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Acute and 90 Days Repeated Dose Toxicity of *Rasa parpati* (an Ayurvedic Mercurial Formulation) in Charles Foster Albino Rats

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Abstract

Rasa parpati is a unique Ayurvedic mercurial formulation extensively prescribed by Ayurvedic physicians to treat different ailments. Considering toxicity concerns of traditional formulations for containing heavy metals like Mercury, Arsenic, Lead etc.; it becomes, imperative to evaluate safety aspects of such formulations. In Ayurveda, metals and minerals are advocated to be processed through the specified guidelines before their therapeutic application. An attempt has been made to evaluate the impact of *Shodhana* on safety aspects of *Rasa parpati* through acute and 90 days repeated dose toxicity of *Rasa parpati* that was prepared by two different samples of raw materials. One by using *Hingulottha parada* and *Shuddha gandhaka* (HRP) while, another with *Ashuddha parada* and *Ashuddha gandhaka* (ARP). Oral acute toxicity of test drugs was carried out at limit dose of 2000 mg/kg orally along with adjuvant in Charles foster female rats. In 90 days repeated dose toxicity, both ARP and HRP were orally administered at therapeutically equivalent dose (22.5 mg/kg), TEDx5 (112.5 mg/kg), TEDx10 (225 mg/kg) levels along with adjuvant for 90 days. Two recovery groups were also studied in the experiment. Acute toxicity revealed that, both the test drugs did not produce any signs and symptoms of toxicity and mortality up to oral dose of 2000 mg/kg in rats. Administration of HRP for 90 days revealed that, the drug at higher dose has potential to produce liver and kidney toxicity however, can be relatively safe at therapeutic dose level. ARP has potential for liver, kidney and GI tract toxicity with deposition in other organs. The recovery study suggests that, after discontinuation of HRP the observed changes are reversible in nature while, in ARP some of the observed changes are irreversible in nature. From present study, it is concluded that HRP was found to be safer than ARP at different dose levels in rats suggesting safe use at therapeutic dose level. It proves that classical procedures like *Shodhana* are mandatorily to be followed in the pharmaceutical processing of these formulations.

Keywords: Ayurveda, Mercury, Parada, *Rasa parpati,* Safety, Toxicity

1. Introduction

The pharmacological section of Ayurvedic system of medicine recognizes three major sources of medicines, those of herbal origin (e.g. herbs), mineral origin (e.g. salts, metals like gold, copper, silver), and animal origin (e.g. milk, honey etc). Although, *Kashthaushadhis* and *Rasaushadhis* are two main groups of medicines, the former is devoid of any metals and minerals and is purely herbal product and can be considered as safest of medicines, and later consists of metals and minerals in the form of *Bhasma* (incinerated metal, minerals etc.). These metallic preparations occupy a significant seat in Ayurvedic pharmacopoeia. These formulations are attributed to be safe and efficacious with several therapeutic benefits when manufactured by following specified pharmaceutical code of conduct and used judiciously as mentioned in the classical literature of Ayurveda. The well-known metals mostly used in Ayurveda include mercury, gold, silver, copper, arsenic, lead and tin. Considering possibility of accumulated toxicity and hazardous effects of heavy metals have made the health scientists to raise concerns against using heavy metals in therapeutics[1–4]. As a result beneficial effects of processed metals and minerals is often viewed with skepticism.

Rasaushadhis are products of Indian classical alchemical procedures that are organo-metallic compounds of certain metals in a very fine powdered form, made in

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complex, elaborate processes. Recent articles pertaining to the alarming level of heavy metals, especially Lead, Mercury, and Arsenic in Ayurvedic formulations have created a lot of controversy regarding the safety and efficacy of Ayurvedic formulations.

Mercury is an important metal, which if properly processed is useful in the therapeutics of many diseases but, if unprocessed is used in therapeutics, is said to be life threatening[5]. Classics emphasized on certain pharmaceutical procedures like *Shodhana, Samskara* etc. to make mercury suitable for its therapeutic use. It is believed that elaborative preparation protocols developed by ancient seers are useful in transforming the raw material into suitable forms enhancing therapeutic benefits. *Rasa parpati* is a classical mercurial formulation widely prescribed by Ayurvedic physicians in the treatment of *Grahani* (irritable bowel syndrome), *Pandu* (anemia), *Arsha* (hemorrhoids), *Kasa* (cough) etc[6]. Though, it is a widely prescribed formulation; safety data on this important metallic formulation is unavailable. Considering this, an attempt has been made to prepare *Rasa parpati* by using *Shodhita* and *Ashodhita* samples of *Parada* and *Gandhaka* and to evaluate their comparative safety profiles in Charles foster albino rats.

2. Material and Methods

2.1 Preparation of *Rasa Parpati*

Two samples of *Rasa parpati* were prepared in the laboratory. The first one labelled as HRP is prepared with *Hingulottha parada* (mercury extracted from cinnabar) and *Shuddha gandhaka* (processed sulphur), while, the second one labelled as ARP is prepared with *Ashuddha parada* (unprocessed mercury) and *Ashuddha gandhaka* (unprocessed sulphur) by following standard classical guidelines. *Ashuddha hingula* (unprocessed cinnabar), *Ashuddha parada* and *Ashuddha gandhaka* were procured from Pharmacy, Gujarat Ayurved University, Jamnagar and authenticated at Department of Rasashastra and Bhaishajya kalpana, Institute of Teaching and Research in Ayurveda, Jamnagar.

Platform of even surface was prepared by fresh cow dung. Fresh *Kadali patra* (banana leaves) were collected from the local garden. *Parada* and *Gandhaka* were mixed together in equal proportion and triturated till black colored fine powder (*Samaguna kajjali*) was formed. *Samaguna kajjali* was prepared by using *Shuddha* and *Ashuddha* samples of *Parada* and *Gandhaka*. In preparation of HRP, *Samaguna kajjali* was taken in stainless steel ladle smeared with ghee and melted over the heat source (LPG stove) maintaining temperature around 125^{0}C. Heating was continued till the contents became semisolid (*Pankawat*) by periodical stirring with stainless steel spatula. *Pankawat kajjali* was immediately poured on the platform prepared with cow dung covered with banana leaf and covered with another banana leaf and compressed gently by using a flat surface of a steel plate. After cooling, solidified flakes of HRP were collected. The same procedure was followed to prepare ARP, but *Kajjali* used here is prepared from *Ashuddha Parada* and *Gandhaka*[7].

2.2 Animals

Animal experimentation was approved by the Institutional Animal Ethics Committee (IAEC/18/2015/28). Charles foster albino rats of either sex weighing 200 ± 20 g of 6-8 weeks age were procured from the animal house of the Pharmacology laboratory, Institute of Teaching and Research in Ayurveda, Jamnagar. While housing the animals, ambient temperature (23 ± 2^{0}C) and humidity (50-60%) were maintained following the approved husbandry conditions. The animals were fed ad *libitum* with Amrut brand rat pellet feed supplied by Pranav Agro Industries and drinking water.

2.3 Dose Fixation and Schedule

Considering therapeutic dose of *Rasa parpati* as 250 mg per day in Rasa classics; rat dose was calculated by referring to the table of Paget and Branes (1964)[7-8]. Therapeutic Equivalent Dose (TED) is found to be 22.5 mg/kg body weight of rat. As Rasa classics advocated the use of *Rasa parpati* with honey as an adjuvant; the test drugs (ARP and HRP) were added with honey (1 ml/kg) as adjuvant and made the animals to lick the drug by placing over the tongue with the help of a cannula.

2.4 Acute Toxicity Study

Organization for Economic Cooperation and Development (OECD) 425 guidelines were followed to perform acute oral toxicity study[9]. Nulliparous and non-pregnant Charle's foster strain albino female rats were selected. They were acclimatized for seven days prior to the experiment. Both the test drugs (ARP and HRP) along with honey as adjuvant were administered orally at limit dose of 2000 mg/kg to overnight fasted rats in sequential manner. Rats were observed closely for the first six hours and thereafter periodically up to 14 days for changes in behavior, toxicity signs, and mortality, if any.

2.5 90 Days Repeated Dose Toxicity Study

OECD Guideline repeated dose 90-day oral toxicity study in rodents was followed in the study[10]. The rats were randomly categorized into nine groups, each comprising of six rats (three male and three females in each group). Animals of Group I received vehicle (1 ml/kg of honey orally) and served as Vehicle Control (VC). Group II to IV received HRP along with adjuvant at TED (22.5 mg/kg orally), TED×5 (112.5 mg/kg orally) and TED×10 (225 mg/kg

orally) dose levels, respectively. Animals of Group V to VII received ARP at the same three dose levels of HRP. Group VIII and IX received HRP and ARP respectively at TED×10 (225 mg/kg orally) along with adjuvant and served as recovery groups. Animals of Group I to VII were sacrificed on 90th day of drug administration, while in Group VIII and IX on 120th day [Table 1].

Table 1. Details on grouping of animals, drug and dose

Groups	Number of animals	Purpose	Drug	Dose level	Dose (mg/kg)	Duration of study
I	6	Vehicle control	Honey	-	1	90 days
II	6	Trial groups	HRP	TED	22.5	
III	6			TEDx5	112.5	
IV	6			TEDx10	225	
V	6		ARP	TED	22.5	
VI	6			TEDx5	112.5	
VII	6			TEDx10	225	
VIII	6	Recovery groups	HRP	TEDx10	225	120 days
IX	6		ARP	TEDx10	225	

HRP: *Rasa Parpati* prepared by using *Hingulottha parada* and *Shuddha gandhaka*, ARP: *Rasa Parpati* prepared by using *Ashuddha parada* and *Ashuddha gandhaka*, TED= Therapeutic equivalent dose

Initial body weight of all the animals was recorded. General behavioral pattern was assessed once in a week by exposing each animal to open arena. On 90th day, all animals of group I to VII were weighed again, supra-orbital plexus was punctured under light anesthesia with diethyl ether. Blood was collected by capillaries in two different tubes, one plain tube for serum biochemical investigations and another containing anti-coagulant fluid for hematological parameters. The rats then were sacrificed with overdose of diethyl ether; a midline incision was made over the abdomen; necropsy changes were recorded followed by dissecting the important visceral organs.

Hematological analysis was performed by using an automatic hematological analyzer (Swelab, Sweden). Total Red Blood Cell (RBC), Hemoglobin (Hb), Mean Corpuscular Hemoglobin (MCH), Hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cell (WBC), Percentage of lymphocytes, Neutrophils, Monocytes and Eosinophils, Packed Cell Volume (PCV) and Platelet count were measured from the blood samples.

Serum biochemical parameters were carried out by using fully automated biochemical random access analyzer (BS-200, Lilac Medicare Pvt. Ltd., Mumbai). The studied parameters are blood urea (Urease – GLDH – Fixed time, Kinetic, enzymatic method), creatinine (Modified Jaffe's reaction), total protein (Biurate Method, End method), albumin (BCG Dye method, end point), globulin, alkaline phosphatase (IFCC Method, Kinetic method), SGOT (IFCC method without Pyridoxal Phosphate), SGPT activity (IFCC method, kinetic without Pyridoxal Phosphate), total bilirubin, direct bilirubin, uric acid, calcium, total cholesterol, high density lipoprotein (HDL)-cholesterol and triglyceride[11–23].

Internal organs viz. Kidney, liver, lungs, heart, stomach, spleen, ileum, thymus, adrenal, lymph node, testes, pituitary, seminal vesicle, prostate, uterus, ovary and skin were carefully dissected. After observing and noting the ponderal changes and signs of gross lesions in major organs; all organs were transferred to 10% phosphate buffered formalin solution for fixation, subjected to dehydrating, wax embedding, sectioning and staining with haematoxylin and eosin for histological evaluation. The slides were observed under trinocular research Carl-Zeiss's microscope at various magnifications to note down the histopathological changes in respective organs.

3. Statistical Analysis

The results are expressed as mean ± Standard Error of Mean (SEM) for each experimental group. Statistical comparisons were carried out by both unpaired student's t test and one-way analysis of variance to compare the mean values of quantitative variables among the groups followed by Dunnett multiple t-test for unpaired data by using Sigma stat software (version 3.5, Systat Software Inc.) to determine significant difference between groups at $P<0.05$.

4. Results

In acute toxicity study, both the test drugs with an adjuvant (honey) up to single oral dose of 2000 mg/kg did not reveal any changes in normal gross behavior and other parameters in any of the animals. All rats were survived and did not show any signs of toxicity or mortality during the experimental period of 14 days. There were no abnormal behavioral changes in *Rasa parpati* treated groups during entire course of 90 days repeated dose toxicity. No toxicity or mortality was observed at TED, TEDx5, TEDx10 dose levels and even in the animals of recovery groups. Normal body weight gain was observed in control, test drug treated

Table 2. Effect of HRP and ARP on body weight of albino rats in 90 days repeated dose toxicity study

Groups	0 day	4th week	8th week	12th week	90th day	120th day
Vehicle Control	183.33 ± 8.4	226.67 ± 5.58***	246.67 ± 7.15***	236.67 ± 9.89**	251.67 ± 11.0**	--
HRP TED	173.33 ± 6.1	208.33 ± 8.72**	223.33 ± 10.54***	233.33 ± 12.5***	220.00 ± 11.2**	--
HRP TEDx5	180.00 ± 10.65	221.67 ± 12.50***	238.33 ± 15.37***	246.67 ± 18.01***	248.33 ± 19.22***	--
HRP TEDx10	180.00 ± 10.33	235.00 ± 11.48***	243.33 ± 10.85***	230.00 ± 20.25*	234.00 ± 14.70*	--
ARP TED	183.33 ± 12.56	221.67 ± 11.38***	246.67 ± 12.02***	241.67 ± 14.70***	263.33 ± 17.64***	--
ARP TEDx5	185.00 ± 7.64	208.33 ± 5.43***	225.00 ± 17.65*	241.67 ± 15.79**	235.00 ± 13.35*	--
ARP TEDx10	175.00 ± 4.28	213.33 ± 16.67	223.33 ± 24.59*	243.33 ± 24.99*	258.33 ± 20.88**	--
HRP Recovery	176.67 ± 9.89	230.00 ± 10.33***	258.33 ± 10.76***	263.33 ± 18.92***	266.67 ± 23.19***	283.33 ± 20.28***
ARP Recovery	171.67 ± 6.01	233.33 ± 9.55***	253.33 ± 11.74***	218.33 ± 8.33**	260.00 ± 8.56***	261.67 ± 10.14***

Data presented as Mean ± SEM, *P<0.05, **P<0.01, ***P<0.001 (Paired t- test), SEM = Standard error of mean

Table 3. Effect on hematological parameters during 90 days repeated dose toxicity study

Parameters	Vehicle control	HRP TED	HRP TED 05	HRP TED 10	ARP TED	ARP TED 05	ARP TED 10	HRP TED 10 R	ARP TED 10 R
Neutrophil (%)	12.33 ± 2.26	19.00 ± 1.57#	23.00 ± 1.46##	24.00 ± 3.52##	20.83 ± 2.41#	17.33 ± 3.03	25.33 ± 5.80*	30.33 ± 2.39**###	29.00± 4.04**##
Lymphocyte (%)	83.50 ± 2.51	76.00 ± 1.77#	71.67 ± 1.45##	71.20 ± 3.22#	75.33 ± 2.51#	79.00 ± 3.30	69.83 ± 5.69*	65.17 ± 2.71**###	69.83 ± 3.96**##
Hb (g%)	14.17 ± 0.34	14.75 ± 0.30	14.87 ± 0.26	14.38 ± 0.24	15.20 ± 0.22#	15.38 ± 0.35*#	14.77± 0.26	14.82± 0.23	15.35 ± 0.22*#
TRBC(103/µL)	7.70± 0.37	8.38 ± 0.29	8.62 ± 0.35	8.03 ± 0.13	8.28 ± 0.19	8.57 ± 0.38	8.15 ± 0.23	8.43 ± 0.19	8.14 ± 0.23

Data presented as Mean±SEM,*P<0.05, **P<0.01 when compared to control group (ANOVA followed by Dunnett's multiple't' test); #P<0.05, ##P<0.01, ###P<0.001 when compared to control group (Unpaired't' test)

Values of other hematological parameters like total white blood cells (10^3/Cu mm), eosinophils (%), monocytes (%), PCV (%), MCV (fl), MCH (pg), MCHC (g/dl) and platelets (10^3/µl) were observed to be within normal range.

as well as in recovery groups [Table 2]. Besides this, insignificant change in relative weight of nine visceral organs after administration of HRP and ARP at different dose levels was noticed.

Significant increase in neutrophils percentage and significant decrease in lymphocyte percentage was observed at low and high dose levels in HRP and ARP and both recovery groups. Significant increase in Hemoglobin and non-significant increase in PCV and RBC level was observed in ARP treated group at TED and TEDx5 dose level and in HRP recovery group. All other groups produced insignificant increase in Hb count in comparison to vehicle control group. [Table 3]

Among eighteen biochemical parameters; administration of HRP resulted in significant decrease in cholesterol at TED dose level and non-significant decrease at TEDx5 and TEDx10 dose levels. Administration of ARP at TEDx5 dose level produced non-significant decrease in cholesterol level when compared to vehicle control group. Blood urea increases significantly in HRP treated groups and ARP at higher dose levels. After discontinuation of both test drugs, there was significant decrease in blood urea level in recovery groups in comparison to control group. Creatinine level was found significantly decreased in animals of HRP group at TEDx5 and in both recovery groups (Group VIII and IX). SGOT level was increased at significant level in ARP at TED 5 and non-significant increase at higher dose levels. Even after discontinuation of ARP in recovery study, still high level of SGOT level observed in comparison to control group. Significant increase in bilirubin was observed in animals of ARP group at TED and TEDx5 dose levels. Calcium level was significantly decreased in HRP at TEDx10 dose level. The changes observed in remaining biochemical parameters were statistically insignificant in comparison to vehicle control group. [Table 4]. Values of other biochemical parameters like triglycerides (mg/dl), VLDL (mg/dl), SGPT (IU/dl), total proteins (g/dl), albumin (g/dl), globulin (g/dl), ALP (IU/dl), direct bilurubin (mg/dl) and uric acid (mg/dl) were found to be within normal range.

Table 4. Effect on serum biochemical parameters in 90 days repeated dose toxicity

Parameters	Vehicle control	HRP-TED	HRP-05	HRP-10	ARP-TED	ARP-05	ARP-10	HRP-10 R	ARP-10 R
BSL(mg/dl)	77.50 ± 6.45	75.00 ± 10.03	74.50 ± 2.38	68.60 ± 5.63	68.00 ± 4.78	61.00 ± 3.15#	70.33 ± 7.91	68.50 ± 5.38	74.17 ± 4.37
Cholesterol (mg/dl)	55.33 ± 3.32	37.33 ± 2.62*##	45.83 ± 3.95	44.00 ± 2.90#	58.00 ± 7.01	46.00 ± 5.05	53.17 ± 4.13	56.83 ± 4.83	55.67 ± 5.71
HDL (mg/dl)	40.5 ± 3.22	27.17 ± 2.33##	41.33 ± 7.88	27.00 ± 5.63#	39.67 ± 4.06	29.67 ± 1.87#	36.17 ± 2.55	34.50 ± 4.43	38.67 ± 4.05
LDL (mg/dl)	5.17 ± 1.54	12.17 ± 2.64#	11.00 ± 3.43	6.80 ± 2.44	9.33 ± 4.22	10.33 ± 3.11	8.50 ± 2.99	5.17 ± 1.47	6.00 ± 1.21
Blood urea (mg/dl)	63.50 ± 1.61	77.50 ± 0.85**##	69.17 ± 1.82#	70.80 ± 1.99#	69.17 ± 3.12	68.50 ± 3.27	74.17 ± 1.94**###	41.00 ± 2.46**###	57.33 ± 2.08#
Creatinine (mg/dl)	0.62 ± 0.04	0.57 ± 0.03	0.47 ± 0.42*#	0.54 ± 0.05	0.57 ± 0.03	0.53 ± 0.03	0.57 ± 0.02	0.40 ± 0.00**###	0.48 ± 0.02*#
SGOT (IU/dl)	115.67 ± 2.29	173.00 ± 38.82	121.00 ± 6.78	123.60 ± 7.47	130.83 ± 7.20	157.83 ± 9.85##	142.00 ± 12.26	177.50 ± 22.15#	141.50 ± 9.27#
T. Bilirubin (mg/dl)	0.33 ± 0.02	0.37 ± 0.02	0.42 ± 0.03	0.36 ± 0.02	0.48 ± 0.04*#	0.57 ± 0.03**##	0.37 ± 0.03	0.43 ± 0.02#	0.33 ± 0.02
S. calcium (mg/dl)	10.15 ± 0.21	9.83 ± 0.06	10.55 ± 0.12	10.06 ± 0.21	10.05 ± 0.09	10.08 ± 0.07	9.47 ± 0.16*#	9.93 ± 0.03	9.93 ± 0.02

Data presented as Mean±SEM,*P<0.05, **P<0.01 when compared to control group (ANOVA followed by Dunnett's multiple't' test); #P<0.05, ##P<0.01, ###P<0.001 when compared to control group (Unpaired't' test)

The microscopic observations in histopathological studies revealed that HRP at TEDX10 treated group along with honey as adjuvant exhibited adverse changes in heart, liver and stomach. But, animals treated with ARP at TEDx10 dose levels showed moderate to severe changes in seven visceral organs viz. heart, kidney, liver, stomach, intestine, spleen and thymus. HRP at TEDx10 dose level produced fatty changes in heart and liver whereas, animals in recovery group (in Group VIII) showed normal cytoarchitecture. [Figures 1 and 2].

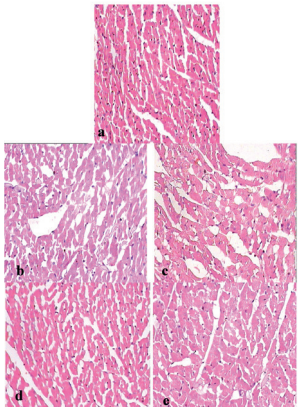


Figure 1. Photomicrographs of sections of the Heart taken at X 400 (a) Normal cytoarchitecture in Vehicle Control (b) Mild fatty changes in HRP TED×10 (c) Severe fatty degenerative changes in ARP TED×10 (d) Almost normal cytoarchitecture in HRP TED×10 Recovery (e) Micro fatty changes with globules in ARP TED×10 Recovery.

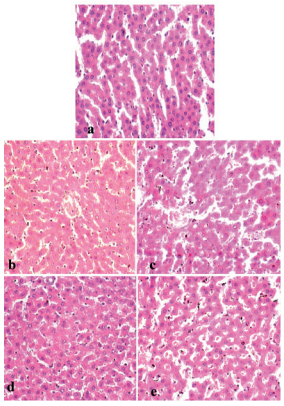


Figure 2. Photomicrographs of sections of the Liver taken at X 400 (a) Normal cytoarchitecture in Vehicle Control (b) Micro fatty changes in HRP TED×10 (c) Fatty changes, loss of cytoarchitecture and black particle deposition in ARP TED×10 (d) Almost normal cytoarchitecture in HRP TED×10 Recovery (e) Fatty changes, cell infiltration and black particle deposition in ARP TED×10 Recovery.

Administration of ARP at TEDx10 (Group VII) produced moderate to severe fatty degenerative changes in heart and fatty changes, black particle deposition in liver, which were also persistent in recovery phase. [Figures 1, 2]

Administration of ARP at TEDx10 (Group VII) also showed severe inflammation and fatty degenerative changes in kidney that were also observed even in recovery groups (Group IX). [Figure 3].

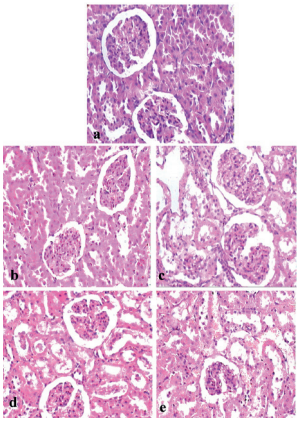


Figure 3. Photomicrographs of sections of the Kidney taken at X 400 (a) Normal cytoarchitecture in Vehicle Control (b) Normal cytoarchitecture in HRP TED×10 (c) Severe inflammation and fatty degenerative changes in ARP TED×10 (d) Normal cytoarchitecture in HRP TED×10 Recovery (f) Inflammation and fatty degenerative changes in ARP TED×10 Recovery.

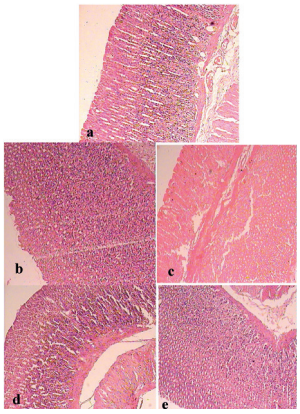


Figure 4. Photomicrographs of sections of the Stomach taken at X 100 (a) Normal cytoarchitecture in Vehicle Control (b) Epithelial erosion in HRP TED×10 (c) Epithelial erosion and sub mucosal inflammation in ARP TED×10 (d) Normal cytoarchitecture in HRP TED×10 Recovery (e) Normal cytoarchitecture in ARP TED×10 Recovery.

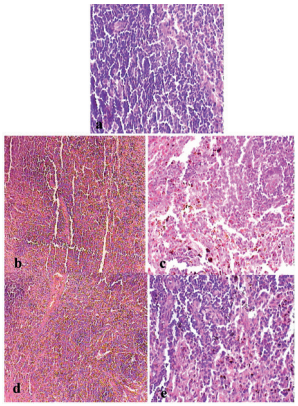


Figure 5. Photomicrographs of sections of the Spleen taken at X 400 (a) Normal cytoarchitecture in Vehicle Control (b) Normal cytoarchitecture in HRP TED×10 (c) Particle deposition and degenerative changes in ARP TED×10 (d) Normal cytoarchitecture in HRP TED×10 Recovery (e) Particle deposition in ARP TED×10 Recovery.

Whereas, HRP at TEDx10 (Group IV) showed normal cyto-atrchitecture. ARP at TEDx10 (Group VII) exhibited epithelial erosion with sub-mucosal inflammation in stomach, whereas these changes were reversed in recovery group. [Figure 4]. HRP showed epithelial erosion with normal cyto-architecture in stomach. Black particle depositions were observed in spleen with administration ARP at TEDx10 (Group VII). [Figure 5].

However, all these changes were not observed in the animals treated with both the drugs at TEDx5 and TED dose levels. The results suggest that, drug has only adverse effects on above organs at TEDx10 dose level. The changes were reversed after discontinuation of drug which suggests the mild toxic potential of test drug at higher dose level after longer administration in rats.

5. Discussion

Ayurveda interventions are gaining popularity and acceptability globally but, numbers of concerns are being raised by scientific community on therapeutic use of metals and minerals in Ayurvedic formulations due to presence of heavy metals in them. Though Ayurvedic seers were well versed about possibility of ill effects caused due to administration of such metallic formulations, if not prepared and processed as per classical guidelines, there is a need to evaluate safety and toxicity profiles of such Ayurvedic metallic formulations by following scientific parameters.

The dose (2000 ml/kg) administered in acute toxicity is several folds higher than the therapeutic equivalent dose. In this study, both the samples of *Rasa parpati* did not produce any observable toxic effects and mortality during entire duration of acute toxicity study. These observations suggest that LD_{50} value of *Rasa parpati* samples may be higher than 2000 mg/kg by oral route. As per UN classification, any substance that has oral LD_{50} of more than 2000 mg/kg is considered as low hazard potential and categorized as UN 6.1 PG III.[24] Thus, HRP and ARP can be categorized as substances with low health hazard potential (Class 4 of GHS and UN 6.1 PG III).

Administration of both ARP and HRP in 90 days repeated dose toxicity showed significant changes in neutrophils, hemoglobin and decrease in lymphocyte percentage but, these changes were not found to be in dose dependent manner and within the normal range. The increase in hemoglobin content in ARP at TED, TEDx5 and HRP TEDx10 recovery groups may be due to increase in the number of circulating red cells, packed cell volume, their concentration of hemoglobin or any combination of these.

Significant decrease in cholesterol and HDL level was found in HRP at TED and TEDx10 dose levels, while LDL is increased at TED dose level. Both the parameters were significantly decreased in ARP at TEDx5 suggesting that test drugs may have an effect on cholesterol turnover and interfere with lipid metabolism in the body. Blood urea value was significantly increased in HRP at all three dose levels and ARP at higher dose level. Serum creatinine was significantly decreased in HRP at TEDx5 dose level and in both recovery groups. But these changes were within normal limits when compared with control group. Blood urea and serum creatinine levels are the indicators of kidney function. Though, these parameters were significantly increased in both treated groups at different dose levels; these levels almost reversed in recovery groups when compared with control group suggesting that both the drugs are devoid of drastic toxicity in rats at therapeutic dose levels. Significant alteration of SGOT, bilurubin and calcium level was observed at different dose levels in ARP treated groups but, these changes were not seen in HRP at all three dose levels. It may suggest that ARP is more prone to affect liver, kidney and heart functions.

Both the samples of *Rasa parpati* revealed adverse changes in cytoarchitecture of visceral organs at higher dose levels of TEDx10 on repeated administration for longer duration. But HRP showed less magnitude of toxicity as only mild changes were observed in three visceral organs viz. heart, liver and stomach. Moderate to severe changes were observed in the seven visceral organs of ARP treated animals at TEDx10 dose levels viz. heart, kidney, liver, stomach, in-

testine, spleen and thymus. However, the observed histopathological changes were not seen at therapeutic dose level and not in the recovery groups. The results of bio-chemical parameters also reveal that, the HRP do not have potential to produce any drastic changes in the liver and kidney function parameters in rats; which suggests that the organ damage as mentioned above in 90 days repeated dose toxicity is of mild intensity at higher dose level, however test drugs can be relatively safe at therapeutic dose level. However, the results observed in ARP treated group suggest that, *Rasa parpati* prepared by *Ashuddha Parada* and *Gandhaka* has potential for adverse changes in majority of the organs particularly liver, kidney and GI tract of rats.

Almost normal cyto-architecture of stomach and intestine was observed in HRP at TEDx10 dose level, while ARP at highest dose level exhibited loss of epithelial, villi with shortened height and fatty changes in intestine. This suggests that HRP has fewer tendencies to bind with alimentary and intestinal mucosa in comparison to ARP, which demonstrate safety of HRP on 90 days administration. Inorganic mercurials are insoluble in lipids rendering them unable to cross the blood-brain or blood-placental barriers; thus they do not produce any toxic potential on brain and foetus.[25] In current study, both ARP and HRP (that are inorganic mercurial) showed normal cyto-architecture in fore, mid and hind brain.

The study suggests that, though *Rasa Parpati* prepared by *Shuddha Parada* and *Gandhaka* is not toxic at therapeutic dose level, has potential to produce adverse effects when administered for longer duration at higher dose levels. Further, *Rasa Parpati* prepared by *Ashuddha Parada* and *Gandhak*a has potential for adverse changes in variety of organs when administered for longer duration at higher dose levels and even after discontinuation of test drugs in recovery study. Hence, *Shodhana* of *Parada* and *Gandhaka* is must for its internal administration. Classical procedures like *Shodhana* are mandatorily to be followed in the pharmaceutical procedures of these formulations.

Traditional formulations as potential source of therapeutics have attained a significant role in healthcare system all over the world not only in the disease condition but also as potential material for maintaining health. The need of the time is to generate safety, toxicity and efficacy profiles of all such formulations following the standard protocols. Based upon the observations, it can be concluded that HRP and ARP did not produce any signs and symptoms of toxicity or mortality during acute toxicity study up to the oral dose level of 2000 mg/kg suggesting that LD_{50} value may be higher than 2000 mg/kg by oral route in rats. Thus, both the drugs (HRP, ARP) can be categorized as substances with low health hazard potential. Administration of HRP for 90 days at therapeutic dose levels is safe to be used. Higher dose of HRP resulted in mild damage to the cyto-architecture of liver and kidney that are reversible after discontinuation of the drug. ARP has greater potential of causing damage to various visceral organs including liver, kidney and GI tract, which are irreversible. The more magnitude of toxic changes with ARP (sample prepared without *Shodhana*) reveal that the classical procedures like *Shodhana* are mandatorily to be followed in the pharmaceutical procedures of the formulations. Non following of such essential classical procedures lead to formulations of poor quality that may further prove to be toxic and bring a bad repute to the system as a whole.

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7. References

1. Mehta V, Midha V, Mahajan R, Narang V, Wander P, Sood R, Sood A. Lead intoxication due to Ayurvedic medications as a cause of abdominal pain in adults. Clin Toxicol (Phila). 2017; 55:97–101. PMid: 27957879. https://doi.org/10.1080/15563650.2016.1259474
2. Saper RB, Russell S, Phillips, Sehgal A, Khouri N, Davis RB, et al. Lead, Mercury and Arsenic in US and Indian manufactured Ayurvedic medicines sold via the internet. JAMA. 2008; 300:915–23. PMid: 18728265 PMCid: PMC2755247. https://doi.org/10.1001/jama.300.8.915
3. Mikulski MA, Wichman MD, Simmons DL, Pham AN, Clottey V, Fuortes LJ. Toxic metals in Ayurvedic preparations from a public health lead poisoning cluster investigation. Int J Occup Environ Health. 2017; 23:187–92. PMid: 29528276 PMCid: PMC6060866. https://doi.org/10.1080/10773525.2018.1447880
4. Sathe K, Ali U, Ohri A. Acute renal failure secondary to ingestion of Ayurvedic medicine containing mercury. Indian J Nephrol. 2013; 23:301–3. PMid: 23960350 PMCid: PMC3741978. https://doi.org/10.4103/0971-4065.114485
5. Mishra GS. Ayurveda Prakasha. Varanasi: Chaukhambha Bharati Academy; 2007.
6. Sharma S. Rasatarangini. New Delhi: Motilal Banarasi das; 2009.
7. Kulkarni DA. Rasaratnasamucchaya. Varanasi: Chaukhamba Bharati Academy; 2011.

8. Paget GE, Branes JM. Toxicity tests. Laurence DR, Bocharach AL, Eds. Evaluation of drug activities: Pharmacometrics. New York: Academic Press; 1964. https://doi.org/10.1016/B978-1-4832-2845-7.50012-8
9. OECD. OECD Guidelines for the testing of chemicals: Acute oral toxicity-up-and-down-procedure. OECD Publishing: 2001.
10. OECD. OECD Guidelines for the testing of chemicals: Repeated dose 90-day oral toxicity study in rodents. OECD Publishing: 1998.
11. Talke H, Schubert GE. Enzymatic urea determination in the blood and serum in the Warburg optical test. Klin Wochenschr. 1965; 43:174–5. PMid: 14258517. https://doi.org/10.1007/BF01484513
12. Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method. Sc and J Clin Lab Invest. 1965; 17:381–7. PMid: 5838275. https://doi.org/10.3109/00365516509077065
13. Tietz NW, Editor. Text book of clinical chemistry. Philadelphia: WB Saunders and Company; 1986.
14. Doumas BT, Arends RL, Pinto PC. Standard methods of clinical chemistry. Vol VII. Chicago: Academic Press; 1972.
15. Wilkinson JH, Boutwell JH, Winsten S. Evaluation of a new system for the kinetic measurement of serum alkaline phosphatase. Clin Chem. 1969; 15:487–95. PMid:5786804.https://doi.org/10.1093/clinchem/15.6.487
16. Tietz NW, Editor. Clinical guide to laboratory tests. 3rd ed. Philadelphia: WB Saunders and Company; 1995.
17. Burtis CA, Ashwood ER, Eds. Tietz textbook of clinical chemistry. 3rd ed. Philadelphia: WB Saunders and Company; 1999.
18. Pearlman FC, Lee RT. Detection and measurement of total bilirubin in serum, with use of surfactants as solubilizing agents. Clin Chem. 1974; 20:447–53. https://doi.org/10.1093/clinchem/20.4.447
19. Kabasakalian P, Kalliney S, Westcott A. Determination of uric acid in serum, with use of uricase and a tribromophenol-aminoantipyrine chromogen. Clin Chem. 1973; 19:522–4. PMid: 4703662. https://doi.org/10.1093/clinchem/19.5.522
20. Moorehead WR, Biggs HG. 2-Amino-2-methyl-1-propanol as the alkalizing agent in an improved continuous-flow cresolphthalein complex one procedure for calcium in serum. Clin Chem. 1974; 20:1458–60. https://doi.org/10.1093/clinchem/20.11.1458
21. Roeschlau P, Bernt E, Gruber WA. Enzymatic determination of total cholesterol in serum. J Clin Chem Clin Biochem. 1974; 12:226.
22. Dominiczak M, McNamara J, Nauk M, Wiebe D, Warnick G. Handbook of lipoprotein testing. Measurement of high-density-lipoprotein cholesterol. Rifai N, Warnick GR, Dominiczak MH, Eds. 2nd ed. Washington, DC: AACC Press; 2000.
23. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem. 1982; 28:2077–80. PMid: 6812986. https://doi.org/10.1093/clinchem/28.10.2077
24. Anonymous. Class 6-toxic and infectious substances. UN recommendations on the transport of dangerous goods-model regulations. Part-2. 12th revised ed. Ch. 2.6. Geneva: United Nations Publications; 2001.
25. Dart RC, Sulliva JB. Medical toxicology. Mercury. Dart RC, Ed. 3rd ed. Philadelphia: Lippincott Williams and Wilkins; 2004.