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ACUTE ORAL TOXICITY OF PRAMEHAKULANTAKA RASA IN WISTER ALBINO RATS

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ABSTRACT

Background: A herbo-metallic Ayurvedic formulation *Pramehakulantaka Rasa* mentioned for the treatment of *Prameha/Madhumeha* in Ayurvedic text was subjected to Acute Oral Toxicity Study according to OECD Guideline 423 for evaluation of its safety. **Material and Method:** Two dose levels of 300 mg/kg and 2000 mg/kg of *Pramehakulantaka Rasa* were selected to evaluate its safety and hence were administered in two groups of three Wistar albino rats each. Each rat was under observation for 14 days. **Observation:** Each rat was observed for behavioral changes at 30min, 4hr, 24 hr, 48 hr, 1st week, and 2nd week in terms of changes in skin & fur, mucous membranes, for the appearance of any abnormal symptoms like salivation, lethargy, sleep, coma, concentration, tremor, diarrhea, morbidity, and motility. At the end of 14th day, hematological analysis was conducted. **Results:** Throughout 14 days of observation, no abnormal behavioral changes were noted in any of the rats also the values of hematological analysis remained under normal limits at the end of the 14th day. **Conclusion:** through the results obtained from the study, it could be concluded that the *Pramehakulantaka Rasa* has no toxic effect in Wister albino rats and hence can be used for further evaluation of its effects.

KEYWORDS: *Prameha, Madhumeha,* Herbometallic formulation, Ayurvedic drug, Preclinical Study, Toxicity study, OECD Guideline 423.

INTRODUCTION

Ayurvedic formulations are being used for the treatment of diseases for centuries. *Pramehakulantaka Rasa* is a herbo-metallic preparation described for the treatment of all twenty types of *Prameha* described in Ayurvedic texts. It contains metals like Tin as *Vanga Bhasma,* Mica as *Abhraka Bhasma,* purified *Parada* (Mercury), purified *Gandhaka* (Sulphur), *Shilajit (Asphaltum punjabinum)* and many other herbs which are used for the treatment of diseases like diabetes and other metabolic disorders. *Parada* (Mercury) which is a content of this drug is listed under schedule E (1) of Drug and Cosmetic Rule 1945 under Ayurvedic (including Siddha) and Unani System of Medicine. So, before subjecting this formulation for evaluation of its effects it was necessary to evaluate its safety. So, this study was conducted to determine its safety according to OECD Guideline 423.

AIM AND OBJECTIVE

To evaluate the safety of *Pramehakulantaka Rasa* in Wistar albino rats through Acute Oral Toxicity according to OECD Guideline 423.

MATERIAL

Test drug

The test drug "Pramehakulantak Rasa" is a herbo-mineral formulation described in *Prameha Rogadhikara* of *Bhaishajya Ratnavali*[1] and was prepared in a Rasayanshala of National Instituted of Ayurveda, Jaipur and issued with batch number C-253 dated Dec, 2019.

Test animals

Healthy Wistar albino rats of both sexes weighing between 100-150 gm were used in the study.

Ethical clearance

Ethical clearance was obtained from the Animal Ethics Committee of Research Centre of Bilwal Medchem and Research Laboratory, Reengus, Rajasthan vide Ref No: BMRL/AD/CPCSEA/IAEC/2020/8/1 dated 10/8/2020.

Place of work

The Animal house of Bilwal Medchem and Research Laboratory Pvt. Ltd. H-9 SKS Reengus Industrial Area, Reengus, Rajasthan.

METHODS

Housing and Feeding of rats

The temperature in the experimental animal room was maintained in the range of 22ºC (±3ºC). The maximum and minimum relative humidity was maintained between 30-70% except during room cleaning but the aim was between 30-50%. Artificial lighting with 12 hours light and 12 hours dark was maintained. For feeding, conventional laboratory diet was used with an unlimited supply of drinking water.

Preparation of animals

The rats were randomly selected and marked with Picric acid H (on Head), B (on Back), and T (on Tail) for individual identification. Marked rats were kept in cages for at least 5 days before dosing to allow them for acclimatization to the laboratory conditions.

Grouping administration of the dose

Two dose levels 300 mg/kg body weight and 2000 mg/kg body weight were selected to access oral acute toxicity according to OECD 423 guideline annex 2.[2] Six Wistar Albino Rats were divided into 2 groups of three rats each. In Group-1 three rats were administered with a single dose of 300 mg/kg aqueous solution of *Pramehakulantaka Rasa* orally and in Group-2 three rats were administered with a single dose of 2000 mg/kg aqueous solution of *Pramehakulantaka Rasa* orally using an oral feeding needle. The rats were fasted before dosing. After fasting, the rats were weighed and then test drug was administered. After test drug administration, food was withheld for 3-4 hours in rats.

OBSERVATION

For assessment of Toxicity, behavioral observation, hematological observation, and histopathological examination are conducted.

1. Behavioral observation

The behavior of each rat of both groups was observed individually for 14 days after dosing and special attention was given during the first 4 hours. Behavioral observation includes changes in skin & fur, mucous membranes, and symptoms like salivation, lethargy, sleep,

coma, concentration, tremor, diarrhea, motility, and mobility which were observed at 30min, 4hr, 24 hr, 48 hr, 1st week, and 2nd week. (Table No. 1, Table No. 2)

Table No. 1: Behavioral observation for test sample at dose 300 mg/kg.

S. No.	Observation	30min.	4hr.	24hr.	48hr.	1w	2w
1	Skin and Fur	N	N	N	N	N	N
2	Eyes	N	N	N	N	N	N
3	Mucous Membrane	N	N	N	N	N	N
4	Salivation	Ab	Ab	Ab	Ab	Ab	Ab
5	Lethargy	Ab	Ab	Ab	Ab	Ab	Ab
6	Sleep	N	N	N	N	N	N
7	Coma	Ab	Ab	Ab	Ab	Ab	Ab
8	Convulsions	Ab	Ab	Ab	Ab	Ab	Ab
9	Tremors	Ab	Ab	Ab	Ab	Ab	Ab
10	Diarrhea	Ab	Ab	Ab	Ab	Ab	Ab
11	Morbidity	Ab	Ab	Ab	Ab	Ab	Ab
12	Mortality	Ab	Ab	Ab	Ab	Ab	Ab

Table No. 2: Behavioral observation for test sample at dose 2000 mg/kg.

S. No.	Observation	30min.	4hr.	24hr.	48hr.	1w	2w
1	Skin and Fur	N	N	N	N	N	N
2	Eyes	N	N	N	N	N	N
3	Mucous Membrane	N	N	N	N	N	N
4	Salivation	Ab	Ab	Ab	Ab	Ab	Ab
5	Lethargy	Ab	Ab	Ab	Ab	Ab	Ab
6	Sleep	N	N	N	N	N	N
7	Coma	Ab	Ab	Ab	Ab	Ab	Ab
8	Convulsions	Ab	Ab	Ab	Ab	Ab	Ab
9	Tremors	Ab	Ab	Ab	Ab	Ab	Ab
10	Diarrhea	Ab	Ab	Ab	Ab	Ab	Ab
11	Morbidity	Ab	Ab	Ab	Ab	Ab	Ab
12	Mortality	Ab	Ab	Ab	Ab	Ab	Ab

2. Hematological observation

Hematological observation including hemoglobin, WBC, RBC, neutrophils, lymphocytes, eosinophils, monocytes, basophils was done on the 14th day. (Table No. 3)

Table No. 3: Hematological analysis of group 1, 2.

S. No.	Parameters	Value (Mean±S.E.M)		Normal Range
		Group 1	Group 2	
1.	Haemoglobin	13.52	14.41	11.5-16.1 gm/dl
2.	WBC	9.48	9.12	6.6-12.6 x 10^3/mm^3
3.	RBC	8.74	9.14	6.76-9.75 x 10^6/ mm^3
4.	Neutrophils	2.58	2.85	1.77-3.38 x10^3/ mm^3

5.	Lymphocytes	8.47	7.56	4.78-9.12 x 10^3/ mm^3
6.	Eosinophils	0.05	0.04	0.03-0.08 x 10^3/ mm^3
7.	Monocytes	0.03	0.02	0.01-0.04 x 10^3/ mm^3
8.	Basophils	0.00	0.00	0.00-0.03 x 10^3/ mm^3

3. Histopathological observation

No animals were found in moribund condition or showed severe pain or enduring signs of severe distress and hence no rat was subjected to necropsy and histopathological examination. The principles and criteria summarized in the Humane Endpoints Guidance Document was taken into consideration.[3]

DISCUSSION

After the test substance administered, behavioral changes were observed at 30min, 4hr, 24 hr, 48hr, 1st week, and 2nd week but none of the rats from any of the two-dose levels showed any changes in their skin and fur, eyes, mucous membrane or showed any abnormal behavior related to salivation, lethargy, sleep, coma, convulsion, tremors, diarrhea, morbidity or mortality.

Hematological tests including hemoglobin, WBC, RBC, neutrophils, lymphocytes, eosinophils, monocytes, basophils were done on the 14th day to access the safety of the trial drug. All hematological test values were found under normal limits.

CONCLUSION

It can be concluded that in the acute oral acute toxicity study, the *Pramehakulantaka Rasa* is found safe at both dose levels of 300 mg/kg and 2000 mg/kg in Wistar albino rats and hence can be used for further evaluation of its therapeutic effects.

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