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Research Article

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Acute and sub acute toxicity study of Siddha formulation of Lavankathi Chooranam in wistar albino rats

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Abstract

Lavankathi Chooranam (LC) is a compound polyherbal Siddha formulation is indicated for Respiratory disease, Fever, Menaregea, Diarrhea, Thirst, Myocardial infaction , ect. This formulation has 30 drugs. Still now no safty profile has been published for this formulation. Present study acute and sub acute toxicity study about the formulation. As a mandatory steps were taken to evaluate safety and efficacy of trial drug LC in wistar albino rats. According to OECD guidelines, acute toxicity single dose of 300mg and 2000 mg LC were administered, and monitor for 14 days. Sub acute toxicity studies were carried out in five groups of six animals. LC administered to rats at the dose 50mg, 100mg, 200mg and 400 mg/kg/day for 20 days. Detailed hemotological, biochemical, histopathological evaluation of different organs was performed in all animals. Histo-pathological analysis revealed that the liver, lung, kidney, heart of treated groups did not show any signs of toxicity. The results obtained, up to the dose level of 300mg/kg/bodyweight, and the P value is (<0.05). LC was non-toxic both acute and sub-acute toxicity study. Previously some article published in the same name LC.

Keywords: Siddha medicine, Lavankathi Chooranam (LC), Acute and Sub Acute toxicity

Introduction

Determination of acute oral toxicity is usually the initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute, sub-acute and chronic toxicity. Acute toxicity is involved in estimation of LD_{50} (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals) (Shetty Akhila, *et al.,* 2007).(1)

Aim of the study

Aim of the study is to evaluate the acute toxic effect of the Siddha drug LAvankathi Chooranam (LC) in Albino rats.

Materials and Methods

Acute oral toxicity of Lavankathi Chooranam is carried out as per the guidelines Organization of Economic Co-operation and Development (OECD) -423 guidelines after the animal ethical clearance from Institutional Animal Ethics Committee.

The albino mice are fasted over night and provided only water, after which the **Lavankathi Chooranam** is administered by gastric intubations to the relevant group of animals orally at the dose of 50 $mg.kg^{-1}$ body weight in Tween-80. The animals are then observed for 14 days and maintained with normal food. A mortality rate of 2 or 3 animals in 14 days is recorded and the dose is said to be toxic dose. But when mortality of one animal is observed, then the same dose is repeated again for confirmation. However, if mortality is not observed, the procedure is repeated for further higher doses such as 300 and 2,000 $mg.kg^{-1}$ body weight. Toxic symptoms are observed for 72 hrs including behavioral changes, locomotion, convulsions and mortality (Shah Ayub, 1997, Bürger, 2005).(2,3).

Cage Side Observations

Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Special attention is directed for the observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.
Body Weight, Food and Water Intake

Body weight, food and water intake are recorded at two-day intervals.

Pathology

Surviving animals are fasted overnight, weighed and humanely killed on the 15^{th} day using anesthetic ether. All test animals are subjected to gross necropsy.

Sub-chronic test for Lavankathi chooranam

Material and Method:

Male and female Wistar rats weighing 180 ± 10 g are used for the present study. The animals are divided into five groups of six animals each. The dose of the preparation is calculated based on the body weight of the animal. The animals in Group I are administered with a single daily dose of 0.5 ml of Tween 80 orally for 20 days. The animals in Group II are administered with 50 $mg.kg^{-1}$b.w. of the Lavankathi Chooranamorally once daily for 20 days. The animals in Group III are administered with 100 $mg.kg^{-1}$b.w. of the Lavankathi Chooranam orally once daily for 20 days. The animals in Group IV and V are administered once daily with 200 and 400 $mg.kg^{-1}$b.w. of the Lavankathi Chooranam respectively for 20 days orally (Pieme,*et al* 2006, Joshi, *et al* 2007, Mythilypriya, *et al.,* 2007).(4,5,6)The animals are then weighed every five days, from the start of the treatment, to record the weight variation. At the end of the treatment, blood samples are collected by puncturing retro orbital plexus after mild anesthesia for biochemical analysis. The collected blood sample is centrifuged within 5 min of collection at 4000 g for 10 min to obtain plasma, which is analyzed for total cholesterol, total triglyceride, HDL-cholesterol levels,LDL-cholesterol,plasma glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and urea.

Results

Acute toxicity study with Lavankathi Chooranam

The acute toxicity of Lavankathi Chooranamwas evaluated using OECD- 423 guidelines. There was no mortality or morbidity observed in animals through the 15-days period following single oral administration at all selected dose levels of the Lavankathi Chooranam(Table-1). The animals did not show any changes in the general appearance during the observation period. Morphological characteristics such as fur, skin, eyes and nose appeared normal. No tremors, convulsion, salivation, diarrhea, lethargy or unusual behaviors such as self mutilation, walking backward and so forth were observed. Gait and posture, reactivity to handling or sensory stimuli, grip strength was also normal.

Table – 01

	Dose ($mg.kg^{-1}$)	Sign of Toxicity ($ST.NB^{-1}$)	Mortality ($D.S^{-1}$)
Group I	0	0/3	0/3
Group II	300	0/3	0/3
Group III	2000	0/3	3/3

Acute toxicity studyof Lavankathi Chooranam on experimental mice. The acute toxicityof Lavankathi Chooranam on experimental mice was tested using OECD-423 guidelines, where ST- sign of toxicity; NB-normal behaviour; D- died; S- survive. Values are expressed as number of animals (n=3).

Effect of Lavankathi Chooranam in Sub chronic Toxicity

Lavankathi Chooranamwere evaluated for sub chronic toxicity.

Effect of Lavankathi Chooranam on body weight changes in rats

The effect of Lavankathi Chooranamwas observed for their effect on the body weight changes from the study it was observed that, there was significant increase ($p < 0.05$) in body weight in all the animals observed. The results are shown in Table 02

Table – 02

Treatment	Day 1	Day 5	Day 10	Day 20
Control	189.19±5.4	189.40 ±6.14	199.10 ±6.30	199.6±6.30
Lavankathi Chooranam **50 mgkg^{-1}**	196.34 ±6.2	199.30 ±6.45	200.48 ±6.75	200.30±6.84*
Lavankathi Chooranam **100 mgkg^{-1}**	189.36 ±6.0	196.43 ±6.40	198.30 ±6.54	200.84±6.70*
Lavankathi Chooranam **200 mgkg^{-1}**	198.25 ±7.0	200.20±6.32	200.48 ±6.58**	208.35±6.72**
Lavankathi Chooranam **400 mgkg^{-1}**	179.54 ±6.34	196.35 ±6.60	198.15 ±6.65**	206.52±6.74**

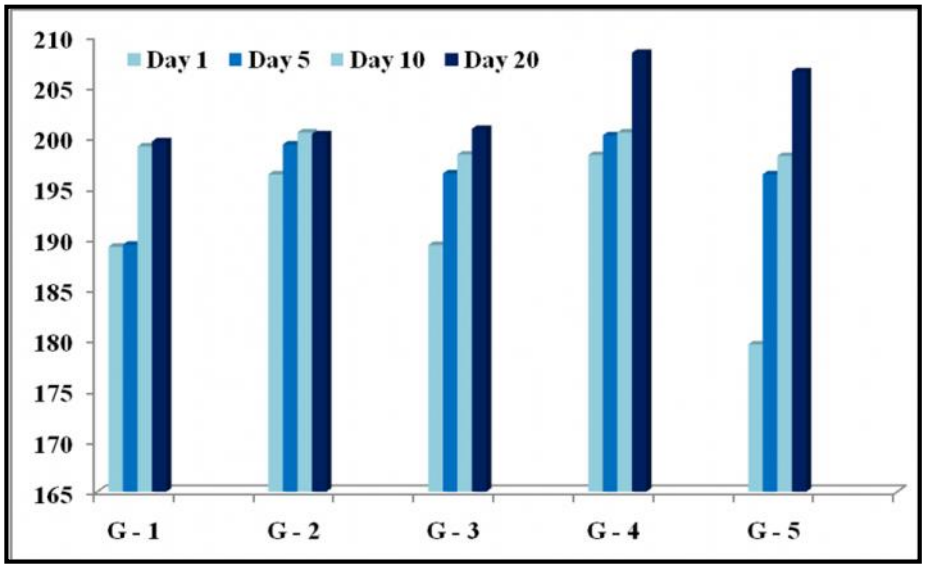

Figure – 01

The effects of **Lavankathi Chooranam** on body weight changes in rats. A study on the effects of **Lavankathi Chooranam** on body weight changes in rats was carried out.. where, group I animals (GPI) were treated with normal saline (5 ml.kg^{-1}), group II animals (GPII) with 50 mg.kg^{-1} of **Lavankathi Chooranam**, group III animals (GPIII) with 100 mg.kg^{-1} of **Lavankathi Chooranam**, group IV animals (GPIV) with 200 mg.kg^{-1} of **Lavankathi Chooranam**, group V animals (GPV) with 400 mg.kg^{-1}**Lavankathi Chooranam**. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01 *P<0.05.

Effect of Lavankathi Chooranam on kidney, heart, liver and brain in rats.

The effects of **Lavankathi Chooranam** on kidney, heart, liver and brainof the rats were observed.From the study it was clear that, significant ($p<0.01$) changes in the weights of various organs of the animals occurred with higher doses of the extract (400 mg.kg^{-1}bwt), but macroscopic examinations did not show any changes in colour of the organs of the treated animals compared with the control. The results are shown in Table.3.

Table - 03

Treatment	Heart (gms)	Kidney (gms)	Liver (gms)	Brain (gms)
Control	0.35 ± 0.04	0.72± 0.03	3.32± 0.14	0.72± 0.05
Lavankathi Chooranam @ 50 mgkg^{-1}	0.36± 0.05	0.82± 0.05	3.42± 0.19	0.70± 0.03
Lavankathi Chooranam @ 100 mgkg^{-1}	0.39± 0.06	0.82± 0.04	3.44±0.21	0.68± 0.08
Lavankathi Chooranam @ 200 mgkg^{-1}	0.34± 0.03	0.75± 0.02	3.36± 0.22	0.76± 0.09
Lavankathi Chooranam @ 400 mgkg^{-1}	0.37± 0.05	0.74± 0.02	3.38± 0.15	0.75± 0.12

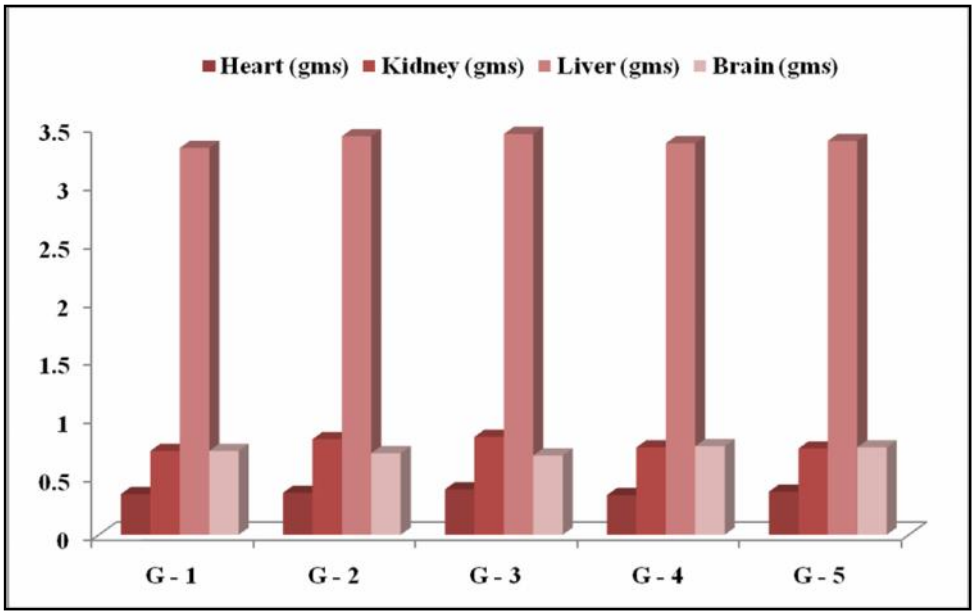

Figure - 2

The effects of **Lavankathi Chooranam**on kidney, heart, liver and brainof the rats. A study on the effects of **Lavankathi Chooranam**on kidney, heart, liver and brainof the rats was tested. where, group I animals (GPI) treated with normal saline (5 ml.kg^{-1}), group II animals (GPII) with 50 mg.kg^{-1} of **Lavankathi Chooranam**, group III animals (GPIII) with 100 mg.kg^{-1} of **Lavankathi Chooranam**, group IV animals (GPIV) with 200 mg.kg^{-1} of **Lavankathi Chooranam**, group V animals (GPV) with 400 mg.kg^{-1}**Lavankathi Chooranam**. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01.

Effect of Lavankathi Chooranam on biochemical profiles of rats

The effect of **Lavankathi Chooranam** on various biochemical parameters of the experimental animal 'rats' were tested.From the study it was evident that, there was significant decrease ($p<0.05$) in the plasma glucose level in treated rats especially at higher dose (400 mg.kg^{-1}) compared with control rats. The control rats were administered only with 5 ml of normal saline. Significant decrease ($p<0.05$) in the plasma total cholesterol (TC), triglyceride (TG) and LDL-cholesterol levels were observed. But a significant increase ($p<0.05$) in HDL-cholesterol levels were observed in all the treated animals compared with the control animals. AST, ALT and ALP levels were also normal in the **Lavankathi Chooranam** treated animals. From the results of biochemical study there was no evidence of severe toxicity associated with the administration of higher concentration of **Lavankathi Chooranam**. The results are shown in Table.4.

Table - 04

Treatment	Glucose ($mg.dl^{-1}$)	Cholesterol ($mg.dl^{-1}$)	Triglyceride ($mg.dl^{-1}$)	HDL ($mg.dl^{-1}$)	LDL ($mg.dl^{-1}$)
Control	99.42±1.74	44.05± 0.62	33.25±1.43	143.45±3.15	80.30±1.85
Lavankathi Chooranam @ 50 $mgkg^{-1}$	97.50±1.62	30.30± 0.36*	16.36± 0.85*	181.40±3.65*	75.75±1.38
Lavankathi Chooranam @ 100 $mgkg^{-1}$	95.44±1.52	27.65± 0.30*	18.32± 0.90*	170.30±3.40*	74.54±1.30
Lavankathi Chooranam @ 200 $mgkg^{-1}$	94.30±1.35**	34.20± 0.38	20.40± 0.92*	189.34±3.70*	51.52±1.18
Lavankathi Chooranam @ 400 $mgkg^{-1}$	97.28±1.43**	35.45± 0.48	23.30±1.15*	187.24±3.66*	50.30±1.05

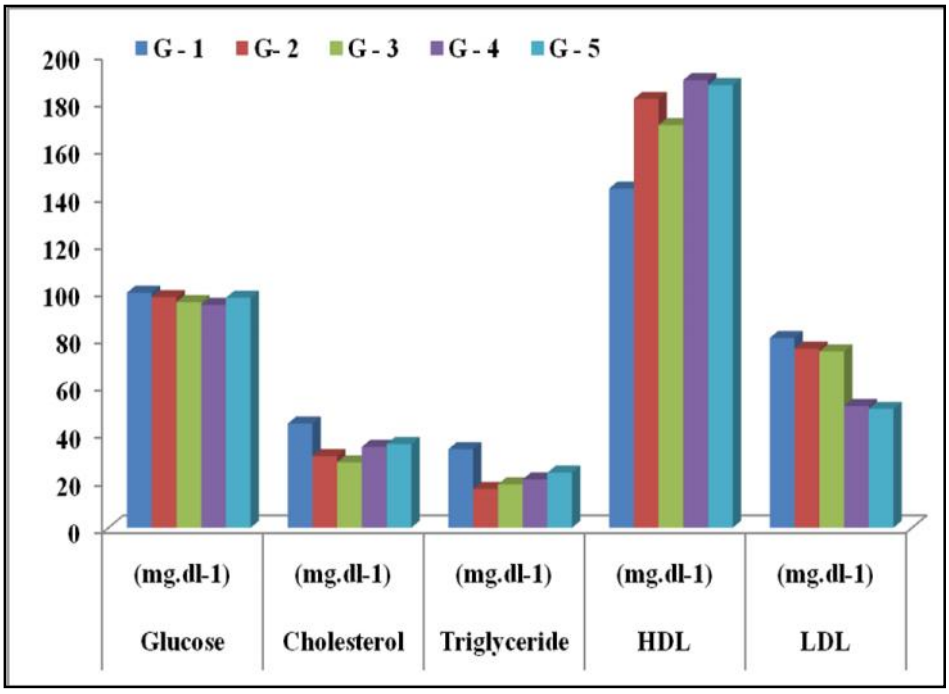

Figure - 03

The effect of **Lavankathi Chooranam** on biochemical parameters such as glucose, cholesterol, triglyceride, HDL and LDL. A study on the effect of **Lavankathi Chooranam**on biochemical parameters such as glucose, cholesterol, triglyceride, HDL and LDL in rats was tested.where, group I animals (GPI) treated with normal saline (5 $ml.kg^{-1}$), group II animals (GPII) with 50 $mg.kg^{-1}$ of **Lavankathi Chooranam**, group III animals (GPIII) with 100 $mg.kg^{-1}$ of **Lavankathi Chooranam**, group IV animals (GPIV) with 200 $mg.kg^{-1}$ of, group V animals (GPV) with 400 $mg.kg^{-1}$**Lavankathi Chooranam**. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01 *P<0.05

Table - 05

Treatment	AST ($IU.l^{-1}$)	ALT ($IU.l^{-1}$)	ALP ($IU.l^{-1}$)	TP ($g.l^{-1}$)	ALBUMIN ($g.l^{-1}$)
Control	320.3±11.60	65.4± 3.42	255.35± 8.60	73.36± 3.28	43.30±2.45
LC @ 50 $mgkg^{-1}$	310.4±10.52**	63.3± 2.90**	257.15±8.75**	73.30±3.20	40.24±2.30
LC @ 100 $mgkg^{-1}$	309.5±10.60**	60.3±2.92**	262.38±8.30**	83.12±3.80	41.30±2.45
LC @ 200 $mgkg^{-1}$	308.5±9.90	57.3± 2.38	258.20±8.36	74.35± 3.65	42.28±2.46
LC @ 400 $mgkg^{-1}$	310.4±9.94	57.6±2.45	258.42±8.44	75.30± 3.75	42.64±2.50

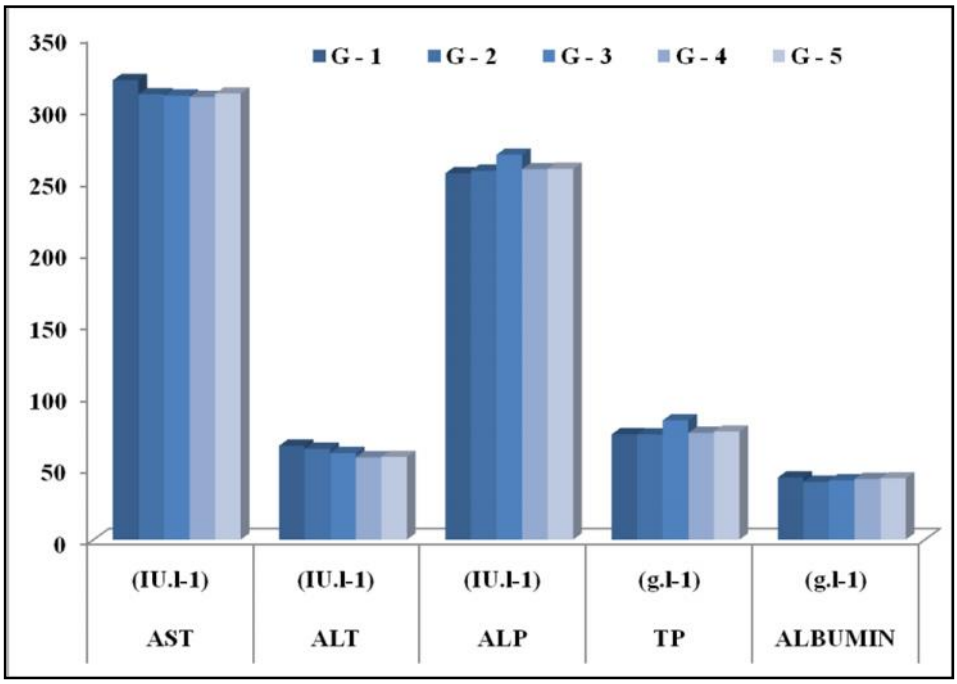

Figure – 4

The effects of **Lavankathi Chooranam** on biochemical parameters such as AST, ALT, ALP, TP and Albumin in rats. A study on the effects of **Lavankathi Chooranam** on biochemical parameters such as AST, ALT, ALP, TP and Albumin rats was tested. where, group I animals (GPI) were treated with normal saline ($5ml.kg^{-1}$), group II animals (GPII) with 50 $mg.kg^{-1}$ of HAEBD group III animals (GPIII) with 100 $mg.kg^{-1}$ of **Lavankathi Chooranam**, group IV animals (GPIV) with 200 $mg.kg^{-1}$ of **Lavankathi Chooranam**, and group V animals (GPV) with 400 $mg.kg^{-1}$**Lavankathi Chooranam** The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01 *P<0.05.

Effect of Lavankathi Chooranam on haematological parameters in rats

The effects of **Lavankathi Chooranam**were observed for its effect onhaematological parameterson the experimental rats. From the study it was evident that, a significant increase ($p<0.01$) were observed in the haemoglobin contents and RBC count in the group treated with 200 $mg.kg^{-1}$ body weight of **Lavankathi Chooranam**and a significant decrease of the parameters occurred in the group treated with 400 $mg.kg^{-1}$ b.w.t compared with the control. There was no significant change in the calcium level in all the treated animals compared to the control.

Table - 06

Treatment	Haemoglobin ($mg.dl^{-1}$)	RBC (10^6 /mm^3)	WBC (10^6 /mm^3)	Calcium ($mg.dl^{-1}$)
Control	13.52±1.28	9.25± 0.93	11.52± 0.90	9.43 ±0.60
Lavankathi Chooranam @ 50 $mgkg^{-1}$	14.3±1.35*	9.39±1.05*	9.31± 0.82*	9.24 ±0.38
Lavankathi Chooranam @ 100 $mgkg^{-1}$	14.21±1.84*	9.49±1.20*	8.34± 0.28*	9.22 ±0.45
Lavankathi Chooranam@ 200 $mgkg^{-1}$	13.26±1.25*	8.39± 0.85*	11.53± 0.83*	9.55 ±0.56
Lavankathi Chooranam@ 400 $mgkg^{-1}$	13.23±1.23*	8.50± 0.92*	10.86±0.75*	9.64 ±0.64

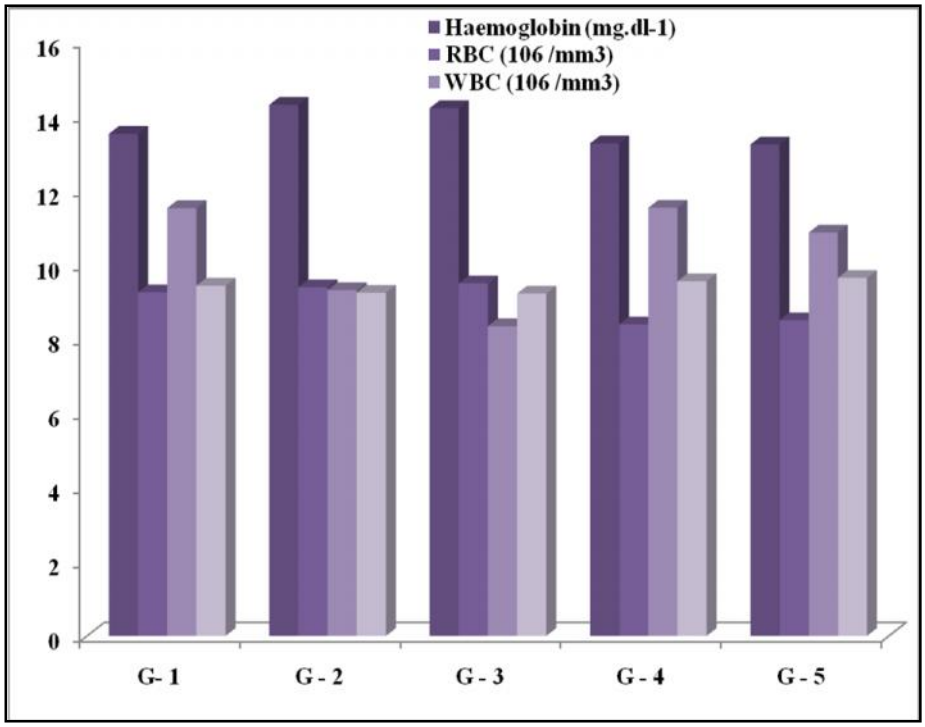

Figure - 5

The effect of **Lavankathi Chooranam** on haematological parameters such as HB, Calcium, RBC and WBC in rats. A study on the effect of **Lavankathi Chooranam** on haematological parameters such as Hb, RBC, WBC, Calcium in rats was tested. where, group I animals (GPI) treated with normal saline (5 $ml.kg^{1}$), group II animals (GPII) with 50 $mg.kg^{-1}$ of **Lavankathi Chooranam**, group III animals (GPIII) with 100 $mg.kg^{-1}$ of **Lavankathi Chooranam**, group IV animals (GPIV) with 200 $mg.kg^{-1}$ of **Lavankathi Chooranam**, and group V animals (GPV) with 400 $mg.kg^{-1}$**Lavankathi Chooranam**. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV and V. The statistical analysis was carried out using one way ANOVA method, where *P<0.05.

Discussion

The evaluation of sub-chronic and chronic dosing in experimental animals may be more relevant in determining the overall toxicity of the plant preparation. The highest overall concordance of toxicity in animals in comparison with humans is with hematological, gastrointestinal, and cardiovascular adverse effects whiles certain adverse effects in humans, especially hypersensitivity and idiosyncratic

reactions, are poorly correlated with toxicity observed in animals (Olson, *et al*., 2000).(7)

In the present study, where the acute toxicity study of **Lavankathi Chooranam** was carried out as per OECD-423 guidelines,no mortality was observed in both the animals of control group as well as animals treated with a maximum dose of 2000 $mg.kg^{-1}$. Hence, $1/10^{th}$ of 2000 $mg.kg^{-1}$ i.e. 200 $mg.kg^{-1}$ of dose was selected as a minimum dose for sub-acutetoxicity study (Abu TahaNael, *et al.,* 2008).(8)

The results of sub-acute toxicity study shows that there was no significant change in animal behaviour due to the absence of toxicity. The animals treated with **Lavankathi Chooranam** showed normal growth pattern and body weight compared with control rats treated with normal saline. So the changes in body weight can be used as an indicator of adverse effects of drugs and chemicals (Tofovic and Jackson, 1999; Raza, *et al.*, 2002; Teo, 2002).(9,10,11)

The changes in enzymes like ALP, AST and ALT levels show liver impairment, due to toxicity (Hayes, 1989).(12) Serum cholesterol and proteins mainly regulated via synthesis in the liver and increase or decrease in serum concentrations of constituents suggest liver toxicity. The results of the present study were assessed after 28 days of administration of **Lavankathi Chooranam**, and it was found that **Lavankathi Chooranam** at all concentrations do not produce liver damage.

There was a slight decrease in plasma glucose level, when higher doses of **Lavankathi Chooranam** (400 $mg.kg^{-1}$) were administered in the treated rats.

Analysis of blood parameters is likely to risk evaluation as the change in hematological system has a higher predictive value for human toxicity, when data are translated from animal studies (Olson, *et al*., 2000).(7) After 28 days of treatment, there were no significant changes in the haematological parameters between control and treated groups. No significant changes in the levels of WBC, RBC were observed between control and test groups following repeated administration of **Lavankathi Chooranam**. Interestingly, significant increase in the levels of hemoglobin was found in treatment with **Lavankathi Chooranam** with a higher dose of 400 $mg.kg^{-1}$. The possible reason could be that one of the constituents **Lavankathi Chooranam** may increase absorption of iron.

The overall results suggest that **Lavankathi Chooranam** are non toxic to the haaematopoietic and leucopoietic system. The haematopoietic and leucopoietic systems are the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animal (Adeneye, *et al*., 2006).(13) Therefore, it is possible to assume that the **Lavankathi Chooranam**is non haematotoxic.

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