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Research Article

Acute Oral Toxicity Study of Ethanolic Extract from Indian Gooseberry (*Phyllanthus emblica* Linn.) in Sprague Dawley rats

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Abstract

Indian Gooseberry (*Phyllanthus emblica* Linn.) is a herbal used in "Ayurvedic" system of medicine. The aim of this study was to investigate the possible toxic effects of the *Phyllanthus emblica* Linn. ethanolic extraction in order to find the acceptably safety level in rats. The study was in compliance with the Organization of Economic Cooperation and Development (OECD) guidelines 420 (Acute Fixed Dose Procedure). For sighting study, *Phyllanthus emblica* Linn. ethanolic extraction at the dose of 2,000 mg/kg body weight was given orally to 8 weeks old female Mlac:SD (Sprague Dawley) rats. The sighting study was presented the oral safety in female Mlac:SD rats. For main study, to confirm the toxicity information after sighting study, the *Phyllanthus emblica* Linn. ethanolic extraction at a dose of 2,000 mg/kg body weight was selected. The *Phyllanthus emblica* Linn. ethanolic extraction was single administered to the treatment rats. The result did not show any toxicity sign during the experimentation period. The *Phyllanthus emblica* Linn. ethanolic 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 *Phyllanthus emblica* Linn. ethanolic extraction had no toxicologically effects on acute oral administration in rats.

INTRODUCTION

Medicinal plants continue to be an important therapeutic aid for ailments of humankind. Over the last 2500 years, there have been very strong traditional systems of
Medicine such as Chinese, Ayurvedic and the Unani (Mamun-or-Rashid *et al.*, 2014). The traditions are still popularity approximately 80% of the people in the developing countries. These systems of medicine for their primary health care needs. A lot of research work has been carried out on some medicinal herbs and they have been found to have definite action on the nervous, circulatory, respiratory, digestive and urinary systems (Nadheesha *et al.*, 2007 and Naik *et al.*, 2006).

Indian Gooseberry (*Phyllanthus emblica*) is an important herbal component of Thai traditional recipes, is believed to slow aging (Nosál'ová *et al.*, 2003). The plant also contains phenolic compounds, tannins, phyllembelic acid, phyllembelin, rutin, curcuminoids, and emblicol. All parts of the plant are used for medicinal purposes, especially the fruit, which has been used in Ayurveda as a traditional medicine for the treatment of diarrhea, jaundice,

and inflammation. The fruit of Indian Gooseberry (*Phylanthus emblica* Linn.) is acidic, bitter tasting and rich in vitamin (Baliga *et al.*, 2011). The aim of this study was to investigate the possible toxic effects of the from *Phyllanthus emblica* Linn. ethanolic extraction in order to find the acceptably safety level of the *Phyllanthus emblica* Linn. ethanolic extraction in rats.

MATERIALS AND METHODS

Animals and Husbandary

Healthy young female and male Sprague dawley (Mlac:SD) rats of body weight range 180 - 220 were obtain from Office of Laboratory Animal Production, NLAC, Mahidol University, Thailand. The animals were kept under standard conditions 12:12 (light : dark cycles) at 22 ± 3^0C and 30 – 70% relative humidity. The animals were housed individually in stainless steel cages with food (082, Perfect Companions, Thailand) and 5-7 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 2011; 8th Edition NIH publication number #85-23, revised 2011) 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). The *Phyllanthus emblica* Linn. ethanolic extraction 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 rats were fasted 15-18 hours to administration of test sample. The *Phyllanthus emblica* Linn. ethanolic 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 rats were 15-18 hours fasted 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 AND DISCUSSION

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

All rats appeared active and healthy or normal behavior during 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. and Table 2).

Body weight, Food and Water consumption

Body weight gain and the percentage increase in body weight of treatment rats were treated with the *Phyllanthus emblica* Linn. ethanolic extraction had no significant difference. The body weight gain and the percentage increase in body weight are shown in Table 3.

Food and water consumption of the treatment rats that oral administration of the the *Phyllanthus emblica* Linn. ethanolic extraction are shown in Table 4 and Table 5, respectively. There have no significant difference was also detected.

Table 1 Clinical observation parameters for the sighting study

Observation parameters	Time				Clinical Observation
	4 hrs	24 hrs	Day 7	Day 14	
Skin and fur	0/1	0/1	0/1	0/1	clinically normal
Eyes and mucus membrane	0/1	0/1	0/1	0/1	clinically normal
Respiratory	0/1	0/1	0/1	0/1	clinically normal
Circulatory	0/1	0/1	0/1	0/1	clinically normal
Somatomotor activity	0/1	0/1	0/1	0/1	clinically normal
Behavior pattern	0/1	0/1	0/1	0/1	clinically normal
Tremors	0/1	0/1	0/1	0/1	clinically normal
Convulsion	0/1	0/1	0/1	0/1	clinically normal
Salivation	0/1	0/1	0/1	0/1	clinically normal
Diarrhea	0/1	0/1	0/1	0/1	clinically normal
Sleep and coma	0/1	0/1	0/1	0/1	clinically normal
Mortalities	0/1	0/1	0/1	0/1	clinically normal

Table 2 Clinical observation parameters for the main study.

Observation parameters	Time				Clinical Observation
	4 hrs	24 hrs	Day 7	Day 14	
Skin and fur	0/5	0/5	0/5	0/5	clinically normal
Eyes and mucus membrane	0/5	0/5	0/5	0/5	clinically normal
Respiratory	0/5	0/5	0/5	0/5	clinically normal
Circulatory	0/5	0/5	0/5	0/5	clinically normal
Somatomotor activity	0/5	0/5	0/5	0/5	clinically normal
Behavior pattern	0/5	0/5	0/5	0/5	clinically normal
Tremors	0/5	0/5	0/5	0/5	clinically normal
Convulsion	0/5	0/5	0/5	0/5	clinically normal
Salivation	0/5	0/5	0/5	0/5	clinically normal
Diarrhea	0/5	0/5	0/5	0/5	clinically normal
Sleep and coma	0/5	0/5	0/5	0/5	clinically normal
Mortalities	0/5	0/5	0/5	0/5	clinically normal

Table 3 Body weights (g) of rats were treated with *Phyllanthus emblica* Linn. ethanolic extraction (2,000 mg/kg body weight) in main study.

Animal	Body weight (g)			Percentage of body weight increase
	Day 1	Day 7	Day 14	
Female No.1	193	206	235	17.87
Female No.2	194	215	230	15.65
Female No.3	196	210	224	12.50
Female No.4	201	224	244	17.62
Female No.5	206	226	249	17.27
Male No.1	196	247	308	36.36
Male No.2	201	252	320	37.19
Male No.3	202	250	308	34.42
Male No.4	205	252	313	34.50
Male No.5	208	256	323	35.60

Table 4 Food consumption (g) of rats were treated with *Phyllanthus emblica* Linn. ethanolic 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	12.0	16.0	14.0	**Male No.1**	16.0	24.0	23.5
Female No.2	12.0	16.0	14.0	**Male No.2**	16.0	24.0	23.5
Female No.3	10.0	16.0	13.0	**Male No.3**	16.5	23.0	24.0
Female No.4	10.0	16.0	13.0	**Male No.4**	16.5	23.0	24.0
Female No.5	12.0	17.0	14.0	**Male No.5**	15.0	23.0	24.0

Table 5 Water consumption (ml) of rats were treated with *Phyllanthus emblica* Linn. ethanolic 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	16.0	22.0	25.0	**Male No.1**	20.5	25.0	30.0
Female No.2	16.0	22.0	25.0	**Male No.2**	20.5	25.0	30.0
Female No.3	14.0	19.0	20.5	**Male No.3**	23.0	28.5	31.0
Female No.4	14.0	19.0	20.5	**Male No.4**	23.0	28.5	31.0
Female No.5	20.0	27.0	18.0	**Male No.5**	17.0	25.0	29.0

DISCUSSION

Phytonutrition has maintained greater popularity all over developing world and the use is rapidly on the increase (Daswani *et al.*, 2006). In compliance with OECD 423 the from *Phyllanthus emblica* Linn. ethanolic extracttion at 5,000 mg/kg body weight did not cause any mortalities and toxicity signs. The result suggested that from *Phyllanthus emblica* Linn. ethanolic extraction was classified in Globally Harmonised System (GHS) for the classification of chemicals which cause acute toxicity category 5 or Unclassified, the LD50 cut off at 5,000 - ∞ mg/kg body weight.

In this study, the *Phyllanthus emblica* Linn. ethanolic extraction at 2,000 mg/kg body weight did not caused any death or acute oral toxic in both female and male 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 *Phyllanthus emblica* Linn. ethanolic 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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