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ACUTE AND SUB ACUTE TOXICITY STUDY OF THE SIDDHA DRUG UTHAMANI CHOORANAM (PERGULARIA DAEMIA)

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ABSTRACT

Atopic Dermatitis (AD) is a chronic, highly pruritic inflammatory skin disease, and is one of the most common skin disorder in children. *Uthamani Chooranam* has been employed as a traditional remedy for Atopic dermatitis (AD), which is a herbal formulation containing whole plant of *Pergularia daemia.* As a mandate, steps were taken to evaluate safety profile of UC in rats using OECD guidelines. Swiss albino rats of either sex weighing 220-240 gm were used. Acute and Sub-acute toxicity studies were carried out as per OECD guidelines 423 and 407. Haematological, biochemical parameters, histo pathological study were performed for all animals. The study concludes that on oral administration of 200 and 400 mg/kg body weight of UC to swiss albino rats, no change in behavioural and characteristic clinical sign of toxicity was observed.The UC was found to be safe in animals. No toxic effect was observed in both acute and sub-acute toxic studies of *Uthamani Chooranam.*

KEYWORDS: *Uthamani Chooranam,* OECD, Atopic Dermatitis.

INTRODUCTION

Atopic dermatitis (AD) or eczema is the most common chronic relapsing skin disease seen in infancy and childhood. It affects 10-30 % of children worldwide and frequently occurs in families with other atopic diseases, such as asthma, allergic rhinitis and food allergy. AD is a

complex genetic disorder that results in a defective skin barrier, reduced skin innate immune responses, and exaggerated T-cell responses to environmental allergens and microbes that lead to chronic skin inflammation [1]. The prevalence of AD has increased over the past 30 years. It is currently estimated that 10- 20 % of children and 1-3% of adults in developed countries are affected by the disorder. AD often starts in early infancy; approximately 45% of all cases begin within the first 6 months of life, 60% during the first year, and 85% before 5 years of age. Up to 70% of these children outgrow the disorder before adolescence. Children with AD are at high risk of developing asthma and allergic rhinitis [2].

The clinical features of AD correlates with the symptoms of *Balakarappan* described in the siddha text book of *Balavagadam*. In siddha literature *Bala karappan* is one of the eighteen types of *"Karappan noi"* that occurs in children [3]. The siddha drug *Uthamani chooranam* is a herbal preparation containing whole plant of *Pergularia daemia.* It's indication for karappan is quoted in the siddha text *Koshayi Anuboga Vaidhya Bramma Ragasiyam* [4]. Preclinical toxicity studies were essential for determining a safe dose for human trial. The present pre-clinical study is aimed at evaluating the acute and sub-acute toxicity of UC. This study provides vital information about the efficacy and safety of *Uthamani Chooranam.*

MATERIALS AND METHODS

The *Uthamani chooranam* contains whole plant of *Pergularia daemia* is known as *"veliparuthi"* belongs to the family *Asclepediaceae*. The plant material was collected from the fertile area of Arakkonam, Vellore district, Tamil nadu, India. The plant was identified and authenticated by the pharmacology experts of Post graduate department of *Gunapadam*, Govt. Siddha Medical College, Arumbakkam, Chennai. Plants of *Pergularia daemia* were air dried under shade, powdered with a mechanical grinder, filtered in fine cloth then the fine powder made into *Chooranam*. This powder was sieved through a clean white cloth and further purified by *Pittaviyal method* (steam boiling with milk) based on siddha classical literature [5].

The *Chooranam* was moistened with cow's milk and made into a solid form. Then it is kept in a clean cloth which is tied to the mouth of a mud vessel containing equal amount of cow's milk and water. Then it is finally covered over with a top vessel and their junction is covered with a cloth, so that vapour does not escape while boiling. After boiling and complete evaporation of liquid, the solid mixture is taken and dried in sunlight and grinded finally and

stored in an air tight container kept at room temperature. This *Chooranam* was labelled as UC and used for the present study.

Animals

Swiss albino rats of either sex weighing about 230-250 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/12 hr 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) at C.L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai, with the approval number: IAEC/XXXIX/12/CLBMCP/2013/ dated 29.6.2013.

Acute Oral Toxicity

The acute oral toxicity test for *Uthamani Chooranam* was evaluated in rats using the procedures described by Organization for Economic Co-operation and Development 423 Guidelines [6]. The control group was given 10ml/kg of normal saline. UC was administered orally 2000 mg/kg body weight of different groups of rats and observed for toxicological study. Animals were fasted approximately 12 hours prior to dosing. Following administration of a single dose of herbal preparation, the animals were observed for behavioural changes and general toxicity signs [Table 1]. Results were recorded for the first 30 minutes and then each hour for the next 24 hours and thereafter for a total of 14 days [7].

Sub-acute Oral Toxicity

Repeated dose oral toxicity was carried out according to OECD Guidelines 407 [8]. The animals were divided into three groups of each 6 animals (3 males and 3 females). Group 1 received 10 ml/kg body weight of normal saline and served as control. Groups 2 and 3 received 200 mg/kg and 400 mg/kg body weight respectively. Mortality, body weights, food consumption as well as observation for general toxicity signs of the animals were evaluated daily for 28 days [Table 2].

Table 1: Dose Finiding 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 colour	No significant colour change
Piloerection	Not observed
Defecation	Normal
Sensitivity response	Normal
Locomotion	Normal
Muscle gripness	Normal
Rearing	Mild
Urination	Normal

Table 2: Food Intake and Body Weight Of Rats Treatment With Uc For 28 Days

Grouping	**Food (g/day/rat)**	**Body weight (g)**
CONTROL		
MEAN	22.17	228.8
SD	2.483	5.382
SE	1.014	2.197
LOW DOSE	**Food (g/day/rat)**	**Body weight (g)**
MEAN	27.5	229.7
SD	4.135	6.022
SE	1.688	2.459
HIGH DOSE	**Food (g/day/rat)**	**Body weight (g)**
MEAN	21.33	227.2
SD	4.412	4.997
SE	1.801	2.04

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

Haematological Analysis

Diethyl ether was used to anaesthetize the animals before blood samples were collected through cardiac puncture into ethylene diamine tetra acetic acid (EDTA) tubes. Haematological parameters such as complete blood count, WBC, RBC, Platelet count, Haematocrit and HB [Table 3&4] were observed and recorded.

Table 3: Hematological Parameters after 28 Days Treatment with Uc in Rats

Grouping	Total red cells count (×10 6 µl)	Total WBC Count (×10 3µl)	Platelet count (×10 3µl)	Packed Cell Volume (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Blood sugar ® (mg/dl)	BUN (mg/dl)
CONTROL									
MEAN	7.333	8.333	565.8	46.5	55.5	21.83	33.83	74.17	13.83
SD	1.033	0.8165	11.18	3.619	3.728	3.971	2.483	5.076	2.317
SE	0.4216	0.3333	4.564	1.478	1.522	1.621	1.014	2.072	0.9458

LOW DOSE	Total red cells count (×10 6µl)	Total WBC Count (×10 3µl)	Platelet count (×10 3µl)	Packed Cell Volume (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Blood sugar ® (mg/dl)	BUN (mg/dl)
MEAN	8.7	10.42	570.2	48.17	61	24.17	43.83	74.83	13.67
SD	0.7874	1.796	5.419	1.835	4.472	5.115	3.764	4.665	2.16
SE	0.3215	0.7332	2.212	0.7491	1.826	2.088	1.537	1.905	0.8819

HIGH DOSE	Total red cells count (×10 6µl)	Total WBC Count (×10 3µl)	Platelet count (×10 3µl)	Packed Cell Volume (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Blood sugar ® (mg/dl)	BUN (mg/dl)
MEAN	8.767	7.983	563.5	42.17	56	21.17	44.17	72.83	13.5
SD	0.367	1.372	5.468	4.119	3.033	4.167	3.545	4.119	3.332
SE	0.1498	0.56	2.232	1.682	1.238	1.701	1.447	1.682	1.36

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

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

GROUPING	HB (G/DL)	NEUTROPHILS (%)	LYMPHOCYTES (%)	EOSINOPHILS (%)	MONOCYTES (%)	BASOPHILS (%)
CONTROL						
MEAN	15	69.5	31.83	1.367	0.8	0
SD	1.349	4.416	3.189	0.3445	0.2608	0
SE	0.5508	1.803	1.302	0.1406	0.1065	0
LOW DOSE	**HB (G/DL)**	**NEUTROPHILS (%)**	**LYMPHOCYTES (%)**	**EOSINOPHILS (%)**	**MONOCYTES (%)**	**BASOPHILS (%)**
MEAN	16.83	65	35.67	1.567	0.7333	0.1667
SD	3.371	4.099	3.777	0.367	0.2066	0.4082
SE	1.376	1.673	1.542	0.1498	0.08433	0.1667
HIGH DOSE	**HB (G/DL)**	**NEUTROPHILS (%)**	**LYMPHOCYTES (%)**	**EOSINOPHILS (%)**	**MONOCYTES (%)**	**BASOPHILS (%)**
MEAN	13.67	65.83	40.33	1.033	0.4833	0
SD	2.066	4.07	5.086	0.5538	0.04082	0
SE	0.8433	1.662	2.076	0.2261	0.01667	0

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

Biochemical Analysis

Serum samples were used for estimation of biochemical parameters. Sample of control and experimental rats were analysed for Total Protein, Albumin, SGOT, SGPT, Serum creatinine, Total Cholesterol, Triglycerides, HDL, LDL, VLDL and estimated as per colorimetric procedure [Table 5& 6].

Table 5: Bio Chemical Parameters after 28 Days Treatment with Uc In Rats

Grouping	**Serum total protein (g/dl)**	**Serum albumin (g/dl)**	**SGOT (AST) (IU/ml)**	**SGPT (ALT) (IU/L)**
CONTROL				
MEAN	5.967	2.517	244	62.33
SD	0.5317	0.4215	6.87	7.174
SE	0.2171	0.1721	2.805	2.929
LOW DOSE	**Serum total protein (g/dl)**	**Serum albumin (g/dl)**	**SGOT (AST) (IU/ml)**	**SGPT (ALT) (IU/L)**
MEAN	4.667	2.783	214.8	70.33
SD	0.7367	0.4446	3.189	3.141
SE	0.3007	0.1815	1.302	1.282
HIGH DOSE	**Serum total protein (g/dl)**	**Serum albumin (g/dl)**	**SGOT (AST) (IU/ml)**	**SGPT (ALT) (IU/L)**
MEAN	4.2	2.467	212.3	72.67
SD	0.6033	0.383	2.658	1.966
SE	0.2463	0.1563	1.085	0.8028

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

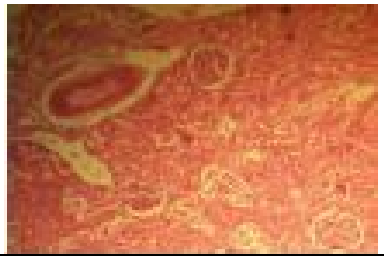
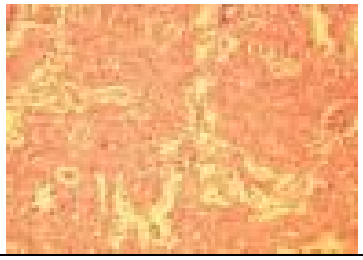
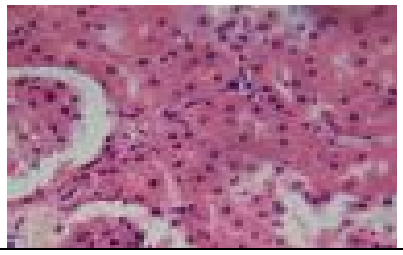
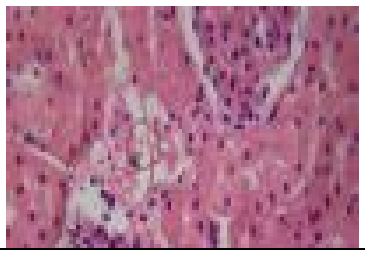
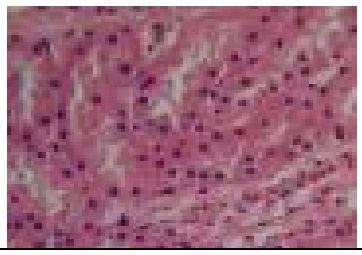
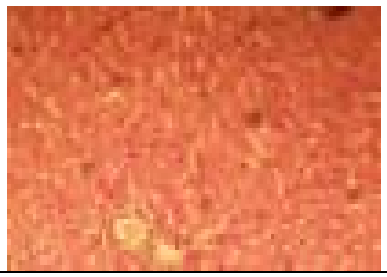
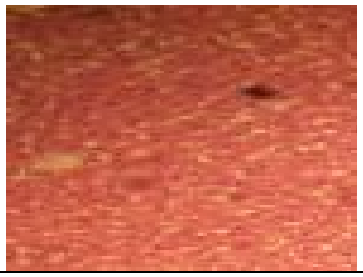
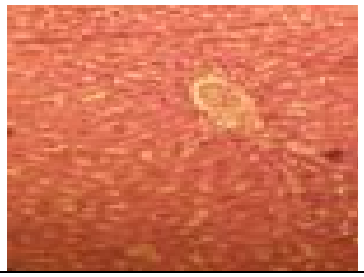
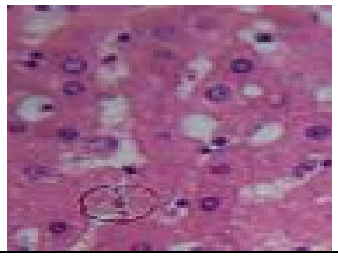
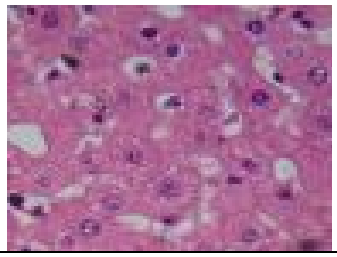
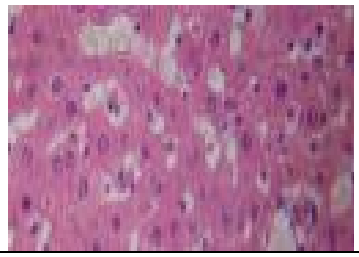
Table 6: Bio Chemical Parameters after 28 Days Treatment with Uc in Rats

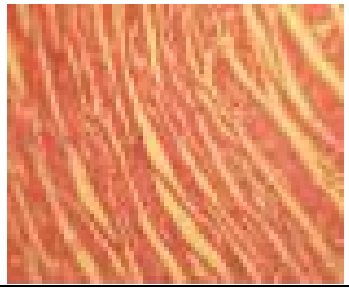
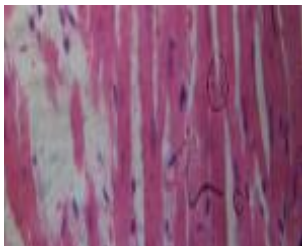
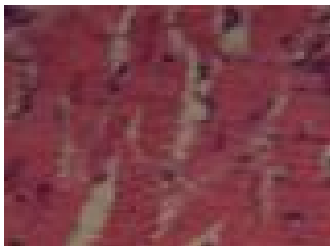
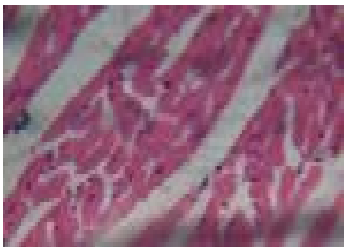
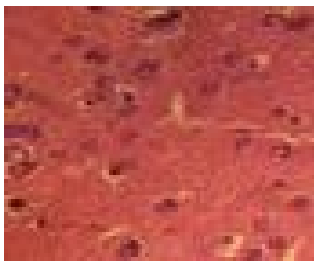
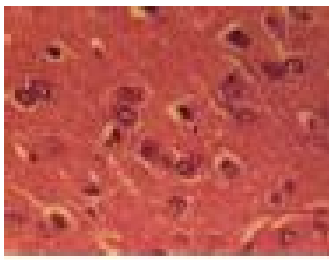
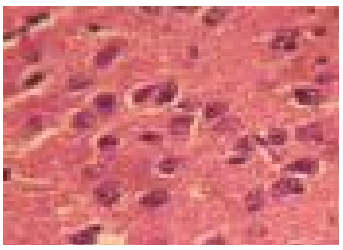
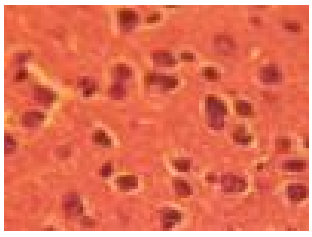
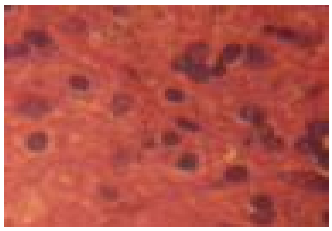
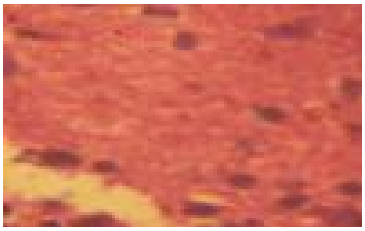
Grouping	Serum creatinine (mg/dl)	Serum total cholesterol (mg/dl)	Serum triglycerides level (mg/dl)	Serum HDL cholesterol (mg/dl)	Serum LDL cholesterol (mg/dl)	Serum VLDL Cholesterol (mg/dl)
CONTROL						
MEAN	0.7	95.33	48	26.17	44.33	34.67
SD	0.1673	2.875	4.98	3.312	4.761	2.805
SE	0.06831	1.174	2.033	1.352	1.944	1.145
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)**
MEAN	0.9667	103.5	48.17	28	46	34.67
SD	0.2338	3.619	3.601	6.356	3.899	3.882
SE	0.09545	1.478	1.47	2.595	1.592	1.585
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)**
MEAN	0.5667	103.7	42.5	30.17	40.67	33.33
SD	0.2066	2.658	1.761	4.119	2.338	3.445
SE	0.08433	1.085	0.7188	1.682	0.9545	1.406

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

Necropsy: All rats were sacrificed after the blood collection. The position, shapes, sizes, and colours of the internal organs were evaluated**.** The Spleen, Testes, Pancreas, Lung, Liver, Brain, Heart, Stomach, Intestine, Bone, Ovary, Kidney tissues were excised from all rats to visually detect gross lesions, and weighted to determine relative organ's weight and preserved in 10% buffered formalin and processed for routine paraffin block preparation. Sections of thickness about 5m were cut and stained with haematoxylin and eosin for histopathological investigation [9].

Table 7: Histo Pathological Analysis of Sub Acute Toxicity Study:

GROUP SAMPLE	CONTROL	LOW DOSE	HIGH DOSE
KIDNEY (Magnification Low power 10X)			
KIDNEY (Magnification High power 45X)			
LIVER (Magnification Low power 10X)			
LIVER (Magnification High power 45X)			

HEART (Magnification Low power 10X)			
HEART (Magnification High power 45X)			
BRAIN (Magnification Low power 10X)			
BRAIN (Magnification High power 45X)			

Statistical Analysis: All the data were expressed as mean ± S.E.M. Statistical significance between more than two groups were determined using One-way Analysis of Variance (ANOVA) followed by dunnet' test. Calculations were done using Graph Pad prism software. A *p* value of 0.05 or less ($p < 0.05$) were taken as significant.

RESULTS AND DISCUSSION

In acute toxicity study animals were not showing any significant toxic clinical signs during the period of 14 days observation [Table 1]. In sub-acute toxicity study no behavioural changes and death were observed at the end of the treatment period. Similarly, no significant differences in food intake and weight gain were observed between control and treated groups during the period of 28 days [Table 2]. The results of haematological investigations such as

Erythrocytes, Total leukocytes count, Platelet count conducted on the 29th day, revealed no significant changes in the values compared with those of respective controls [Table 3&4]. Serum creatinine, SGPT, SGOT, Total Protein, Albumin were within the limits [Table 5&6]. In histo pathological examination, Liver shows normal hepato cellular architecture and hepatic veins. No signs of necrosis or cirrhosis. Heart shows normal myocardial cells with no major signs of abnormalities in all three groups. Kidney shows normal lumen with no signs of bundle shrinkage or disruption. Brain shows normal inter neuronal distance with no signs of degeneration and oedema.

CONCLUSION

The acute and sub- acute toxicity study of *Uthamani Chooranam* revealed that no toxicity by oral route over the period of 28 days. So this study concluded that *Uthamani Chooranam* is suitable for therapeutic use in human with the dosage of recommendations of upto 400 mg/kg body weight p.o.

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