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Acute Fixed Dose Oral Toxicity Study of Ethanolic Extracts from Galanga (*Alpinia galanga* L.) in Sprague dawley (SD) rats

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Abstract: *Alpinia galanga* L. is commonly used in traditional medicine in the preparations of food and cosmetics. The aim of this study was to investigate the possible toxic effects of the ethanolic extracts from *Alpinia galanga* in order to find the acceptably safety level of the *Alpinia galanga* extraction in rats. The study was in complance with the Organization of Economic Cooperation and Development (OECD) guidelines 420 (Acute Fixed Dose Procedure). The *Alpinia galanga* extraction at the dose of 2,000 mg/kg body weight was given orally to 8 weeks old female Mlac:SD (Sprague Dawley) rat for sighting study. Our earlier study demonstrated the oral safety in female SD rats to confirm the toxicity information after single administration of the *Alpinia galanga* extraction at a dose of 2,000 mg/kg body weight was selected for the main study. The *Alpinia galanga* extraction was single administered to the treatment rats. The result did not show any toxicity sign during the experimentation period. The *Alpinia galanga* extraction was classified in Globally Harmonized System of Classification and labelling of Chemicals (GHS) category 5, the LD50 cut off at 5,000 mg/kg body weight. The results from the study suggest that the *Alpinia galanga* extraction had no toxicologically effects on acute oral administration.
Key words: Acute Oral Toxicity, Fixed Dose, Galanga , *Alpinia galanga* L., medicinal plants, traditional medicine.

Introduction

The medicinal plants of the native flora are consumed with little or no knowledge of their pharmacological properties. The use of medicinal plants and preparations derived from them as dietary supplements, nutraceuticals, functional foods and herbal medicinal products has become more widely accepted in developing countries. Therefore, it is important to evaluate the adverse effects and preparations of these plants to increase the confidence in its safety to human, particularly for use in the development of pharmaceutical products. The medicinal plant selected and investigated for present study *Alpinia galanga* L. has a very strong botanical history endemic in south east Asia[1,2,3]. Herbal prescriptions and natural products are commonly used for treatment of various diseases in developing countries particularly in Thailand. According to

World Health Organization (WHO), it has recommended the use of herbal drugs as an alternative medicine, because almost 80% of the world's population uses medicine from herbal origin for primary health care [4].

Alpinia galanga L. (Fam: Zingiberaceae) is a herb used in Ayurvedic system of medicine in the name of Rasna, Sugandhamula and Greater galangal. It is also a part of Unani, Chinese and Thai folk medicine in the preparations of food and cosmetics. The pungent, spicy and zinger like odour of the drug is due to rich essential oils like cineole, methyl cinnamate, myrecene and methyl eugenol[5,6]. Recent studies confirmed that the rhizome of *Alpinia galanga* L. possessed significant anti-inflammatory, anti ulcer and anti calculi activities[7].

The aim of this study was to investigate the possible toxic effects of the *Alpinia galanga* extraction related to different doses in order to find the acceptably safety level of the *Alpinia galanga* extraction in rats.

Material and Method

Animals and Husbandary

Healthy young female and male Sprague dawley (SD) rats of body weight range 200 ± 10% mean were obtain from Office of Laboratory Animal Production, NLAC, Mahidol University, Thailand. The animals were kept under standard conditions 12:12 (day:night cycles) at 22±2 ^{0}C and 40–65% relative humidity. The animals were housed individually in stainless cages with food (082, Perfect Companions, Thailand) and 5-6 ppm chlorinated water *ad libitum*. All the animals were acclimatized for at least 5 days prior to the study. Guidelines of "Guide for the care and use of laboratory animals" (Institute of laboratory animal resources, National academic press 1996; NIH publication number #85-23, revised 1996)[8] were strictly followed throughout the study. The study was approved by National Laboratory Animal Center Animal Care and Use Committee (NLAC-ACUC), Mahidol University; Thailand.

Acute Toxicity: Sighting Study

Acute oral toxicity was in compliance with the Organization of Economic Cooperation and Development (OECD) guidelines 420 (Acute Fixed Dose Procedure) [9]. The selection of *Alpinia galanga* ethanolic extract was done based on the quantity of the extracts and the presence of phytoconstituents in it. The sighting study was a stepwise procedure with 1 female rat. The animal were fasted overnight prior to administration of test sample. The *Alpinia galanga* extraction at the dose of 2,000 mg/kg body weight was given orally to the rat by gavage using a ball-tipped stainless steel feeding needle. The rat was observed for toxic effects at the first 30 minutes with special attention given during the first 4 hours, periodically during the first 24 hours. If no signs of toxic effects or mortalities observed on rats within the first 24 hours, then administrated with the dose level 2,000 mg/kg body weight for main study.

Acute Toxicity: Main Study

The main study was a stepwise procedure with 5 female rats and 5 male rats. The animal were fasted overnight prior to administration of test sample. The extraction at the dose of 2,000 mg/kg body weight was given orally to the rat by gavage. Rats were observed closely for body weight, signs of toxicity and mortality were observed after the administration at the first hour and once daily for next 14 days.

Observation

Clinical observations which include changes in skin and fur, eyes and mucus membrane, respiratory, circulatory, somatomotor activity, behavior pattern, tremors, convulsion, salivation, diarrhea, sleep and coma were observed for all the experimental rats after administration of doses and thereafter once a day for 14 days.

Results

Acute Toxicity

The studies were carried out according to the OECD guidelines 420. Clinical observation of treated rats throughout the study indicated that did not showed signs of toxic effect. No mortalities were observed in any rats. Necropsy examinations were confirmed whether not the organs or tissues had been damaged (data not show).

Observation

Clinical observation of treated rats throughout the study indicated that did not showed signs of toxic effect include changes in skin and fur, eyes and mucus membrane, respiratory, circulatory, somatomotor activity, behavior pattern, tremors, convulsion, salivation, diarrhea, sleep and coma. No mortalities were observed in any rats (Table 1.).

Table 1 Clinical observation parameters for the sighting and main study.

Observation parameters	Sighting study				Main study			
	4 hrs	24 hrs	Day 7	Day 14	4 hrs	24 hrs	Day 7	Day 14
skin and fur	0/1	0/1	0/1	0/1	0/10	0/10	0/10	0/10
eyes and mucus membrane	0/1	0/1	0/1	0/1	0/10	0/10	0/10	0/10
respiratory	0/1	0/1	0/1	0/1	0/10	0/10	0/10	0/10
circulatory	0/1	0/1	0/1	0/1	0/10	0/10	0/10	0/10
somatomotor activity	0/1	0/1	0/1	0/1	0/10	0/10	0/10	0/10
behavior pattern	0/1	0/1	0/1	0/1	0/10	0/10	0/10	0/10
tremors	0/1	0/1	0/1	0/1	0/10	0/10	0/10	0/10
convulsion	0/1	0/1	0/1	0/1	0/10	0/10	0/10	0/10
salivation	0/1	0/1	0/1	0/1	0/10	0/10	0/10	0/10
diarrhea	0/1	0/1	0/1	0/1	0/10	0/10	0/10	0/10
sleep and coma	0/1	0/1	0/1	0/1	0/10	0/10	0/10	0/10
mortalities	0/1	0/1	0/1	0/1	0/10	0/10	0/10	0/10

Table 2 Body weights (g) of rats were treated with *Alpinia galanga* extraction (2,000 mg/kg body weight) in main study.

Sex	Body weight (g)			Sex	Body weight (g)		
	Day 1	Day 7	Day 14		Day 1	Day 7	Day 14
Female No.1	191	218	244	**Male No.1**	199	251	320
Female No.2	196	217	237	**Male No.2**	200	252	312
Female No.3	197	219	239	**Male No.3**	202	257	317
Female No.4	200	214	235	**Male No.4**	203	260	333
Female No.5	205	224	240	**Male No.5**	207	256	319

Table 3 Food consumption (g) of rats were treated with *Alpinia galanga* extraction (2,000 mg/kg body weight) in main study.

Sex	Food consumption (g)			Sex	Food consumption (g)		
	Day 1	Day 7	Day 14		Day 1	Day 7	Day 14
Female No.1	11.0	14.5	16.5	**Male No.1**	16.5	23.5	23.5
Female No.2	11.0	14.5	16.5	**Male No.2**	16.5	23.5	23.5
Female No.3	11.5	14.0	13.5	**Male No.3**	16.0	24.0	22.0
Female No.4	11.5	14.0	13.5	**Male No.4**	16.0	24.0	22.0
Female No.5	12.0	16.0	12.5	**Male No.5**	16.0	20.0	21.0

Table 4 Water consumption (ml) of rats were treated with *Alpinia galanga* extraction (2,000 mg/kg body weight) in main study.

Sex	Water consumption (ml)			Sex	Water consumption (ml)		
	Day 1	Day 7	Day 14		Day 1	Day 7	Day 14
Female No.1	15.0	19.5	22.5	**Male No.1**	20.5	24.5	26.5
Female No.2	15.0	19.5	22.5	**Male No.2**	20.5	24.5	26.5
Female No.3	25.5	24.0	24.5	**Male No.3**	24.5	28.0	29.0
Female No.4	25.5	24.0	24.5	**Male No.4**	24.5	28.0	29.0
Female No.5	18.0	25.0	21.0	**Male No.5**	19.0	22.0	26.0

Body weight, Food and Water consumption

Body weight gain of treatment rats were treated with the *Alpinia galanga* extraction had no significant difference was also observed on Day 1, 7 and 14. (Table 2) The percentage increase in body weight of female and male rats were treated with the *Alpinia galanga* extraction found 17.24 % and 36.85%, respectively.

Food and water consumption of the treatment rats that oral administration of the the *Alpinia galanga* extraction were shown in Table 3 and Table 4, respectively. There have no significant difference was also detected.

Discussion

Phytonutrition has maintained greater popularity all over developing world and the use is rapidly on the increase[10]. In Ayurveda the use of the plant in preparations like arishtas, asavas, kasayas, churnas and tailas to cure vata and kapha is highly recommended. Being a member of Zingiberaceae family the plant is rich in volatile oils. The extracts of the rhizomes were evaluated for the presence of phytoconstituents before the anti-inflammatory study and the results were as given positive reactions for alkaloids, glycosides, tannins, flavonoids and saponins, whereas the chloroform extract for terpenoids and petroleum extract for phytosterols, volatile oils and fixed oils. The animals tested in acute toxicity had no sign of toxicity for 14 days. In compliance with OECD 420 the methanolic extracts from *Alpinia galanga* at 5,000 mg/kg body weight did not cause any mortalities and toxicity signs. The result suggested that methanolic extracts from *Alpinia galanga* was classified in GHS (Globally Harmonized System of Classification and labelling of Chemicals) category 5 or Unclassified, the LD50 cut off at 5,000 - ∞ mg/kg body weight[6].

In this study, the *Alpinia galanga* extraction at 2,000 mg/kg body weight did not caused any death or acute toxic in rats and did not show any toxicity signs such as variations in body weight, changes in skin and fur, eyes and mucus membrane, respiratory, circulatory, somatomotor activity, behavior pattern, tremors, convulsion, salivation, diarrhea, sleep and coma, food and water consumption.. The result suggested that the the *Alpinia galanga* extraction was classified in GHS (Globally Harmonized System of Classification and labelling of Chemicals) category 5 or Unclassified, the LD50 cut off at 5,000 mg/kg body weight.

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