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## TOXICOLOGICAL ASSESSMENT OF SIDDHA HERBOMINERAL PREPARATION KASHA GULANTHAGA MATHIRAI IN ANIMAL MODELS

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### ABSTRACT

Efforts have been made to prove the safety of the traditional Siddha medicine, Kasha Gulanthaga Mathirai. Effects of Kasha Gulanthaga Mathirai in model organisms were not reported till date. This paper appraises a detail study of physio-chemical properties, phytochemical constituents and heavy metal contents of the selected drug [KGM]. The ICPOES reveals that the main metals present in the tablet are Mercury and Arsenic. HRSEM analysis has been used to study particle size shape and distribution. The acute and long term toxicity studies were carried out in Swiss albino mice and Wister albino rats as per WHO guidelines. Histo pathological studies, hematological and biochemical parameters were done to find out any metal toxicity. Toxicity studies reveal the dose mentioned in siddha text kundriyalavu (130mg ) is the safety dose for human consumption.

**KEYWORDS:** Kasha Gulanthaga Mathirai, ICPOES studies, Swiss albino mice, Wister albino rats, Kundriyalavu



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## INTRODUCTION

Siddha is one of the traditional medical systems which are the part of human health care system. Unlike other system of Indian medicine it includes the number of minerals, metals and animal products, in the same manner as that of herbs <sup>(1)</sup>. It also employs numerous method of purification for every single metal and minerals used. Thereby it ensures the safety in siddha system itself. Due to lack of standardization of the drugs of siddha system of medicine, there are reports on the presence of heavy metals that may be toxic for human consumption <sup>(2)</sup>. These reports cause the unnecessary anxiety and panic among the large population. This is probably due to lack of scientific studies to document the safety profile of standardized drugs. In siddha system a large number of formulations are available for the management of Swasakasam (bronchial asthma) <sup>(3)</sup>, Shayam (tuberculosis) <sup>(3)</sup>, Suram (fever) <sup>(3)</sup> one such herbo- mineral formulation is Kasha Gulanthaga Mathirai which is found to be practiced in southern parts of India a long time since time memorial. There seems to be no literature available in siddha as well as in modern medicine with regards to its toxicity and pharmacological activities. Hence in the present study the herbo mineral formulation (KGM) was evaluated for its toxicity and prove their safety of the tablet which is mentioned in siddha text kannusamiyam paramparai vaithyam <sup>(4)</sup>.

## MATERIALS AND METHODS

### (i) Preparation of Standardized Kasha Gulanthaga Mathirai

KGM was prepared as per the methodology mentioned in the sasthric siddha text recognized by drugs and cosmetic act 1940 <sup>(4)</sup>. The raw drugs were procured from various standard raw drug stores in Chennai and Nagarcovil, Tamilnadu, India. The plant drugs were authenticated by botanist, Department of Medicinal Botany, NIS, Chennai and the mineral drugs were authenticated Department of Pharmacognacy, SCRI Chennai. Each drug

was purified by the purification methods mentioned in various siddha texts <sup>(5; 6; 7; 8; 9)</sup>. The tablet was prepared by grinding the raw materials with ginger juice. They are usually prescribed in the dose of 260 mg/day (130mg B.d) and recommended to be taken with ginger juice.

### Ingredients

Lingam ( <i>Redsulphide of mercury</i> ) <sup>(6)</sup>	- 35 gms
Manosilai ( <i>Arsenic di sulphide</i> ) <sup>(6)</sup>	- 35 gms
Karu naabi ( <i>Aconitum ferox</i> ) <sup>(9)</sup>	- 35 gms
Milagu ( <i>Piper nigrum</i> ) <sup>(9)</sup>	- 35 gms
Thalagam ( <i>Arsenic tri sulphide</i> ) <sup>(6)</sup>	- 35 gms
Vengaram ( <i>Sodium bi borate</i> ) <sup>(6)</sup>	- 35 gms
Chukku ( <i>Zingiber officinal</i> ) <sup>(9)</sup>	- 35 gms
Thippili ( <i>Piper longum</i> ) <sup>(10)</sup>	- 35 gms
Ginger juice <sup>(11)</sup>	- required amount

### (ii) Elemental analysis and physio chemical analysis

Analysis of KGM was performed using Optima 5300 DV ICP-OES equipped with a Sea Spray concentric nebulizer (Glass Expansion, Pocasset, MA) and cyclonic spray chamber. Following parameters were introduced: nebulizer flow, 0.8 l min<sup>-1</sup>; radio frequency power, 1450 W; sample introduction, 1.5 ml min<sup>-1</sup>; flush time, 20 s; delay time, 10 s; read time, 10 s; wash time, 30 s; and replicates, three. Standards were prepared by dilution of 1000 mg l<sup>-1</sup> stock solutions and the calibration curve were obtained using five to ten points including the blank <sup>(12)</sup>. The other physio chemical analyses were carried out by using the standard procedures.

### HRSEM analysis

The particle size was determined by using the HR- SEM analysis. JEOL ASM 3500 SEM was used for the analysis. A representative portion of each sample was sprinkled onto a double side carbon tape and mounted on aluminum stubs in order to get a higher quality secondary electron image for SEM examination. All the analytical procedures were carried out in SAIF, IIT, and MADRAS. Chennai36.

**(iii) Experimental animals & housing condition**

6 weeks old Wister albino rats (weight 150-200 gm) and Swiss albino mice (weight 20-25 gm) of both sexes were obtained from the King Institute, Chennai. The animals were adapted to the condition of the animal house for a week before the experiment. Animals were maintained in the standard environmental condition ( $22\pm 3^{\circ}\text{C}$ ). They were allowed to free access to water and standard pellet diet (Sai meera pvLtd, Bangalore) the principles of the laboratory animal care were followed. All experiments were conducted after getting the clearance from IACE of NIS (1248\ac\cpcsea\03\iaec). All the animal studies were conducted in Department of Pharmacology, NIS

**(iv) Drug dosing**

The animal dose was derived from the human therapeutic dose indicated in the siddha text<sup>(4)</sup>. The dose level was calculated according to body weight and surface area of the animal. Since the clinical dose is 260 mg/day, the acute toxicity study was carried at the dose level of about 4.68 mg/animal body weight, and the long-term toxicity study was carried out at different dose level of (1)4.68mg, 23.4 mg, and 46.8 mg per animal body weight.

**(v) Acute toxicity study**

The acute toxicity study was carried out as per WHO guidelines (1993)<sup>(13)</sup>. It is a single dose study observed for the period of 14 days. 10 male and 10 female mice were used for the study. They were separated into 2 groups (5/group). The control group received 10% of aqueous tween 80 solution. The test group received a single dose of KGM. After the administration of drug the animals were monitored for the period of 14 days for any abnormal signs.

**(vi) Long-term toxicity study**

Wister albino rats were randomly divided into four groups as per guidelines<sup>(13)</sup>. Each group consists of 3 male and 3 female rats. KGM was suspended in 10% aqueous tween 80 solution. It was administered to Group II, III and IV corresponds to three doses of the test drug treated rats. (i.e. 4.68mg, 23.4 mg, 46.8 mg) respectively. Group I serve as vehicle

control animals they received aqueous tween 80 solution. The test drug was administered by oral gavages once daily for a period of 14 days. The observations such as body weight, feed intake and water intake were noted followed by hematological, biochemical and histopathological analysis.

**Physiological, hematological and biochemical analysis**

The body weight change was recorded weekly with simultaneous observations of clinical manifestation and mortality. The clinical signs such as alertness, aggressiveness, passivity grooming, gripping strength, touch responses, restlessness, tremors, convulsions, pain response, righting reflex, pinna reflex, corneal reflex, papillary size, lacrimation, salivation, urination, writhing, skin colour was noted once a day for the period of 14 days. Food and water intake was measured once in a day for 14 days. On 15<sup>th</sup> day blood was collected through cardiac puncture under ether anesthesia, portion of the blood was immediately used for hematological estimation. The hematological parameters such as Hb, Trbc, Twbc were evaluated. The remaining blood was subjected to serum separation and it was used for the further biochemical analysis (lipid profile, renal and hepatic parameters). After the blood collection, the animals were sacrificed by decapitation and the brain, liver, kidneys were carefully dissected and weighed. Then a portion of each organ was fixed in 10% formalin solution. The fixed tissues were routinely processed, sectioned and stained with hematoxylin and eosin for further histopathological examination.

**(viii) Statistical analysis**

The clinical signs of intoxication, body weight changes, food consumption, hematological and biochemical parameters were subjected to computer software programme insta v3 version. The results were presented as MEAN  $\pm$  SEM for 3 male and 3 female in each group. The results were analyzed statistically by one way ANOVA followed by dunnett's test. P value < 0.05 was taken to indicate a statistical significant difference between the groups being compared.

## RESULTS

### (i) *Elemental and Physio chemical analysis*

The physicochemical property of KGM formulation was tabulated in table1-3 .The preliminary phytochemicals present in the KGM was tabulated in table 4.

**Table 1**  
***Colour characters of Kasha Gulanthaga Mathirai***

S No	Solvent used	Under light	ordinary	Under light	ultra violet
1	PM*	Light Brown		Light Brown	

\*Powdered material

**Table 2**  
***Physicochemical properties of Kasha Gulanthaga Mathirai***

S No.	Parameters	Values (%w/w)	obtained	Heavy/ toxic metals
1	Total Ash value	9.52		Lead BDL
2	Acid insoluble ash	0.95		Cadmium BDL
3	Water soluble ash	5.1		Mercury 3.124
4	Moisture content	7.20		Arsenic 7.824
5	Foreign organic matter	8.2		Volatile oil BDL
6	Alcohol soluble extractive	6		
7	Water soluble extractive	9.67		
8	Loss of weight at 105°C	6.20		
9	Hardness	6.3 GPa		

**Table 3**  
***Colour, nature and percent yields of extracts of Kasha Gulanthaga Mathirai***

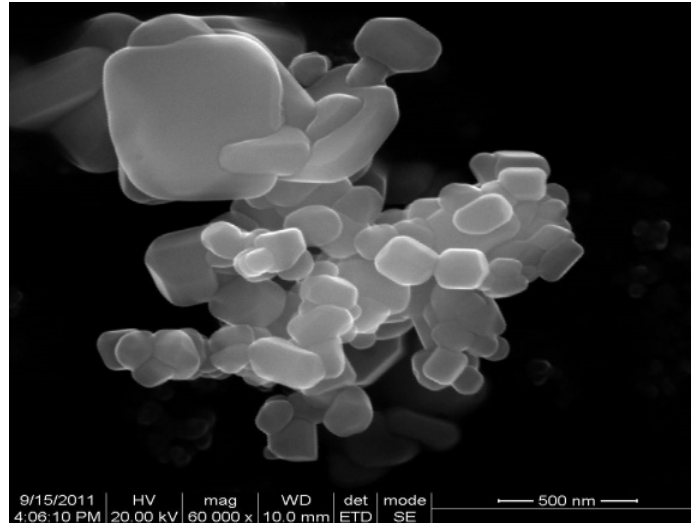
S.No	Extract solvent	Colour	Nature	% (w/w)	yield	pH	particle size in micron
1.	Water	Light Brown	Solid	45		7.1-7.5	0.1-1

**Table 4**  
***Preliminary phytochemical analysis of different extracts of Kasha Gulanthaga Mathirai***

S.No	Phytoconstituents	Ethanol
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Tannins	+
5	Glycosides	+
6	Carbohydrates	-
7	Amino acids	-

**(ii) HRSEM Analysis**

Particle Size: 0.1-1 $\mu$   
 Shape: rectangular  
 Distribution: cumulative distribution  
 Surface: smooth



**Figure 1**

**HR-SEM image shows the size, shape distribution and surface of KGM formulation Size: 0.1-1 $\mu$  Shape: Rectangular Distribution: cumulative distribution Surface: smooth**

**(iii) Toxicity studies**

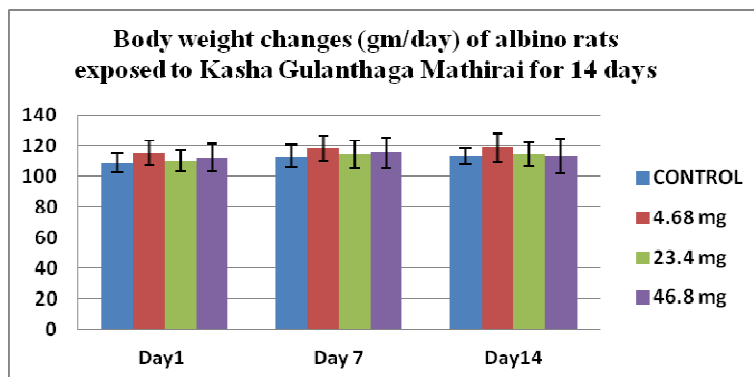
**a. Acute toxicity study**

There was no mortality over a period of observation for 14 days in animals treated with a single over dose of 4.68mg/ animal body weight. There were no other signs of toxicity noted.

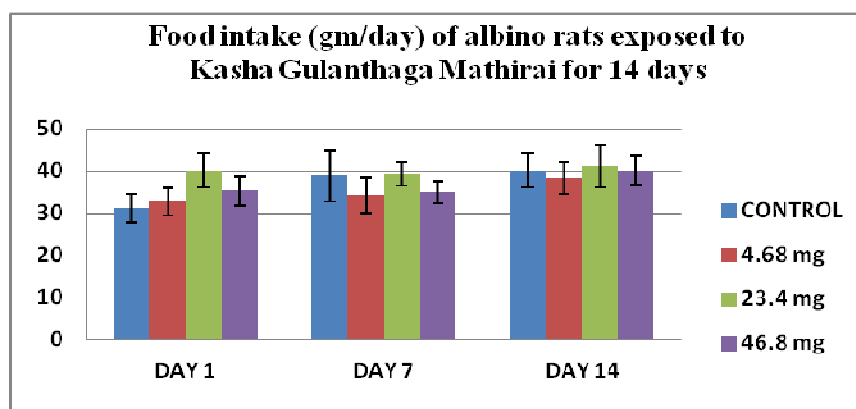
**b. Long-term toxicity study**

Significant behavioral changes and mortality were not observed during the period of 14 days of KGM administration. Water and feed intake were found to be insignificant in all test drugs treated animals compared to the control animals. All the animals gained body weight during the course of the study and the body weight changes was not found to be significant as compared to the vehicle control animals. (Graph1- 3)

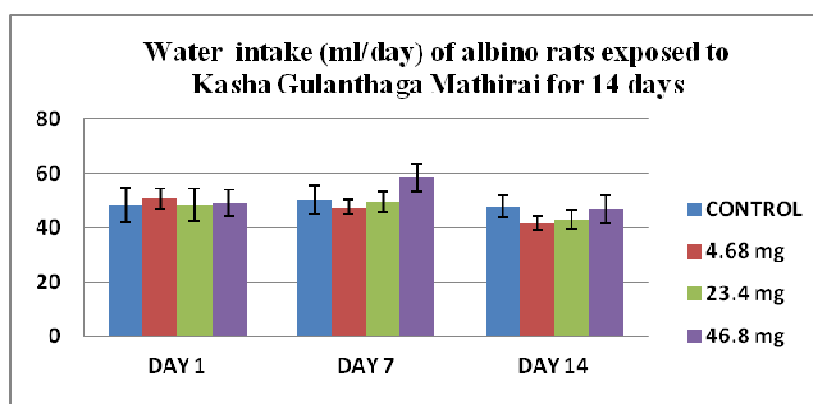
**Graph 1  
 BODY WEIGHT CHANGES**



**Graph 2**  
**FOOD CONSUMPTION**



**Graph 3**  
**WATER CONSUMPTION**



**i. Effect of KGM on hematological profile**

The hematology profile of vehicle and KGM treated rats are shown in table 5 significant alterations were not observed in the hematological parameters.

**Table 5**  
**Effect of KGM on hematological profile\***

INVESTIGATION	GROUP I	GROUP II	GROUP III	GROUP IV
Hb%	11.8 ±1.5	17.9±4.8	17.6±2.9	19.8±5.8
T c cells/ $\mu$ l	4750±353.5	4750±1930.5	6900±3686.7	4433.3±1482.8
Polymorphs%	36.5±26.2	58±2.5	46.8±17.5	38.6±20.5
Lymphocytes%	58±19.8	41.5±2	52.5±16.9	59.3±9.03
Esinophils%	2.1±2.9	0.7±0.8	0.5±0.8	0.4±0.5
TRBC millions/ $\mu$ l	3.8±.3	6±1.6	5.8±1	6.5±2

\*Data are expressed in mean  $\pm$  sem, n=6 in each group, Dunnet't' test

**ii. Effect of KGM on renal and hepatic markers**

The renal and hepatic markers of the vehicle and test drug treated rats are shown in table 6 and 7. In the present study significant changes were not observed in control and test group animals.

**Table 5**  
**Effect of KGM on hepatic markers\***

INVESTIGATION	GROUP I	GROUP II	GROUP III	GROUP IV
T.bilirubin mg/dl	0.7±.1	0.7±0.13	0.71±0.18	0.65±0.1
Dir. Bilirubin mg/dl	0.2±0.08	0.3±0.06	0.36±0.10	0.28±0.07
Ind. Bilirubin mg/dl	0.4±0.07	0.36±0.13	0.35±0.10	0.36±0.08
SGOT U/L	54±7.1	73.8±24.6	50.1±5.0	63.1±19.0
SGPT U/L	61±8.4	82.5±33.6	66.3±12.5	75.8±20.3
Alk.ph U/L	141±7.8	142.6±15.5	149.3±18.5	140.3±8.5
Total protein g/dl	6.6±0.4	7.1±0.37	6.8±0.62	6.7±0.3
Albumin g/dl	3.05±0.35	3.4±0.25	3.4±0.35	3.5±0.33
Globulin g/dl	3.55±0.07	3.6±0.48	3.4±0.41	3.1±0.24

\*Data are expressed in mean ± sem, n=6 in each group, Dunnet't' test

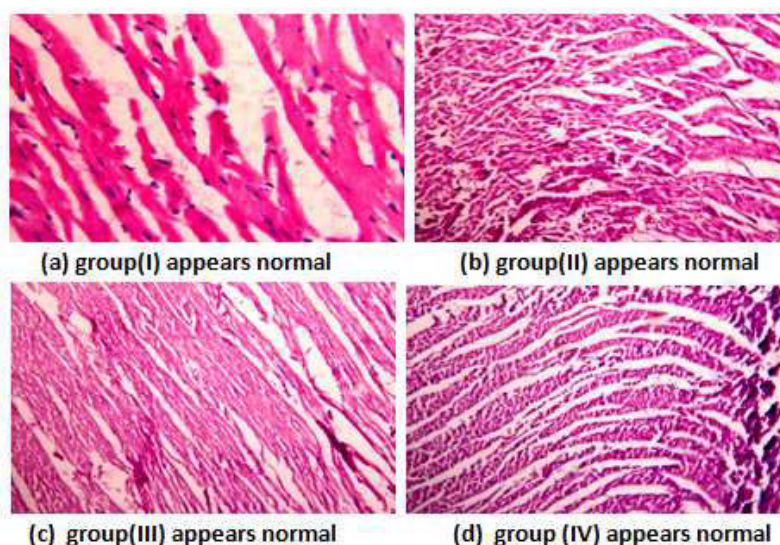
**Table 6**  
**Effect of KGM on renal markers\***

INVESTIGATION	GROUP I	GROUP II	GROUP III	GROUP IV
Urea	28±15.5	34.2±4.2	24±7.7	26.8±2.7
Creatinine	0.67±.12	0.76±0.08	0.63±0.08	0.7±0.1
Uric acid	2.44±.1	2.3±.35	2.3±0.63	2.4±.54
Calcium	8.7±0.8	8.7±0.67	8.5±1.1	9.7±0.48
Phosphorous	2.85±.07	3.1±.62	2.6±0.92	3.7±0.58
Calcium	8.7±0.8	8.7±0.67	8.5±1.1	9.7±0.48

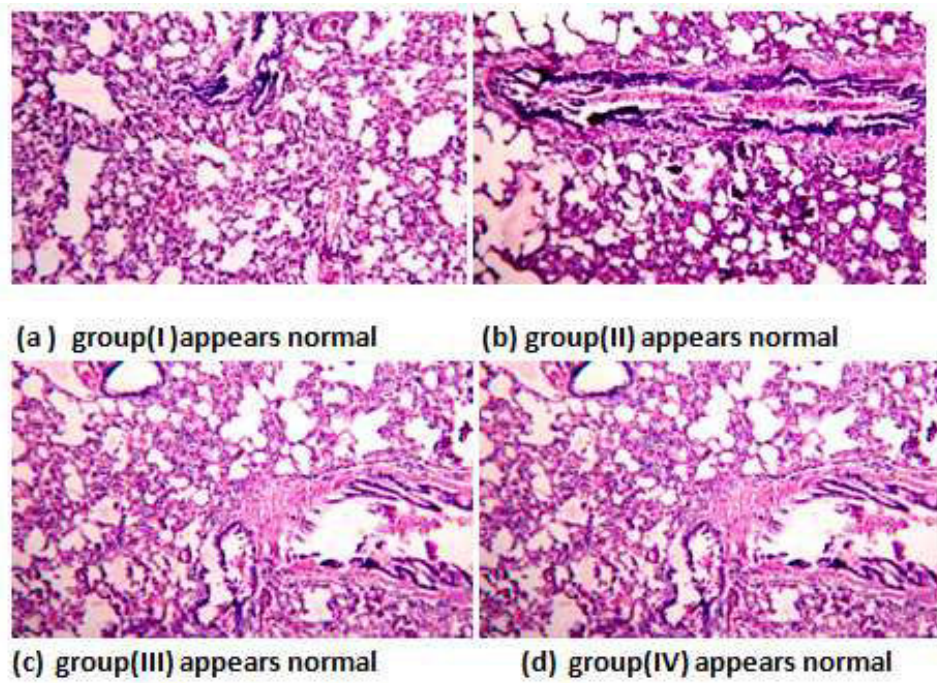
\*Data are expressed in mean ± sem, n=6 in each group, Dunnet't' test

### iii. Histopathological analysis

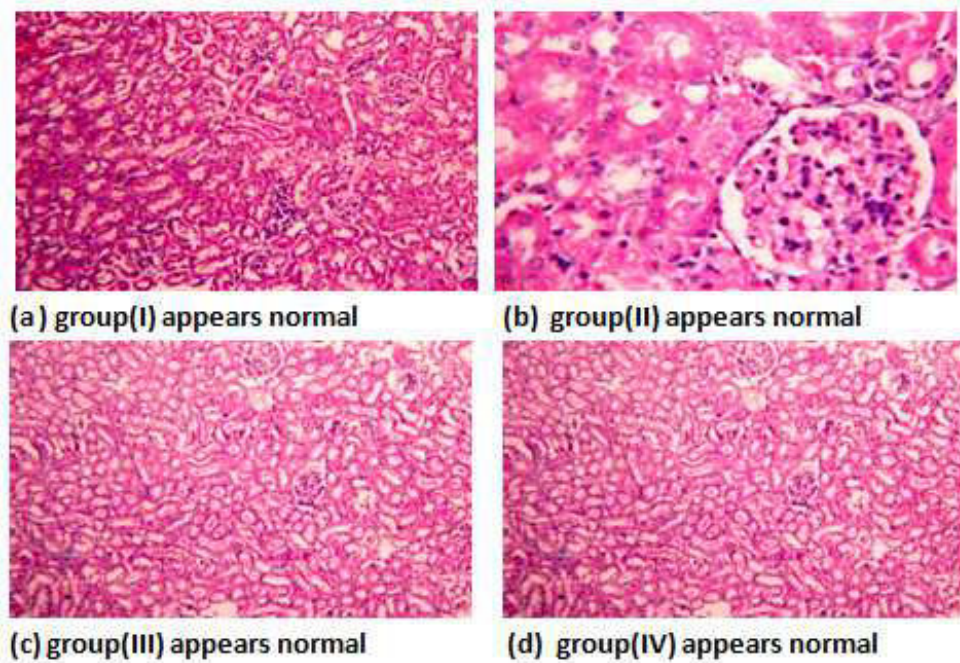
Histopathological analysis of heart, kidney, lungs, brain and liver are shown in fig (2-5). Gross pathological examination of animals doesn't reveal any abnormalities in control and test groups. The histopathological study of the organs such as heart, lungs, kidney, brain and liver was normal in Group I, II&III animals. In Group IV animals the liver shows fatty changes. The lungs, heart, brain, kidney shows normal histology.



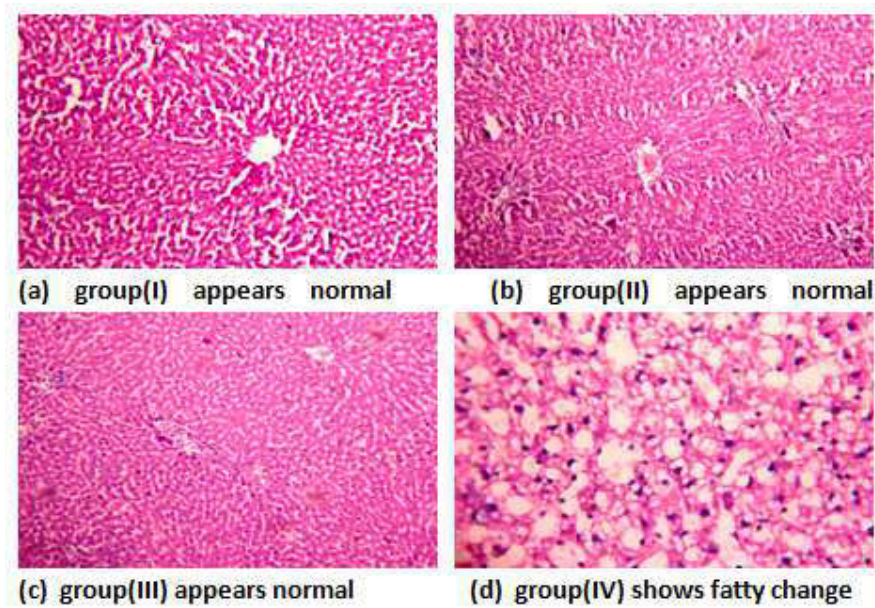
**Figure 2**  
**Histological analysis of heart sections in control and experimental Groups**



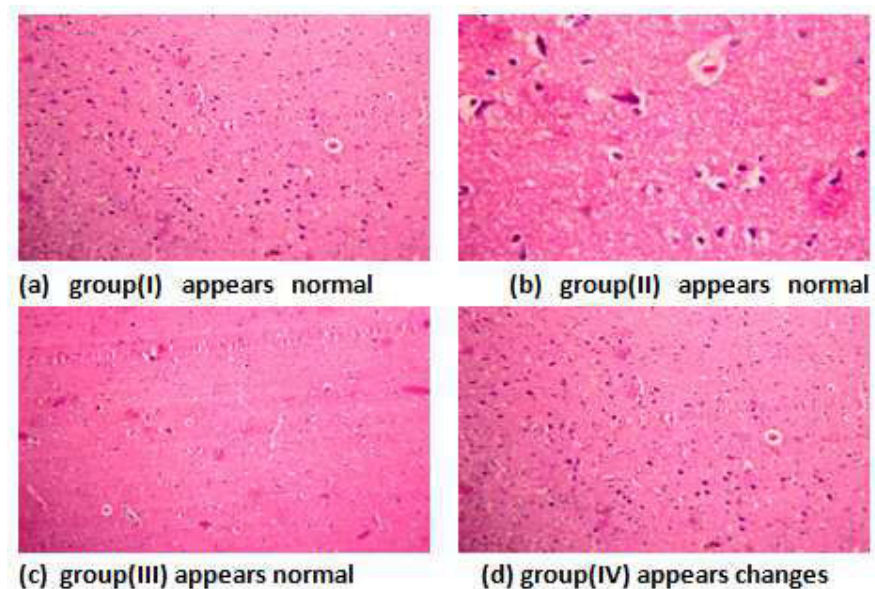
**Figure 3**  
*Histological analysis of lung sections in control and experimental groups*



**Figure 4**  
*Histological analysis of kidney sections in control and experimental group*



**Figure 5**  
*Histological analysis of liver sections in control and experimental group*



**Figure 6**  
*Histological analysis of brain sections in control and experimental group*

## DISCUSSION

In recent times there is growing and increasing interest in siddha medicine consequently they have greater attention as an alternative to clinical therapy leading to increased demand <sup>(14)</sup>. A world health organization survey indicates that about 70-80% of the world's populations rely on non-

conventional medicine mainly of herbal source in their primary healthcare <sup>(15)</sup>. In spite of the widespread use very few scientific studies have been done to ascertain the safety and efficacy of traditional remedies. There by the toxicological evaluation is necessary to determine whether the drug is

safe for human use. KGM is the traditional siddha medicine which is prescribed for Shayam

(pulmonary tuberculosis), Swasakasam (bronchial asthma), and Suram (fever) and is found to be effective against the diseases but there are no scientific studies available to study its toxic profile. In the present study there were no toxic or harmful phytochemicals identified in the drug which proves that it is not toxic and does not have any side effects. Physicochemical analysis of KGM has given the results that, the pH of the drug was 7.1-7.5 i.e. weakly alkaline. Hence, in the oral administration of the drug it may not irritate the gastrointestinal mucosa like strong alkali drugs. The mean ash value (%) was found to be 9.52 (total), 5.1 (water soluble ash), 0.9 (acid insoluble ash) implies the presence of inorganic compounds in the preparation; Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards (Kokate et al., 2006). The yield value implies the purity of the drug. Percent weight loss on drying was 6.20 %, which is not too high, hence could discourage bacterial, fungal, or yeast growth (African Pharmacopoeia, 1986). Foreign organic matter in the preparation was 8.2%; this may be due to first hand collection of raw material from non polluted area (Khandelwal, 2005). The moisture content was found to be 7.2%. The less value of moisture content could prevent bacterial, fungal and yeast growth<sup>(16)</sup>. ICPOES studies showed the presence of heavy metals such as mercury, arsenic within their permissible limits.

HR-SEM analysis indicated the particle size of Kasha Gulanthaga Mathirai as 0.1-1  $\mu$ ; the particles were rectangular in

shape, cumulatively distributed and have smooth surface which indicated the even finishing of the drug. Hence the drug will give better absorption. In Acute toxicity study there was no abnormal signs and no mortality reported at the dose level of 4.68 mg/animal body weight. Long term toxicity study revealed that there was no significant change in body weight, water and food intake, hematological and biochemical parameters. Obvious changes were noted in Hb and mild changes in other hematological parameters compared to control group. The histopathological study revealed the liver shows fatty changes and the other organs were normal.

## CONCLUSION

From the results of acute and long term toxicity study, the test drug Kasha Gulanthaga Mathirai does not produce any toxic signs and mortality up to its maximum dose level of 4.68 mg/animal body weight in Swiss albino mice and 46.8 mg/animal body weight in Wistar albino rats. As the qualitative and quantitative analysis reveals that the presence of heavy metals are within limits and the toxicity studies indicate no mortality, no changes in behavior, hematological parameters, biochemical parameters and histopathology up to the dose level of 10 times that of therapeutic dose. Based on these results it can be concluded that, the dose level of Kasha Gulanthaga Mathirai 130 mg (kundriyalavu) mentioned in the Siddha Literature Kannusamiyam Paramparai Vaithyam is the safety dose for human consumption.

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