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Research Article

PHARMACEUTICAL PREPARATION AND TOXICOLOGICAL STUDY OF *RASA BHASMA*

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ABSTRACT

The traditional use of Ayurvedic formulations is a widely accepted therapeutic option especially in chronic diseases. The quality of *Bhasma* depends on the manufacturing procedures and dosage schedule mentioned as per the classical texts. The Ayurvedic formulations are being considered as therapeutic potential agent as they have been evaluated based on experimental analysis. However for global acceptance the safety is a concern, the WHO has recognized *Ayurveda* as a part of integrated system of medicine. In view of developments an investigation is undertaken to evaluate the safety of a *Bhasma*. *Rasa Bhasma*, an organo-metallic compound is one such compound described in *Rasa Shastra* for treating acute and chronic diseases. It is prepared in several steps, which increases its therapeutic potential and eliminates its metallic toxicity. Among the various *Bhasma* preparations a popularly recommended and used formulation of *Rasa Bhasma* was selected for the toxicology study. In the present investigation it is proposed to undertake a short term pre clinical acute toxicity test on Rats, to obtain the safety profile. The proposed experiment has been under taken after taking the approval of Institutional Approval Ethical Committee, SIPRA Labs to conduct the experiment at their centre. Acute toxicity study on 15 Swiss Albino Rats, have been randomizingly selected and divided into two groups to receive the test compound in a vehicle and identified as a TD and a vehicle group to receive Honey, Water for a period of 7 days and identified as VC. 8 (3VC+5TD) animals after 48 hours and remaining 7(2VC+5TD) animals after 7 days were subjected for haematology, clinical chemistry and necropsy observation. After 7 days of exposure to the test compound of recommended therapeutic dose, no significant changes in haematological parameters & clinical chemistry parameters were found. No Pre-terminal deaths occurred in rats, which received test compound at therapeutic dose levels. No abnormalities in physical, physiological, clinical chemistry, hematological parameters and no gross necropsy changes were observed on administration (oral) of test compound prepared according to the classical literature. At this level of study, it may be concluded that *Rasa Bhasma* is safe as per toxicological study and can be subjected for further study of clinical evaluation.

KEY WORDS: *Rasa Bhasma,* Toxicological study, Hematological parameters.

INTRODUCTION

A careful scrutiny of the Rasatantric texts would reveal that almost all the treatises on *Rasashastra* contain references related to *Parada* Marana. However, these references a '*Rasasadhaka*' may make his mind in selecting a suitable procedure for *Parada Marana*. A planning for evaluation of these methods regarding their perfectness as well as planning in the sphere of research can also be worked out. To facilitate for such a planning it is obvious that all such references should be compiled at one place and scrutinised scientifically and carefully. There are many more methods in the published treatises on *Rasashastra*. It is quite possible that various unpublished and unnoticed manuscripts on *Rasashastra* will have some more methods of preparation of *Parada Bhasma*. In various traditions also, it is possible that various methods of Parada *Bhasma* preparations will prevail which traditional Rasa *Sadhakas* who are hardeners never disclose. Wherever the methods of *Parada Bhasma* preparations are described scientific explanation or the explanation of the interaction between the ingredients or the changes undergone through various processes have been made. The compilation has been classified in all five categories have been made based on the colour of *Parada Bhasma.*

Specification of Colour

1. White coloured *Parada Bhasma*
2. Red coloured *Parada Bhasma*
3. Yellow coloured *Parada Bhasma*
4. Black coloured *Parada Bhasma*
5. *Parada Bhasma* of unspecified colour.

Properties of *Parada Bhasma*

A careful screening of the *Rasatantric* literature would reveal that various types of therapeutic effects and pharmacological actions of *Parada Bhasma* have been mentioned by various authorities. Though the consideration of these pharmacological or therapeutic properties does not make the part of the present study, even then it will provide a background for future worker on this subject.

Rasa Bhasma is prepared in 2 methods, one is taken from *Rasa Manjari* and the other is taken from *Khanija Bhasmarajam.* Three methods were followed for mercury purification and purified mercury is named as M1, M2 and M3 from which they obtained *Rasa Bhasma* named respectively RB 1 to RB 6, and processing has been divided into following steps.

Method-I

A. *Shodhana* of raw materials
   i. *Parada*
   ii. *Gandhaka*
B. Preparation of *Kajjali* and *Bhavana* with *Grhitakumari*
C. Preparation of *Samputayantra* and *Bhudhara Yantra*

Method-II

A. *Shodhana* of raw materials
   i. *Parada*
   ii. Vanga
B. Preparation of *Baddha Parada*
C. Preparation of *Samputa* and *Puta*

MATERIALS AND METHODS

1. Preparation of *Rasa Bhasma*
2. Purification of raw material
3. Preparation of *Kajjali*
4. Toxicological Study

Method-I:

1. *Shodhana* (purification) of *Parada*:

Purification of *Parada* was carried out with three different procedures from various texts such as *Rasatarangini*, *Rasa Ratnakar Riddhikhand* and from *Bharata Bhaisajya Ratnakar.*

Procedure-1

1 kg of *Parada* was taken with 1 kg of *Chuna* (lime powder) in granite *Khalva* and trituration was done for eight hours in a day for 3 days. After complete trituration it was filtered through a double folded cloth to obtain the *Parada*. Because of its heaviness, *Parada* passed through the cloth and lime powder retained. Thus obtained *Parada* was filtered by a cloth. Thus, the clear 980 gm.. of Parada was obtained.

Obtained *Parada* was taken into a *Khalva yantra* and equal quantity of garlic paste (980 gm.) and half the amount of *Saindhava lavana* (490 gm.). are added. Trituration was again applied until whole mixture became black colour (paste of garlic). The washing and decanting was applied with the help of hot water for several times to get a clear Parada (920 gm.). *Parada* so obtained was completely pure[1]. All types of blemishes, which it had in the beginning are now removed through the process.

PROCEDURE-2

250g. of *Hingula* was taken in *Khalva* and was made powdered, added lemon juice as *Bhavana* dravya and triturated continuously for 8 hrs. In between the process citrus juice was added whenever necessary. After triturating *Hingula* paste is applied in inner bottom part of *Ghata* and was made *Urdhvapatana Yantra*. It was heated on *Vidhyadhara Yantra* with mild (300^0C), moderate (400^0C) and intensive (600^0C) Agni for 6 hours. In intensive period (last 2 hours) cold pads were kept over the top of the *Urdhvapatana Yantra*. After that left the *Yantra* for getting self-cooling. (Rasa Ratnakara Riddhikhanda*. 2/53*)

Next day, seal was removed carefully and *Parada* was collected by rubbing with brush at the *Parada* containing surface of the upper utensil, and filtered it by clean cotton cloth. 160 g. of *Parada* was obtained in this procedure.

PROCEDURE-3

1 kg. of *Parada* was taken in *Khalva*, 1 kg. of garlic paste was added to the mercury, triturated for 8 hrs. It was washed with hot water and filtered through double folded cloth. Obtained *Parada* (810 gm.) was again triturated with juice of betel leaf for 8 hrs, washed with hot water and filtered. 780 gm. of obtained *Parada* again triturated with *Triphala* decoction (3 myrobalans), washed with hot water and filtered. The weight of *Parada* thus obtained was 720 gms[2].

Sodhana* (Purification) of *Gandhaka

2 lit. of milk was boiled and taken in a vessel, the mouth of the vessel was covered with a clean cloth piece and tied around. 500 gm. of *Gandhaka* was melted with *Sarshapa* (mustard) oil in an iron spoon and immediately poured into the milk through the cloth.

The *Gandhaka* on cooling was collected from the vessel, in the form of large granules. It was washed with warm water, dried and then powdered[3].

Importance of usage of *Taptakhalva* in *Parada Samskara*

Nityanatha Siddha mentioned in his Rasa *Ratnakara Riddhi Khanda* in second chapter to use the *Tapta Khava*. He revealed that it is ideal to use *Taptakhalva* in *Mardana, Shodhana, Charana, Jarana*, *Murchana, Bandhana* and Marana of *Parada* (R.R.Ri.2/45). It is also stated that *Loha Kharal* (Iron mortor) is ideal for the *Taptakhara*; and in the absence of *Loha Kharal* we can use stone *kharal* (R.R.Ri.2/47).

Preparation of *Kajjali*

Kajjali was the chief source of this procedure. Therefore, *Kajjali* was prepared at first.

The purified mercury and sulphur alone or in combination with other metals/drugs recommended in the compound are triturated without liquids so as to be converted into a smooth, blackish powder, free from any shining. It is known as *Kajjali*[4].

Ingredients

a. Purified *Parada* – 100 gm. (Each M_1, M_2, M_3 separately)

b. Purified Sulphur – 50 gm.

Method of preparation

1. In this procedure, *Kajjali* was prepared 3 times with purified mercury M_1, M_2, M_3 and sulphur, and obtained *Kajjali* was named as K_1, K_2, K_3.
2. Purified mercury and sulphur were taken in 2:1 ratio and were put in an iron mortar (*Taptakalva*) and grinded well.
3. The grinding was continued until the total mixture is converted into a fine, black, smooth, tasteless powder.
4. Weight of prepared *Kajjali* K1, K2 and K3 are respectively 140 gm. 138 gm. and 135 gm.

PREPARATION OF RASA BHASMA

Requirements

- Kajjali K_1- 140 gm.
  K_2 – 138 gm.
  K_3 – 135 gm.
- Ghrita Kumari (Aloe vera) Swarasa – q.s.
- Apparatus used – Khalva, musal, Bhudara Yantra etc.

Bhudara Yantra

The pit was made in the earth with the measurements of 6 inches of depth and 8 inches of width. The pit was filled with sand upto 2 inches and *Samputa Yantra* was placed on the sand and the total pit was filled with the sand. On the surface of the sand cow dung cakes were placed and subjected to heat continuously for 8 hrs.

Process

Kajjali was taken in *Khalva*, added with *Kumari Svarasa* (juice of Aloe vera L.) as *Bhavana dravya* and triturated for 8 hrs. Then made into bolus and dried in the sunlight. The bolus was named as B_1, B_2, B_3 and its weights were 200 gm. 190 gm. 193 gm. respectively. These bolus were kept into *Samputa Yantra*, seal both edges of the vessels and dried in sunlight. The *Samputa Yantra* (S_1, S_2, S_3) were kept in *Bhudhara Yantra* and subjected to heat continuously for 8 hrs. After *Svangashita* the *Samputa Yantras* were taken out, the boluses were crushed into powder, and obtained *Rasa Bhasma* were named as RB_1, RB_2 and RB_3. (Rasa Manjari /11)

Result

Weight of *Rasa Bhasma* 1 – 125 gm.

Weight of *Rasa Bhasma* 2 – 120 gm.

Weight of *Rasa Bhasma* 3 – 110 gm.

VANGA SODHANA

Procedure

1 kg. of *Vanga* (tin) is taken into an iron cauldron and heated over moderate fire till the complete *Vanga* is melted. This melted *Vanga* is poured into limewater and allowed to settle. This is taken out of limewater and washed off to remove crystals or sediments of lime deposited over it. For proper purification of *Vanga*, the process of *Dhalana* was carried out seven times[5].

Process No.	Quantity of Vanga taken	Quantity obtained	Loss during the process
1.	1000 gm.	990 gm.	10 gm.
2.	990 gm.	985 gm.	5 gm.
3.	985 gm.	970 gm.	15 gm.
4.	970 gm.	960 gm.	10 gm.
5.	960 gm.	945 gm.	15 gm.
6.	945 gm.	920 gm.	25 gm.
7.	920 gm.	895 gm.	25 gm.

Net weight of Sodhita Vanga obtained – 895 gm.

Preparation of *Rasa Bhasma*

Date of commencement: 25-8-2006

Date of completion: 15-09-2006

Ref: Khanija Bhasma Rajam, Page No.105

Process

50 gm. of purified *Vanga* was taken in an iron spoon, kept on fire and 50 gm. of purified mercury was added to the melted *Vanga*, mixed and poured into the earthen pot filled with 100 gm. of ghee.

After cooling, solidified *Vanga* mixed *Parada* was obtained from the ghee, then made into seven pieces, 125 gm. of *Dugdhika* powder was taken in a cloth, placed these seven places of *Vanga* mixed *Parada* powder and spread over with 125 gm. of *Dugdhika* powder and corners of the cloth. It was rolled and applied 12 layers of mud pasted cloth and dried. Then it was subjected to *Puta* with 300 cow dung cakes (each 50 gm. weight), on the next day, *Samputa* was collected from *Puta* and Rasa *Bhasma* and *Vanga* were separated.

The same procedure was done three times with 50 gm. of purified mercury M_1, M_2 and M_3 each time respectively.

RESULT

Weight of *Rasa Bhasma* RB_4 = 4 gm.

Rasa Bhasma RB_5 = 0 gm.

Rasa Bhasma RB_6 = 0 gm.

Weight of remained *Vanga* V_1 = 50 gm.

V_2 = 50gm.

V_3 = 50 gm.

RASA BHASMA – ACUTE TOXICITY TEST

In the present investigation, it is proposed to undertake a short-term pre clinical acute toxicity test on Rats to obtain the safety profile.

MATERIALS AND METHODS

Test compound

Among the various *Bhasma* preparations, a popularly recommended and used formulation Rasa *Bhasma* 2 has been selected.

Dosage schedule:

Dose (clinical)	:	120 mg/day/Adult
Duration	:	3-4 Weeks
Route	:	oral
Dosage experiment	:	10.8 mg/kg
Duration	:	7 days
Route	:	oral
Vehicle	:	Honey & Water (3:2)

IAEC approval

The proposed experiment has been under taken after taking the approval of Institutional Approval Ethical Committee, SIPRA Labs to conduct the experiment at their centre.

Species

Swiss Albino (I.B) Rat have been obtained from registered breeder. The initial weights were recorded and conditioned for 5 days to initiate the experiment.

Housing

Animals were housed in groups of two in standard suspended polycarbonate cages with top grill having facilities for pelleted feed and drinking water in glass bottles with stainless steel sipper tubes. The bottom of the cage was also stainless steel grilled to facilitate free dropping of feces and urine to prevent coprophagy.

Husbandry

The environmental conditions were maintained at 21 ± 2°C, with 10 – 15 air changes per hour and relative humidity of 50 – 55% with a 12 hour light/dark cycle.

Feed and Water

The animals had free access to sterile pelleted feed of standard composition containing all macro and micronutrients. Water that was passed through activated charcoal filter and exposed to UV rays (Aqua guard on-line water filter-cum-purifier) was provided.

Age at start of treatment

4 - 6 weeks

Body weight range at the start of the experiment

Rats having body weight of 120 – 150 gm. were selected for the acute study.

Animal Identification

Animals receiving the same treatment were housed in groups of two with appropriate identification (label) on cages. Animals in each cage were identified by standard ear marking (Right cut, Left cut & No cut for 3 animals) and (Right cut, Left cut for 2 animals) procedures for rats and by the labels on the cage. The Unique ID no's were given. In addition, cage cards were also marked.

Study design

Since it is a acute toxicity study 15 Rats have been randomizingly selected and divided into two groups to receive the test compound in a vehicle and identified as a TD and a vehicle group to receive Honey, Water for a period of 7 days and identified as VC. (Table No.1)

Table 1: Test groups

S.No	Test Group	Duration of Exposure	Study Parameters	
			48 Hrs after last exposure	7 Days after Last Exposure
1	Vehicle Control (05)	7 Days	Hematology Biochemistry Necropsy Findings (03)	Hematology Biochemistry Necropsy Findings (02)
2	Test group (10)	7 Days	Hematology Biochemistry Necropsy Findings (05)	Hematology Biochemistry Necropsy Findings (05)

( ) = No of animals

The animals were observed for lethality and mortality after test compound exposure followed by daily observation for 14 days.

i. The Pre-terminal deaths if were recorded every day till 14th day of the test compound exposure.

ii. In case of death of any animal during the observation period the autopsy were proposed to collect all vital organs like brain, liver, kidney, heart, lung, productive organs, intestine will be collected for studying histopathological changes.

iii. 8 (3VC+5TD) animals after 48 hours and remaining 7(2VC+5TD) animals after7 days of last were subjected for haematology, clinical chemistry and necropsy observation.

Table 2: Dosage Schedule (RAT)

S.No	Group details	Human dose in mg per day	Compound details +	Dosage schedule* per 200 gm rat / day	
				Strength mg / 1ml	Strength mg / kg
1.	Vehicle Control	-	Sterile distilled water and honey (2:3)	HW	
2.	Therapeutic dose	120	RB2 + HW	4.32mg	10.8 mg.

* Route of administration = oral

* volume of administration = 0.5ml

Table 3: Mortality & Body Weights of Rats in acute study

S. No	Pre-terminal deaths	Activity	Animal Body Weights (gm)				
			1st day	4th day	8th day	11th day	14th day
1	X	1	141.7	154	160.0	E	E
2	X	1	137.6	151	157.0	E	E
3	X	1	136.3	154	159.0	E	E
4	X	1	139.3	153	158.0	163	169
5	X	1	149.3	163	16.6	172	180
6	X	1	140.1	142	149.0	E	E
7	X	1	134.5	140	149.0	E	E
8	X	1	121.5	130	135.0	E	E
9	X	1	118.1	124	125.0	E	E
10	X	1	138.4	155	163.0	E	E
11	X	1	130.0	149	154.0	158	159
12	X	1	131.4	150	146.0	148	149
13	X	1	119.0	142	144.0	147	145
14	X	1	148.5	158	174.0	178	181
15	X	1	133.7	137	149.0	152	151

* 1 – Active, 2 – Inactive E -- Euthenised

Table 4: Live phase of animals

Groups	Days*	General behaviour		Water intake	
		Active	Not active	Adequate	Inadequate
VC	Baseline	100 %	Nil	100 %	Nil
	7	100 %	Nil	100 %	Nil
	14	100 %	Nil	100 %	Nil
TD (1X)	Baseline	100 %	Nil	100 %	Nil
	7	100 %	Nil	100 %	Nil
	14	100 %	Nil	100 %	Nil

Table 5: Cage Side Observation

Groups	Days	Home cage activity (%)				
		Lying on side	Resting	Sitting	Alert & oriented towards investigator	Circling purposelessly
VC	Baseline	Nil	70 %	30 %	Nil	Nil
	7	Nil	60 %	40 %	Nil	Nil
	14	Nil	100 %	Nil %	Nil	Nil
TD (1X)	Baseline	Nil	60 %	40 %	Nil	Nil
	7	Nil	70 %	30 %	Nil	Nil
	14	Nil	100 %	Nil	Nil	Nil

Table 6: Cage Side Observation (Contd..)

Groups	Days	Feces output (%)		Feces consistency (%)	
		Normal	Abnormal	Normal	Abnormal
VC	Baseline	100 %	Nil	100 %	Nil
	7	100 %	Nil	100 %	Nil
	14	100 %	Nil	100 %	Nil
TD (1X)	Baseline	100 %	Nil	100 %	Nil
	7	100 %	Nil	100 %	Nil
	14	100 %	Nil	100 %	Nil

Table 7: Cage Side Observation (Contd..)

Groups	Days	Urine output (%)		Urine colour (%)	
		Normal	Abnormal	Normal	Abnormal
VC	Baseline	100 %	Nil	100 %	Nil
	7	100 %	Nil	100 %	Nil
	14	100 %	Nil	100 %	Nil
TD (1X)	Baseline	100 %	Nil	100 %	Nil
	7	100 %	Nil	100 %	Nil
	14	100 %	Nil	100 %	Nil

Table 8: Cage Side Observation (Contd.)

Groups	Days	Behaviour while removal from cage (%)				
		Animal quiet easily removed	Runs around cage	Orient towards investigator	Aggressive	Vocalisation
VC	Baseline	Nil	100 %	Nil	Nil	Nil
	7	80 %	10 %	Nil	10 %	Nil
	14	100 %	Nil	Nil	Nil	Nil
TD (1X)	Baseline	10 %	90 %	Nil	Nil	Nil
	7	90 %	10 %	Nil	Nil	Nil
	14	90 %	10 %	Nil	Nil	Nil

Table 9: Physical Examination

Groups	Days	Hair coat (%)		Piloerection (%)		Respiratory rate (%)	
		Clean groomed	Slightly soiled	Normal	Abnormal	Normal	Increased
VC	Baseline	100 %	Nil	100 %	Nil	100 %	Nil
	7	100 %	Nil	100 %	Nil	100 %	Nil
	14	100 %	Nil	100 %	Nil	100 %	Nil
TD (1X)	Baseline	100 %	Nil	100 %	Nil	100 %	Nil
	7	100 %	Nil	100 %	Nil	100 %	Nil
	14	100 %	Nil	100 %	Nil	100 %	Nil

Table 10: Physical Examination (Contd..)

Groups	Days	Res. character (%)		Lacrimation (%)	Salivation (%)
		Normal	Abnormal	None	None
VC	Baseline	100 %	Nil	100 %	100 %
	7	100 %	Nil	100 %	100 %
	14	100 %	Nil	100 %	100 %
TD (1X)	Baseline	100 %	Nil	100 %	100 %
	7	100 %	Nil	100 %	100 %
	14	100 %	Nil	100 %	100 %

Table 11: Physical Examination (Contd..)

Groups	Days	Eye prominence (%)		Eye lid (s) closure (%)
		Normal	Abnormal	Wide open
VC	Baseline	100 %	Nil	100 %
	7	100 %	Nil	100 %
	14	100 %	Nil	100 %
TD (1X)	Baseline	100 %	Nil	100 %
	7	100 %	Nil	100 %
	14	100 %	Nil	100 %

( ) No of animals

Table 12: Physical Examination (Contd..)

Groups	Days	Convulsions (%)	Biting (%)	Tremors (%)	
		None	None	None	Body tremors only
VC	Baseline	100 %	100 %	100 %	Nil
	7	100 %	100 %	100 %	Nil
	14	100 %	100 %	100 %	Nil
TD (1X)	Baseline	100 %	100 %	100 %	Nil
	7	100 %	100 %	100 %	Nil
	14	100 %	100 %	100 %	Nil

( ) No of animals

Table 13: Neurological Examination

Groups	Days	Locomotor activity (%)		Rearing Activity (%)		
		Resting	Scratching casually	Did not rear	Rear on hind limbs with the use of tail	Falls to side
VC	Baseline	80 %	20 %	100 %	Nil	Nil
	7	60 %	40 %	100 %	Nil	Nil
	14	100 %	Nil	100 %	Nil	Nil
TD (1X)	Baseline	70 %	30 %	100 %	Nil	Nil
	7	60 %	40 %	100 %	Nil	Nil
	14	90 %	10 %	100 %	Nil	Nil

Table 14: Neurological Examination (Contd..)

Groups	Days	Tail elevation (%)		Static limb position (%)	Abnormal gait (%)	Ataxic gait (%)
		Lifts while walking	Occasionally lifts	Limbs normal	None	None
VC	Baseline	100 %	Nil	100 %	100 %	100 %
	7	100 %	Nil	100 %	100 %	100 %
	14	100 %	Nil	100 %	100 %	100 %
TD (1X)	Baseline	100 %	Nil	100 %	100 %	100 %
	7	100 %	Nil	100 %	100 %	100 %
	14	100 %	Nil	100 %	100 %	100 %

Table 15: Neurological Examination (Contd..)

Groups	Days	Head Position (%)		Pinna touch response (%)		
		Head without tilt	Head With tilt	No response	One touch	More than one touch
VC	Baseline	100 %	Nil	Nil	80 %	20 %
	7	100 %	Nil	Nil	80 %	20 %
	14	100 %	Nil	Nil	70 %	30 %
TD (1X)	Baseline	100 %	Nil	Nil	80 %	20 %
	7	100 %	Nil	Nil	50 %	50 %
	14	100 %	Nil	Nil	70 %	30 %

Table 16: Clinical Chemistry

Parameters	VC		TD (1X)	
	7 days(03)	14 days(02)	7 days(05)	14days(05)
Glucose (mg/dl)	73.0 ± 22.45	-	56.6 ± 18.27	-
Albumin (g/dl)	3.9 ± 0.21	4.2 ± 0.18	3.1 ± 1.30	3.9 ± 0.26
Total Protein (g/dl)	6.7 ± 0.54	7.3 ± 0.00	5.7 ± 2.30	6.8 ± 0.53
Alkaline phosphatase (IU/L)	148.0 ± 28.44	180.5 ± 29.50	163.1 ± 67.72	248.4 ± 66.06
SGPT (ALT) (U/L)	41.3 ± 4.03	41.5 ± 8.50	36.2 ± 13.33	25.8 ± 4.87
SGOT (AST) (U/L)	127.3 ± 13.70	153.0 ± 8.00	111.0 ± 40.67	143.8 ± 6.79
BUN mg/dl	34.7 ± 7.72	42.0 ± 0.00	32.2 ± 11.57	35.2 ± 1.83
BIT mg/dl	0.1 ± 0.09	0.3 ± 0.03	0.2 ± 0.07	0.2 ± 0.16
BID mg/dl	0.1 ± 0.03	0.1 ± 0.01	0.1 ± 0.03	0.1 ± 0.06

( ) = No of animals Values are mean ± SD

Table 17: Hematology

Parameters	VC		TD (1X)	
	7 days(03)	**14 days(02)**	**7 days(05)**	**14 days(05)**
WBCX 10^3/cu.mm	7.2 ± 2.36	7.9 ± 0.70	6.3 ± 1.36	8.0 ±2.11
RBCx10^6/cu.mm	5.4 ± 0.38	5.1 ± 0.28	5.3 ± 0.36	4.9 ± 0.65
Hemoglobin (g/dl)	11.1 ± 0.65	10.6 ± 0.65	10.8 ± 0.54	9.5 ± 1.40
HCT %	32.2 ± 2.13	29.6 ± 1.50	30.6 ± 1.70	27.4 ± 4.22
MCV μ^3	59.2 ± 2.14	57.9 ± 0.25	58.1 ± 1.16	55.5 ± 2.12
PLT X10^3	544.7 ± 104.38	434.0 ± 50.00	556.8 ± 131.21	422.8 ± 109.85

Values are expressed as Mean ± SD ( ) No of animals

Table 18: Organ Weights (gr.)

Parameters	7 days		14 days	
	VC(03)	**TD(05)**	**VC(02)**	**TD(05)**
Heart	0.5 ± 0.02	0.5 ± 0.04	0.5 ± 0.00	0.5 ± 0.06
Kidney (L+R)	1.0 ± 0.07	0.9 ± 0.05	1.0 ± 0.02	0.9 ± 0.09
Liver	5.6 ± 0.26	4.8 ± 0.48	5.6 ± 0.40	4.9 ± 0.60
Lung	1.2 ± 0.11	1.1 ± 0.40	1.2 ± 0.04	1.1 ± 0.11
Spleen	0.9 ± 0.13	0.7 ± 0.12	0.8 ± 0.08	0.8 ± 0.07

Values are mean ± SD ( ) No of animals

Table 19: Necropsy Findings

DOSAGE	VC	TD (1X)
No. of Animals	05	10
No. dead during treatment	0	0
No. moribund and sacrificed	0	0
No. finally sacrificed	5	10
No. examined for gross pathology	5	10
No. showing gross pathology	0	0
Visceral organ pathology	X	X

Results

1. No mortality, morbidity, toxic signs, weight loss or abnormal behavior were recorded till 14 days after 7 days exposure of the test compound of recommended therapeutic dose.
2. No significant treatment related effect on food intake; body weight, clinical signs or behavioral activity etc was observed and were compared from vehicle group.
3. No significant changes in hematological parameters & clinical chemistry parameters were found.
4. No specific test compound induced pathological changes in various organs were observed.

Discussion

As per the toxicological study, due to paucity of time an acute toxicity test on swiss albino Rats has been planned following the approval of ethical committee of Sipra Labs in collaboration with NIN Hyderabad under guidance of Dr. B. Dinesh Kumar. The drug administration was done in 10.8 mg/kg body weight for a period of 7 days via oral route. The vehicle of honey and water was given to the control group. 15 rats have been randomisingly selected and divided into two groups as test dose group and vehicle group. In both groups, haematological, biochemical investigations were carried out at the end of 7 days after 48 hrs after last exposure and again after 7 days after last exposure, same investigations have been carried out apart from the necropsy observations done in all organs of animals. It is observed that no preterminal deaths occurred and every animal was active, there is no loss of body weight. Water intake was adequate. In cage side observation they were all either resting or sitting and there was no lying on side, alert and orient towards investigator and circling purposelessly. There was no abnormality in faeces output and faeces consistency. Urine output and colour was also normal in all animals. In test dose group, in the cage side observation there was no change like orient towards investigator, aggressiveness or vocalisation in the animals. In both the groups there was no hair coat change, piloerection and respiratory rate. Respiration character, lacrimation and salivation were not disturbed. There was no abnormality in eye prominence and eyelid closure. There were no significant changes in haematological parameters and clinical chemistry parameters were found. No specific test compound induced pathological changes in various organs were observed.

CONCLUSION

No Pre-terminal deaths occurred in rats received test compound at therapeutic dose levels. No abnormalities in physical, physiological, clinical chemistry, hematological parameters and no gross necropsy changes were observed on administration (oral) of test compound prepared according to the classical literature. At this level of study, it may be concluded that Rasa Bhasma is safe as per toxicological study and can be subjected for further study of clinical evaluation.

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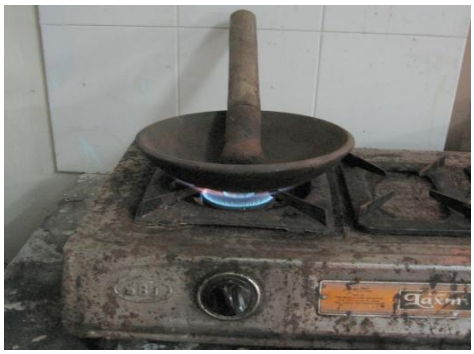
PREPARATION OF RASA BHASMA

METHOD – I






METHOD – II