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RESEARCH ARTICLE

Toxicological Evaluation Of *Pancha Lavana Dravagam*: A Siddha Poly Mineral Distillate.

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ABSTRACT

Siddha system is an ancient system of medicine widely practiced in southern part of India. The system has developed a rich and unique treasure of drug knowledge in which use of metals and minerals is very much advocated. *Pancha Lavana Dravagam* is one such classical distillate siddha formulation mentioned in Siddha System of Pharmacopoeia. It is indicated for liver and gastrointestinal disorders. To ensure the safety of *Pancha Lavana Dravagam*, acute and sub-acute toxicity studies were carried out based on the OECD guidelines 423 and 407. There was no significant behavioral changes and mortality during acute toxicity studies. There were also no significant changes in internal organs and haematological parameters in sub-acute toxicity study. Based on the toxicological evaluation, it is evident that the drug *Pancha Lavana Dravagam* is safe, and it can be further subjected to pharmacological studies to elucidate its therapeutic efficacy.

Keywords: Toxicological evaluation, Siddha, Pancha Lavana Dravagam, Distillate.

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INTRODUCTION

Siddha system of medicine is one among the traditional healthcare systems practiced widely in India. It was explained by various personalities called *Siddhars* with intellectual and spiritual wisdom. It is an age old medical system with enormous formulations to treat various co morbid conditions. It uses higher minerals and metals for preparing medicines which do not deteriorate with time [1].

Toxicity testing is paramount in the screening of newly developed drugs before it can be used on humans. But in case of traditional system of medicines, the drugs are time proven and no such toxicological evidence has been provided. Though it is widely used by humans, according to the context of WHO International Drug Perspective, safety of a drug is more important than its efficacy. Hence the toxicity studies were conducted based on the OECD guidelines.

The essence of toxicity testing is not just to check how safe a test substance is; but to characterize the possible toxic effects it can produce. It also resolves the general misconception among the physicians, researchers and public as well regarding the safety of taking Siddha medicines [2].

Pancha Lavana Dravagam is a poly mineral formulation obtained from minerals by distillation process in a traditional distillating apparatus called "Dravaga Vaalai Iyanthiram". It is indicated for *Gunmam* (Gastritis), *Eri gunmam* (Dyspepsia), *Soolai* (Colic), *Kalleeral / Maneeral Veekam* (Enlargement of Liver or Spleen), *Soodhaga vali* (Dysmenorrhea), *Soodhaga vaayu* (Amennorhea) [3].

There was no scientific evidence available to assess the safety of *Pancha Lavana* Dravagam. Hence, acute and sub-acute oral toxicity studies have been carried out to ensure its safety on long term.

MATERIALS AND METHODS

Ingredients: [3]

Purified *Vediyuppu* (Potassium nitrate)	– 600 g
Purified *Kariyuppu* (Sodium chloride)	– 600 g
Purified *Padigaram* (Aluminum potassium sulfate)	– 600 g
Purified *Vengaram* (Borax)	– 300 g
Purified *Navacharam* (Ammonium chloride)	– 300 g

Experimental Animals

Wistar Albino strain healthy rats (*Rattus norvegicus*) weighing 150 to 200 g were obtained from Tamil Nadu Veterinary and Animal sciences University, Madhavaram milk colony, Chennai-51, Tamil Nadu, India. These animals were housed at the Animal house, National Institute of Siddha at standard environment with controlled temperature of 22°C ⍰⍰3°C and relative humidity between 55% ⍰ 5% with a 12–12 h light–dark cycle. The animals were provided with standard pellet food (VRK Nutritional Solutions, Maharastra) and water *ad libidum* during the entire study period. Animals were allowed an acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment.

All the experiment protocols employed herein was approved by Institutional Animal Ethical Committee of National Institute of Siddha, Chennai-47, Tamil Nadu, India. (NIS/IAEC – VII/28082018/04 dated 28.08.2018) and conducted in accordance with the guidelines established by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for laboratory animal facilities [4].

Acute Oral toxicity

The acute oral toxicity was carried out based on the Organisation for Economic Co-Operation and Development (OECD) guideline 423 [5].

Experimental Design

Pancha Lavana Dravagam was suspended in water and administered to the groups of wistar albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle i.e., Water. Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three Female animals were used for each group. The dose level of 2 ml/kg body weight was administered. After the substance has been administered, food was withheld for further 3 - 4 hours. The principles of laboratory animal care were followed. Observations were made and recorded systematically and continuously as per the guideline after substance administration. The visual observations included skin changes, mobility and aggressiveness, sensitivity to sound and pain, as well as respiratory movements. Finally, the number of survivors was noted after 24 hours and these animals were then monitored for further 14 days and observations were made daily. The toxicological effect was assessed on the basis of mortality.

Sub-Acute Oral toxicity (28 days)

The Sub- acute oral toxicity was carried out based on the Organisation for Economic Co-Operation and Development (OECD) guideline 407 [6].

Experimental Design

Wistar Albino Rats (20 M + 20 F) were selected and divided into 4 groups as shown in Table 1. Each group consists of 10 animals (5 Male, 5 Female). First group was treated as a control and other three groups were treated with test drug (low, mid and high doses) for 28 days. Each animal was marked with picric acid for identification. The females were nulliparous and non-pregnant.

As per OECD guidelines, three dose levels will be usually selected for the study. They are low dose (X), mid dose (2X) and high dose (4X). X is calculated by multiplying the therapeutic dose (15 drops, i.e. 0.75 ml) and the body surface area of the rat (0.018) i.e. X dose is 0.5 ml/kg, 2X dose is 1 ml/ kg, 4X dose is 2 ml/kg (Paget and Barnes, 1964)

Table: 1 Grouping of Animals

GROUPS	NUMBER OF RATS
Group I Vehicle control (Water)	10 (5 M , 5 F)
Group II PLD* – Low dose X (0.5 ml/kg b.w)	10 (5 M , 5 F)
Group III PLD- Mid dose 2X (1 ml/kg b.w)	10 (5 M , 5 F)
Group IV PLD- High dose 4X (2 ml/kg b.w)	10 (5 M , 5 F)
TOTAL	40 (20 M , 20 F)

*PLD – Pancha Lavana Dravagam

The animal was monitored 28 days for the following: Body weight, food and water intake for every week; unusual behaviors and clinical signs; mortality. On 29th day, the animals were fasted for approximately 18 h, then sacrificed with over dose of ether and blood samples were collected from the caudal vena cava into two tubes: one with EDTA for immediate analysis of Hematological parameters, the other without any anticoagulant and was centrifuged at 3000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20°C until analyzed for biochemical parameters.

On 28th day of the experiment, 24 h urine samples were collected by placing the animals in the metabolic cage with free access to tap water but no feed was given.

The urine was free from fecal contamination. Toluene was used as a preservative while collecting the sample. The sediments present in the urine were removed by centrifugation and the collected urine was used for biochemical estimations.

Organs will be collected from all animals, preserved in 10% buffered neutral formalin for 24 h, and washed in running water for 24 h. The organs were sliced into 5 or 6µm sections and were dehydrated in an auto technician and then cleared in benzene to remove solute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubicle block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Hematoxylin-eosin red. The organs included Heart, Lung, Liver, Stomach, Spleen, Kidney, Brain, Uterus, Ovaries and Testes.

RESULTS

Acute Oral Toxicity

The results of Acute Toxicity Study of *Pancha Lavana Dravagam* were shown on Tables 2, 3 and 4. *Pancha Lavana Dravagam* didn't show any change in general behavior, home cage activity, hand held behaviors and did not produce any toxic symptoms and mortality (Table 5) during the 24 hours of oral administration and 14 days of observation. From the dose administered in acute toxicity study, the three doses 0.5, 1 and 2 ml/kg b.wt were selected for further sub – acute toxicity study.

Table 2: Behavioral signs of Acute Oral Toxicity

S.NO	OBSERVATION	CONTROL GROUP	TEST GROUP (2 ml/kg b.wt)
1.	Body Weight	Normal	Increased Normally
2.	Assessments Of Posture	Normal	Normal
3.	Signs Of Convulsions	Absent	Absent
4.	Ptosis	Normal	Normal
5.	Stupor Reaction	Normal	Normal
6.	Salivation	Normal	Normal
7.	Change In Skin Color	No significant color change	No Significant Color Change
8.	Piloerection	Normal	Normal
9.	Defecation	Normal	Normal
10.	Sensitivity Response	Normal	Normal
11.	Locomotion	Normal	Normal
12.	Muscle Gripness	Normal	Normal
13.	Rearing	Mild	Mild
14.	Urination	Normal	Normal

Table 3: Home Cage Activity

S.NO	FUNCTIONAL AND BEHAVIOURAL OBSERVATION	CONTROL GROUP	TEST GROUP (2 ml/kg b.wt)
1.	Body Position	Normal	Normal
2.	Respiration	Normal	Normal
3.	Clonic Involuntary Movement	Nil	Nil
4.	Tonic Involuntary Movement	Nil	Nil
5.	Palpebral Closure	Normal	Normal
6.	Approach Response	Normal	Normal
7.	Touch Response	Normal	Normal
8.	Pinna Reflex	Normal	Normal
9.	Tail Pinch Response	Normal	Normal

Table 4: Hand Held Observation

S.NO	FUNCTIONAL AND BEHAVIOURAL OBSERVATION	CONTROL GROUP	TEST GROUP (2 ml/kg b.wt)
1.	Reactivity	Normal	Normal
2.	Handling	Normal	Normal
3.	Palpebral Closure	Normal behaviour	Normal behavior
4.	Lacrimation	Normal	Normal
5.	Salivation	Normal behaviour	Normal behavior
6.	Piloerection	Normal	Normal
7.	Pupillary Reflex	Normal	Normal
8.	Abdominal Tone	Normal	Normal
9.	Limb Tone	Normal	Normal

Table 5: Mortality

GROUPS	DOSES (p.o)	MORTALITY
CONTROL GROUP	Water	0 of 3
TEST GROUP	2 ml/kg b.wt	0 of 3

Sub-Acute Oral Toxicity (28 days)

Effect of PLD on Biochemical investigations

In Sub-Acute Oral Toxicity study of *Pancha Lavana Dravagam,* the body weight, food intake and water intake of each group of rats were recorded on 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} days after the drug administration. The effect of PLD on the body weight after 28 days of drug administration was given in Table 6. There was no significant change in body weight of the animals in treated groups when compared to the animals in control groups during the 28 days treatment.

The effect of PLD on the food and water intake on 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} days after the drug administration was given in Table 7 and 8. There was no significant change in food and water intake of the animals in treated groups when compared to the animals in control groups during the 28 days treatment. This shows that the drug didn't have any influence on food and water intake of the animals.

Table 9 and 10 shows the effect of PLD on haematological parameters like RBC, WBC, Platelet counts and Hb after 28 days of drug administration. From the table it was evident that there were no significant changes in RBC, WBC, Platelet counts and Hb of the treated group of animals when compared to the control group.

Table 11 shows the effect of PLD on Differential Count after 28 days of drug administration. From the table it was clear that there were no significant changes in Differential count of the treated group of animals when compared to the control group.

On evaluating the hepatic function, Table 12 shows the effect of PLD on liver enzymes SGOT, SGPT, Total Bilirubin and Total Cholesterol after 28 days of drug administration. The drug didn't show any significant changes in the liver enzymes of the treated group of animals when compared to the control group.

On assessing the renal function, Table 13 shows the effect of PLD on BUN and Serum Creatinine after 28 days of drug administration. The drug didn't show any significant changes in the BUN and Serum creatinine values of the treated group of animals when compared to the control group.

Table 14 shows the effect of PLD on urinary parameters which indicated that there was no significant change in the color, specific gravity of the urine. It was also noted that there was no elimination of sugars, albumins and RBC's in urine.

Table 6: Effect of *Pancha Lavana Dravagam* (PLD) on body weight during 28 days drug administration in rats.

GROUPS	DRUG TREATMENT (p.o)	BODY WEIGHT (gms)				
		0th Day	7th Day	14th Day	21st Day	28th Day
I	Control group (Distilled water)	164.23±14.1	167.23±9.2	172.44± 18.4	176.71± 12.7	178.13± 8.1
II	Low Dose PLD (0.5 ml/kg b.wt)	165.18± 10.60	169.09±13.2	172.54± 19.9	175.41± 11.7	179.74± 9.1
III	Mid Dose PLD (1 ml/kg b.wt)	164.51± 16.2	168.64± 10.6	172.24± 18.1`	176.74± 9.8	180.09± 15.1
IV	High Dose PLD (2 ml/kg b.wt)	164.38± 9.6	167.84± 11.0	171.25± 15.8	175.19± 14.9	179.58± 17.8

Values are expressed as Mean ± Standard Deviation (n=10) with one way ANOVA followed by Dunnett's test. P value is not significant when comparing the treated groups with control group. (NS – Not Significant)

Table 7: Effect of *Pancha Lavana Dravagam* (PLD) on food intake during 28 days drug administration in rats.

GROUPS	DRUG TREATMENT (p.o)	FOOD INTAKE (gms/rat)				
		0th Day	7th Day	14th Day	21st Day	28th Day
I	Control group (Distilled water)	32.65±1.6	35.19±2.1	39.51±1.9	42.36±2.3	45.87±2.7
II	Low Dose PLD (0.5 ml/kg b.wt)	30.58±3.1	33.97±2.7	38.94±2.4	42.47±1.9	44.21±2.7
III	Mid Dose PLD (1 ml/kg b.wt)	32.28±2.7	36.84±2.7	38.74±1.5	41.64±1.7	43.22±1.3
IV	High Dose PLD (2 ml/kg b.wt)	33.91±1.5	36.14±2.5	39.71±1.8	41.23±2.9	45.01±2.1

Values are expressed as Mean ± Standard Deviation (n=10) with one way ANOVA followed by Dunnett's test. P value is not significant when comparing the treated groups with control group. (NS – Not Significant)

Table 8: Effect of *Pancha Lavana Dravagam* (PLD) on water intake during 28 days drug administration in rats.

GROUPS	DRUG TREATMENT (p.o)	WATER INTAKE (ml/rat)				
		0th Day	7th Day	14th Day	21st Day	28th Day
I	Control group (Distilled water)	44.96±3.4	45.67±2.4	46.95±2.3	48.09±2.5	49.27±1.4
II	Low Dose PLD (0.5 ml/kg b.wt)	43.32±2.7	45.21±1.7	47.67±3.4	48.16±2.7	50.42±2.9
III	Mid Dose PLD (1 ml/kg b.wt)	43.14±1.8	45.38±3.6	48.23±2.0	50.06±1.7	50.79±3.4
IV	High Dose PLD (2 ml/kg b.wt)	44.67±2.4	46.19±3.1	47.01±1.9	49.49±2.6	50.27±2.4

Values are expressed as Mean ± Standard Deviation (n=10) with one way ANOVA followed by Dunnett's test. P value is not significant when comparing the treated groups with control group. (NS – Not Significant)

Table 9: Effect of *Pancha Lavana Dravagam* (PLD) on RBC, WBC and Hb during 28 days drug administration in rats.

GROUPS	DRUG TREATMENT (p.o)	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Hb (gm/dl)
I	Control group (Distilled water)	7.35±0.80	9.18±1.12	14.02±0.28
II	Low Dose PLD (0.5 ml/kg b.wt)	7.49±1.23	9.14±0.12	13.89±0.61
III	Mid Dose PLD (1 ml/kg b.wt)	7.53±0.80	8.99±0.47	12.48±0.21
IV	High Dose PLD (2 ml/kg b.wt)	7.61±0.94	8.61±0.08	14.68±0.19
	P value	NS	NS	NS

Values are expressed as Mean ± Standard Deviation (n=10) with one way ANOVA followed by Dunnett's test. P value is not significant when comparing the treated groups with control group. (NS – Not Significant)

Table 10: Effect of *Pancha Lavana Dravagam* (PLD) on Platelet counts during 28 days drug administration in rats.

GROUPS	DRUG TREATMENT (p.o)	PLATELET COUNT (10^3/mm^3)	P Value
I	Control group (Distilled water)	198.24±6.27	NS
II	Low Dose PLD (0.5 ml/kg b.wt)	204.84±5.70	NS
III	Mid Dose PLD (1 ml/kg b.wt)	187.65±7.21	NS
IV	High Dose PLD (2 ml/kg b.wt)	194.18±7.50	NS

Values are expressed as Mean ± Standard Deviation (n=10) with one way ANOVA followed by Dunnett's test. P value is not significant when comparing the treated groups with control group. (NS – Not Significant)

Table 11: Effect of *Pancha Lavana Dravagam*(PLD) on Differential counts during 28 days drug administration in rats.

GROUPS	DRUG TREATMENT (p.o)	DIFFERENTIAL COUNTS (10^3/mm^3)			
		Neutrophils	Eosinophils	Monocytes	Lymphocytes
I	Control group (Distilled water)	2.01±2.3	0.04±1.1	0.01±2.8	6.74±1.5
II	Low Dose PLD (0.5 ml/kg b.wt)	3.14±1.0	0.05±2.8	0.02±3.1	5.93±3.7
III	Mid Dose PLD (1 ml/kg b.wt)	2.93±2.7	0.04±2.1	0.02±2.9	6.19±1.9
IV	High Dose PLD (2 ml/kg b.wt)	2.75±1.6	0.06±1.7	0.01±1.2	7.02±0.9
	P Value	NS	NS	NS	NS

Values are expressed as Mean ± Standard Deviation (n=10) with one way ANOVA followed by Dunnett's test. P value is not significant when comparing the treated groups with control group. (NS – Not Significant)

Table 12: Effect of *Pancha Lavana Dravagam* (PLD) on LFT during 28 days drug administration in rats.

GROUPS	DRUG TREATMENT (p.o)	SGOT (U/L)	SGPT (U/L)	TOT. BILIRUBIN (mg/dl)	TOT. CHOLESTEROL (mg/dl)
I	Control group (Distilled water)	58.9±8.33	22.52±3.48	0.28±0.09	98.51±3.64
II	Low Dose PLD (0.5 ml/kg b.wt)	55.27±9.21	21.93±3.16	0.28±0.16	94.12±7.43
III	Mid Dose PLD (1 ml/kg b.wt)	59.78±3.10	21.64±3.29	0.27±1.3	94.75±5.08
IV	High Dose PLD (2 ml/kg b.wt)	59.99±4.34	22.37±3.33	0.31±0.16	96.01±3.33
	P Value	NS	NS	NS	NS

Values are expressed as Mean ± Standard Deviation (n=10) with one way ANOVA followed by Dunnett's test. P value is not significant when comparing the treated groups with control group. (NS – Not Significant)

Table 13: Effect of *Pancha Lavana Dravagam* (PLD) on RFT during 28 days drug administration in rats.

GROUPS	DRUG TREATMENT (p.o)	BUN (mg/dl)	S.CREATININE (mg/dl)	P Value
I	Control group (Distilled water)	17.7±1.57	0.4±0.08	NS
II	Low Dose PLD (0.5 ml/kg b.wt)	16.7±1.50	0.35±0.17	NS
III	Mid Dose PLD (1 ml/kg b.wt)	16.53±1.01	0.35±0.14	NS
IV	High Dose PLD (2 ml/kg b.wt)	16.94±1.70	0.33±0.20	NS

Values are expressed as Mean ± Standard Deviation (n=10) with one way ANOVA followed by Dunnett's test. P value is not significant when comparing the treated groups with control group. (NS – Not Significant)

Table 14: Effect of *Pancha Lavana Dravagam*(PLD) on Urinary Parameters during 28 days drug administration in rats.

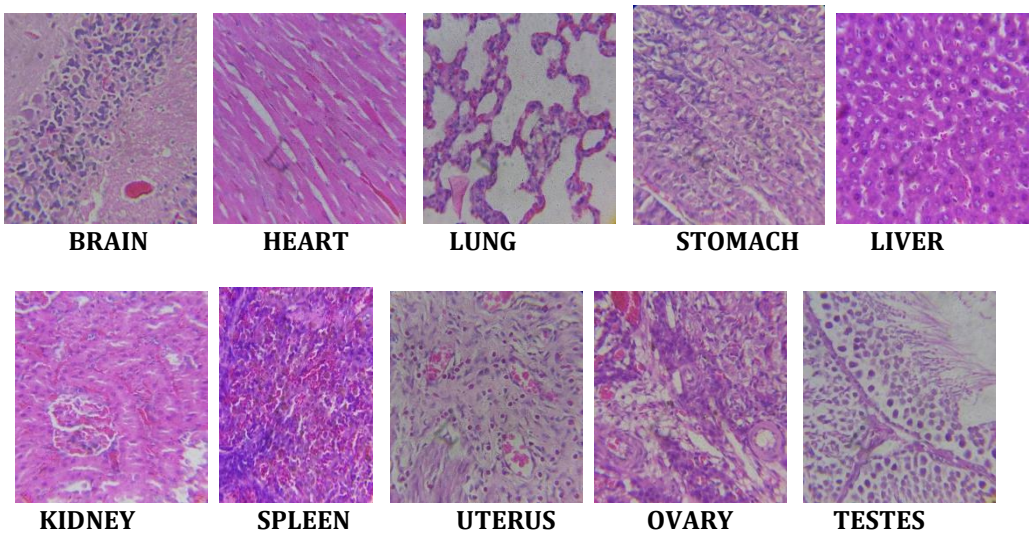
S.NO	PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
1.	Color	Yellow	Pale yellow	Colorless	Pale yellow
2.	Specific Gravity	1.01	1.01	1.01	1.02
3.	Glucose	Nil	Nil	Nil	Nil
4.	Proteins	Nil	Nil	Nil	Nil
5.	RBC	Nil	Nil	Nil	Nil
6.	Epithelial Cells	Nil	Nil	Nil	Nil

Histopathological Evaluation

The examination of the tissues obtained from the organs collected from control and *Pancha Lavana Dravagam* treated rats didn't show any massive changes in their cyto architecture. The Brain of rats from the high dose group i.e., 2 ml/kg bw showed regular marginal alignment on the neurons with promising histology. Their heart had the normal histological structure of myocardium. There was a regular arrangement of alveoli and alveolar sac in their lungs. The surface epithelium, mucosa and sub-mucosa layers were normal in their stomach. Liver showed normal Hepatic cords with radiating morphology. Lumen of distal convolutes tubule and collecting duct was normal in Kidneys. No signs of immunological activities were noted in spleen. The arrangement of stratum basale, functionale and surface epithelium seemed normal in uterus. In ovary, follicular cells, cytoplasm and nucleus appeared normal. Section of testes of showed normal interstitial

connective tissue with ovoid or polygonal leydig cells (arrow) and flat myoid cells. The sections of the organs were depicted in Figure 1.

Figure 1: Histopathological images of internal organs treated with *Pancha Lavana Dravagam*(PLD)



DISCUSSIONS

Acute Oral Toxicity Study

Pancha Lavana Dravagam was subjected to acute oral toxicity study with a dose of 2 ml/ kg b wt. It didn't produce any toxic signs, changes in general and functional behavior and mortality during the study. There were also no abnormal changes in body weight and necropsy findings of the treated group of rats. This ensures that the dosage given was below the toxic limit and confirms the safety of drug. Based on this dosage, further sub-acute toxicity study was carried out.

Sub-acute Oral Toxicity Study

Pancha Lavana Dravagam was administered to the wistar albino rats to carry out the repeated 28 days oral toxicity at the dose levels of 0.5, 1 and 2 ml/kg b.wt. The drug treated animals survived throughout the study period of 28 days and they did not show any treatment related major abnormal clinical signs.

The overall percentage of gain in body weight of rats treated with the drug was found to be normal indicating that the test animals were in a healthy condition during the 28 days of observation period. There was also no significant changes in the heamatological evaluation of drug treated animals.

The necropsy studies showed no notable changes. In histopathological analysis, *Pancha Lavana Dravagam* treated rats didn't show any significant abnormalities.

This strongly emphasis the fact that the drug *Pancha Lavana Dravagam* has no toxic effect on the body metabolism.

CONCLUSION

Safety of a drug is more important than their therapeutic efficacy. Hence, *Pancha Lavana Dravagam* was subjected to toxicity studies before carrying out the pharmacological activity studies. On analyzing the toxicity profile of *Pancha Lavana Dravagam* for acute and 28 days repeated dose oral toxicity, the drug didn't produce any toxic symptoms and mortality. This ensures the safety of *Pancha Lavana Dravagam.*

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Conflicts of Interest

None declared.

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