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Research Article

EVALUATION OF ACUTE TOXICITY OF MODIFIED *TRIVIKRAMA RASA*: A RESEARCH REPORT

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ABSTRACT

Trivikrama Rasa is a classical Ayurvedic formulation indicated in the management of *Ashmari*. The present study was undertaken as a part of thesis work to evaluate the acute toxicity profile of a modified preparation of *Trivikrama Rasa*. In addition to the classical method of preparation, a modified sample was developed by administering seven *Bhawana* of *Trina Panchamoola Kwatha* to potentially enhance its pharmaceutical and therapeutic attributes. The acute oral toxicity study of the modified formulation was carried out in vivo in accordance with OECD guideline 423, with observations recorded over a period of 14 days. The results obtained during the study period are presented in this manuscript and indicate that the modified formulation did not produce any toxic manifestations at the tested dose levels: 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg. There were no behavioural and neurological changes, no signs of systemic toxicity, no significant variations in body weight, no gross pathological findings and no mortality was recorded. The findings suggest that the modified *Trivikrama Rasa* is safe to use however, further long-term safety evaluations are warranted to substantiate its clinical applicability.

INTRODUCTION

Trivikrama Rasa is a classical Ayurvedic formulation containing *Aja Dugdha pachit Tamra Bhasma, Shuddha Parada, Shuddha Gandhaka* and *Nirgundi Swarasa* as *Bhavana drav.*

MATERIAL AND METHODS

Preparation of Modified *Trivikrama Rasa*

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Trivikrama Rasa was prepared according to the reference provided in AFI[1] (Sharangdhar Samhita)[2]. For potentiation of *Trivikrama Rasa, Kwatha* of *Trina Panchamoola* was prepared as described in *Rasatarangini[3].* Fresh *Kwatha* was prepared daily for the purpose of *Bhawana,* and trituration was carried out for 8 hours each day until the material became completely dry.

This sample was named as modified *Trivikrama Rasa.* In total, seven *Bhawana* were administered. The quantity of *Bhawana drava* used for each *Bhawana* is presented below.

Table 1: Quantity of *Bhawana Drava* added in *Trivikrama Rasa*

Trivikrama Rasa	**150gm**
Kwatha used in first *Bhawana*	50ml
Kwatha used in second *Bhawana*	46ml
Kwatha used in third *Bhawana*	40ml
Kwatha used in fourth *Bhawana*	35ml
Kwatha used in fifth *Bhawana*	30ml

Kwatha used in sixth *Bhawana*	30ml
Kwatha used in seventh *Bhawana*	25ml

Ethical Clearance

The Acute Toxicity Study protocol was submitted to the Animal Ethical committee of Central Preclinical (Animal) Research Facility, Datta Meghe Institute of higher Education and Research. The experiment was performed after obtaining permission from the Institutional Animal Ethical Committee (Approval number-DMIHER/IAEC/24-25/53) as per guideline of CPCSEA, India.

Preparation of test animals

Fifteen experimental Animals (female wistar rats, between 8-12 weeks) were used for oral acute toxicity study. Study was done as per the OECD Guidelines 423. Rats were divided into five groups, each group containing three rats. Group 1 was normal control without any intervention and the duration of the study was 14 days.

The animals were housed three per cage in the polypropylene cages in the animal house. All animals were kept in experimental room. The room was well ventilated (> 10 air changes per hour) with 100% fresh air. A 12-hr light/dark photoperiod was maintained. Room temperature and relative humidity was set to be maintained between 22±3°C and 30-70% respectively according to guidelines. The animals had free access to pelleted feed of standard composition. Water from (Aqua guard on-line water filter-cum-purifier) was provided ad libitum. All fifteen female rats were randomly selected, marked with Picric acid H (mark on head), B (Mark on Back), T (mark on Tail) for individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions.

Dose Calculation for experimental Rats

The dose for experimental animals selected in Acute Toxicity was calculated by applying the Human dose to animal dose (based on the body weight) as per OECD guideline 423.[4] Group 1 was normal control without any intervention.

Table 2: Dosing Chart

Group 2 (5 mg/kg)			
Marking	**Weight (gm)**	**Dose (mg)**	**Dose (10 ml/kg) 1 %w/v solution**
H	280	1.4	0.04
B	230	1.15	0.05
T	176	0.88	0.048

Group 3 (50mg/kg)			
Marking	**Weight (gm)**	**Dose (mg)**	**Dose (10 ml/kg) 1%w/v solution**
H	270	13.5	0.46
B	270	13.5	0.45
T	250	12.5	0.49

Group 4 (300mg/kg)			
Marking	**Weight (gm)**	**Dose (mg)**	**Dose (10 ml/kg) 1%w/v solution**
H	260	78	0.31
B	230	69	0.27
T	250	75	0.28

Group 5 (2000mg/kg)			
Marking	**Weight (gm)**	**Dose (mg)**	**Dose (10 ml/kg) 1%w/v solution**
H	230	460	1.30

B	200	400	1.82
T	230	460	1.92

Preparation of test material

The finely powdered modified *Trivikrama Rasa* was taken in requisite quantity in separate small porcelain mortar for every group and few drops of 5% gum acacia suspension were added, the mixture was further grounded for 5 minutes and the volume was made up with in 10ml of distilled water.

Administration of test material

The test substance has been administered in a single dose to each group animals by gavage using an oral feeding needle. Animals had been fasted prior to dosing. Following the period of fasting, the animals were weighed and the test substance administered. All groups are dosed in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000mg/kg. After the substance has been administered, food withheld for a further 3-4 hours in rats.

Investigation of serum has done for total protein, albumin, creatinine, total bilirubin, Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), and cholesterol. For gross pathology 1 animal from each group was sacrificed to detect any pathological signs of toxicity. Lungs, brain, liver and heart were examined for any gross pathological changes.

OBSERVATIONS & RESULTS

General Behavioural changes

The general behaviour of the animal was continuously monitored for 1 h after dosing, periodically during the first 24 h (with special attention given during the first 4h), and daily thereafter for a total of 14 days. Rats were observed for toxic signs daily and for preterminal deaths. Food and water intake was observed daily. Individual body weights were recorded once in a week. No death was recorded in the 14 days of observation period even in the animals given 2gm/kg of modified *Trivikrama Rasa* orally. The animals did not show any changes in the general appearance during the observation period.

Table 3: Weight Changes of group 1, 2, 3, 4 and 5

Weight changes (gm)										
Marking	**Group 1**		**Group 2**		**Group 3**		**Group 4**		**Group 5**	
	7th day	**14th day**	**7th day**	**14th day**	**7th day**	**14th day**	**7th day**	**14th day**	**7th day**	**14th day**
H	286	290	280	280	270	270	265	270	235	240
B	278	280	230	225	275	280	230	230	200	200
T	256	260	178	182	250	250	250	250	220	230
Mean	**273**	**268**	**276**	**250**	**229**	**250**	**229**	**250**	**265**	**218**

Hematology, Biochemistry & Gross Pathology

After 14 days of observation Blood analysis, Biochemistry & Gross Pathology was done. Blood was collected from retro orbital vein of rats from each group. Hematology analysis was performed using an automated hematology analyzer (ABX Vet abc hematology analyzer; Horiba Medical Ltd). The following parameters were recorded: RBC, Hb, WBC, MCV, MCH, MCHC and Platelets count. Clinical biochemistry measurements were made using commercial kits procured from Yucca Diagnostics with the aid of a clinical chemistry analyser, Prietest Touch semi-automatic biochemistry analyser (Robonik India Pvt Ltd).

Table 4: Haematological Parameters of group 1, 2, 3, 4 and 5

Haematological parameters of all five groups						
Name of Test	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**References Value**
RBC (x10 /mm^3)	15.15	12.96	14.07	12.82	16.96	**7-10**
	9.95	12.42	14.94	13.75	17.00	
	7.49	9.45	16.33	8.38	12.98	
Mean	**10.86**	**11.61**	**15.11**	**11.65**	**15.65**	
Hb (g/dl)	29.1	23.6	28.3	24.9	29.0	

	17.6	22.6	27.4	26.8	27.2	**11-18**
	14.2	16.5	24.3	15.2	22.0	
Mean	**20.3**	**20.9**	**26.67**	**22.3**	**26.07**	
WBC ($X10^3/mm^3$)	8.7	8.5	10.2	8.0	5.5	**6-17**
	5.9	10.2	8.3	10.3	23.6	
	6.4	11.9	10.3	6.8	7.2	
Mean	**7**	**10.2**	**9.6**	**8.37**	**12.1**	
MCV	59	57	58	57	55	**53-59**
	57	55	58	57	54	
	57	57	56	56	52	
Mean	**57.67**	**56.33**	**57.33**	**56.67**	**53.67**	
MCH	19.2	18.2	20.1	19.4	17.1	**17-22**
	17.6	18.2	18.3	19.5	16.0	
	18.9	17.4	21.9	18.1	16.9	
Mean	**18.57**	**17.93**	**20.1**	**19**	**16.67**	
MCHC	32.8	32.1	34.8	33.9	31.1	**30-41**
	31.1	32.9	31.4	34.0	29.6	
	33.5	30.8	39.1	32.5	32.2	
Mean	**32.47**	**31.93**	**35.1**	**33.47**	**30.97**	
Platelets ($X10^3/mm^3$)	326	440	269	388	188	**500-1300**
	452	560	368	347	283	
	462	738	738	671	513	
Mean	**413.33**	**579.33**	**458.33**	**468.67**	**328**	

Table 5: Biochemical Parameters of group 1, 2, 3, 4 and 5

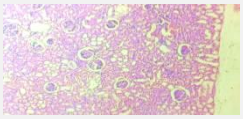
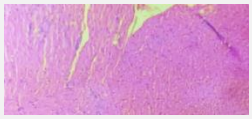
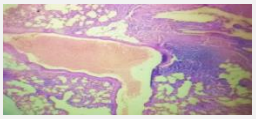
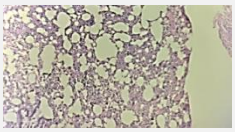
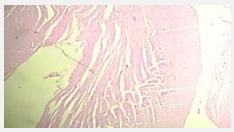
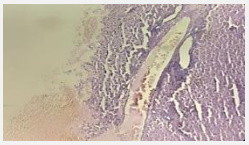
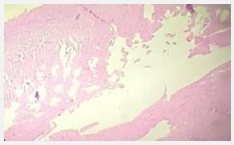
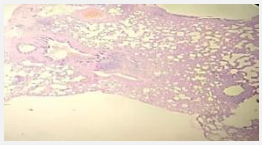
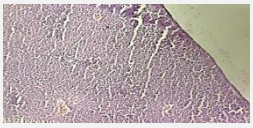
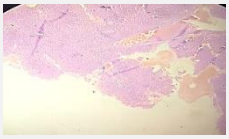
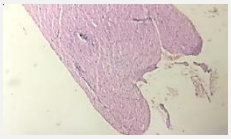
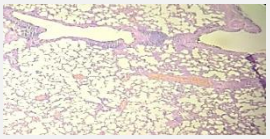
Biochemical parameters of all five groups						
Name of Test	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Reference Value**
Protein (g/dl)	**8.2276**	7.4561	7.2753	7.6887	**8.7183**	**5.6-7.6**
	7.4315	7.0638	7.7168	**8.6647**	**7.9898**	
	8.0170	**8.0837**	6.2168	**8.3268**	6.8874	
Mean	**7.892033**	**7.534533**	**7.069633**	**8.226733**	**7.865167**	
Albumin (g/dl)	3.7957	4.6951	3.7864	4.1193	4.9508	**2.8-4.8**
	4.3278	3.9120	4.4394	4.5650	2.9566	
	3.8782	3.9450	4.6088	4.3359	4.2591	
Mean	**4.000567**	**4.184033**	**4.2782**	**4.340067**	**4.0555**	
Creatinine (mg/dl)	0.3437	0.2621	0.2447	0.3379	0.4350	**0.2-0.8**
	0.2913	0.3146	0.3398	0.3864	0.3262	
	0.3650	0.3165	0.3845	0.3884	0.4078	
Mean	**0.333333**	**0.297733**	**0.323**	**0.3709**	**0.389667**	
Bilirubin	**1.0597**	0.3246	0.3246	0.6114	0.3819	**0.2-0.55**

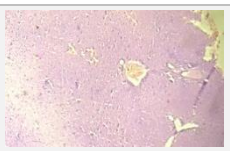
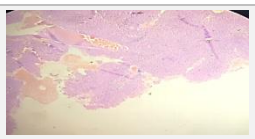
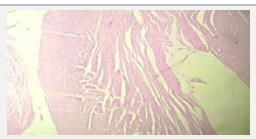
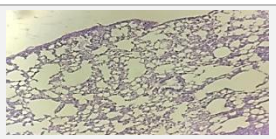
(mg/dl)	0.3985	0.4378	0.3397	0.3850	0.3034	
	1.0416	0.4016	0.5163	0.5646	0.4227	
Mean	**0.833267**	**0.388**	**0.393533**	**0.520333**	**0.369333**	
SGOT(IU/l)	99.281	97.923	96.911	112.486	117.092	**50-150**
	142.758	**168.177**	116.080	125.475	88.285	
	89.749	117.73	123.509	119.222	111.687	
Mean	**110.596**	**127.9433**	**112.1667**	**119.061**	**105.688**	
SGPT(IU/l)	40.717	29.72	43.960	56.308	44.553	**24-60**
	35.6395	35.154	37.855	52.222	56.739	
	30.0268	40.033	39.7473	26.655	43.9091	
Mean	**35.4611**	**34.969**	**40.52077**	**45.06167**	**48.40037**	
Cholesterol (mg/dl)	110.214	73.1901	89.2231	76.4628	68.5289	**40-130**
	94.9752	81.851	81.8843	71.9008	64.7603	
	121.685	67.4711	93.3223	69.7521	71.6694	
Mean	**108.9581**	**74.17073**	**88.14323**	**72.70523**	**68.31953**	

Gross Histopathology Results

Histopathology of Liver, Lung, Brain and Kidney of animals of Group 1, 2, 3 revealed no toxic changes. There were foci of ballooning degeneration & dilated blood vessel were found in liver of group 4 (300mg/kg). Histopathology of kidney revealed mild oedema in group 4 (300mg/kg). There were normal histopathology findings in group 5 (2000mg/kg dose)

Histopathology Results

Liver	**Kidney**	**Heart**	**Lungs**
			
Group 1- U/R in morphology	**Group 1- U/R in morphology**	**Group 1- U/R in morphology**	**Group 1- U/R in morphology**
			
Group 2- U/R in morphology	**Group 2- U/R in morphology**	**Group 2- U/R in morphology**	**Group 2- U/R in morphology**
			
Group 3- U/R in morphology	**Group 3- U/R in morphology**	**Group 3- U/R in morphology**	**Group 3- U/R in morphology**
			

Group 4- foci of ballooning degeneration & dilated blood vessel	**Group 4- U/R renal parenchyma with focal edematous stroma focus**	**Group 4- U/R in morphology**	**Group 4- U/R lung parenchyma with dilatation of alveoli**
			
Group 5- Normal Findings +dilated blood vessels	**Group 5- U/R in morphology**	**Group 5- U/R in morphology**	**Group 5- U/R lung parenchyma with dilated blood vessels & cystic dilatation of alveoli**

DISCUSSION

In this study, a modified sample of *Trivikrama Rasa* was prepared by subjecting it to seven successive *Bhawana* of *Trina Panchamoola Kwatha*. The rationale behind selecting *Trina Panchamoola* has well-established description in Ayurvedic classics for the management of *Ashmari*. Classical texts describe the constituents of *Trina Panchamoola* under *Mootravirechaniya* and *Veeratarvadi Gana,* indicating their role in facilitating urinary excretion and alleviating urinary calculi. Owing to their *Ashmari Bhedana* and *Mutrala* properties, these drugs are traditionally believed to aid in fragmentation and expulsion of urinary stones while also reducing the chances of recurrence. Therefore, *Bhawana* of *Trina Panchamoola Kwatha* was expected to potentiate the therapeutic attributes of classical *Trivikrama Rasa*. The acute oral toxicity assessment of Modified *Trivikrama Rasa* (MTR) was performed according to OECD guideline 423 in female Wistar rats using graded dose levels of 5mg/kg, 50mg/kg, 300mg/kg, and 2000mg/kg body weight. Throughout the 14-day observation period, none of the experimental animals exhibited mortality or severe toxic manifestations, including those administered with the highest dose of 2000mg/kg. The absence of mortality at this limit dose suggests that the formulation possesses a comparatively wide margin of safety under acute exposure conditions.

Continuous monitoring of behavioural and physiological parameters revealed no treatment-related abnormalities. The animals maintained normal locomotor activity, feeding behaviour, water intake, and general appearance during the entire study period. No signs such as tremors, convulsions, salivation, diarrhoea, lethargy, or respiratory distress were observed, indicating that the formulation did not adversely influence the central nervous system or general physiological functioning. Body weight changes recorded during the experimental period also remained within normal range, without any remarkable decline in treated groups. Maintenance of body weight is considered an important indicator of the non-toxic nature of a test substance, as significant reduction generally reflects systemic toxicity or metabolic impairment. The present findings therefore support the tolerability of the modified formulation.

Evaluation of haematological parameters demonstrated that values of RBC, Hb, WBC, MCV, MCH, MCHC, and platelet count remained largely within physiological limits in all groups. Likewise, biochemical investigations related to hepatic and renal functions, including serum protein, albumin, creatinine, bilirubin, SGOT, SGPT, and cholesterol, did not reveal any significant dose-dependent alterations. These observations indicate that administration of Modified *Trivikrama Rasa* did not produce marked impairment of liver or kidney function during the acute study period. Histopathological examination of vital organs such as liver, kidney, lung, and brain further substantiated the safety profile of the formulation. No pathological alterations were observed in groups receiving lower doses or in the highest dose group. Mild oedematous changes in kidney tissue and focal ballooning degeneration with vascular dilatation in the liver were noted only in the 300mg/kg dose group. Interestingly, similar findings were absent in the 2000mg/kg group, making a direct dose-dependent toxic correlation unlikely. These isolated observations may therefore be attributed to physiological stress or incidental variation rather than a consistent toxic effect of the formulation.

Taken together, the results of the present investigation suggest that modified *Trivikrama Rasa* is relatively safe in acute oral administration and does not produce significant toxic effects even at higher dose levels. The study provides preliminary scientific

evidence supporting the safety of the modified preparation. However, further studies involving repeated dose toxicity, chronic toxicity, and clinical evaluation are necessary to comprehensively establish its long-term safety and therapeutic utility.

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