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Toxicological Study of *Rasamanikya* by L.D. 50 Method

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Abstract

Rasamanikya is a single drug formulation of *Hartal* (Arsenic Trisulphide).*Hartal* is a drug categorised as one of the two *dhatuvisha* described by AcharyaSushruta. *Rasmanikya* is a drug which is useful in several diseases. It precipitates the need of toxicity study of *Rasamanikya*. In present study, *Rasamanikya* has been prepared as per the classical text and then its analytical as well as toxicity studies have been carried out. The SEM (Scanning Electron Microscope) with EDAX (Energy Dispersive X-Ray Analysis) analytical study shows 56.80% of Arsenic and 43.20% of Sulphur in the *Rasmanikya*. The toxicity study did not show any toxicity signs or mortality in Albino mice when given in normal dose.

Keywords

Rasamanikya, Hartal, Toxicity study, Dhatuvisha

Greentree Group

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INTRODUCTION

Agadatantra is a science that deals with the signs and symptoms along with the management of poisoning due to bites of snake, insects, worms, spiders, rodents etc. and various other poisons produced by improper combinations of substances or drugs[1]. In *Agadtantra*, detailed description of the visha (poison), its classification and examinations, diseases caused, different treatment principles, preventive measures etc. of the poisons are mentioned.

*Hartal*is categorized under *Sthavara-Dhatuvisha*and *Uparasa*. It is also included in one of the two *Dhatuvisha*by AcharyaSushruta. *Rasamanikya*, prepared using *Hartal*, is the *Rasakalpa* which is widely used in *Vatarakata, Firanga*, various types of *Kushtha*, and *Nadivrana*. It is also effective in *Nasaroga* and *Mukharoga*, as per the reference from Rasatarangini.[2]

Ancient *Rasasiddhas* have given proper guidelines for the pharmaceutical procedures with the light of micro to macro levels of the product referred to bioavailability of the medicament. According to modern science, *Hartal* i. e.., Yellow Orpiment (As_2S_3) is a compound of arsenic. Arsenic is a heavy metal poison and if taken orally, produces acute gastro-intestinal disturbances.

If *Hartal* is not purified by *shodhan* process, it shows toxic effects on body, produces *Vataj* & *Kaphaj vyadhi* and also can be fatal, as per reference from Rasatarangini.[3] In present study, *Rasamanikya* was prepared as per the standard method mentioned in Rasatarangini. As per this reference, shodhan (Purification) of *Hartal* was done. Such shodhit (Pure) *Hartal* was further used for the preparation of *Rasmanikya*.

Prepared *Rasmanikya* was tested analytically for its physico-chemical tests, confirmative test and for SEM with EDS test. Along with these analytical tests, in the present international circumstances, conduction of animal experimentations to verify the potential, safety of *Ayurvedic* formulations is also quite important. It precipitates the need of the toxicity studies of medicinal formulations with mineral ingredients. So in present study, *Rasamanikya* was subjected for its toxicity study.

Toxicity studies on experimental phases are carried out on small sized animals which are similar to human beings in drug activity. Albino mice are among the commonest and most standardized of all the laboratory animals suitable for experimental work,

because of its small size and greater sensitivity to many drugs. Hence albino mice are used as experimental animals during this study. To test the safety of *Rasamanikya* with modern parameters and to assure modern scientific community, conduction of this study to determine acute oral toxicity of *Rasamanikya* in albino mice was done, following OECD guidelines for testing of chemicals no.423. Determination of acute oral toxicity is an important initial step in identifying the toxicological properties of *Rasamanikya* if any. It may provide information on health risks resulting from a single oral intake.

The performed experiment includes study of behavioural pattern, mortality, body weight changes, salivation, diarrhoea, convulsion, coma, histopathology changes and autopsy of mice.

Study will provide much needed information regarding the toxic effects of *Rasamanikya* on the basis of the above mentioned parameters.

MATERIAL AND METHODS

Rasaamanikya were prepared as per the reference obtained from*Rasatarangini*[4]

Following this reference, present study has been completed in following steps –

1. *Shodhan* of *Hartal*
2. Preparation of *Rasamanikya*
3. Standardization of *Rasamanikya*
4. Acute Toxicity of *Rasamanikya* in Albino mice

***Shodhana*of *Hartal*:**

Raw Materials-(Figure 1)

1. *Ashuddha* (Impure) *Hartal* (Orpiment)
2. Fruit of *Kushmanda* (Benincasahispida)
3. Fruit of *Nimbu* (Citrus acida)

Ashuddha Hartalchurna (Powder) was taken in a *khalvayantra* (Mortar and pestle).*Kushmand Swarasa* was poured in it and the mixture was triturated well till *Hartal* absorbs the *swaras*. The mixture was then dried. Similar procedure was repeated for another 6 times. Then *Hartal* was dried well. Then seven nos. of *bhavana* of *Nimbu swaras* were given to the above powder of *Hartal***(Figure 2)**. After drying, this *Shuddha Hartal* powder was used for the preparation of *Rasamanikya*.

Preparation of *Rasamanikya*-(Figure 3)

Shodhit Hartal was spread on a Mica sheet and then covered by another sheet of Mica. The joint of the Mica sheets was sealed using 'U' pins. Then it was held with a pair of tongs and heated on LPG stove. During the heating procedure emission of fumes took place. When the mixture inside the

sheets become reddish in colour, heating was stopped. Then the Mica sheets were separated and *Rasamanikya*, which was sticked to the Mica sheet, was scratched and taken out. Thus prepared *Rasamanikya* was stored in an airtight container.

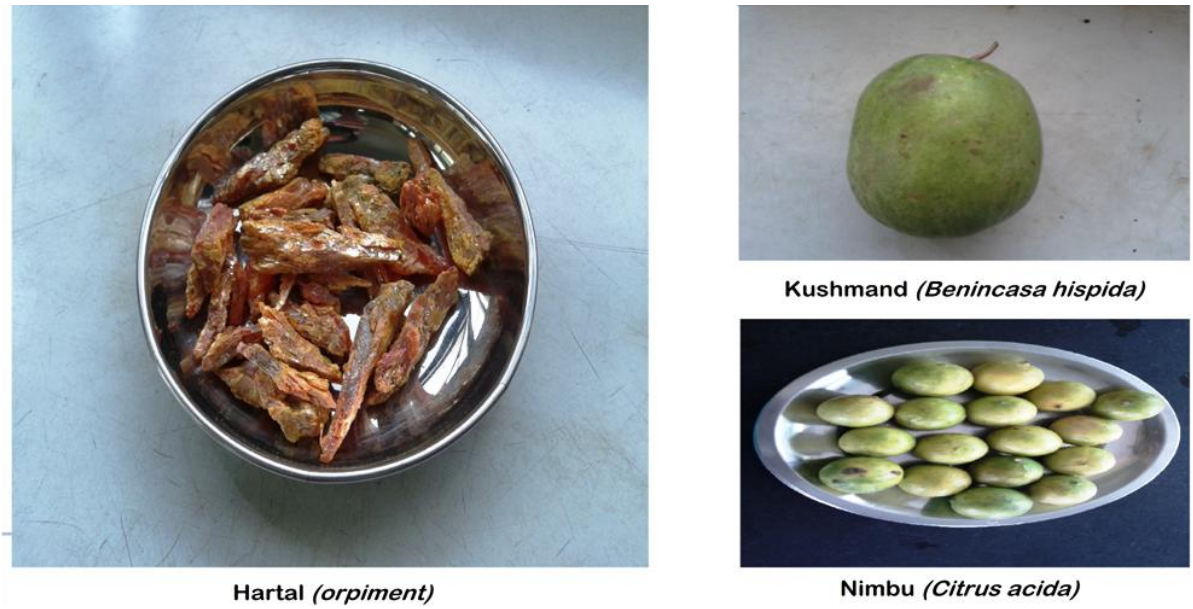
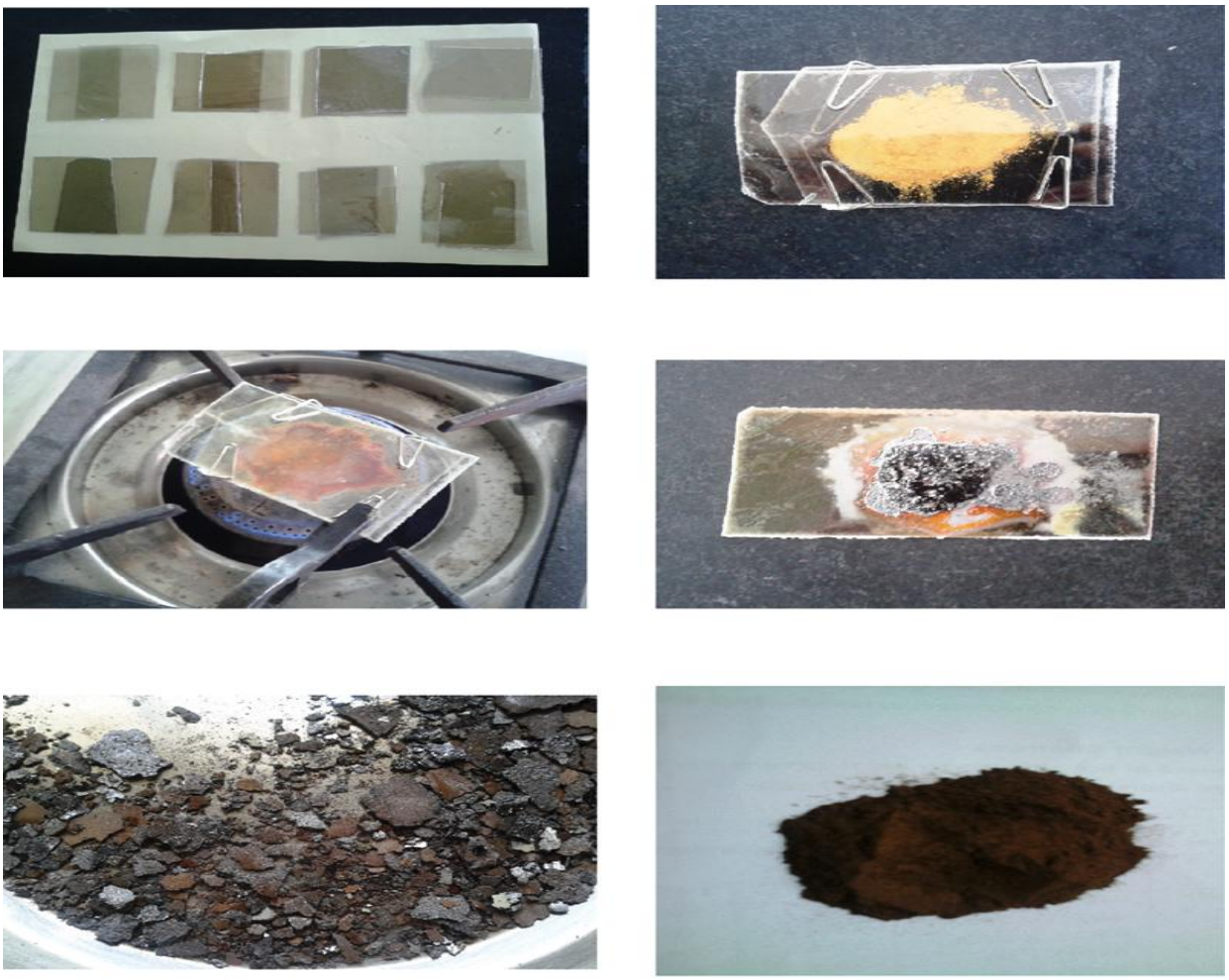


Figure 1 Raw material

Figure 2 Shodhan of Hartal



Figuer 3 Preparation of Rasamanikya

Standardization of *Rasamanikya*:

Rasamanikya was subjected to analysis by employing various suitable parameters and methods as mentioned below:

1. Ash value

This test was conducted to evaluate the ash content of the sample drug.The crucibles were initially cleaned with distilled water and dried in oven at 105^0C for 2 hours. One gram accurately weighed

sample was taken in a pre-weighed dried crucible. It was incinerated in a muffle furnace up to 750^0C.The crucible was taken out, self-cooled and weighed immediately.The percentage of ash obtained was calculated from the weight of the ash obtained and expressed as % w/w.

2. **Acid Insoluble Ash**

The acid insoluble ash content test was conducted to assess the percentage of inorganic content of the sample, which is insoluble in dilute acid.

The ash of the test drug was taken with 25 ml of dilute hydrochloric acid in a beaker, boiled for few minutes and cooled. It was then filtered through 41 no. Whatman's filter paper (ash less) and washed with distilled water repeatedly till it became chloride free. The filter paper in the glass funnel, along with its residue was kept for drying in the oven. The dried paper along with the residue was shifted to a pre-weighed crucible, kept in muffle furnace and heated up to 750^0C, till constant weight was obtained. On cooling, it was weighed and the acid insoluble ash content was calculated from the weight of residua obtained and expressed as % w/w.

3. Loss on Drying

This test was conducted to evaluate the moisture content of the sample drug.

Silica crucible was cleaned with distilled water and dried in oven at 105^oC for 2 hours. One gram of drug sample was taken in a pre-weighed dried petridish. It was dried in oven at 105^oC till reaching a constant weight.The crucible was taken out, self-cooled and weighed immediately.The weight loss, i.e., loss on drying was calculated and expressed as % w/w.

Confirmative test for *Rasamanikya*:

1. Determination the presence of As^{+++} in *Rasamanikya*

1. One ml of O.S. (original solution) i.e., *Rasamanikya* solution was taken in test tube. Then 2ml of sodium hydroxide (NaOH) was added and after that 2ml of Copper Sulphate ($CuSO_4$) added in it.

Confirmation- Formation of green ppt indicated presence of As in Rasamanikya. On addition of excess of NaOH and boiling it turned red.

2. One ml of O.S. i.e., *Rasamanikya* solution was taken in test tube and to it 2ml of $NaHCO_3$ was added. After that 3ml of 10% Iodine solution was added to the above mixture. It was shaken vigorously for 10 min.

Confirmation- Iodine colour decolourised.

2. Determination the presence of Sulphurin *Rasamanikya*

1. O.S. i.e., 0.5 gm of *Rasamanikya* solution was taken in test tube. One ml 1N HCL was added to it. After heating this, H_2S (has smell of rotten eggs) gas was evolved which passed over lead acetate paper (formed by inserting paper in lead acetate solution).

Confirmation- Lead acetate paper was turned to black due to evolution of H_2S gas

2. O.S. i.e., 0.5 gm of *Rasamanikya* solution was taken in test tube. One ml 1N HCL was added in it. After heating this, gas was evolved which passed over silver nitrate paper (formed by inserting paper in silver nitrate solution).

Confirmation- Silver nitrate paper turns to black.

SEM with EDAX

SEM (Scanning Electron Microscope):-

The SEM is an instrument that produces a largely magnified image by using electron instead of light to form an image.

EDAX (Energy Dispersive X-ray Analysis)

Instrument: - Ultra thin sapphire window Si(Li) detector with 135 eV resolution at 5.9 keV.

It is an analytical technique used for the elemental analysis of chemical characterization of a sample.

Acute Toxicity study of Rasamankya in Albino mice

This study was conducted to establish the toxicity of *Rasamanikya* when administered by the oral route to the albino mice according to the OECD Guidelines No. 423, Adopted on 17th Dec. 2001 at pharmacy college. The Acute Toxicological study was conducted as per the protocol approved by the Institutional Animal Ethical Committee (SGRS/IAEC/03/2012-13).

MATERIALS

Sample- *Rasamanikya*

Animal- Mice

Vehicle-0.5% Carboxy Methyl Cellulose (CMC).

Other apparatus-

1 Syringe with oral feeding needle.
2 Test tubes
3 Cage.
4 Gloves.

Study Design:

Test Drug has been described in **Table 1**

Test Animal has been described in **Table 2**

In each step the animals were weighed, marked for identity and the dose was calculated per kg body weight.

Table 1 Test Drug

Test Drug	
Test drug	*Rasamanikya*.
Prepared at	Rasashastra Lab.
Physical state	Solid state, powder form.
Colour	Brownish black.
Date of Mfg	16-5-2013
Date of test	14-8-2013.

Table 2 Test Animal

Test Animal	
Species	Mice
Strain	Swiss Albino
Source	Animal house of Pharmacy College
Weight range	22-26gm
Age	6-8wks
Sex	Female
Number	12 Mice
Housing	3 of similar sex(Female) per cage
Diet	Pellets Feed
Water	Distilled water provided ad libitum
Room Temperature	20-40 ^{o}C
Relative Humidity	40-60^{o}C
Light cycle	12hrs light and 12hrs dark
Acclimatization	7 days prior to dosing

Marking of Animals

The Albino mice were marked using picric acid. The marking were done at Neck (N), Paw (P) and Tail (T) region of the animals in each group, so that each animal should be identified and observed during the experimental procedure.

Preparation of Dosage

The dose used in each step was as per the OECD Guidelines No. 423 for Acute Oral Toxicity Study. The doses used were 300mg/kg, 2000mg/kg and 5000mg/kg of body weight. In each step the dose of *Rasamanikya* was calculated in individual animal according to their body weights.

Preparation of suspension

As *Rasamanikya*was in the form of powder, it was not possible to administer it in animals directly. So CMC as a vehicle was used to prepare the suspension of *Rasamanikya*, which can be administered in the mice through the syringe with oral dosing needle.

Experimental Procedure

Total 12 no.of female mice were assigned to the vehicle control and experimental (test) group. The test material (*Rasamanikya*) was administered once orally by syringe to the mice.

The mice were deprived of feed 3-4 hours and 1-2 hrs after administration of test material (*Rasamanikya*), water was allowed ad *libitum*.

This study was performed in a stepwise manner

Step I

Vehicle control (CMC 0.5%)

A group of three female mice were administered CMC to show that CMC did not cause any toxicity. The vehicle caused no signs of intoxication after dosing till the end of the study.

Step II

Three female mice were administered *Rasamanikya* diluted in CMC at the dose of 300 mg /kg body weight.

Step III Three female mice were administered the test material diluted in

CMC at the dose of 2000 mg / kg body weight.

Step IV

Three female mice were administered the test material diluted in CMC at the dose of 5000 mg / kg body weight.

The groups which were used for the study are shown in **Table 3.**

Table 3 Groups of Mice used for the study

Sr.	Group No.	No. of mice	Dose (mg/kg)
1.	I	3	Vehicle control
2.	II	3	300
3.	III	3	2000
4.	IV	3	5000

OBSERVATIONS

- Animals were observed during first 4 hours with special attention for immediate signs and daily thereafter for delayed signs up to total of 14 days.
- Mice were observed for 14 days for following signs and symptoms:

Weight changes, Behavioral pattern, Salivation, Diarrhea, Convulsions, Coma, and Mortality

Weight changes

- Weight was taken once in a week.

Necropsy

- All the animals were sacrificed at the termination of the study i.e., after 14 days and a gross necropsy was performed on all animals.

Histopathology

- Histopathology of liver and kidney of all mice were done at the end of study.

OBSERVATIONS AND RESULTS

***Rasamanikya*:**

Observations of the prepared *Rasamanikya*are are stated in **Table 4**

Net weight of obtained*Rasamanikya* was 24 gm.

Table 4: Observations of *Rasamanikya*

Colour	Dark brownish
State	Solid powder form
Taste	Tasteless
Odour	Odourless
Touch	Crystalline soft

Standardization of *Rasamanikya*:

1. Physico-chemical tests

Rasamanikya was analysed physico-chemically. The findings are given in **Table 5.**

2. C.T. (confirmative test)

Confirmative test results are given in **Table 6.**

3. SEM with EDS test

Results are given in **Table 7**

Acute toxicological study

1) Individual Body Weight Changes

The body weight changes of the animals in each group on 0^{th} day, 7^{th} day and 14^{th} day are mentioned in **Table 8**

Table 5 Physico-chemical Tests

Sr. No.	Test	Result (w/w)
1.	Ash value	Negligible (0.06%)
2.	Acid insoluble ash	Negligible (Nil)
3.	Loss on drying	Not more than one (0.22%)

Table 6 Confirmative Test

Test Drug	Confirmation test	Result
Rasamanikya	As^{+++}	Positive
	S^{--}	Positive

Table 7 SEM with EDS test

Sr. No.	Elements	Result %
1.	As	56.80
2.	S	43.20

Statistical Analysis of Body Weight Changes

The difference in body weight change before the dosing and after 14days of dosing was recorded and it was statistically analysed by student's'‘t’ test.

1. Paired‘t’ test

It was applied to determine the significance in the body weight gain in all the groups before the dosing and after 14 days of dosing. The level of significance was set at 5% or 0.05

The result are shown in **Table no.9**

The above table reveals that there is significant body weight gain in all the groups before and after the dosing.

2. Unpaired‘t’ test

It was applied to compare the effect on the body weight gain in vehicle control group and test groups. The level of significance was set at 5% or 0.05.

Table 8 Body weight changes

Group	Sex	Animal no.	Weight (in gm) recorded on day 0^{th}	7^{th}	14^{th}	Total Weight gain (gm)
I (Vehicle Control)	Female	01(N)	23.2	26.4	29.0	5.8
		02(P)	24.1	27.3	30.2	6.1
		03(T)	22.8	25.3	28.5	5.7
II (300 mg/kg)	Female	01(N)	24.2	27.1	29.9	5.7
		02(p)	22.7	24.9	28.7	6.0
		03(T)	23.6	25.7	29.8	6.2
III (2000 mg/kg)	Female	01 (H)	22.6	25.4	28.2	5.6
		02 (P)	24.7	27.2	30.6	5.9
		03 (T)	23.1	26.3	29.1	6.0
IV (5000 mg/kg)	Female	01 (H)	24.3	27.2	30.1	5.8
		02 (P)	23.6	26.4	29.4	5.8
		03 (T)	23.1	25.8	29.3	6.2

a) Group I (Vehicle Control) compared with group II for body weight gain-

Results are shown in **Table 10**

b) Group I (Vehicle Control) compared with group III for body weight gain

Results are shown in **Table 11**

c) Group I (Vehicle Control) compared with group IV for body weight.

Results are shown in **Table 12**

Table 9 Paired‘t’ test

Groups	Mean observed difference	S.D.	S.E.	‘t’ value	P value	Result
I	5.87	0.20	0.11	53.36	<0.05	Significant
II	5.97	0.25	0.14	42.64	<0.05	Significant
III	5.84	0.20	0.11	53.09	<0.05	Significant
IV	5.94	0.23	0.13	45.69	<0.05	Significant

Table 10 Unpaired ‘t’ test

Groups	Difference of mean	S.D.	S.E.	‘t’ value	‘P’ value	Result
I &II	0.10	0.22	0.17	0.58	>0.05	Insignificant

Table 11 Unpaired ‘t’ test

Groups	Difference of mean	S.D.	S.E.	‘t’ value	‘P’ value	Result
I&III	0.03	0.20	0.16	0.18	>0.05	Insignificant

Table 12 Unpaired ‘t’ test

Groups	Difference of mean	S.D.	S.E.	‘t’ value	‘P’ value	Result
I&IV	0.07	0.21	0.17	0.41	>0.05	Insignificant

The above study shows that there is no difference or insignificant difference in the body weight gains in all the test groups when compared with vehicle control group i.e., there is no harmful effect on body weight gain in the test groups when compared with that of vehicle control group.

1. Behavioural signs

These are described in **Table 13**

No any definitive sign of toxicity was seen.

2. Salivation, Diarrhoea, Coma, Convulsion and Mortality

Animals were observed for first 4 Hours and thenobserved daily with an interval of 24 hours up to 14 days. No signs of salivation,Diarrhoea, Coma, Convulsion and Mortality were found.

Autopsy finding:

Autopsy findings are shown in **Table 14**

After sacrificing all mice at the end of the study, autopsies were done.

There were no any deformities observed in autopsy of any mice.

Histopathology:

1. Histopathology of kidney

Findings are shown in **Table 15**

2. Histopathology of Liver

Findings are shown in **Table16**

Table 13 Behavioural patterns

Group	Animal no	Behavioural patterns observed after dosing																	
		First 4 Hours				Days													
		1	2	3	4	1	2	3	4	5	6	7	8	9	10	11	12 1	13	14
I	01 (N)	A	A	A	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	02 (P)	A	A	A	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	03 (T)	A	A	A	A	A	N	N	N	N	N	N	N	N	N	N	N	N	N
II	01 (N)	A	A	A	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	02 (P)	A	A	A	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	03 (T)	A	A	A	A	A	N	N	N	N	N	N	N	N	N	N	N	N	N
III	01 (N)	A	A	A	A	A	N	N	N	N	N	N	N	N	N	N	N	N	N
	02 (P)	A	A	A	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	03 (T)	A	A	A	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N
IV	01 (N)	A	A	A	A	A	N	N	N	N	N	N	N	N	N	N	N	N	N
	02 (P)	A	A	A	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	03 (T)	A	A	A	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N-Normal
A- Anxiety (maybe due to oral feeding needle and process of dosing)

Table 14 Autopsy findings

Group	Dose(mg/kg)	Sex	Finding	No. of animals
I	(Vehicle)	Female	NAD	3
II	300	Female	NAD	3
III	2000	Female	NAD	3
IV	5000	Female	NAD	3

NAD - No Any Deformity

L.D.50 Value-

L.D. 50 of *Rasamanikya*was found to be > 5000mg/kg

Previous study on toxicological study of *Rasamanikya* prepared by different methods also shows that *Rasamanikya* prepared using *Abhrakpatra* and that using *sharavasamputa* showed minimal histopathological changes in Albino rats proving its non-toxicity.[5]

Table 15 Histopathology of Kidney

Groups	Animal no.	Glomerulopathy	Degeneration of tubules	Cellular infiltration

I	01 (N)	0	0	0
	02 (P)	0	0	0
	03 (T)	0	0	0
II	01 (N)	0	0	0
	02 (P)	0	0	0
	03 (T)	0	0	0
III	01 (N)	+	+	0
	02 (P)	0	+	+
	03 (T)	+	+	+
IV	01 (N)	+	+	+
	02 (P)	+	++	+
	03 (T)	++	+++	++

Note:
0: no abnormality detected
+: damage/ active changes up to less than 25 %
++: damage/ active changes up to less than 50 %
+++: damage/ active changes up to less 75 %

Table 16 Histopathology of Liver

Groups	Animal no.	Swelling of hepatic cords	Degenerative changes	Cellular infiltration
I	01 (N)	0	0	0
	02 (P)	0	0	0
	03 (T)	0	0	0
II	01 (N)	0	0	0
	02 (P)	0	0	0
	03 (T)	0	0	0
III	01 (N)	+	0	0
	02 (P)	0	+	+
	03 (T)	+	0	0
IV	01 (N)	+	+	+
	02 (P)	+	+	+
	03 (T)	+	+	+

Note:
0: no abnormality detected
+: damage/ active changes up to less than 25 %
++: damage/ active changes up to less than 50 %
+++: damage/ active changes up to less 75 %

DISCUSSION

Rasamanikya is having a single ingredient i.e., *Hartal* which has been described as a *dhatuvisha*. While describing the preparation of *Rasamanikya*, firstly *shodhan* of *Hartal* has been stated. Being a *dhatuvisha* and an Arsenic compound, *shodhan* of *Hartal* is necessary.So *shodhan* of *Hartal* by giving *bhavana* of *Kushmanda swaras* and *Nimbu swaras* was done as per the reference. Properties of *Kushmanda swaras* and *Nimbu swaras* may be incorporating in *Hartal* after *bhavana* which may be making it compatible for human.

ShodhitHartal was used for the preparation of *Rasamanikya*. *Abhrakpatra* were used for the preparation of *Rasamanikya.Abhrak patra* act only as a barrier during heating the *Hartal*. Indirect heat could be given through *Abhrakpatras*. After heating the sandwitch of *Hartal* and *Abhrakpatra*, prepared *Rasamanikya* had to be scratched off from the *Abhrakpatra.*

By C.T. (Confirmative tests) of *Rasamanikya*, it was found that *Rasamanikya* contains Arsenic (As^{+++}) and sulphide (S^{--}). When *Rasamanikya* was incinerated in a muffle furnace at 750^0C, Ash value found was negligible may be because, it was not containing organic matter and Arsenic sulphide has property to sublimation below the melting point(325^0C). A mere 0.22% of moisture was recorded while measuring loss on drying at 105^0C. These values were matched with standard values of *Rasamanikya*, mentioned in CCRAS.

SEM with EDAX study showed that *Rasamanikya* contains 56.80 % of Arsenic and 43.20% sulphur. Standard value of *Hartal*shows Arsenic (As) - 60.90 % Sulphur (S) - 39.10 %.

Thus prepared *Rasamanikya*, which fulfilled all the criteria mentioned in Ayurvedic texts, was tested for its acute oral toxicity in albino mice as per the OECD guidelines No.423.

In acute toxicity study, single dose of the drug was used in each animal on one occasion only for the determination of gross behaviour and LD50 dose i.e., the dose which kills 50% of the animals of a particular species.

The OECD (Organization for Economic Cooperation and Development) guidelines for testing of chemicals are collection of most relevant internationally agreed testing methods used by government, industry and independent laboratories to assess the safety of chemical products. Owing to its international acceptance and guidance on selection of the most appropriate methods for a given purpose, the present study was conducted as per the OECD guidelines. As the purpose of this study was to study the acute toxicity, the OECD guidelines No.423 for acute oral toxicity study were selected for the present study.

Present study was conducted on Wistar albino mice. Albino mice have similarity of structures, functions and drug activity to human beings. They are the smallest laboratory animals that can be breed uniformly. They are cheap and easy to

handle and being very small, they are sensitive to small doses of substance.

In order to administer the test substance orally by syringe it should be liquid or semi liquid at room temperature. As *Rasamanikya* was solid, powdery in nature, it was not possible to administer it orally to mice. Thus, *Rasamanikya* was needed to be dissolved or suspended in a suitable vehicle. For this purpose CMC, a standard vehicle according OECD guidelines was selected as a vehicle to form suspension for dosing in mice. It is an inert compound and having readily suspension property.

In this study, four groups were formed.

Group I

It was taken as a control group. To rule out the toxicity of vehicle, CMC was administered to Group I as a control group. Control group helps to find out if any significant change happened because of medicine or not.

Group II, III & IV

These were taken as testing /dosing groups.

The starting dose level should be that one which is most likely to produce mortality in some of the dosed animals. But when there is no information available on a substance to be tested, for animal welfare reasons, it is recommended to use the starting dose of 300mg/kg body weight. As no such previous informationon *Rasamanikya* was available, the starting dose level was selected 300mg/kg body weight.

As the dose level of 300mg/kg body weight did not cause any mortality, further testing was carried out as per the guidelines by using the dose levels of 2000 mg/kg. But these dose levels also had not caused mortality.

As *Rasamanikya* is a medicinal preparation, its testing results have direct relevance to human health. Hence additional upper dose level of 5000 mg/kg was selected.

In Ayurveda texts it is mentioned to administer *Rasamanikya* by oral route, so an acute oral toxicity study was conducted.

The animals in each group were observed for signs of toxicity and mortality, if any. The animals were observed for behavioural changes, weight gain or loss, salivation, diarrhea, convulsion, coma and mortality.

The observations were made for individual animal first 4 hours for immediate signs of toxicity or death. Then the animals were observed daily with an interval of 24 hours for delayed signs of toxicity upto 14 days. However no signs of toxicity or mortality were observed after administration of *Rasamanikya* at all dose levels starting from

300 mg/kg body weight to 5000 mg/kg body weight in all groups.

The record of body weight of each group was maintained prior to the dosing and consequently after 7 days and 14 days of dosing. The results of body weight changes were analysed by statistical methods.

The statistical analysis on applying unpaired‘t’ test shows that the changes in body weights in vehicle control group and test groups are statistically insignificant ($P>0.05$). There is no difference in body weight gain in control and test groups. It shows that the higher dose levels of *Rasamanikya* did not cause considerable weight changes as compared to that of control group.

The statistical analysis of weight gain in each group on applying paired ‘t’ test shows that the gain in body weight in vehicle control group and test groups is statistically significant ($P<0.05$). This shows the normal weight gain in both the control and test groups. This weight gain is attributed to the normal weight gain after 14 days.

In behavioural pattern, mice showed anxiety in first four hours maybe because of oral feeding needle and dosing process, which is new thing for mice.

All the animals dosed with *Rasamanikya* were alive after 14 days of study without showing any signs of toxicity.

No abnormality was detected in the liver, kidney, stomach and spleen on gross necropsy of all the animals after 14 days. It reveals that there were no adverse effects or signs of toxicity, after the dosing with *Rasamanikya*.

Histopathology of liver and kidney showed no any pathological changes of both organs in group I (control) and group II (300mg/kg) group. But only at higher dose i.e., in group III (2000mg/kg) and group IV (5000mg/kg) showed pathological changes. Kidney showed more pathological changes than liver, this reveals that, *Rasamanikya* in higher dose, affects more on kidney than liver.

So, it can be said that, in concern of acute toxicity, *Rasamanikya*is safe for internal use.

In the current era, there is demand for the assurance about the safety and purity of the medicinal compounds. Hence the need for toxicity study of Ayurvedic mineral preparations like *Rasamanikya* is necessary. For clinical practitioners, knowledge of particular toxicity profile of Ayurvedic medicines used in clinical practice is

necessary for the medicinal preparations containing metals, minerals and herbo-mineral compounds. Mineral preparations are excellent in therapeutic efficacy but it also must be safe for treatment.

Hence it is essential to evaluate the margin of safety between the dose levels which produces therapeutic effects to the level that produces adverse effects i.e., to provide the benefit as to the risk assessment. For this, the toxicity profile of Ayurvedic mineral preparations by animal experiments is the only way through which this assessment can be made. Present study will be definitely beneficial while using *Rasamanikya* for the treatment. Also it can be concluded that if the medicine is properly prepared following the classical/textual reference, it doesn't show toxic effects on the body if consumed in the given dose of that medicine.

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