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Toxicity Evaluation of Aqueous Extracts of *Allium cepa* and Coriandrum sativum in Rats

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Abstract

Objectives: *Allium cepa* and *Coriandrum sativum* are reported in the Ayurvedic literature for hypolipidemic activities. Aqueous extracts of *A. cepa* and *C. sativum* prior to the assessment of the efficacy of the extracts for antiobesity potential were investigated for the safety of acute oral exposure in rats. **Materials and Methods:** Aqueous extracts of *A. cepa* or *C. sativum* was administered orally in a sequential manner to one animal each at 2000 and 5000 mg/kg limit test of sighting study followed by four animals at 5000 mg/kg in the main study as per Organisation for Economic Cooperation and Development guideline 420. The experimental animals were observed for mortality, adverse clinical signs, alterations in the body weights, and gross pathological lesions at necropsy. Similar procedure was adopted for the other test substance. **Results:** Aqueous extracts of *A. cepa* or *C. sativum* treated rats survived throughout the study period and did not exhibit any treatment-related adverse clinical signs at the tested dose levels. The overall percent body weight gain in rats treated with aqueous extracts of *A. cepa* or *C. sativum* was found to be normal during the 14 days observation period. At necropsy, the rats did not reveal any major pathological lesions. **Conclusion:** In summary, acute oral toxicity testing of aqueous extracts of *A. cepa* and *C. sativum* did not demonstrate any treatment-related adverse effects up to the dose level of 5000 mg/kg body weight, and hence the tested products were labeled unclassified in the hazard category according to Globally Harmonized System.

Keywords: Acute Oral Toxicity, *Allium cepa, Coriandrum sativum*, Organisation for Economic Cooperation and Development, Rats

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1. Introduction

Traditional herbal medicines are getting significant attention globally. During the past few decades, clear shift in the public interests toward herbal medicine has been observed, and the acceptance of these herbal remedies is increasing considerably in both developing and the developed countries. It is reported that 80% of the worldwide population utilize herbs and as per the World Health Organization estimates the global market is approximately $83 billion annually (Ekor, 2014; Pelkonen, 2014). The prevalence and the market size for herbal medicines provide an evidence of the growing interests in herbal remedies (Rivera *et al.*, 2013). Several important factors viz., undesirable side effects of the synthetic medicines, cost of the drugs, perception that herbal medicines are generally safe, etc., promulgated the public globally to rely on the herbal medicines for primary health care (Hussain *et al.*, 2009). Although it is believed that herbal medicines are natural, inherently safe, and nontoxic, it is not always true, and toxicological screening remains important to ascertain the safety of the herbal remedies (Wachtel-Galor and Benzie, 2011). Thus, the potential herbs that provide a multitude of health

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benefits should be screened before use to treat or prevent various health ailments in humans and animals.

Ayurvedic system of medicine reports medicinal plants as a source of reducing body fat and lipid levels. *Allium cepa* and *Coriandrum sativum* are two such plants reported for hypolipidemic activity (Joshi and Jain, 2014). Before assessing these plants for antiobesity and associated effects, this study was aimed at screening these two plant extracts for toxic potential. Onion (*A. cepa*. Linn), belonging to family liliaceae commonly used in the daily diet, has been extensively studied for its therapeutic uses (Bora and Sharma, 2009). Onion has been reported to effectively decrease the lipid levels in experimental animals (Bang *et al.,* 2009). In addition, the phytoactives of onion has been reported to possess a multitude of health benefits such as antibiotic, fibrinolytic, anticarcinogenic, antithrombotic, and antiasthmatic activities (Abou El-Soud and Khalil, 2010).

C. sativum L. (Coriander), an herbal plant, belonging to the family Apiaceae, is valued for its culinary and medicinal uses. Aerial parts and seeds of coriander are a rich source of lipids and essential oil. These bioactives are reported for a wide array of pharmacological activities which include antidyslipidemic, antimicrobial, antioxidant, antidiabetic, antidepressant, anxiolytic, antiepileptic, antimutagenic, anti-inflammatory, antihypertensive, neuroprotective, and diuretic (Sahib *et al.,* 2013). Hence, the two herbs possess pharmacological activities and could be potential antiobesity therapeutics. However, prior to efficacy assessment, safety evaluation is essential and as a primary step in the characterization of the toxic potential of these two herbs, assessment of acute oral toxicity was undertaken. This study was aimed at toxicological evaluation of aqueous extracts of *A. cepa* bulb and *C. sativum* aerial parts on acute oral exposure in rats according to Organisation for Economic Cooperation and Development (OECD) guidelines for the testing of substances.

2. Materials and Methods

2.1 Plant Materials

Plant materials *A. cepa* bulb and *C. sativum* aerial part were procured from local commercial suppliers and were identified and authenticated at the National Institute of Science Communication and Information Resources, New Delhi.

For the preparation of aqueous onion extract, the bulbs were washed thoroughly, cut into small pieces, and subsequently subjected to extraction with water (1:0.5). The extract obtained was filtered and the filtrate obtained was spray dried.

For the preparation of coriander extract, dried aerial parts were subjected to extraction by refluxing with boiling water (1:3) for 45 min thrice. The filtrate obtained was concentrated under vacuum at Not More Than (NMT) 70°C temperature to thick paste and subsequently added to ethanol (1:5) for precipitation and then centrifuged. The supernatant obtained was concentrated and then dried under vacuum at NMT 70°C. Parallely, dried coriander seeds were subjected to extraction by refluxing with boiling water (1:3) for 60 min thrice, the filtrate obtained was concentrated under vacuum to 20% total solids and spray dried. The aerial part extract and seed extract were mixed in a ratio of 1:1 to get the final extract.

2.2 Animals

Female nulliparous and nonpregnant albino Wistar rats (8–12 weeks) were housed in polypropylene cages with stainless steel grill tops and provided with bedding of clean paddy husk. Animals were housed under standard laboratory conditions of 12 h/12 h light/dark cycle at 25°C ± 2°C with 30–70 % relative humidity and were provided free access to standard laboratory rodent pelleted feed (M/s Amrut Laboratory Animal Feeds, India) and ultraviolet purified and filtered water *ad libitum*. The animal experiment was conducted taking into consideration the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines and after approval by the Institutional Animal Ethics Committee.

2.3 Experimental Procedure

The study was performed as per the OECD guideline for the testing of chemicals (guideline No. 420, Section 4: Health effects), acute oral toxicity-fixed dose procedure, adopted on 17 December 2001 (OECD, 2001a). The test substance was solubilized in demineralized water and was orally administered in a sequential manner to one animal each at 2000 and 5000 mg/kg limit test of sighting study and four animals at 5000 mg/kg in the main study. The experimental animals were fasted overnight before dosing. On the day of dosing, all the animals were observed for

mortality and clinical signs for first 10 min, 30 min, 1 h, 2 h, 4 h, and 6 h after dosing and thereafter, twice daily for mortality and once a day for clinical signs, for 14 days. The body weights of animals were recorded individually before dosing and at weekly intervals thereafter. Changes in skin, fur, eyes, and mucous membrane; circulatory, respiratory, central, and autonomic nervous systems; somatomotor activity; and behavior pattern, if any, were recorded. Particular attention was directed to observations of tremors, convulsions, lethargy, sleep, salivation, coma, and diarrhea. At the termination of the study, rats were sacrificed for complete gross pathology examination. A similar procedure was adopted for the other test substance.

3. Results

The aqueous extract of *A. cepa* treated rats in the sighting, and main studies did not show any mortality or adverse signs of intoxication immediately following dosing and during the observation period of 14 days. The overall weight gain in all treated rats was found to be normal at the end of 14 days. On necropsy, no major gross pathological changes were observed in any of the treated rats (Tables 1 and 2). Therefore, the aqueous extract of *A. cepa* was found to be safe up to the dose level of 5000 mg/kg body weight.

The aqueous extract of *C. sativum* treated rats in the sighting and main studies survived throughout the study period. In the sighting study, rats did not show any major adverse clinical signs immediately following dosing and during the observation period of 14 days except for hypoactivity, mild abdominal discomfort for a transient period only on the day of dosing at the dose of 5000 mg/kg. In the main study, the animals exhibited clinical signs viz., hypoactivity, laxation, mild abdominal discomfort, piloerection, and polyuria only on the day of dosing. However, the overall weight gain of the animals during the 14 days observation period in the sighting and main studies were found to be normal. On necropsy, no major gross pathological changes were observed (Tables 3 and 4). The aqueous extract of *C. sativum* was found to be safe up to the dose level of 5000 mg/kg.

4. Discussion

In the past few decades, the safety associated with the synthetic antiobesity medications has been unsuccessful and huge unmet need has arisen for safe antiobesity therapeutics (Cheung *et al.*, 2013). Antihyperlipidemic

Table 1. Mortality, clinical signs, and gross pathology findings observed in female Wistar rats treated with aqueous extract of *Allium cepa*

Study	Dose (mg/kg)	Mortality (died/total)	Cage side observations		Gross pathology findings
			Signs	Period (onset-recovery)	
Sighting (n=1)	2000	0/1	Nil	-	No abnormality detected
Sighting (n=1)	5000	0/1	Nil	-	No abnormality detected
Main (n=4)	5000	0/1	Nil	-	No abnormality detected
	5000	0/1	Nil	-	No abnormality detected
	5000	0/1	Nil	-	No abnormality detected
	5000	0/1	Nil	-	No abnormality detected

n=Number of animals

Table 2. Effect of aqueous extract of *Allium cepa* on body weight and body weight gain in female Wistar rats

Study	Dose (mg/kg)	Body weight (g)			Percent body weight gain		
		Day 0	Day 7	Day 14	Days 0–7	Days 7–14	Days 0–14
Sighting (n=1)	2000	158	185	202	17.09	9.19	27.85
Sighting (n=1)	5000	159	192	208	20.75	8.33	30.82
Main (n=4)	5000	163	196.25	210	20.4	7.01	28.83

n=Number of animals

Table 3. Mortality, clinical signs, and gross pathology findings observed in female Wistar rats treated with aqueous extract of *Coriandrum sativum*

Study	Dose (mg/kg)	Mortality (died/total)	Cage side observations		Gross pathology findings
			Signs	Period (onset-recovery)	
Sighting (n=1)	2000	0/1	Nil	-	No abnormality detected
Sighting (n=1)	5000	0/1	Hypoactive and mild abdominal discomfort	30 min - 1 h postdosing	No abnormality detected
Main (n=4)	5000	0/1	Hypoactive	1 h 30 min - 3 h postdosing	No abnormality detected
	5000	0/1	Hypoactive	1 h 30 min - till the end of observation period 2 h 40 min postdosing	No abnormality detected
			Laxation	3 h 5 min postdosing	
	5000	0/1	Hypoactive and mild abdominal discomfort	40 min - 3 h postdosing	No abnormality detected
			Piloerection	2 h 10 min - 3 h postdosing	
	5000	0/1	Hypoactive	1 h 30 min - 3 h postdosing	No abnormality detected
			Piloerection and polyuria	2 h 10 min - 2 h 40 min postdosing	

n=Number of animals

Table 4. Effect of aqueous extract of *Coriandrum sativum* on body weight and body weight gain in female Wistar rats

Study	Dose (mg/kg)	Body weight (g)			Percent body weight gain		
		Day 0	Day 7	Day 14	Days 0–7	Days 7–14	Days 0–14
Sighting (n=1)	2000	158	192	206	21.52	7.29	30.38
Sighting (n=1)	5000	163	175	205	7.36	17.14	25.77
Main (n=4)	5000	161.25	195	214	20.93	9.74	32.71

n=Number of animals

herbal substances remain a promising alternative approach. The penchant for herbal interventions is rising as they are efficacious and generally regarded as safe (Lee *et al.,* 2015). Nevertheless, there is a lack of scientific evidence on the toxic/adverse effects of herbal remedies. Therefore, acute toxicity is imperative to identify clinical signs/mortality elicited by the substance under investigation and also for subsequently identifying the range of doses that could be used in evaluating the efficacy of substances.

In the current study, the aqueous extracts of *A. cepa* and *C. sativum* were screened to evaluate the safety by acute oral toxicity study. Acute oral toxicity is the preliminary step and provides information on the health hazards that may arise from acute exposure. This study employed OECD guidelines for testing of chemicals, test guideline 420, acute oral toxicity-fixed oral dose procedure. Although several internationally accepted protocols are available for acute oral toxicity testing, keeping in view, that the fixed dose procedure avoids using the death of the animals as an endpoint and is relied on the observations of the clear signs of toxicity, involves use of fewer animals and causes less suffering than the traditional methods of acute toxicity testing, fixed dose procedure is adopted. Further, this procedure is reproducible and can be used to rank and classify the substances according to the globally harmonized system (OECD, 2001a).

It is generally known that *A. cepa* (onion) and *C. sativum* (coriander) are consumed by humans, they are assumed to be nontoxic. Therefore, based on the history of use, the substances were tested at a very high dosage level of 2000 mg/kg rat body weight. As 2000 mg/kg did not reveal any toxicity symptoms, a sequential dose of 5000 mg/kg was tested in the sighting study followed by the main study. In this study, single acute oral administration of test substances (aqueous extracts of *A. cepa* (bulb) and *C. sativum* (aerial parts) to five female albino Wistar rats at the dose level of 5000 mg/kg body weight did not cause any mortality, and the median lethal dose was found to be more than 5000 mg/kg body weight. The extracts tested did not cause mortality, stages leading to death or any conditions such as pain, showing severe and enduring distress that necessitates humane killing (Stokes, 2002). Further, all the rats administered with both the test substances survived for a period of 14 days demonstrating the safe nature of the tested substances.

Changes in the external physical appearance, clinical and behavioral signs following test substance administration immediately, and until the completion of the study period indicates a deviation from the animal's normal state and can be correlated with the perturbations in the physiological process (OECD, 2000). Although few clinical signs such as hypoactive, laxation, and mild abdominal discomfort were observed during the study period, the signs lasted for transient duration and the animals survived with no effects on body weight and gross pathology. Hence, these signs are considered incidental and do not indicate any toxicological relevance. The tested extracts did not reveal any major treatment-related adverse clinical signs either immediately following dosing or until the end of the study period; the results indicate that the extracts are safe.

A sensitive and most critical objective sign of deteriorating condition of the animals due to pain and distress is significant changes in body weight gain of individual rats. Weight loss may point to wasting diseases, pain, distress, inability to eat, and weight gain may indicate conditions such as large tumors (OECD, 2000). No such changes related to body weight were observed during the study period reinstating the safety of the tested extracts".

As specified in various regulatory guidelines for acute toxicity studies (OECD, 2001a; OECD, 2001b; OECD, 2001c), necropsy examination plays an important role in identifying the general and target organ specific toxic effects of the test substance under study (OECD, 2000; Johnson and Gad, 2007; Frame et al., 2007). The internationally accepted protocols recommend testing animals (including those that die during the test or are removed from the study for animal welfare reasons) in gross necropsy. In this study, as no mortality or no conditions necessitating humane killing were encountered, all the surviving animals were sacrificed at the end of the study period and were subjected to gross pathological changes. The absence of any such treatment-related gross pathological changes in the organs of the treated rats suggests wide harmless nature of the test substances. Microscopic examination of organs is recommended by test guideline in case of any evidence of gross pathology in animals; however, microscopic examination was not conducted as the extracts did not show any remarkable gross pathological findings.

Toxicity studies reported elsewhere on *A. cepa* (Kojima *et al.,* 1993; Bhanot and Shri, 2010) and *C. sativum (*Patel *et al.,* 2012; Burdock and Carabin, 2009) also indicate the safe nature of extracts reinstating these study findings. The acute toxicity test of onion-coat extract performed in (C57BL/6 × C3H) F1 mice administered with doses of 2500, 5000, 7500, and 10,000 mg/kg body weight orally maintained for 14 d demonstrated a tolerated dose between 5000 and 7500 mg/kg body weight which is in accordance with the current study (Kojima *et al.,* 1993). Similar to this study findings, Patel *et al.* (2012) also did not report any mortality or abnormal clinical/behavioral signs in the rats treated with *C. sativum* extract at 5000 mg/kg body weight, and the LD_{50} was reported to be higher than 5000 mg/kg body weight.

5. Conclusion

Acute oral toxicity testing of aqueous extracts of *A. cepa* bulb and *C. sativum* aerial parts did not cause any untoward effects and hence can be labeled unclassified in the hazard category according to Globally Harmonized System and thus can be considered safe.

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