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Acute and subacute toxicity profiles on Siddha drug Thulasi Ennai in wistar rats

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ABSTRACT

Medicinal plants have been used in traditional medicines for their unmatched availability of bioactive compounds. Asthma is the most common chronic disease among children worldwide. It is ranked 16th among the leading causes of years lived with disability. Medicinal plants have played a vital role in the siddha system of medicine over centuries to cure acute and chronic illness. The aim of the present study was to investigate toxicity analysis to evaluate safety of the siddha drug Thulasi Ennai in vivo in wistar albino rats. Thulasi Ennai is a polyherbal siddha formulation mentioned in the ancient siddha books and literature, indicated to cure childhood bronchial asthma. In this study, Thulasi Ennai administered orally at a single dose of 2000mg/kg body weight and monitored for 14 days. For subacute toxicity study, Thulasi Ennai was orally administered in different doses of 200,400mg/kg body weight, daily for 28 days. At the end of each study physical parameters, hematological, biochemical and histopathological analysis were evaluated. No animals in each group of acute or subacute toxicity study showed mortality or clinical signs of toxicity throughout the study. Hence, the results of the study indicate a safe toxicological profile of Thulasi Ennai.

Keywords: Thulasi Ennai. Childhood bronchial asthma, Acute toxicity, Sub-acute toxicity.

INTRODUCTION

Medicinal plants are considered a repository of numerous types of bioactive compounds possessing varied therapeutic properties. According to the World Health Organisation survey report approximately 80% of the people in developing countries depend on traditional herbal related formulations to cure diseases [1]. Asthma is one among the top 20 chronic conditions of disability-adjusted life years in children. In India it was estimated that 1.9 disability adjusted life year (DALYs) are lost every year due to asthma per thousand children under 15 year of age [2]. Due to severity of the disease, children are frequently hospitalized and causing elementary school absenteeism which impaired the child academic achievement [3, 4].

Siddha is an ancient traditional system of medicine predominantly followed in south India for treating acute and chronic medical illness [5]. Botanicals constitute major part of these traditional medicine and standardization for herbal formulations is an essential factor in order to assess the quality of the drugs based on the concentration of their active principle and to ensure safety profile of each medicinal plants [6]. A pre-clinical toxicity study is mandatory in determining a safety dose and to identify adverse effects of the drug before initiating the human trial [7]. Thulasi Ennai is a poly herbal Siddha formulation containing *Ocimum tenuiflorum* (*karunthulasi*), *Plastoma menthoids* (*Nilathulasi*), *Ocimum americanum* (*kanjankorai*), *Artemisia nilagirica* (*masipathri*), *Aegle marmelos* (*vilvam*), *Allium sativum* (*vellulli*), *Zingiber officinale* (*Chukku*), *Piper nigrum* (*Milagu*), *Piper longum* (*Arisi thipilli*), *Ricinus communis* (*Amanakku*) and honey. The interventional study drug Thulasi Ennai has been quoted in Siddha literature "Anuboga vaithiya navaneetham" for childhood bronchial asthma. The purpose of the present study aimed at evaluating the acute and sub-acute toxicity of Thulasi Ennai in wistar albino rats.

MATERIALS AND METHODS

The ingredients of TE were authenticated by the Department of Medicinal botany and pharmacognosy, Government Siddha Medical College, Chennai, Tamil Nadu, India. The study was authorised by the Institutional Animal Ethical Committee of Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India. The entire preclinical study followed the guidelines of Organization for Economic Co-operation and Development (OECD).

Preparation of the Thulasi Ennai

650ml of castor oil *amanakku* (*Ricinus communis*), 336ml of *karunthulasi* (*Ocimum tenuiflorum*) leaf

extract, 168 ml of *nilathulasi* (*Plastoma menthoids*), *kanchakorai*, (*Ocimum americanum*), *masipathi* (*Artemisia nilagirica*), *vilvam* (*Aegle marmelos*) leaf extracts and 168 ml of garlic extracts (*Allium sativum*). The dried and powdered form of 8.4grams of *chukku* (*Zingiber officinale*), *milagu* (*Piper nigrum* and *arisi thipilli* (*Piper longum*) and finally 84 ml of honey. The preparations followed as per Siddha literature.

Acute toxicity study

The acute oral toxicity study for TE was carried out as per OECD guideline 423 [8]. The study was observed by administering a single oral dose of Thulasi Ennai in experimental rats.

Animal

The healthy adult Wistar albino rat weighing between 180-200grams were selected for the study. The animals were housed in polypropylene cages ventilated with 100% fresh air by using an air handling unit (AHU). A12 hours light/12 hours dark cycle was maintained at a standard room temperature 22±2°C with relative humidity was 50-65%. The rats provided food (supplied by Amruth, kvat, Hyderabad, India) and water ad libitum. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study.

Study description

6 adult female rats selected to perform acute toxicity study. 2000mg/kg dose utilized for evaluation of the study which is considered higher than that of normal therapeutic dose. The test drug Thulasi Ennai administered to the rat was calculated as the body weight of the rat per ml of the test drug was found to be 1.8gm/ml. The animals were continuously observed inside the cages for the first 24 hours and it is continued once daily for 14 days to rule out the emerging signs of behavioural changes, body weight changes and for mortality. Attention was directed to observations of Lacrimation, salivation, animal

appearance, open field behaviour, cage behaviour, convulsion, laxative action, sensory responses, mobility/balancing, skin tone, CNS abnormalities, CVS abnormalities, respiratory distress, muscle strength/coordination, urine analysis, faecal pellet consistency and mortality.

Sub-acute toxicity study

This study was implemented as per OECD guidelines-407 [9]. Totally 18 rats with both genders were utilized for the study. The animals are randomly assigned into three groups. Each group consists of 3 males and 3 female rats (n=6). The group-1 rats considered has control were received only normal saline 5ml/kg b.w (p.o). Group-II rats received low dose of test drug Thulasi Ennai in 200mg/kg b.w(p.o) and finally group III were received 400mg/kg b.w(p.o). All three groups administered with assigned study drug dose once daily for 28 days. During the period of the trial, the rats weighed periodically and the intake of food and water consumption, excretory materials, behaviour pattern of the animals were monitored. At the end of 28th day of the trial the animals were fasted for overnight with free access to water. The next day the animals were sacrificed with excess anaesthesia. Blood samples were collected from aorta and stored in EDTA (ethylenediamine-tetraacetate) for haematological and biochemical analysis. The vital organs including heart, brain, lungs, spleen, kidneys, liver, stomach, testes, and ovary were harvested and carefully examined for gross lesions. The organs were preserved in 10% formalin for histopathological assessment and interpretation.

FAECAL PELLETS ANALYSIS

Methodology

Rats of three groups were allowed to travel in an open field sterile stainless-steel tray to collect the pellets. The collected pellets were analysed for consistency, colour, shape and presence of blood cells.

Table 1: Faecal pellet analysis of rats exposed to Thulasi Ennai

Acute Toxicity Study				
Analysis	Thulasi Ennai			
Consistency	Pasty			
Shape	Irregular			
Colour	Pale greenish			
Mucous Shedding	Absent			
Blood Cells	Absent			
Signs of Infection	None Observed			
Sub-Acute Toxicity Study				
Analysis	Control	Low Dose	High Dose	
Consistency	Rigid	Pasty	Pasty	
Shape	Oblong	Irregular	Irregular	
Colour	Greenish	Pale greenish	Pale greenish	
Mucous Shedding	Absence	Absence	Absence	
Blood Cells	Absent	Absent	Absent	
Signs of Infection	None Observed	None Observed	None Observed	



Figure 1



Figure 2

Hematological analysis

Toxicity analysis of hematological parameters included Packed cell volume (PCV), Red blood cell count (RBC), White blood cell count (WBC), Platelet count (PLT), Haemoglobin (HGB), Mean corpuscular haemoglobin concentration (MCHC), Mean corpuscular Volume (MCV), Mean corpuscular haemoglobin (MCH), Mean platelet volume (MPV), Neutrophils, Eosinophils, Lymphocytes and Monocytes. The analysis was done using an automated Bayer Hematology analyzer.

Biochemical analysis ^[10]

Serum samples were collected from the rats to find out toxicity changes in the biochemical parameters namely high-density lipoprotein (HDL), low density lipoprotein (LDL), very- low density lipoprotein (VLDL), triglycerides(TGL), total cholesterol, blood urea nitrogen (BUN), creatinine, albumin, total protein, glucose, uric acid, aspartate transaminase (AST), alanine amino transaminase (ALT) and alkaline phosphatase (ALP) using mind ray auto analyzer model BS 120.

Histopathological evaluation ^[11]

Histopathological investigation of the vital organs was done. The organs such as heart, brain, lungs, spleen, kidneys, liver, stomach, testes and ovary were removed carefully and the relative organ weights were calculated for each organ separately from all the animals. Histology slides of all the organs were made to observe pathological lesions under the microscope. The examinations done in the preserved tissues with particular emphasis on those which showed gross pathological changes.

Statistical analysis

Data were expressed in mean \pm standard error mean (SEM). Comparison between the means was done by analysis of variance (ANOVA) followed by dunnett-t-test using (GraphPad prism 5.0). The value less than 0.05 ($P < 0.05$) was considered as significant.

RESULTS AND DISCUSSION

Acute toxicity study

The gross behavioural changes of rats (n = 6) were monitored after administering Thulasi Ennai (Table 2). There were no toxic lesions and mortality observed at the maximum dose level of 2000mg/kg body weight in acute oral toxicity. The body weight of rats observed on the initial day and the 14th day. The values were expressed in mean \pm SEM with significant analysis done between control and treatment groups using one -way ANOVA followed by Dunnett's test. The body weight of the initial day of rats shows (183.7 \pm 0.91) and the final day was (190.3 \pm 3.36) ($P > 0.05$). This result expresses the healthy body condition of the dose treated animals.

Sub-acute toxicity study

The results from 28-day repeated oral toxicity study in dose treated groups 200, 400mg/kg body weight of wistar albino rats indicates there are no changes in general behaviour and no mortality was observed throughout the entire period of study. Further there was significant body weight gain (Table 2) was noted compared to the initial day of assessment. It can be stated that Thulasi Ennai did not interfere with the

normal metabolism of animals. There were no alterations in intake of feed and water consumptions (Table 3) exhibited between the groups. Similarly, the results of this study revealed no significant changes were observed between the groups in the relative organ weight (Table 4) and gross histopathological examination (Figure 3) of brain, heart, lungs, stomach, liver, spleen, kidneys, testes, uterus, and ovary. This result showed that Thulasi Ennai in 28 days repeated oral dose produced no effect on the normal growth, or no signs of toxicity developed in dose treated groups.

Table 2: Effect of Thulasi Ennai on body weight of rats in sub-acute toxicity study

Dose(mg/kg/day)	Body weight in grams	
	Initial	Final
Control	185.7 \pm 0.42	216.7 \pm 1.76
200	182.5 \pm 0.56	217 \pm 3.67
400	184.7 \pm 0.66	216.2 \pm 1.81

Values are mean \pm SEM (n = 6 per group). Dunnett's test. ^{ns} $P > 0.05$.

Table 3: Quantitative data on food and water intake of rats treated with Thulasi Ennai for 28 days in sub-acute toxicity study

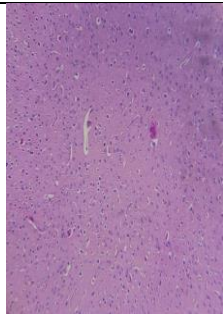
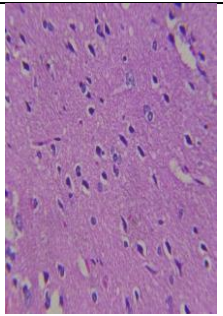
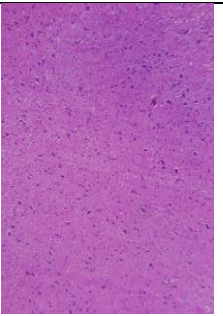
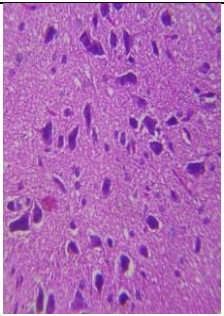
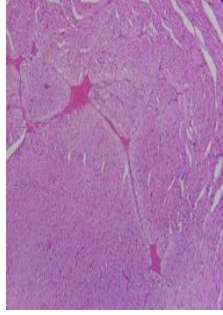
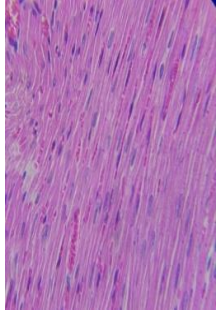
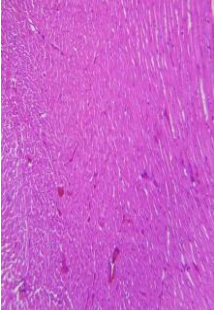
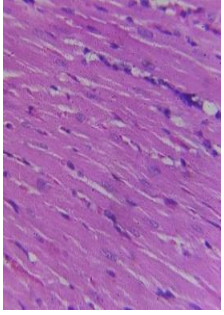
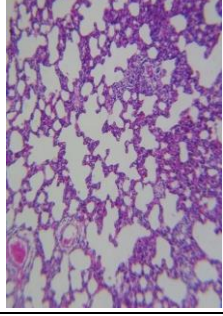
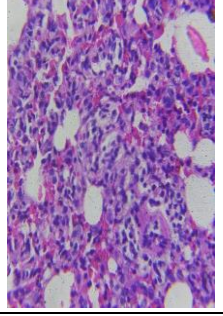
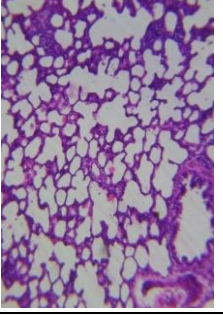
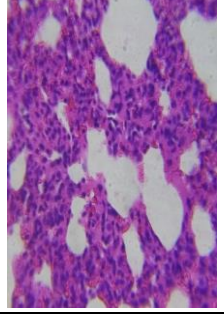
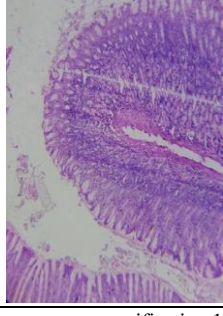
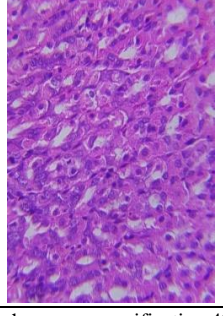
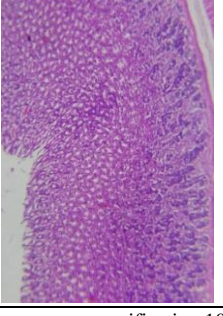
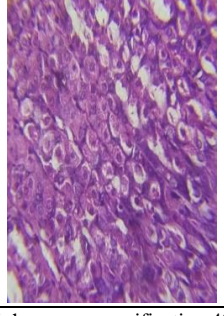
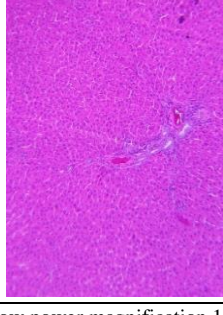
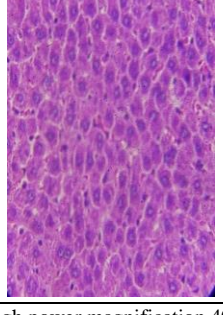
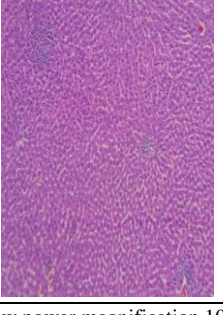
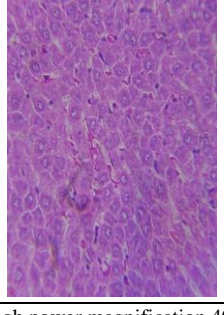
Dose(mg/kg/day)	Average feed and water intake	
	Feed intake in grams	Water intake in ml
Control	15.33 \pm 0.98	23.5 \pm 0.76
200	16.5 \pm 0.76	23.83 \pm 1.07
400	16.17 \pm 0.79	25.67 \pm 0.61

Values are mean \pm SEM. (n = 6 per group). Dunnett's test. ^{ns} $P > 0.05$.

Table 4: Effect of Thulasi Ennai in sub-acute oral administration on absolute organ weight of control and treatment group rats

Dose(mg/kg)	Wistar Rats	Control	200mg/kg	400mg/kg
Brain(g)	Male	1.76 \pm 0.03	1.72 \pm 0.07	1.57 \pm 0.06
	Female	1.53 \pm 0.06	1.63 \pm 0.06	1.58 \pm 0.01
Heart(g)	Male	0.59 \pm 0.04	0.56 \pm 0.01	0.51 \pm 0.06
	Female	0.58 \pm 0.08	0.52 \pm 0.02	0.69 \pm 0.03
Lung(g)	Male	1.56 \pm 0.15	1.33 \pm 0.28	0.93 \pm 0.06
	Female	1.60 \pm 0.04	2.79 \pm 0.48	1.30 \pm 0.07
Stomach(g)	Male	1.51 \pm 0.17	1.47 \pm 0.08	1.36 \pm 0.04
	Female	1.39 \pm 0.08	1.79 \pm 0.16	1.53 \pm 0.14
Liver(g)	Male	4.37 \pm 0.23	3.92 \pm 0.13	3.68 \pm 0.17
	Female	3.97 \pm 0.20	4.91 \pm 0.30	4.25 \pm 0.41
Spleen(g)	Male	0.34 \pm 0.03	0.40 \pm 0.07	0.28 \pm 0.03
	Female	0.67 \pm 0.11	0.67 \pm 0.10	0.65 \pm 0.15
Kidney (g)	Male	1.22 \pm 0.10	1.09 \pm 0.03	0.98 \pm 0.05
	Female	1.00 \pm 0.07	1.09 \pm 0.10	1.12 \pm 0.12
Testes (g)	Male	2.93 \pm 0.40	2.22 \pm 0.61	1.41 \pm 0.16
Uterus (g)	Female	0.26 \pm 0.03	0.17 \pm 0.07	0.40 \pm 0.15
Ovary (g)	Female	0.09 \pm 0.00	0.06 \pm 0.02	0.19 \pm 0.09

Values are mean \pm SEM (n = 6 per group). Control and treatment groups were compared. Dunnett's test. ^{ns} $P > 0.05$.

	Control		High dose	
Brain				
	Low power magnification 10x	High power magnification 40x	Low power magnification 10x	High power magnification 40x
Heart				
	Low power magnification 10x	High power magnification 40x	Low power magnification 10x	High power magnification 40x
Lungs				
	Low power magnification 10x	High power magnification 40x	Low power magnification 10x	High power magnification 40x
Stomach				
	Low power magnification 10x	High power magnification 40x	Low power magnification 10x	High power magnification 40x
Liver				
	Low power magnification 10x	High power magnification 40x	Low power magnification 10x	High power magnification 40x

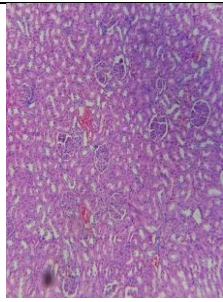
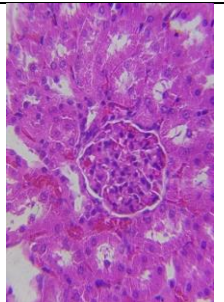
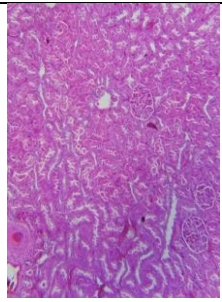
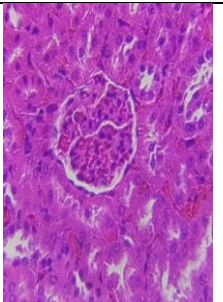
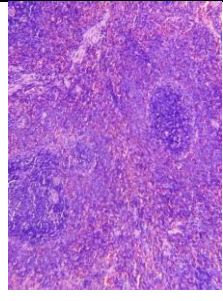
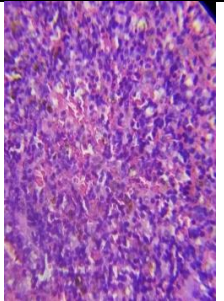
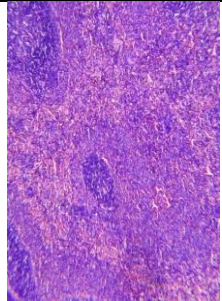
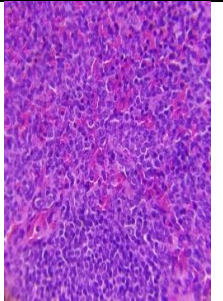
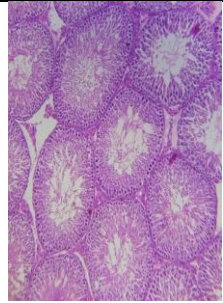
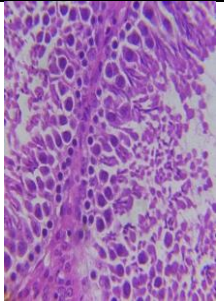
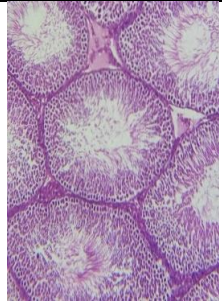
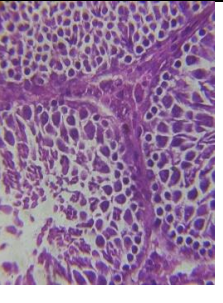
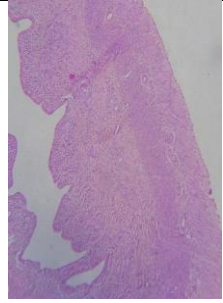
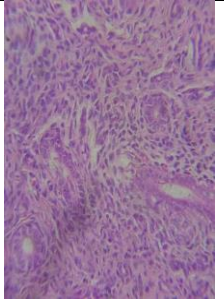
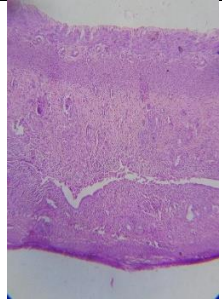
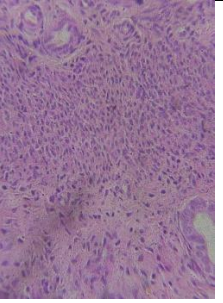
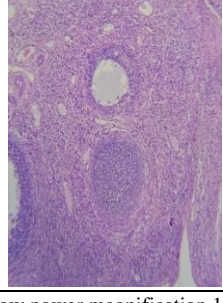
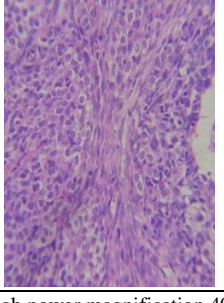
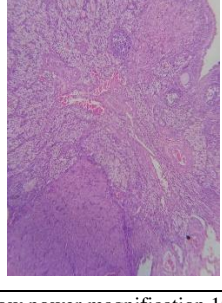
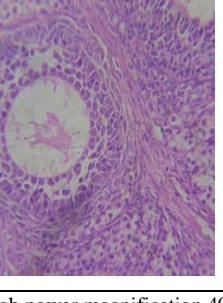
Kidney				
	Low power magnification 10x	High power magnification 40x	Low power magnification 10x	High power magnification 40x
Spleen				
	Low power magnification 10x	High power magnification 40x	Low power magnification 10x	High power magnification 40x
Testes				
	Low power magnification 10x	High power magnification 40x	Low power magnification 10x	High power magnification 40x
Uterus				
	Low power magnification 10x	High power magnification 40x	Low power magnification 10x	High power magnification 40x
Ovary				
	Low power magnification 10x	High power magnification 40x	Low power magnification 10x	High power magnification 40x

Figure 3: Histopathology of vital organs

Hematological and blood biochemical analysis

In the haematological parameters (Table 5), there was a marked change in platelet count noted in the treatment group ($P<0.05$). Studies suggest that platelets are activated in bronchial asthma [12]. There is also an increase in haematocrit value ($P<0.05$) in the dose treated group compared to the control group and there is no alteration in other haematological parameters. The hemopoietic system serves as an important index for toxic compounds to rule out pathological

conditions in humans and animals [13]. From the biochemical analysis (Table 6) report the LDL parameter showed significance ($P<0.01$) in the animals treated with TE. The transaminases SGOT and SGPT could be used as a parameter to detect liver damage. In this study there were no changes in the levels of SGOT and SGPT in Thulasi Ennai treated groups. This result proves there are no alterations done in the liver metabolism. Based on the results of 28 days repeated oral toxicity there were no signs of toxicity in the Thulasi Ennai treated groups and no mortality was observed up to the dose level of 400mg/kg body weight.

Table 5: Haematology parameters after 28 days study with Thulasi Ennai in wistar rats

S.no	Haematology parameters	Control	Low dose (200mg/kg)	High dose (400mg/kg)
1	RBC ($\times 10^6 \mu\text{l}$)	6.76 \pm 0.49	7.25 \pm 0.41	6.80 \pm 0.49
2	WBC ($\times 10^3 \mu\text{l}$)	8.96 \pm 1.23	7.80 \pm 1.01	7.03 \pm 0.86
3	PLT ($\times 10^3 \mu\text{l}$)	754.17 \pm 35.03	525.00 \pm 42.34*	607.83 \pm 67.79*
4	Hgb (g/dl)	11.23 \pm 0.29	12.76 \pm 0.34*	12.00 \pm 0.66
5	MCH (pg)	19.78 \pm 0.76	19.26 \pm 1.33	16.00 \pm 1.91
6	MCV (fl)	61.06 \pm 1.96	63.93 \pm 2.04	64.50 \pm 0.51
7	Neutrophils $10^3/\text{mm}^3$	2.75 \pm 0.42	2.50 \pm 0.19	2.81 \pm 0.14
8	Eosinophils (%)	1.41 \pm 0.07	1.41 \pm 0.12	1.48 \pm 0.13
9	Basophils (%)	0.17 \pm 0.16	0.17 \pm 0.16	0.17 \pm 0.16
10	Lymphocyte (%)	72.73 \pm 5.30	68.86 \pm 4.32	75.86 \pm 2.29
11	Monocyte (%)	3.06 \pm 0.51	2.26 \pm 0.34	3.05 \pm 0.35

Values are mean \pm SEM (n=6 animals per group) * $P<0.05$; ** $P<0.01$ vs control

Table 6: Biochemical analysis after 28 days study with Thulasi Ennai in wistar rats

S.no	Parameter	Control	Low dose (200mg/kg)	High dose (400mg/kg)
1	BUN (mg/dl)	12.67 \pm 1.25	14.67 \pm 0.98	13.17 \pm 0.94
2	Serum Creatinine ($\times 10^3 \mu\text{l}$)	0.60 \pm 0.05	0.60 \pm 0.05	0.60 \pm 0.05
3	Total Bilirubin(mg/dl)	0.41 \pm 0.07	0.50 \pm 0.09	0.41 \pm 0.06
4	SGOT (IU/ml)	88.83 \pm 7.67	83.33 \pm 3.71	85.00 \pm 6.06
5	SGPT(IU/ml)	27.50 \pm 1.76	31.67 \pm 2.61	24.50 \pm 2.93
6	Total cholesterol(mg/dl)	111.50 \pm 2.21	118.95 \pm 7.41	103.71 \pm 16.58
7	HDL (mg/dl)	61.33 \pm 2.12	53.50 \pm 3.55	56.83 \pm 3.93
8	LDL (mg/dl)	36.17 \pm 1.70	50.50 \pm 4.47*	48.67 \pm 3.93*
9	VLDL (mg/dl)	14.00 \pm 1.06	14.95 \pm 0.87	14.88 \pm 1.51
10	TG (mg/dl)	34.17 \pm 4.12	29.33 \pm 3.39	30.67 \pm 1.82

Values are mean \pm SEM (n=6 animals per group) * $P<0.05$; ** $P<0.01$ vs control

CONCLUSION

In conclusion, Thulasi Ennai in acute toxicity study at a maximum dose of 2000mg/kg and sub-acute toxicity study in high doses 400mg/kg suggests no toxic effects and no mortality was observed. So, the evidence of the study recommends Thulasi Ennai can be safe to prescribe for therapeutic use in humans for long term therapy.

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