RESEARCH ARTICLE

Evaluation of Acute Oral Toxicity of the *Emblica officinalis* Phytosome Formulation in Wistar Rats

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Received: 12th October, 2022; Revised: 10th November, 2022; Accepted: 27th November, 2022; Available Online: 25th December, 2022

ABSTRACT

Emblica officinalis, commonly known as amla, belongs to the *Euphorbiaceae* family. The herbal medicine phytosome was used to create a novel drug delivery system to extract *E. officinalis* fruit. *E. officinalis* fruit extract phytosome (EOP) formulation was tested for toxicity, and the LD_{50} cut-off value was determined using female wistar rats weighing 95 to 105 g. Acute toxicity testing was performed on a total of 12 female wistar rats, with three rats in each of the four groups as per 423 organisation for economic co-operation and development (OECD) guidelines. A single oral dose of the EOP formulation at 300, 300, 2000, and 2000 mg/kg body weight was administered to groups I, II, III, and IV. For 14 days, we monitored all of the animals to see if any of them died, developed any clinical symptoms, or put on any significant weight. There were no fatalities among the animals, and notable distinctions between the two groups were not found in terms of observed clinical symptoms or animal body weight. At the time of the gross necropsy, no abnormalities were discovered. The phytosome (EOP) formulation of *E. officinalis* has been shown to be non-lethal and tolerable at 2000 mg/kg bw. 5000 mg (mg/kg) of LD_{50} were selected for this investigation.

Keywords: 423 OECD guidelines, Acute toxicity, *Emblica officinalis* phytosome formulation.

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.4.14

How to cite this article: Mane VB, Killedar SG, More HN, Tare HL, Evaluation of acute oral toxicity of the *Emblica officinalis* Phytosome Formulation in Wistar Rats. International Journal of Drug Delivery Technology. 2022;12(4):1566-1570.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Some plants were deemed hazardous, while others were deemed safe by ancient humans. Traditional medicine has gotten a lot of interest for *in-vivo* studies during the last few decades.[1] The traditional (herbal) therapy method has received much attention in recent years. Toxicology is both a science and a craft, similar to medicine. Toxicology is a branch of pharmacology that studies the negative effects of herbal compounds on living creatures before they are used as drugs or chemicals in clinical settings. It entails collecting and analyzing observational data in order to anticipate the results of exposure in humans and animals. Toxicity is the science of poisons at its most basic level. Several studies have focused on toxicity analyses to ensure that medicinal plants and their products are safe. Living tissues are injured or killed as a result of toxic phytochemical interactions. In comparison to allopathic pharmaceuticals, it is assumed that ayurvedic medicines (drugs), which are popular in our nation, have fewer negative effects. As a result, much effort has gone into finding non-toxic plants that humans can consume.[2] Toxicity testing is necessary since some herbs may have harmful effects, and many reports of toxicity induced by long-term herb use have been recorded. The chemical properties and cell membrane of a toxicant can have an impact on how it causes toxicity in humans. It might take place in the cytoplasm, on the cellular surface, within the cytoplasm, or in the extracellular matrix surrounding the cell. In accordance with the Organization for Economic Co-operation and Development (OECD) recommendations, it is not possible to evaluate the efficacy or safety of a newly developed pharmaceutical without first conducting toxicological testing on mice, rats, guinea pigs, dogs, rabbits, and primates. Toxicological investigations can aid in deciding whether or not a novel treatment should be used in clinical practice. OECD rules such as 401, 423, and 425 prohibit using a medicine in a therapeutic setting without undergoing a clinical study and toxicity testing.[3]

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Acute, sub-acute, and chronic toxicological studies are distinguished by the duration of the animal's exposure to the drug under study. For the OECD, "acute toxicity" means an effect that develops rapidly after a single chemical dosage is administered orally or after numerous doses are administered within 24 hours. Acute toxicity can also develop after multiple doses are administered within 24 hours. The LD_{50}, median lethal dose, and overall behavior are determined. To conduct sub-acute testing, researchers give increasing doses to animals (usually rats and dogs) over two to three days. Chronic trials last for six months and involve the daily dosing of two rodents and one non-rodent.

Emblica officinalis is known as amla. The extract of *E. officinalis* fruit *contains a* higher amount of gallic acid, ellagic acid, rutin, and quercetin flavonoids, vitamins, tannins, and amino acids. It has wide applications and is used for the treatment of neurological disorders, cancer, inflammatory conditions, hypertension, infectious disorders, osteoporosis, etc.[5] A novel drug delivery system of the herbal drug such as phytosome was used to make the formulations of *E. officinalis* extract using phospholipid to increase extract bioavailability.

The first thing that must be done to determine whether or not the bioactive chemicals present in plant component extracts and formulations pose a threat is to demonstrate that they are poisonous when consumed in small doses.[6] In the published article, an examination of the toxicity of *E. officinalis* extract is described. An acute toxicity test was used in this investigation to determine the median fatal dosage, or LD_{50}, in female wistar rats using a phytosomal preparation of fruit extract from *E. officinalis*.

MATERIAL AND METHODS

Material

Arjuna Remedies in Kerala provided the standard hydroalcoholic *E. officinalis* fruit extract. VAV Life Sciences Pvt. Ltd., Mumbai, provided LECIVA-S70 (phospholipid).

Method for Preparation of Phytosome Formulation

E. officinalis phytosome formulation was made by combining 1 gm of *E. officinalis* extract with 3 gm of Leciva S70 phospholipid in 60 mL ethanol in a 250 mL round bottom flask. The reflux condenser was attached to the round bottom flask (RBF), and RBF was kept on the magnetic stirrer. For 3 hours, the solution was agitated and refluxed at 60°C. To eliminate any traces of solvent, the resultant solution was evaporated and kept overnight in a vacuum desiccator. The dried formulation was made and stored at room temperature for future usage in an amber-colored bottle.

Acute Oral Toxicity Test

Experimental Animals

E. officinalis phytosome (EOP) formulation LD_{50} was estimated using acute oral toxicity studies. During the study, we used adult female wistar rats weighing somewhere in the range of 95 to 105 g. The Institutional Animal Ethical Committee at Crystal Biological Solutions in Pune approved the study, and it was given the approval number CRY/2122/070.

Housing and Food Availability

The selected animals were housed in groups in stainless steel grill-top polypropylene cages with access to feeding stations. The space afforded by the cage allowed for unobstructed views of all the creatures within. Cage cards were used to identify each enclosure. The information on this card included the subjects' cage numbers, study codes, dates, group numbers, marks, sexes, and doses. In the chamber where the animals were being tested, there was a cycle of light and dark that lasted for 12 hours, and the temperature was maintained at 22.3°C with 55.5% relative humidity. Polypropylene water bottles connected to stainless steel tubing held RO-filtered water, and pellets of food that are available for purchase were given to the animals (provided by Nutrivet Pvt Ltd).[7,8]

Animal Selection and Assignment

The female rats were divided evenly among four groups. Group I: (head) H, (back) B, (tail) T.; Group II: (head back) HB, (back tail) BT, and (head tail) HT; Group III: (front left leg) FLL, (front right leg) FRL, and (hind left leg) HLL; Group IV: (right side legs) RSL, (left side legs) LSL, and (without) W, was used to identify each animal. After being brought into the experimental animal room, the animals were maintained in their cages for 8 days to acclimate to the environment.[9-11]

Preparation and Administration of Doses

The standards that are described in OECD 423 were adhered to during the course of the testing that was conducted to determine the apparent acute oral toxicity. To administer the *E. officinalis* phytosome (EOP) formulation, a suspension was made by dissolving the EOPs in a 0.5% CMC solution. Even though the animals had access to water at all times, they were fasted the night before the dose was given. After the animals' fasting time ended, their weights were recorded.

Dose Administration

Step 1: In group I, the animals received a 300 mg/kg body weight dose of an EOP suspension made in 0.5% CMC.
Step 2: To ensure the safety of the animals, formulation EOP was administered to group II at the same dose (300 mg/kg body weight) as in step 1.
Step 3: To ensure the safety of the EOP formulation, a substantially greater dose of 2,000 mg/kg bw was provided to the third group of animals after an initial dose of 300 mg/kg bw.
Step 4: Animals from group IV were given the same dose (2000 mg/kg of body weight) as had been used in step 3 to validate the animals' safety.

Size 16 oral feeding needles were used to give the EOP formulation orally. The aforementioned protocols were followed in the care of the animals. After each treatment, the animals in each group went without food for another three hours. Water was provided at all times during the testing. Following the initial four hours of intensive observation, the animals were monitored for an additional 14 days.[10,12,13]

Table 1: Brief overview of the mortality data

Step	*Group*	*EOP formulation Dose in mg/kg bw*	*Number of female rat treated*	*Terminally sacrificed*	*Number of Dead animal*
1	I	300	3	3	0
2	II	300	3	3	0
3	III	2000	3	3	0
4	IV	2000	3	3	0
Total			12	12	0

Observations

Clinical Signs and Symptoms

Clinical manifestations and mortality, including changes in the respiratory, circulatory, autonomic, and central nervous systems; skin, fur, eyes, and mucous membranes; somatomotor activity; and behavioral pattern, were tracked for each animal for the first 30, 60, 120, 180, and 240 minutes after dosing, and then once daily for the next 14 days. With considerable curiosity, we noted the patient's drooling, drowsiness, trembling, convulsions, diarrhoea, sleep, and coma. Data on hydration and feeding patterns was also recorded.[10,14]

Body Weight

Each animal's weight was measured at the beginning of the experiment (during the fasting period prior to the administration of the EOP formulation) and again on days 7 (after treatment) and 14.[15]

Necropsy and Pathology

Every animal had a full-scale necropsy performed on it. In a gross necropsy, the animal is opened up and examined with the naked eye to look for abnormalities in normally-functioning organs. Each animal was inspected thoroughly, including its external orifices, brain, chest, abdomen, and skin. During this stage, important organs were identified, including the liver, lungs, ovaries, kidneys, adrenal gland, spleen, pancreas, heart, and brain.[16]

RESULT AND DISCUSSION

Mortality

300 mg/kg body weight and 2,000 mg/kg body weight were given to 12 female rats to determine the safety of the EOP formulation. In none of the test groups did anyone experience a fatality. Table 1 provides a high-level summary of the mortality statistics. After each dose, the animals in each group were monitored closely for four hours, then daily for 14 days.

Table 2: Clinical manifestations seen in female wistar rats administered 300 and 2000 mg/kg bw

Observational variables	*30 minutes*				*4 hours*				*14 days*			
	Group				*Group*				*Group*			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Alertness	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Eyes	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Touch response	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Lacrimation	A	A	A	A	A	A	A	A	A	A	A	A
Tremor	A	A	A	A	A	A	A	A	A	A	A	A
Convulsion	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Salivation	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Diarrhoea	A	A	A	A	A	A	A	A	A	A	A	A
Lethargy	A	A	A	A	A	A	A	A	A	A	A	A
Sedation	A	A	A	A	A	A	A	A	A	A	A	A
Skin & Fur colour	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Mucous membrane	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Itching	A	A	A	A	A	A	A	A	A	A	A	A
Comma	A	A	A	A	A	A	A	A	A	A	A	A
Gripping	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Urination colour	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Haematuria	A	A	A	A	A	A	A	A	A	A	A	A
Moribund state	A	A	A	A	A	A	A	A	A	A	A	A
Respiration	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Heart Rate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Grooming	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd: not detected and A: Absent

Table 3: Changes in female wistar rats' body weight as a result of exposure to an EOP formulation

Group number and dose	*Animal identification marking*	*Day 0*	*Day 7*	*Day 14*
Group I- 300 mg/kg bw (EOP formulation)	H	96.5	105.0	114.0
	B	98.5	106.0	115.0
	T	101.5	110.0	119.5
	Mean	98.83	107.00	116.17
	SD	2.52	2.65	2.93
Group II- 300 mg/kg bw (EOP formulation)	HB	98.5	107.0	116.5
	BT	100.0	109.5	118.5
	HT	102.5	110.5	119.0
	Mean	100.33	109.00	118.00
	SD	2.02	1.80	1.32
Group III- 2000 mg/kg bw (EOP formulation)	FLL	103.0	111.5	120.0
	FRL	99.5	108.0	117.5
	HLL	101.5	110.5	119.0
	Mean	101.33	110.00	118.83
	SD	1.76	1.80	1.26
Group IV- 2000 mg/kg bw (EOP formulation)	RSL	99.0	107.5	116.5
	LSL	102.5	111.0	120.0
	W	103.5	112.5	121.5
	Mean	101.67	110.33	119.33
	SD	2.36	2.57	2.57

Table 4: Gross necropsy result

Group number	*Mark on animal*	*EOP formulation Dose in mg/kg bw*	*Fate TS/FD*	*Gross necropsy observation*
I	H	Group I- 300	TS	NAD
	B		TS	NAD
	T		TS	NAD
II	HB	Group II- 300	TS	NAD
	BT		TS	NAD
	HT		TS	NAD
III	FLL	Group III- 2000	TS	NAD
	FRL		TS	NAD
	HLL		TS	NAD
IV	RSL	Group IV- 2000	TS	NAD
	LSL		TS	NAD
	W		TS	NAD

TS: Terminally Sacrificed, FD: Found Dead and NAD: No Abnormalities Detected

Clinical Signs and Symptoms

Clinical manifestations seen in female wistar rats administered 300 and 2000 mg/kg of body weight, and the resulting clinical signs and symptoms are presented in Table 2. In all groups, no clinical symptoms of toxicity were seen within 30 minutes of dosing, during the first 4 hours of observation, or up to 14 days of daily observation at doses of 300 and 2000 mg/kg bw. All the animals in the four different groupings at two doses showed no clinical signs of intoxication. In none of the tested animal communities, this posed a life-threatening risk.[17]

Body Weight

In an assessment of acute toxicity, Table 3 depicts changes in female wistar rats' body weight as a result of exposure to an EOP formulation. On day 0 (while fasting) and days 7 and 14 after receiving the formulation, the body weight of the experimental animals was recorded. There was no discernible variation in the rate of weight increase among the test animals. Over the course of 14 days, the experimental animals' body weights increased properly and gradually[16,17]

Gross Necropsy and Pathology

Gross necropsies of organs of the cranial cavity, thoracic cavity, external orifices, abdominal cavity, and external surface were performed on the test animals. All of the organs from the female rats used in the experiments showed no obvious abnormal changes. Table 4 displays the gross necropsy results.

CONCLUSION

The acute toxicity of a phytosome formulation of *E. officinalis* was evaluated according to OECD 423 criteria. During the research on acute toxicity, the animals were monitored in order to look for any signs of impending death. Over the course of the study, the animals showed no discernible shifts in either body weight or clinical symptoms. When necropsy results were analysed, no aberrant changes were found in the organs under scrutiny. When the LD_{50} value of the formulation was determined to be between 2000 and 5000 mg/kg bw under laboratory conditions, with an LD_{50} cutoff value of 5000 mg/kg bw, it was pronounced safe for usage. To conclude, the prepared *E. officinalis* phytosome formulation has been shown to be safe and non-toxic even at a higher dose, which was 2000 mg per kg of bw. A deeper look into the subacute and chronic toxicity of this phytosome formulation is still required to corroborate these results.

ACKNOWLEDGMENT

Both the Bharti Vidyapeeth College of Pharmacy in Kolhapur and the Satara College of Pharmacy in Satara deserve praise for approving the conduct of this study and allowing permission for it to be carried out. The authors would also like to thank Crystal Biological Solutions, Pune, for their support during the course of this research.

Potential for conflict of interest

According to the authors, this work does not involve any potential conflicts of interest.

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