xanthocarpum) and cow's ghee-650 ml along with the paste of raw drugs namely *Sittrarathai*-9 gm (Alpinia officinarum), *Sittramutti* -9 gm (Sida cordifolia) , *Nerunjil ver* -9 gm (Tribulus terrestris), *Chukku*-9 gm (Zingiber officinale), *Milagu*-9 gm (Piper nigrum), *Thippili*-9 gm (Piper longum) respectively.

MATERIALS AND METHODS:

Preparation:

First the *Kandangkathiri* leaves are washed well, then crushed using mixer grinder and the leaf juice is extracted. Next the raw drugs are purified and grounded into a fine powder. Then it is mixed along with water into a thick paste. Now the leaf juice, ghee, and the paste are mixed well in a container and heated. It is allowed to boil in a low flame and stirred well until sand like consistency is obtained. Then it is allowed to cooled down and filtered and preserved in a clean container.

Species

Male and female Wistar rats of age 6 – 8 weeks old weighed between 200-250gm were used for experimental study. The animals were obtained from The King Institute of preventive medicine, Alanthur Road , SIDCO Industrial estate , Chennai-600 032, Tamil Nadu.

Environmental Conditions

Air conditioned rooms, temperature was between 22 ± 2° C and illumination cycle set to

12 hours light and 12 hours dark.

Accomodation

Standard polypropylene rat cages with stainless steel top grill.Cleaned paddy husk were used as the bedding material. Animals were housed in groups of three animals of similar sex.

Sanitation

Bedding material and water bottles were changed daily.

Diet and water

Standard pellet feed was provided. Potable water *ad libitum* passed through rat feeding bottles with stainless steel sipper tubes.

Acute toxicity study in rats

Procedure

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co-operation and Development) 423.Healthy female rats weighing 220–240 gm were used for this study. Studied carried out on female rats divided in to two groups of 3 animals each under fasting condition (16 hrs prior to test animals deprived of food not for water), signs of toxicity was 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 daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Observation: Animals were observed for possible signs of toxicity related to CNS, ANS and CVS

Acute toxicity study Grouping

Group I : Control Group: 3 female rats

Group II : Treatment group : 3 female rats

Dose : 2000mg/kg , Route: Oral route (Single dose administration)

Sub-Acute toxicity study in rats

Procedure

Sub-acute toxicity study was carried out as per OECD guideline (Organization for Economic Co-operation and Development, Guideline-407 .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 given dose 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 200mg/kg and Group III treated with high dose of the test drug 400mg/kg, per oral by gastric intubation

technique. All animals were observed daily for clinical signs of toxicity. The time of onset, intensity and duration of the symptoms, if any, were recorded. Body weight , food intake ,water intake and other general behaviour of the animals will be monitored and recorded once in a week for the entire duration of the study.

Sub- Acute toxicity study Grouping

Group I : Control Group: 6 rats (3 male and 3 female)

Group II : Treatment group : 6 rats(3 male and 3 female) - Low dose 200mg/kg

Group III : Treatment group : 6 rats(3 male and 3 female)- High dose 400mg/kg

Dose : Low dose 200mg/kg and high dose 400 mg/kg

Route: Oral route (repeated dose administration)

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

Necropsy

All animals were sacrificed by cervical dislocation on 29th day. Necropsy of all animals were carried out and the weight 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.

Parameter checked

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	Solid consistency
Sensitivity response	Normal
Locomotion	Normal
Muscle grip ness	Normal
Rearing	Normal
Urination/Color	Normal

Effect of Test drug 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

Effect of Test drug on Mortality rate of the study 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

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

Effect of test drug on Hematological and Biochemical analysis

CONTROL	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)
Mean	7	9.667	550.2	47.5	57.17	29.33	44	72.83	16.5
Std. Deviation	1.549	0.8165	55.12	3.271	4.535	5.046	3.578	3.061	3.937
Std. Error	0.6325	0.3333	22.5	1.335	1.851	2.06	1.461	1.249	1.607

LOW DOSE	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)
Mean	6.5	10.33	511	55.17	64	30.33	39.33	83.67	22.5
Std. Deviation	1.517	2.422	20.45	1.941	4.817	3.615	5.785	2.875	3.391
Std. Error	0.6191	0.9888	8.347	0.7923	1.966	1.476	2.362	1.174	1.384
HIGH DOSE	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)
Mean	6.833	8.833	541.3	51.5	59.83	31.67	43.5	86.5	20.17
Std. Deviation	1.835	0.9832	9.832	6.156	6.795	3.777	5.541	3.271	3.656
Std. Error	0.7491	0.4014	4.014	2.513	2.774	1.542	2.262	1.335	1.493

Effect of test drug on Serum creatinine and lipid profile

CONTROL	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)	Serum total protein (g/dl)
Mean	0.9167	100.7	46.67	21.67	50.67	35.5	5.35
Std. Deviation	0.2639	5.888	5.354	2.875	1.966	3.728	0.5468
Std. Error	0.1078	2.404	2.186	1.174	0.8028	1.522	0.2232
LOW DOSE	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)	Serum total protein (g/dl)
Mean	1.2	104.8	54.17	27.5	52.33	37.5	6.667
Std. Deviation	0.2	2.787	1.472	4.506	5.61	3.886	3.141
Std. Error	0.08165	1.138	0.6009	1.839	2.29	1.586	1.282
HIGH DOSE	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)	Serum total protein (g/dl)
Mean	1	97	51.5	24.5	53.33	35.67	8.667
Std. Deviation	0.4336	1.897	5.612	2.739	3.777	2.251	1.966
Std. Error	0.177	0.7746	2.291	1.118	1.542	0.9189	0.8028

Effect of test drug on Albumin and Liver enzymes analysis

CONTROL	Serum albumin (g/dl)	SGOT (AST) (IU/ml)	SGPT (ALT) (IU/L)
Mean	3.15	138.5	74.33
Std. Deviation	0.3146	12.76	2.503
Std. Error	0.1285	5.207	1.022
LOW DOSE	Serum albumin (g/dl)	SGOT (AST) (IU/ml)	SGPT (ALT) (IU/L)
Mean	4.833	115.8	69.5

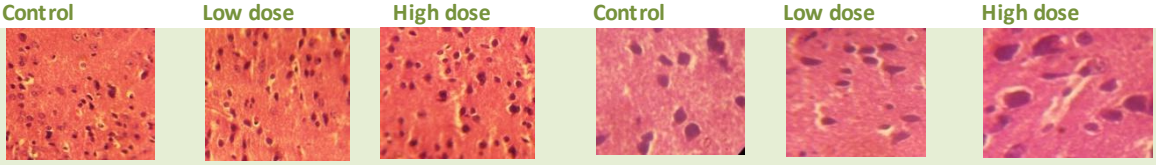
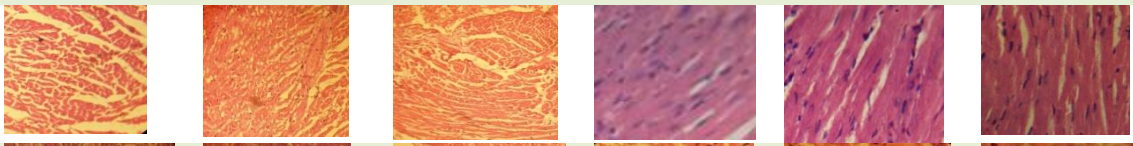
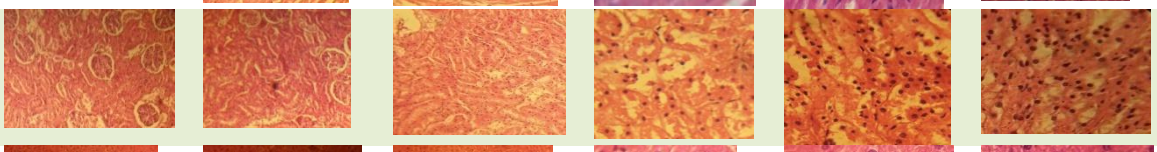
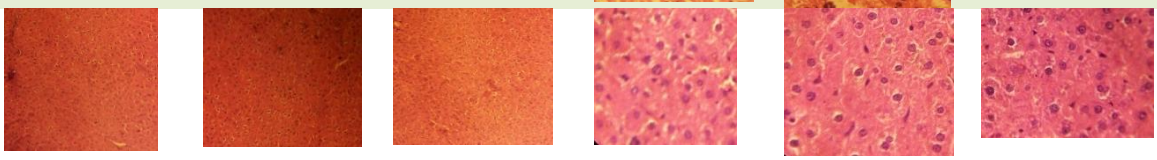
Std. Deviation	0.7528	1.472	5.891
Std. Error	0.3073	0.6009	2.405
HIGH DOSE	Serum albumin (g/dl)	SGOT (AST) (IU/ml)	SGPT (ALT) (IU/L)
Mean	4.833	119.2	64.83
Std. Deviation	1.329	4.916	3.189
Std. Error	0.5426	2.007	1.302

Effect of test drug on Blood cell count

CONTROL	HB (g/dl)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)	Basophils (%)
Mean	16	75.5	36	1.45	0.8833	0.1667
Std. Deviation	1.414	2.258	3.742	0.2881	0.1602	0.4082
Std. Error	0.5774	0.922	1.528	0.1176	0.0654	0.1667
LOW DOSE	HB (g/dl)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)	Basophils (%)
Mean	15.83	72.5	33.67	1.317	1.983	0.3333
Std. Deviation	0.7528	2.588	5.164	0.2563	3.443	0.5164
Std. Error	0.3073	1.057	2.108	0.1046	1.406	0.2108
HIGH DOSE	HB (g/dl)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)	Basophils (%)
Mean	16.5	75.5	32.67	1.633	1.517	0.3333
Std. Deviation	1.643	2.588	1.966	0.2066	0.4665	0.5164
Std. Error	0.6708	1.057	0.8028	0.08433	0.1905	0.2108

Effect of test drug on Body weight and Food consumption

CONTROL	Food (g/day/rat)	Body weight (g)
Mean	23.67	235.2
Std. Deviation	3.141	2.317
Std. Error	1.282	0.9458
LOW DOSE	Food (g/day/rat)	Body weight (g)
Mean	22.83	230.5
Std. Deviation	2.787	3.271
Std. Error	1.138	1.335
HIGH DOSE	Food (g/day/rat)	Body weight (g)
Mean	25	234
Std. Deviation	1.673	3.033
Std. Error	0.6831	1.238

Sample	Low dose group 10x			High dose group45x		
	Group			Group		
	Control	Low dose	High dose	Control	Low dose	High dose
Brain						
Heart						
Kidney						
Liver						

Pathologist report:

Sample	Observation
Kidney	Lumen of the kidney appears normal in all the three groups. Appearance of also arrangement of nephrotic bundle in all the three groups also normal. Renal cortex and medulla appears normal. Arrangement of nephrotic bundles appears regular and highly intact.
Heart	Nuclear arrangement was linear and appears normal no signs of content leakage. Myocardial cells appears with intact and prominent nuclei with no major signs of abnormalities in all the three groups.
Liver	Hepatic Parenchymal lining appears normal. Arrangement of hepatocytes was intact with prominent nuclei stained. Hepatic veins appears normal. No signs of necrosis or cirrhosis. No signs of inflammation in all the three groups.
Brain	No signs of edema and Inter neuronal distance appears regular with no signs of degeneration.

Results and Discussion of Acute toxicity study

Acute toxicity effect of the test drug was estimated by close observation of animals for about 24 hours after single dose administration of the test drug and it was observed that there is no significant signs of C.N.S related toxicity like convulsion, locomotion, muscle strength and A.N.S related toxicity like salivation, lacrimation etc was observed in treatment group

At the end of the study period all animals were sacrificed and the organs was isolated and observed for change in structural morphology. There is no significant change in the organ necropsy of the animals treated with the test drug.It shows that the test drug hasn't produce any internal hemorrhage or organ related toxicity.

Results and Discussion of Sub - Acute toxicity study

Sub acute toxicity for the given test drug was carried out as per the OECD guideline 407 by repeated dose administration of the test drug in animals and further animals were closely monitored for the emergence of toxicity.

Since, there were no significant adverse effects on the hematological, biochemical and histopathological parameters it may be concluded that the test drug at both the dose level of 200mg/kg and 400mg/kg may be considered as relatively safe, as it did not cause either mortality or produce severe toxicological effects on selected body organs, biochemical indices and hematological and histopathological markers of rats during the sub-acute periods of study.

CONCLUSION

In acute toxicity study rats do not showed abnormal behaviour for initial 4 hours after drug administration. In sub acute toxicity study, no significant adverse effect on the hematological, biochemical and histopathological parameters. *Kandangkathiri kirutham* have a broad safety margin in experimental animals.

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