Research Article

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SAFETY PROFILE OF CHAPALA BHASMA WITH SPECIAL REFERENCE TO BISMUTH AND SELENIUM

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ABSTRACT

Rasashastra is the branch of elemental biology in Ayurveda and has utilized almost every substance in nature, ranging from simplest forms like mud, fossils, and resin, to the most precious things like gold, gems etc. Chapala is a rasa dravya included in the group of Maharasa. It is a controversial drug, some authors consider Chapala as Bismuth, and some believe it is Selenium. So, to resolve the controversy of Chapala, a study has been conducted. Aim: To understand the Safety profile of Chapala bhasma (clax) with reference to Bismuth and Selenium through oral toxicity study. Material and Methods: Clax of Bismuth and Selenium, and the study was carried out according to the OECD guidelines. Results: Bismuth Clax has shown considerable changes in histopathology reports and fewer changes in Selenium clax. Conclusion: The Chapala bhasma prepared by Bismuth and Selenium was nontoxic, and Selenium clax showed more safety profile than Bismuth clax.

Keywords: Bismuth bhasma (clax), Selenium bhasma (clax), OECD guidelines, Physical observation, Histopathology.

INTRODUCTION

Rasashastra is one of the branches of Ayurveda that deals with mineral and metallic, medicinal preparations. These drugs are categorized or classified into different groups like Maharasa, Uparasa, Sadharanarasa, Dhatu, Upadhatu, Ratna, etc. Maharasa varga is among the rasa vargeekarana, and among these, Chapala is the 7th one, considered a controversial drug.

In the Present era, the authors of Rasashastra have different opinions regarding Chapala. Some of them mentioned Bismuth as Chapala; others opine Selenium as Chapala. To overcome the controversies, it is necessary to carry out research in this area, and a study was planned to identify Chapala through classical and modern parameters, pharmaceutical, pharmacological and effect. A safety study was also carried out as a part of the research. To understand the safety profile of Bismuth and Selenium clax, we have chosen an Oral Acute toxicity study for 14 days with a lower and higher dose.

Toxicity is the study of the harmful effects of chemical, physical, and biological agents on living organisms and the environment and their prevention and mitigation. The degree to which a material can harm an organism is known as toxicity [1]. Toxicology studies investigate how a substance affects organ structure and/or function to characterize its toxicity profile. This includes determining toxicity severity and reversibility, dose ranges and their relationship to exposure [2].

Aim and Objectives: To do the Oral Acute toxicity study of *bhasmas* prepared from Bismuth and Selenium according to OECD guidelines 423.

MATERIALS AND METHODS

The clax prepared from Bismuth and Selenium was used, and for animal administration, the samples were diluted with distilled water and Carboxy methyl cellulose (CMC).

Institutional Animal Ethics committee (IAEC) Approval: As per the IAEC approval, the study was commenced in healthy young adult albino Wistar rats of both genders. All experiments and protocols described in the present study were approved by the Institutional Animal Ethics Committee (IAEC) of the Pharmacology department, Parul Institute of Pharmacy and Research and with permission from the Committee for Control and Supervision of Experiments on Animals (CPCSEA).

Protocol No. 984/2019: 14
Permitted Animals: 12

Oral Acute Toxicity Study

Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance or multiple doses given within 24 hours. Delayed death means an animal does not die or appear moribund within 48 hours but dies later during the 14-day observation period. The dose is the amount of test substance administered. The dose is expressed as the weight of the test substance per unit weight of the test animal (e.g., mg/kg). [3]

Animal selection: Wister albino rats of either sex are taken, and rats weighing 200-250 gm are used for the study. The animal was maintained under ideal husbandry conditions. All animals were

exposed to the same environmental conditions and maintained a standard diet and drinking water. All females should be nulliparous and non-pregnant.

Housing: Rats were housed in autoclave standard polypropylene IVC cages. Autoclaved paddy husk was used as bedding material. The paddy husk bedding was changed at least twice a week. Three rats were housed per cage.

Environment conditions: The rats were kept clean with 12 hours of light and 12 hours of the dark cycle. The air was conditioned at 22 °C (± 3 °C), and relative humidity was maintained between 30 - 70 % with a 100 % exhaust facility.

Feeding and watering schedule: Animals were given pelleted fed regularly. Autoclave drinking water was provided ad libitum in polypropylene bottles with a stainless-steel sipper tube throughout the study. The microbial analysis of the autoclave water was maintained at the test facility.

Preparation of animal: The animals are randomly selected and marked individually for identification and kept in their cages for at least five days before dosing to allow for acclimatization to the laboratory condition.

Labelling and Identification: Identification was made by marking the body of the rats using a picric acid solution made in water. A label on the cage contains information about the animal sex, group number, cage number and animal number of rats.

Test items details: Name: *Chapala Bhasma*
Appearance: Ash
Colour: Dark Black
Vehicle: 0.5% CMC
Storage conditions: Room temperature
Test systems details:
Species: Rat Strain: Wister albino,
Sex: Male and Female
Source: Animals were procured from Sun Pharma Advance Research Centre Tandalja, Vadodara, India
Age at start of dosing: 8-12 weeks
Body weight range: It fell between ± 20 % of the mean body weight at the time of initiation of the dosing.

Study Design

Oral Acute Toxicity Study: OECD-423 guidelines were followed for the acute toxicity study. Based on the previous studies, the initial limit test dose was fixed as 300 mg/kg. Three animals were used in each group. The formulation was given at the dose of 300 mg/kg of body weight orally to each animal. The animals were observed every 30 minutes for the initial 24 hours, after which the animals were kept once daily for the next 14 days. After 14 days of dose administration, biochemical and histopathology studies were conducted. If the dose was nontoxic, then the dose was increased to 2000 mg/kg of body weight. The animals were observed in a similar pattern previously observed. [4-6]

Dosing schedule: The single dose of the drug was orally administered and then observed for 14 days. After completion of the treatment periods of 14 days, each animal was sacrificed on day 15.

Dosing details
300 mg/kg
1st Group (Test Group): Bismuth Ash.
2nd Group (Test Group): Selenium Ash.
2000 mg/kg:
1st Group (Test Group): Bismuth ash
2nd Group (Test Group): Selenium ash

Dose Preparation: The dose was prepared at the required concentration before dosing; 0.5% CMC was used for dose preparation. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. The dose must be prepared shortly before being administered unless the stability of the preparation is over. The period it was used is known and shown to be acceptable. In rodents, the volume should not normally exceed 1 ml/100 gm of body weight. However, in the case of an aqueous solution

2 ml/100 gm body weight can be considered. The preparation of different doses varies in concentration to allow a constant dose volume.

Route of administration and its justification: Oral (P.O.) route was selected for this study, as the intended route of administration of the test item was oral in human beings. [8]

Administration

The administration was done by oral gavage using an intuition needle (stainless steel, 20 gauge) fitted into a polypropylene disposable syringe. The concentration was adjusted accordingly to the body weight.

Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily after that, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. The times when signs of toxicity appear and disappear are essential, especially if there is a tendency for toxic symptoms to be delayed. All observations were systematically recorded, with individual records being maintained for each animal. Additional observations were necessary if the animals continued to display signs of toxicity. Observations included changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic, and central nervous systems, and somatomotor activity and behaviour patterns. Attention is directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, and coma.

The Histopathology studies of vital organs were conducted on the 15th day of the dose.

OBSERVATIONS AND RESULTS

Acute Toxicity
The initial dose for the acute toxicity study was 300 mg/kg, and no change in the skin, fur, eyes, and mucous membrane was observed.
There were no tremors, convulsions, increased salivation, diarrhoea, lethargy, sleep, or coma.
There was no change in body weight and eating and drinking habits.
No mortality was noted. (Table 1)

When 300 mg/kg was found to be non-toxic, an acute toxicity study was performed at a 2000 mg/kg dose.
No skin, fur, eyes, and mucous membrane change was observed.
There were no tremors, convulsions, increased salivation, diarrhoea, lethargy, sleep, or coma.
There was no change in body weight or eating and drinking habits.

No deaths occurred. (Table 2)
Body Weight: The weight of each animal was recorded one day before the initiation of the dosing (Day 0) and at weekly intervals throughout the study. (Table 3)

Food Intake: The quantity of the feed offered was based on the requirement of the animals in each cage, and the same was recorded. The intake of the feed was recorded weekly once. The feed consumed, i.e., animal cage/week, was calculated by subtraction of the left-over feed from the total quantity of the feed provided during the week Skin and Fur Condition: After giving a single dose of the drug, there was no significant change in the skin, fur, and eyes and in mucus membrane throughout the entire acute toxicity study period. (Table 4)

Urine and Faeces observation: After being given a single drug dose, there were no significant changes in urine and faeces throughout the acute toxicity study. (Table 5)

Histopathology reports of various organ after Acute Toxicity Study

Group A – Oral Acute toxicity report of BISMUTH CLAX
215/22, A: Lungs show interstitial haemorrhage with mild chronic inflammation.
215/22 B, C – Liver shows Periportal inflammation with focal fatty changes.
215/22 D, E, F, G, H, I – Heart, stomach, and kidney showed normal histology.

Group B – Oral Acute toxicity report of SELENIUM CLAX
215/22 J, K – Lungs show congested blood vessels and focal haemorrhage.
215/221 M – Liver shows fatty changes.
215/22 O, P, Q, R, S, T, U – Heart, stomach, kidney and uterus shows normal histology.

Table 1: Observations of Animal behaviours after 300 mg/ Kg dose

Parameters	Observation of control and test groups					
	30 min	4 hours	24 hours	48 hours	7 days	14 days
Salivation	N	N	N	N	N	N
Somatomotor activity and behaviours pattern	N	N	N	N	N	N
Sleep	N	N	N	N	N	N
Convulsion and tremors	N	N	N	N	N	N
Itching	N	N	N	N	N	N
Coma	N	N	N	N	N	N
Mortality	N	N	N	N	N	N

*N: Normal

Table 2: Animal behaviours after 2000 mg/ Kg

Parameters	Observation of control and test groups					
	30 min	4 hours	24 hours	48 hours	7 days	14 days
Salivation	N	N	N	N	N	N
Somatomotor activity and behaviours pattern	N	N	N	N	N	N
Sleep	N	N	N	N	N	N
Convulsion and tremors	N	N	N	N	N	N
Itching	N	N	N	N	N	N
Coma	N	N	N	N	N	N
Mortality	N	N	N	N	N	N

*N: Normal

Table 3: Observation table for body weight scale

Group	0 days	7 days	14 days
Bismuth ash group (300 mg/kg)	245 gm	251 gm	267 gm
Selenium ash group (300 mg/kg)	256 gm	263 gm	268 gm
Mean	250.5 gm	257 gm	265.5 gm
Bismuth ash group (2000 mg/kg)	251 gm	258 gm	266 gm
Selenium ash group (2000 mg/kg)	237 gm	241 gm	248 gm
Mean	244 gm	249.5 gm	257 gm

Table 4: Skin, fur and eye condition

Group	0 days	7 days	14 days
Bismuth ash group (300 mg/kg)	N	N	N
Selenium ash group (300 mg/kg)	N	N	N
Bismuth ash group (2000 mg/kg)	N	N	N
Selenium ash group (2000 mg/kg)	N	N	N

*N: Normal

Table 5: Changes in urine and faeces condition

Group	0 days	7 days	14 days
Bismuth ash group (300 mg/kg)	N	N	N
Selenium ash group (300 mg/kg)	N	N	N
Bismuth ash group (2000 mg/kg)	N	N	N
Selenium ash group (2000 mg/kg)	N	N	N

*N: Normal

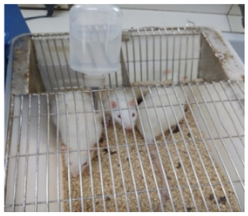

Figure 1: Caging of animals

Figure 2: Labelling of groups

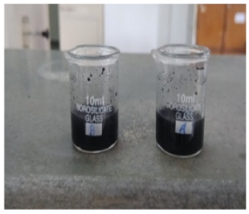

Figure 3: Dosing of medicine

Figure 4: Collections of viscera for histopathological studies

TOXICITY STUDY

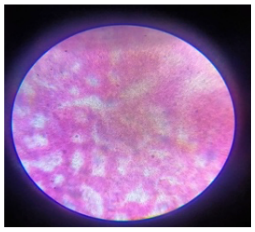

Figure 5: Heart GRP – A

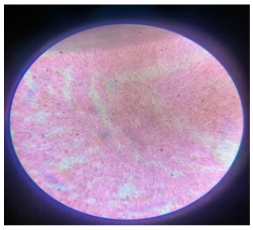

Figure 6: Heart GRP - B

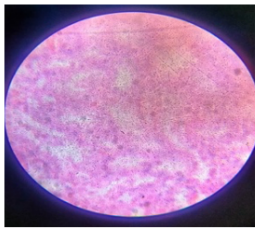

Figure 7: Kidney GRP - A

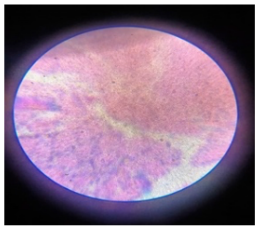

Figure 8: Kidney GRP - B

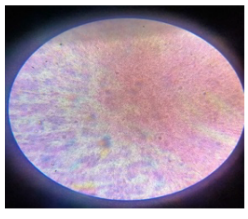

Figure 9: Stomach GRP – A

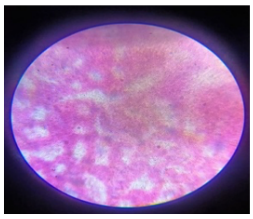

Figure 10: Stomach GRP - B

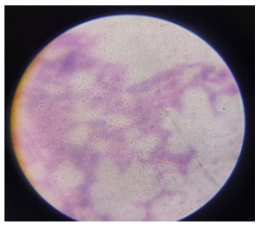

Figure 11: Lungs GRP - A

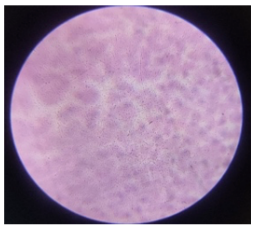

Figure 12: Lungs GRP - B

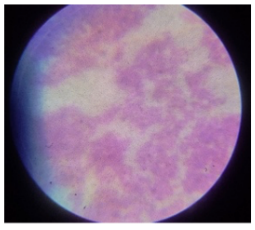

Figure 13: Liver GRP - A

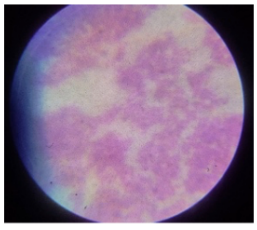

Figure 14: Liver GRP - B

Figure 15: Uterus GRP - A

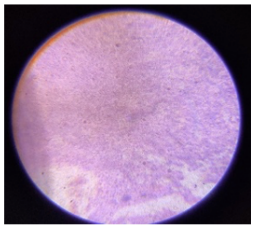

Figure 16: Uterus GRP - B

HISTOPATHALOGICAL SLIDES OF GROUP A (BISMUTH) & B (SELENIUM) AT THE DOSE OF 2000 mg/ Kg

DISCUSSION

The study was conducted in two different doses. Based on previous studies, the trial dose was fixed as 300mg/ kg. Both Selenium and Bismuth were administered. The dose continued for 14 days, and Salivation, Somato motor activity and behaviour pattern, sleep, convulsion and tremors, itching, coma, and mortality were observed. The observations proved that the dose was nontoxic, and then the dose was increased to 2000 mg/ kg. Continued the dose for again 14 days with clax of Selenium and Bismuth and did all the observations. It was also proved as nontoxic through the observations. The histopathology reports also showed nontoxicity of Selenium, and the reports of Bismuth show some changes at the dose of 2000 mg/kg. Like the lungs lead interstitial haemorrhage with mild chronic inflammation, the liver shows Periportal inflammation with focal fatty changes, heart, stomach, and kidney show normal histology. And Selenium clax also showed slight changes like congested blood vessels of the lungs and focal haemorrhage, and the liver showed fatty changes.

CONCLUSION

The OECD guidelines 423 helped to prove the safety profile of *Chapala bhasma* concerning Bismuth and Selenium. Bismuth showed some markable changes, and selenium showed more safety profile. Hence Selenium was safer than bismuth.

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