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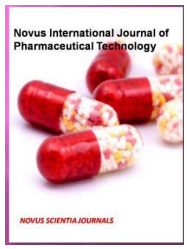

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Acute oral toxicity study of Hypercum on wistar albino rats

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ABSTRACT

High blood pressure has emerged as a leading cause for death and disability worldwide. Management of hypertension effectively has been a distant reality. Hypercum, a rationale combination of herbs has shown to reduce BP effectively along with other benefits as studied in the ancient literatures and research publication.

Conclusion:

From the present study, it can be concluded that the Hypercum (Batch No. : 649AYU) is non toxic up to 2000 mg/kg body weight when administered as a single dose by oral gavage to Wistar Rats and was classified GHS category 5/Unclassified according to the Globally Harmonized System (GHS) for classification of chemicals.

KEY WORDS: Hypercum, Hypertension, Blood pressure, co-morbid conditions.

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INTRODUCTION

The Test item Hypercum (Batch No.: 649AYU) obtained from Natural Solutions, Maharashtra, India, was evaluated for acute oral toxicity in Wistar albino rats as per the OECD guideline for the testing of chemicals, “Acute Oral Toxicity - Fixed dose procedure”, Test No. 420, adopted by the council on 17 December 2001.

A starting dose of 300 mg/kg was selected from the fixed dose levels of 5, 50, 300 and 2000 mg/kg body weight as per the sequential dose selection flow chart provided in the Annexure-2 of the OECD Test No. 420 guideline.

The Sighting study was conducted in one female rat by administering 300 mg/kg body weight

of Shunshine Yellow through oral gavage. There were no clinical signs noticed in Sighting study Step-I at 300 mg/kg body weight. As per the guideline, Sighting study Step-II was conducted in another female rat by administering 2000 mg/kg body weight. The animal did not reveal any clinical signs of toxicity and mortality for 24 hours. Hence as per the Annexure-3 of the guideline, Main study was conducted in another set of 4 female rats by administering a dose of 2000 mg/kg body weight of test item through oral gavage as a single dose. All the animals in the study were observed for 15 days. Body weights of all the animals were recorded on Day 1(pre-dose), 8 and 15 of the Sighting and Main study. On Day 15, the animals were subjected to gross necropsy.

The animals at 2000 mg/kg body weight did not reveal any clinical signs of toxicity and mortality. There were no treatment related changes observed in body weights and body weight gain up to 2000 mg/kg body weight. There were no external and internal gross pathological changes noticed during the necropsy of animals.

Based on the results of the study, it can be concluded that the test item Hypercum (Batch No. : 649AYU) is non toxic up to 2000 mg/kg body weight when administered as a single dose through oral gavage to Wistar rats and can be classified GHS category 5/Unclassified according to the Globally Harmonized System (GHS) for classification of chemicals.

STUDY COMPLIANCE

The study was performed in accordance with the following:

- The study was conducted following the OECD Guidelines for Testing of Chemicals (No. 420, Section 4: Health Effects) on conduct of “Acute Oral Toxicity – Fixed Dose Method" (Adopted: 17th December 2001).
- The Standard Operating Procedures at Liveon Biolabs Pvt. Ltd. and as per the mutually agreed study plan with the sponsor.
- The recommendation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animal facility and approved by Institutional Animal Ethics Committee (IAEC) protocol.

AMENDMENTS AND DEVIATION

There were no amendments and deviations during the conduct of study.

SAFETY PRECAUTIONS

General safety precautions like wearing of gloves, head cap, face mask and slippers were used in addition to other protective body garments to ensure adequate personnel health and safety.

OBJECTIVE

The objective of the present study was to assess the toxicity Hypercum (Batch No. : 649AYU) administered through oral route by gavage as a single dose to female rats at defined single doses and to classify the test item according to Globally Hormonised System (GHS) for the classification of chemicals which cause acute toxicity.

MATERIALS AND METHODS

Test item Information

The following is the test item information provided by the sponsor:

Common Name	Hypercum
Name to be used in Report	Hypercum
Test Item Code by test facility	LBPL/S17/TI086
Physical Appearance	Light Brown Color Powder
Batch No.	649AYU
Storage conditions	+18 to +36 °C (Ambient)
Batch produced on (Date)	IHPL/08
Retest/Expiry date	Not Provided

Test System

Animal species	Rats
Strain	Wistar
Justification for selection of species	Rat is one of the recommended species by regulatory agencies for conducting pre clinical Toxicological studies among rodents.
Source	In-house bred animals
Body weight range	135.4 – 143.4 g
Age at treatment	8-12 weeks
Number of animals / group	Sighting study - 1 animal per step Main study – Four animals A Total of 6 females were utilized (Females were nulliparous and non-pregnant)
Identification	Cage cards

Test Performance

Husbandry Conditions: Animals were housed under standard laboratory conditions, air-conditioned with adequate fresh air supply (Air changes 12-15 per hour), room temperature 22 ± 3^{O}C, relative humidity 30-70 %, with 12 hours light and 12 hours dark cycle. The temperature and relative humidity was recorded once daily.

Housing: Maximum of two animals were housed in a standard polypropylene cage (Size: L 430 x B 270 x H 150 mm) with stainless steel mesh top grill having

facilities for holding pelleted food and drinking water in water bottle fitted with stainless steel sipper tube. Clean sterilized paddy husk was provided as bedding material.

Acclimatization: The animals were acclimatized for a minimum of five days to laboratory conditions and were observed for clinical signs daily.

Diet : The animals were fed *ad libitum* with AMRUT Laboratory Animal Feed manufactured by Pranav Agro Industries Limited, Sangli, and Maharastra throughout the acclimatization and study period.

Water : Water was provided *ad libitum* throughout the acclimatization and study period. Deep bore-well water passed through Reverse osmosis unit was provided in plastic water bottles with stainless steel sipper tubes.

Study Design

The following is the study design for the present study:

Sighting Study

The sighting study was conducted to select appropriate dose for the Main study. A starting dose of 300 mg/kg body weight (sighting study Step-I) was selected from the fixed dose levels of 5, 50, 300 and 2000 mg/kg body weight as per the sequential dose selection flow chart provided in the guideline because of unavailability of sufficient toxicological data on the test item. Flow chart for the sighting study is presented as an Annexure No. 01.

The test item was administered through oral gavage in a single dose of 300 mg/kg body weight to single female rat for sighting study step-I. There were no clinical signs of toxicity or mortality noticed and another single female rat was selected randomly for sighting study step-II at 2000 mg/kg body weight, no clinical signs of toxicity or mortality noticed at 2000 mg/kg body weight. For each sighting study steps a period of 24 hours observation was allowed for any clinical signs and mortality to conduct the Main study.

Main Study

In the absence of clinical signs of toxicity or mortality in the Sighting study step-II, the Main study was conducted by using four female rats which was administered through oral gavage in a single dose of 2000 mg/kg body weight.

Justification for Selection of Vehicle

Distilled water was used as a vehicle as per the sponsors specification

Route of test item administration and justification

The test was administered orally to each animal in a single dose by using gavage needle. As per the OECD 420 guideline the oral route of administration was selected for the present study.

Formulation Details

Required quantity of test item was weighed as per the dose levels. The weighed test item was made soluble in distilled water to get the desired concentration as per the dose levels. Formulation of the test item was prepared shortly before dosing. Formulation details are presented in Annexure No. 04

Stability of the test item

The Stability of the test item rests with the sponsor.

Treatment

The animals were fasted overnight prior to dosing (about 16-18 hours). Water was provided during fasting period. The test item was administered through oral gavage route to each rat as a single dose. The dosage volume administered to individual rat was adjusted according to its body weight recorded on the day of dosing. The dose volume was 10 ml/kg body weight for all animals. Food was offered 3-4 hours followed by dosing.

Duration of the study

The total duration of the study was 23 days.

OBSERVATIONS

The following observations were made during the study period.

Body Weight

Individual animal body weights were recorded on the day of receipt, on Day 1 (before test item administration) and on Day 8 and 15 during the study period.

Clinical observations

All the animals were observed for clinical signs and mortality at 30-40 min, 1 hr (±10 min), 2 hr (±10 min), 3 hr (±10 min) and 4 hr (±10 min) on Day 1 following dosing and thereafter once daily for clinical signs and twice daily for mortality during 15 days observation period.

Pathology

After the completion of the study period, the animals were subjected to following pathological observations

Gross Necropsy

At the end of study period, all the animals were sacrificed using carbon dioxide exposure method and subjected to necropsy and detailed gross pathological examination. The gross pathological findings were recorded.

ANIMAL DISPOSAL

After the completion of necropsy, the entire carcass was stored in deep freezer until disposal and sent to incineration through SembRamky Biowaste Management Pvt Ltd.

REPORT PREPARATION

The computer printouts of the data (in the form of appendix) were verified with the raw data. All individual animal data were summarized and presented as tables. All findings were presented in the report as per the standard reporting procedures.

RESULTS

The following are the results observed during the present study:

Table 1: Summary of body weight (g) and body weight gain (%)

Study Type	Dose (mg/kg)	No. of Animals	Sex	Body weight on days			% Body weight gain	
				1	8	15	1-8	1-15
Sighting Study - Step-I	300	1	Female	143.5	169.8	170.1	18.33	18.54
Sighting Study - Step-II	2000	1	Female	146.8	159.6	171.2	8.72	16.62
Main Study	2000	4	Female	143.78	160.23	170.25	11.42	18.42
				±3.23	±6.20	±3.47	±2.29	±0.71

Values are in Mean ± SD

Table 2: Summary of clinical signs and mortality

Study Type	Dose (mg/kg)	No. of Animals	Sex	Clinical signs	Mortality
Sighting Study - Step-I	300	1	Female	N	0/1
Sighting Study - Step-II	2000	1	Female	N	0/1
Main Study	2000	4	Female	N	0/4

N-Normal

Table 3: Summary of gross pathological findings

Study Type	Dose (mg/kg)	No. of Animals	Sex	Necropsy findings	
				External	Internal
Sighting Study - Step-I	300	1	Female	NAD	NAD
Sighting Study - Step-II	2000	1	Female	NAD	NAD
Main Study	2000	4	Female	NAD	NAD

NAD: No Abnormalities Detected

Body weight

There were no treatment related changes in body weight and percent body weight gain noticed over the study period at all the doses tested.

Clinical Signs and Mortality

There were no clinical signs of toxicity and mortalities noticed in the doses tested.

Pathology

There were no gross pathological changes noticed in any of the animals sacrificed at the end of the study.

Appendix 1: Individual animal body weight (g) and body weight gain (%)

Study Type	Dose (mg/kg)	Animal No.	Sex	Body weight on days			% Body weight gain	
				1	8	15	1-8	1-15
Sighting Study - Step-I	300	1	F	143.5	169.8	170.1	18.33	18.54
Sighting Study - Step-II	2000	2	F	146.8	159.6	171.2	8.72	16.62
	2000	3	F	142.9	159.2	168.6	11.41	17.98
Main Study		4	F	146.7	162.3	174.3	10.63	18.81
		5	F	145.9	167.1	171.7	14.53	17.68
		6	F	139.6	152.3	166.4	9.10	19.20

F :Female

CONCLUSION

From the present study, it can be concluded that the Hypercum (Batch No. : 649AYU) is non toxic up to 2000 mg/kg body weight when administered as a single dose by oral gavage to Wistar Rats and was classified GHS category 5/Unclassified according to the Globally Harmonized System (GHS) .

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