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Acute oral toxicity study of turmeric based herbal product in Sprague Dawley rats

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***Abstract*---**The purpose of this study is to evaluate the acute oral toxicity study of the Turmeric based herbal product in the sprague dawley rats. 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. The LD50 value, defined as the statistically derived dose that, when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given period, is currently the basis for toxicologic classification of chemicals. The test item, Turmeric based herbal product was evaluated for Acute Oral Toxicity in Sprague Dawley Rats as per the OECD Guideline No. 425 -Acute Oral Toxicity - Up-and-Down Procedure. Based on the results, it may be concluded that the LD50 of test item is >5000 mg/kg body weight as per OECD Guideline No. 425-Acute Oral Toxicity-Up-and-Down Procedure.

***Keywords*---**acute toxicity study, histopathology, OECD guidelines.

Introduction

Acute toxicity is usually defined as the adverse change(s) occurring immediately or a short time following a single or short period of exposure to a substance or substances or as adverse effects occurring within a short time of administration of a single dose of a substance or multiple doses given within 24 hr. An adverse effect is "any effect that results in functional impairment and/or biochemical lesions that may affect the performance of the whole organism or that reduce the organ's ability to respond to an additional challenge" [1]. Consequently, a

chemical that enters the organism via the oral route during a restricted time and produces any adverse effect with little delay is orally and acutely toxic. However, the term acute oral toxicity is most often used in connection to lethality and LD50 determinations. Studies of acute systemic toxicity attempt to determine the dose-dependent adverse effect that may occur and various appropriate data may be collected when determining the comprehensive acute toxicity profile of a substance. This may include the incidence of lethality. It has been claimed that when properly performed and closely observed, an acute toxicity test can give more information about the biologic properties of a chemical compound than any other single test, and even if the incidence of lethality were never computed as a consequence of such a test, one would only have lost a small proportion of the available information [2]. If the dose dependent lethality incidence is determined in a precise manner, it is usually expressed as an LD50. This is defined as the statistically derived dose that, when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given period [3]. For a classical LD50 study, laboratory mice and rats are species typically selected. Often the use of both sexes and a route of exposure anticipated to be the most probable route of exposure for humans are necessary for regulatory purposes. When oral administration is combined with parenteral, information on the bioavailability of the tested compound is obtained. Please write literature on toxicity studies done on other turmeric based product Add materials procurement: Sir this portion not required as per manuscript content.

Method

As per available reference (406900G – Turmeric Extract H.GL.-MS - Centerchem), the LD50 of test item was 12200 mg/kg body weight for rats. Hence, the limit dose of 5000 mg/kg body weight was selected as a starting dose. All three animals were dosed in a sequential manner with limit dose of 5000 mg/kg body weight. The next dose level was selected by using self-contained software for OECD Guideline 425 AOT425Statpgm [4-8]. The time interval between the doses was at least 48 hours. The first animal was dosed with 5000 mg/kg body weight, no mortality was observed. Hence, the second animal was dosed with the same dose of 5000 mg/kg body weight, no mortality was observed. Hence, the third animal was dosed with same dose level i.e. 5000 mg/kg body weight, no mortality was observed.

Dose Formulation

The required quantity of test item was received as per dose. A small quantity of vehicle was added to test item and mixed well by using mortar and pestle and thereafter transferred the formulation into measuring cylinder. Again, a small quantity of vehicle was added to the mortar and rinsed and transferred into the measuring cylinder. Finally the volume was made up to required quantity with vehicle to get a desired volume. Freshly prepared test item formulation was used for administration. The homogeneity of the test item formulation was maintained by continuous stirring on a magnetic stirrer during dosing. The formulation preparation details are as indicated in the below table.

Treatment	Dose (mg/kg body weight)	Concentration (mg/mL)	Dose volume (mL/kg body weight)	Quantity of test item (mg)	Volume made up with vehicle (mL)
1st Animal	5000	500	10	5000.3	10
2nd Animal	5000	500	10	5000.2	10
3rd Animal	5000	500	10	5000.1	10

Administration of test item

Animals were fasted overnight (16 to 18 hours) prior to dosing. Water was provided *ad libitum* during fasting period. The freshly prepared test item formulation was administered by oral gavage to each rat as a single dose using intubation cannula. The dose volume administered to individual rat was calculated based on the body weight recorded on the day of dosing. The dose volume was 10 mL/kg body weight. Feed was offered between 3 to 4 hours followed by dosing. The homogeneity of the test item formulation was maintained by continuous stirring on a magnetic stirrer during dosing.

Result

All the animals were observed for clinical signs of toxicity and mortality at 30 to 40 min, 1 hr (±10 min), 2 hrs (±10 min), 3 hrs (±10 min) and 4 hrs (±10 min) on day 1 (after dosing) and once daily for clinical signs of toxicity and twice daily for mortality during the 14 days observation period. Duration of observations were determined by the toxic reactions, rate of onset and length of recovery period. Observations included changes in skin, fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour pattern. A special attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The clinical sign of toxicity and mortality (Table 02), Body weight (g) and percent change in body weight with respect to day 1 (Table 03) and gross pathological findings (Table 04) have been reported and tabulated.

Table 1
Clinical signs of toxicity and mortality record

Dose (mg/kg body weight)	Animal No.	Sex	Day 1	Days																
			30 to 40 Min	1 hr (±10 min)	2hrs (±10 min)	3hrs (±10 min)	4hrs (±10 min)	2	3	4	5	6	7	8	9	10	11	12	13	14	15
5000	Rc1423	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5000	Rc1424	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5000	Rc1425	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N: Normal; F: Female; min: minutes; hr(s): hour(s)

Table 2
Body weight (g) and percent change in body weight with respect to day 1

Dose (mg/kg body weight)	Animal No.	Sex	Body Weight (g) on Days			Percent Change in Body Weight with Respect to Day 1	
			1	8	15	1 to 8	1 to 15
5000	Rc1423	F	171.28	186.38	200.49	8.82	17.05
5000	Rc1424	F	180.47	196.75	210.14	9.02	16.44
5000	Rc1425	F	183.41	198.24	214.14	8.09	16.75

F: Female

Table 3
Gross Pathological Findings

Dose (mg/kg body weight)	Animal No.	Sex	Fate	Gross Pathological Findings	
				External	Internal
5000	Rc1423	F	TS	NAD	NAD
5000	Rc1424	F	TS	NAD	NAD
5000	Rc1425	F	TS	NAD	NAD

NAD: No Abnormality Detected; F: Female; TS: Terminal Sacrifice

Discussion

Total three female rats were used for the experiment. The animals were fasted overnight approximately 16 to 18 hours prior to dosing and feed was offered in between 3 to 4 hours followed by dosing. The required quantity of test item was weighed as per the dose and formulated in corn oil to get the desired concentration. The freshly prepared dose formulation was administered based on individual animal body weight on treatment day (day 1) by oral gavage to each rat as a single dose, using rat intubation cannula at a dose volume of 10 mL/kg body weight. The test item was administered by oral gavage to each rat as a single dose by sequential manner. All the surviving animals were observed for clinical signs of toxicity and mortality during the 15 days observation period. Body weight was recorded at receipt and on day 1 before test item administration and on day 8 and 15 during the experimental period. At the end of observation period on day 15, all animals were euthanized under carbon dioxide anaesthesia and subjected to necropsy.

As per available reference (406900G – Turmeric Extract H.GL.-MS - Centerchem), the LD50 of test item was 12200 mg/kg body weight for rats. Hence, the limit test of 5000 mg/kg body weight was performed with three animals. The first animal was dosed with limit dose of 5000 mg/kg body weight, no mortality was observed. Hence, the second animal was dosed with same dose of 5000 mg/kg body weight, no mortality was observed. Hence, the third animal was dosed with same dose of 5000 mg/kg body weight and no mortality was observed. Based on the outcome of the results, no further testing was carried out. The testing was stopped, since the stopping criteria were met and the study was completed by using three animals at limit test at the dose of 5000 mg/kg body weight. All the animals were observed for clinical signs of toxicity at 30 to 40 min, 1 hr (±10 min), 2 hrs (±10 min), 3 hrs (±10 min) and 4 hrs (±10 min) on treatment day 1 and thereafter once daily for clinical signs of toxicity and twice daily for mortality during the 14 days observation period. The body weight was recorded on day 1 before test item application and on days 8 and 15. At the end of the observation period, all the animals were sacrificed under carbon dioxide anaesthesia and subjected to necropsy and detailed gross pathological examination.

No treatment related clinical signs were noted. No treatment related changes in body weight and percent change in body weight with respect to day 1 were noted. Normal increase in body weights was noted during the observation period. No gross pathological changes were noted in any of the animals at the limit dose of 5000 mg/kg body weight. The Up-and-Down Procedure method described by OECD test guideline is intended to allow the calculation of a precise LD50 value. The LD50 was estimated using self-contained software for OECD Guideline No. 425 “AOT425StatPgm”.

Conclusion

The LD_{50} of test item is>5000 mg/kg body weight as per OECD Guideline No. 425-Acute Oral Toxicity-Up-and-Down Procedure. The test item is “Unclassified” as per the Globally Harmonized System of Classification and Labelling of

Chemicals (GHS). There were no treatment related clinical signs and mortalities noted at limit dose of 5000 mg/kg body weight.

Conflict of interest

The authors declare that we have no conflict of interest.

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