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Toxicity Evaluation of Siddha Herbo-Mineral Drug Pancha Lavana Mezhugu (PLM) in Rat Model

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ABSTRACT

Siddha Medicine is one of the ancient traditional medicinal system that originated in southern part of India in the Tamil Language. Its literature, however, is considerably extensive and spreads over several centuries. Using of imperishable herbals, minerals and metals as the raw drugs for preparing high order pharmaceutical forms of medicines like parpam, chendurum, kattu etc, are the special features of siddha system. PANCHA LAVANA MEZHUGU (PLM), a Siddha herbo-mineral formulation made out of five types of karasaram (salts) and five types of herbals was evaluated for acute and subacute toxicity in adult swiss albino rats. Acute toxicity evaluation was carried out according to Organization For Economic Corporation And Development (OECD) guideline 423 for 14 days. For the study of acute toxicity, PLM was given by oral route in various doses (5, 50, 300, 2000 mg/kg). LD-50 value was determined. PLM did not produce death even at high dose (2 g/kg). 2gm/kg is 30 times of dose quoted to humans. The results showed no signs of toxicity such as change in general behavior, mortality, or change in gross appearance of internal organs. Subacute toxicity (repeated dose 28-day oral toxicity) was carried out according to Organization For Economic Corporation And Development (OECD) guideline 407 for 28 days. It was studied by giving PLM in oral route of 100 mg/kg as low dosage, 400mg/kg as intermediate dose, 750 mg/kg as high dose for 28 days. The results showed no abnormalities in sensory reactivity to stimuli, grip strength, motor activity. No changes were observed in body weight, organ weight, food and water consumption. Hematological and clinical biochemical values were in limits. Neither gross necropsy abnormalities nor histopathological abnormalities were observed. So PLM was found to be safe.

INTRODUCTION

Siddha Medicine is one of the ancient traditional medical systems that originated in southern part of India in the Tamil Language. Its literature, however, is considerably extensive and spreads over several centuries. Siddhars, the intellectual giants are founders and followers of Siddha system of medicine with their higher level of perception which emphasis on healthy and happy life with good mind, body and soul[1]. Using of imperishable herbals, minerals and metals as the raw drugs for preparing high order pharmaceutical forms of medicines like parpam, chendurum, kattu etc, are the special features of siddha system[2]. Karasaram (salts) are the one type of mineral used extensively in Siddha medicine preparation[2]. PANCHA LAVANA MEZHUGU (PLM), the trial drug is a Siddha herbo-mineral formulation made out of five types of karasaram (salts) and five herbs. PLM was quoted in SIGICHA RATHNA DEEPAM ENNUM VAIDHYA NOOL and indicated for VADHA DISEASES (musculoskeletal disorders)[3]. Accurate identification and good quality control of raw drugs, toxicity study provides assurance to the safety and quality of medicine. Minerals that are transformed into drug must have excellent therapeutic efficacy[4]. But it is essential to evaluate the margin of safety between the dose level that produces the therapeutic effects and that produces the adverse effects. Animal experimentation with toxicity study is the only way through which this assessment can be made. In this present paper, the studies on safety and toxicity profile of Pancha Lavana Mezhugu have been enlightened.

OBJECTIVE: To carry out the acute and subacute toxicity study of the Siddha herbo-mineral trial drug, PANCHA LAVANA MEZHUGU**.**

MATERIALS AND METHODS

STANDARD OPERATING PROCEDURE FOR THE PREPARATION OF PANCHALAVANA MEZHUGU [3]:

Ingredients

- INDHUPPU *(Rock salt)-35gm*
- KALLUPPU *(Sodium chloride)-35 gm*
- KARIYUPPU *(Sodium chloride)-35 gm*
- VALAIYALUPPU *(Sodium silicate)-35 gm*

• VEDIYUPPU *(Potassium nitrate)-35 gm*

• PIRANDAI CHARU *(Cissus quadrangularis)-QS*

• KUPPAIMENI CHARU *(Acalypha indica)-QS*

• MURUNGAIPATTAI CHARU *(Moringa olifera)-QS*

• NOCCHI ILAI CHARU *(Vitex negundo)-QS*

• KUMARI CHARU *(Aloe vera)-QS*

• THAEN *(Honey)-QS*

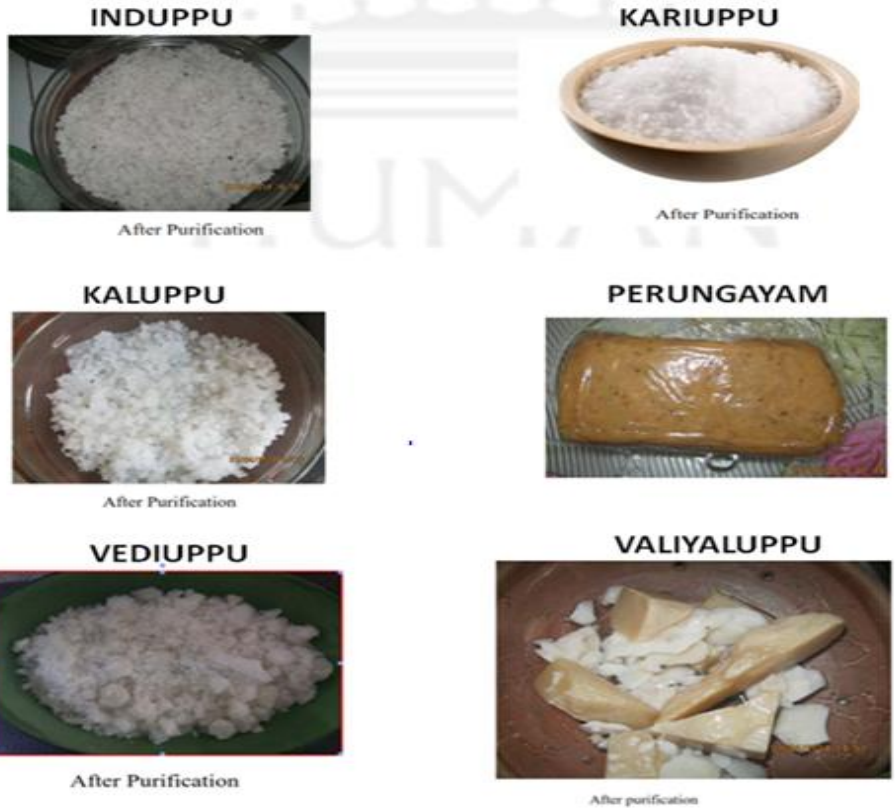
PERUNGAYAM (Ferula asafoedita)-QS

AUTHENTICATION:

Authentication of the materials was done by Pharmacognosist and Chemist in SCRI, Chennai

PURIFICATION:

The above procured raw drugs were purified according to the standard siddha text literature.[4]



ALOE VERA (Figure 4.2.8)

ALYPHA INDICA -(Figure 4.2.9)

MORINGA OLEIFERA (Figure 4.2.10)

VITEX NEGUNDO (Figure 4.2.11)

CISSUS QUANDRAGULARIS (Figure 4.2.12)

Five Salts were powdered well separately in the stone mortar.

- ❖ The powered five salts were mixed and grinded well.
- ❖ The herbs were grinded to get juice.
- ❖ The herbal juices were added one by one and grinded slowly.
- ❖ The juices were added little by little and grinded well for 2 *samam* (6 hrs) in each herbal juices totally 30 hours.

WHILE GRINDING WITH HERBAL JUICES

 Villais (small, round and flattened shape) were made with this paste.

 let it to dry for one day.

 35 gm of *vllais* were placed in the mud plate, the lid was closed and sealed with *seelai* (mud pasted cloth). Let it dry for one day.

SEALED MUD PLATE

 sealed and dried mud plate was introduced to incineration process *(Pudam)* by using 15 numbers of cow dung cakes .

INCINERATION PROCESS

 After the incineration process, the sealed mud plate were allowed to come to atmospheric temperature

 Seal was opened carefully.

 All the villais are joined together and in the colour of while with red tinched.

AFTER PUDAM

FINAL PRODUCT

 The final product was taken and powdered again in the stone mortor.

 It was weighed

 Ferula asafoetida (*Perungayam)* was fried and powdered well

 Ferula asafoetida of ¼ weight was added with the final product. All were grinded well to get the homogenous mixture.

 Honey was added little by little and grinded in the mortor till it comes to waxy consistency (mezhugu padham)

ORAL TOXICITY STUDY – OECD GUIDELINES

The Organization For Economic Corporation And Development (OECD) panel of experts defines acute toxicity as "the adverse effect occurring within a short time of (oral) administration of a single high dose of a substance". And subacute toxicity as "the advance effects occurring as result of the repeated daily (oral) dosing of a substance for 28 days".

Registration number and date of registration:

IAEC Reg No. 321/PO/C/01/CPCSEA
Date: 03/01/2013

Selection of animal species:[5]

The preferred rodent species is Swiss Albino female rat

- weighing about 220–240 gm
- Healthy young adults
- nulliparous and non-pregnant
- age between 8 and 12 weeks old

Housing and feeding conditions:[6]

- The temperature in the experimental animal room should be 22ºC (+3ºC).
- Lighting should be artificial, the sequence being 12 hrs light, 12 hrs dark.

PREPARATION OF ANIMALS:

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions.

ACUTE ORAL TOXICITY – OECD GUIDELINES – 423[7]

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co - operation and Development, Guideline-423)

Acute toxicity study was carried out on three female rats under fasting condition. Depending on the mortality and/or the morbidity status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of PLM. Morbid animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on
test. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step; sufficient information is obtained on the acute toxicity of the test substance to enable its classification.

The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; − no further testing is needed − dosing of three additional animals with the same dose − dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

PLM was given in oral route in various doses (5, 50, 300, 2000 mg/kg) to the swiss albino female rat. Signs of toxicity were observed for every one hour for first 24 hours and every day for about 14 days from the beginning of the study. Observations were made and recorded systematically and continuously after PLM administration. The body weights were recorded individually on 0^{th}, 7^{th}, and 14^{th} day.

SUB ACUTE TOXICITY:[8]

The purpose of this study was to assess the sub-acute toxicity profile of the PLM. Acutely nontoxic compounds may be toxic on prolonged exposure even at low dose levels due to cumulation, changes in enzyme level and disruption of physiological and biochemical homeostasis. It provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time, including effects on the nervous, immune and endocrine systems.

A 28- day study is considered a subacute study, which is well accepted for eliciting any toxicity on long-term feeding. It gives valuable information on the cumulative toxicity of a substance on target organs or physiological and metabolic effects of the compound at low dose on prolonged exposure. A wide variety of adverse effects can be detected from subacute toxicity studies. The long-term safety level of PLM can be predicted from acute or subacute studies.

PLM is orally administered daily 100 mg/kg as low dosage for one group, 400mg/kg as intermediate dose for one group, 750 mg/kg as high dose for one group for a period of 28 days. During the period of administration, the animals are observed closely, each day for signs of toxicity. Observations were made and recorded systematically and continuously after PLM administration

COLLECTION AND ANALYSIS OF BLOOD AND ORGANS:[9]

At the end of the treatment period, the overnight fasted (water allowed) animals were anesthetized, and blood samples were collected by retro-orbital puncture in heparinized (for hematological) and nonheparinised tubes (for biochemical analysis). The blood without the anticoagulant was allowed to clot before centrifugation to obtain serum, which was collected and stored at −20°C until assayed for biochemical parameters the next day.

Detailed hematological, biochemical, necropsy and histopathological evaluation of organs was performed for all animals.

After collecting blood, the rats were quickly dissected and the organs were removed, freed of fat and connective tissue, blotted with clean tissue paper, and then weighed on a balance. Organs

such as brain, lung (right and left), heart, stomach, liver, kidney, thymus, spleen, ovary, and testis were weighed and relative organ weights were calculated. Portions of the tissue from collected organs were used for histopathological examination.

GROSS NECROPSY :[9]

All animals in the study shall be subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their content.

HISTOPATHOLOGICAL STUDY[10]: Microscopic examination of sections of vital organs (liver, kidney, spleen, etc.) from treated groups was observed under microscope at different magnifications.

RESULTS AND DISCUSSION

Observations were made and recorded systematically and continuously after PLM administration

Food and water intake	Normal
Body weight	Normal
Assessments of posture	Normal
Signs of Convulsion Limb paralysis	Absence of sign (-)
Body tone	Normal
Lacrimation	Absence
Salivation	Absence
Change in skin color	No significant color change
Piloerection	Normal

Defecation	Normal
Sensory reactivity to the stimuli	Normal
Locomotion	Normal
Muscle gripness	Normal
Rearing	Mild
Urination	Normal
Respiration	Normal
Motor activity	Normal

Dose mg/kg	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
2000	+	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-

1. Alertness 2.Aggressiveness 3. Pile erection 4. Grooming 5.Gripping 6. Touch Response 7. Decreased Motor Activity 8.Tremors 9 Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13.Hypnosis 14.Analgesia15.Lacrimation 16. Exophthalmos 17. Diarrhea 18. Writhing 19 Respiration 20. Mortality.

EFFECT OF PLM ON GROSS NECROPSY:

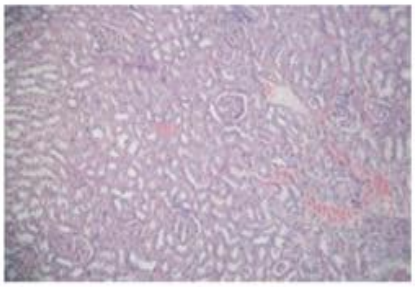
Gross necropsy did not reveal any abnormal pathology in any of the animals.

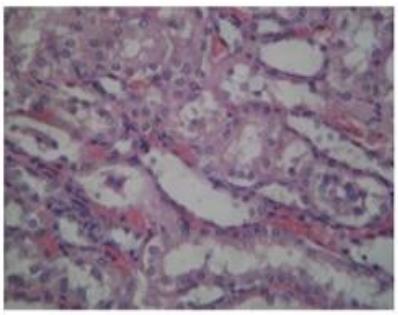
EFFECT OF PLM ON HISTOPATHOLOGY OF VITAL ORGANS:

Microscopic examination of sections of vital organs (liver, kidney, spleen, etc.) from treated groups was observed under microscope at different magnifications.

Gross necropsy did not reveal any abnormal pathology in any of the animals.

HISTOPATHOLOGY OF KIDNEY

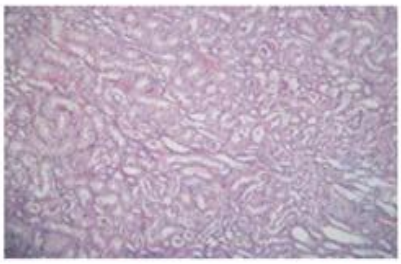


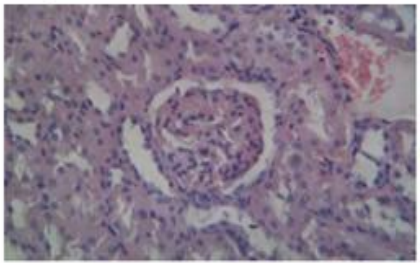


10 x magnification

40 xmagnification

Panchalavana Mezhugu 100mg





10 x magnification

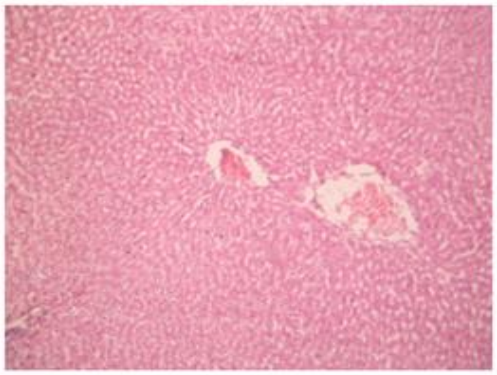
Panchalavana Mezhugu 750mg

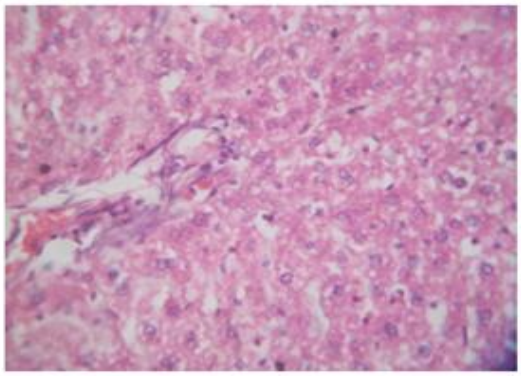
40 x magnification

Report: Kidney shows normal arrangement of nephrotic bundle, glomerulus and renal tubules.

HISTOPATHOLOGY OF LIVER

Figure 5.3.2.

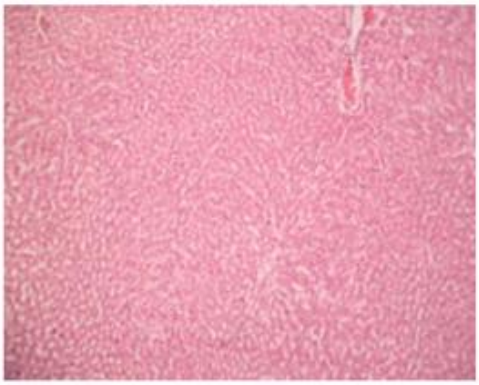




10 x magnification

40 x magnification

Panchalavana Mezhugu 100 mg



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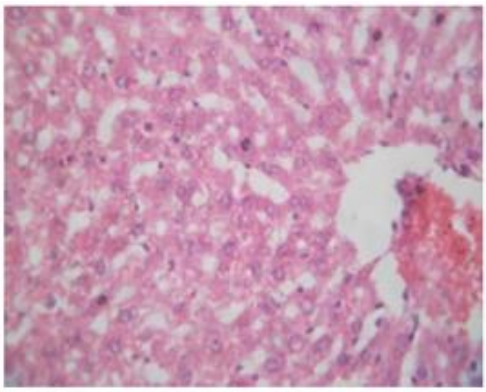
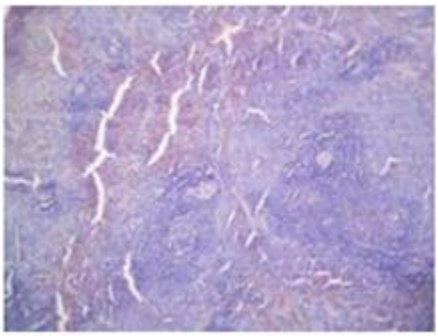


40 x magnification

Report : Histopathology shows normal lobular pattern. Lumen of hepatic veins appears normal. No signs of necrosis.

HISTOPATHOLOGY OF SPLEEN

Figure 5.3.3.



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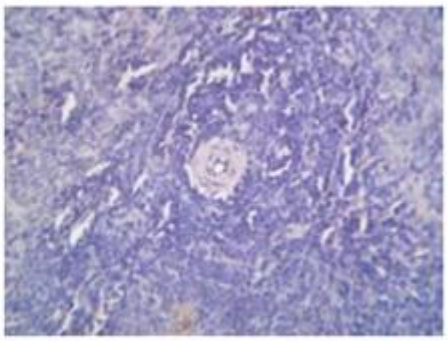
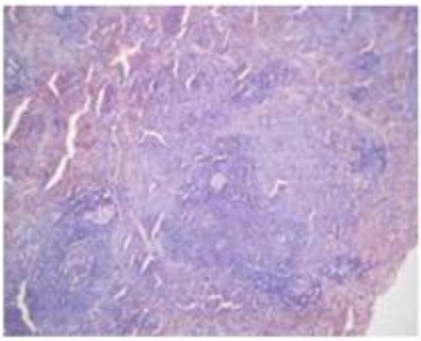


40 x magnification

Panchalavana Mezhugu 100 mg



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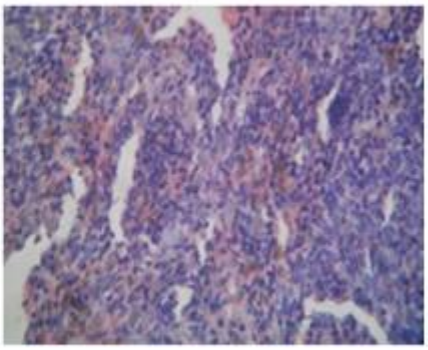

40 x magnification

Panchalavana Mezhugu 750 mg

Report: Section studied shows plenty of intraparenchymal hemosiderin-laden macrophages.

Heart shows no signs of lesion or infracts was observed .Brain shows normal histology with regular neuronal alignment further there was no considerable observation of signs of edema or degeneration.

Post treated values of the hematological parameters biochemical parameters:

HAEMATOLOGY

CBC

WBC : 10,100 cells/cumm

Differential Count
NEUTROPHILLS : 23 %
LYMPHOCYTES : 76 %
EOSINOPHILS : 01 %
MONOCYTES : 00 %
RBC : 6.45 millions/cumm
HB : 12.4 gms%
PCV : 34.8 %
MCV : 54.0 fL
MCH : 19.2 pg
MCHC : 35.6 Grams/dl
PLATELET : 2.16 Lakhs/cumm

BIOCHEMISTRY

Blood sugar : 88 mg/dl
BUN : 25.2 mg/dl
Creatinine : 0.8 mg/dl
SGOT : 208 U/L
SGPT : 97 U/L
ALP : 169 U/L

T.Protein : 8.5 grams/dl

Albumin : 3.1 grams/dl

LIPID PROFILE

T. Cholesterol: 105 mg/dl

Triglycerides: 75 mg/dl

HDL: 25 mg/dl

LDL: 65 mg/dl

VLD : 15.0 mg/dl

Ratio 1(T.CHO/HDL): 4.2

Ratio 2(LDL/HDL): 2.6.

In acute toxicity study, the test drug PLM up to single oral dose of 2000 mg/kg of b.w. did not reveal any abnormal clinical signs in any of the animals. All rats survived and no treatment-related mortality occurred during the period of 14 days. It can be mentioned that the approximate LD50 values are more than 30 times of the quoted human dose

In repeated oral toxicity study all the animals were well oriented and active during the trial period and survived until 28-day treatment period. No signs of major or significant intoxication were observed in animals from lower to higher dose groups during the dosing period of 28 days. No signs of clinical toxicity attributable to PLM were observed throughout the study. All the animals up to 750 mg/kg survived throughout the period of 28 days. No treatment-related mortality occurred during the period of administration of PLM.

It was observed that the animals fed with PLM were healthy.

No differences were found in growth in the animals fed with different doses of PLM. Neither weight gain nor loss was observed throughout the study.

No change in the behavior

Post treated Hematological and Biochemical values are in limits.

Ophthalmoscopic examination reveals normal study.

Food consumption was normal.

Gross pathological examination did not reveal any abnormality.

Histopathological study shows no signs of lesion in different magnification.

CONCLUSION

Our results had demonstrated that PLM poses no toxicity effects up to the dosage of 2000 mg /kg b.w in acute toxicity study and 750 mg/gm b.w in subacute toxicity study as indicated in our rat model. No mortality was seen throughout the study. No abnormality is seen in gross necropsy, histopathological study, haematological and biochemical parameters. So dosage of 60 mg as quoted in sigicha rathna deepam can be followed in clinical trials. Being karasaram (salts) are added in PLM chronic toxicity study should be conducted to ascertain the toxicity effect of PLM.

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