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Research Article

TOXICITY PROFILE OF *CELASTRUS PANICULATUS* SEEDS: A PRECLINICAL STUDY

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ABSTRACT

Objective: The objective of the study was to evaluate the toxicity profile of *Celastrus paniculatus* (CP) by performing a preclinical study on Swiss albino mice and demonstrate a safety description through monitoring their autonomic, neurological, behavioral, physical, and biochemistry profiles.

Methods: The toxicity profiles (acute and subacute) of CP were evaluated using Swiss albino mice in which they were divided into four groups: Group I received 1% Tween 20 and dimethyl sulfoxide. Group II, III, and IV received CP seed oil orally, at doses of 300, 2000, and 5000 mg/kg body weight for both acute and subacute toxicity studies in accordance with Organization for Economic Cooperation and Development guidelines No. 423. Special attention was given during the first 4 h and daily thereafter for a total of 14 days. Behavioral profile, physical state changes, and other parameters such as tremors, convulsion, lethargy were noted. Clinical signs were observed daily during the 28 days of the treatment period. Body weights were measured once a week. On the 29th day, the animals were kept to overnight and blood samples were collected through retro-orbital puncture for biochemical analysis.

Results: In both acute and subacute toxicity studies, the treatment with CP did not affect the normal health status of animals. It is suggestive that CP is considered practically non-toxic.

Conclusion: The toxicity profile of CP seed oil was evaluated and found to be safe until 2000 mg/kg dose.

Keywords: *Celastrus paniculatus*, Acute toxicity, Subacute toxicity, Treatment, Safety.

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INTRODUCTION

Herbal plants have been used from ancient times for the treatment of several diseases in the indigenous system of medicine [1]. *Celastrus paniculatus* (CP) Wild. (Family: Celastraceae) [2], commonly known as Malkangni (in Hindi) or Jyotishmati (in Sanskrit) and commonly known as black oil plant, climbing staff tree (in English) [3] is a long established medicinal plant which has been used extensively in the Ayurvedic system for its recognized analgesic, anti-inflammatory, and marked central effects such as memory boosting and antiepileptic effects [4]. The use of the plant parts in the treatment of several ailments can be attributed to the potential effects of its various phytoconstituents. The seeds of the plant when extracted with petroleum ether yield dark brown oil known as *Celastrus* oil or Malkangni oil are known to have an effect on the central nervous system, mainly the memory-enhancing activity and stimulating effects [3].

The neuropharmacological effects of this herb are striking and the seeds have been reported to possess antidepressant [5], anxiolytic [6], antioxidant [7], hypolipidemic, anti-atherosclerotic, anti-stress, anti-spermatogenic, nootropic activities, and relaxant effects on smooth muscles [8]. *Celastrus* oil therapy in mentally retarded children results in an improvement in their intelligence quotient as it contains a number of fatty acids such as oleic, linoleic, linolenic, palmitic, stearic, benzoic, and acetic acid as volatile acids and their glycerol esters mainly α, α' dipalmitoyl glycerol. They also contain sesquiterpene alkaloids – celapanin, celapanigin celapagin, and malkangunine [9].

Toxicology is an important aspect of pharmacology that deals with the adverse effects of bioactive substances on living organisms before the use as drug or chemical in clinical use. Plants or drugs must be ensured to be safe before they are employed as medicines because the potential toxicity of herbal plants has been recorded [10]. A key stage in ensuring the safety of drugs is to conduct toxicity tests in appropriate animal models.

Although animal toxicity studies of the CP seeds have not yet been established before, it is widely used because of its many beneficial effects and this research is focused on establishing the safety profile of the plant so that they may be credibly employed for various therapeutic applications in medicine.

METHODS

Plant collection and extraction

CP (greenwood essential) seed oil was purchased from a registered, authentic source. The seed oil is administered orally as o/w emulsion, prepared by the wet gum method.

The vehicle employed was phosphate buffer saline, prepared using 4 g of sodium chloride, 100 mg of potassium chloride, 0.72 g of sodium hydrogen phosphate, 120 mg of potassium hydrogen phosphate, and 500 ml distilled water. All the components were initially dissolved in 400 ml distilled water, then the pH was adjusted to 7.4 and was diluted to 500 ml with distilled water [11].

Phytochemical screening

Phytochemical screening for carbohydrate, protein, amino acid, alkaloid, tannins, steroid, terpenoid, volatile oil, glycoside, and fixed oil had been carried out.

Preparation of 100 ml CP o/w emulsion by wet gum method

The proportion of oil:water:gum for preparing primary emulsion is 4:2:1. The required quantity of acacia was weighed and powdered in a dry and clean glass mortar. To this, 28.56 ml water was added to the powdered acacia slowly with trituration to form a smooth mucilage followed by the addition of 0.28 ml CP seed oil and triturated to obtain an even mixture. Then, 56.84 ml coconut oil was introduced in small portions with rapid trituration until a clicking sound was produced and the emulsion becomes white or nearly white to form the primary emulsion after which more water in small portions to the primary

emulsion with trituration to produce the required volume. Next, the mixture was stirred thoroughly so as to form a uniform emulsion. It was then transferred and stored in a cool and dry place [12].

Experimental animals

Ten weeks old healthy Swiss albino mice were selected in this study (n=4), weighing around 20–25 g was purchased from disease-free small animal house, Kerala Veterinary College, Mannuthy. Animals were housed separately in groups of 4 per cage under laboratory conditions with alternating light and dark cycle of 12 h each having free access to food and water. The animals were kept fasting 2 h before and 2 h after drug administration. The animals were acclimatized for at least 5 days before the actual dosing schedule started between 09:00 and 17:00 h. The laboratory animals for using in this experimental study were approved by the Institutional Animal Ethics Committee of NCP (Registration No. 1411/PO/Re/S/11/The Committee for the Purpose of Control and Supervision of Experiments on Animals).

Acute toxicity study

Acute oral toxicity study for the extract was conducted in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines No. 423. The female mice were used for the study. Sixteen female mice were divided into four groups, with each containing four animals. Group I received 1% Tween 20 and dimethyl sulfoxide. Group II, III, and IV received CP seed oil orally, at doses of 300, 2000, and 5000 mg/kg body weight, respectively. After dosing, each animal was observed carefully, at least once during the first 30 min, periodically during the first 24 h. Special attention was given during the first 3 h and daily thereafter for a total of 14 days [13,14]. Behavioral profile (alertness, restlessness, irritability, and fearfulness), autonomic profile (defecation and urination), neurologic profile (locomotion, reactivity, touch, and pain response), physical states such as changes in skin, fur, eyes, mucous membranes, including respiratory, circulatory, central nervous systems, and somatomotor activity, and any lethality or death were also considered during observation. Animals were also carefully observed for tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. The body weight, food, and water intake were monitored weekly.

Subacute toxicity study

The repeated dosing in oral toxicity study has been conducted as the bedside observation as per the OECD 407 for 28 days. Albino young, healthy male mice and non-pregnant female mice were used in this study. Animals were divided into four groups with four animals in each group (n=16; 8 female and 8 male). Group I received 1% Tween 20 and dimethyl sulfoxide. Group II, III, and IV received CP seed oil orally at doses of 300, 2000, and 5000 mg/kg body weight, respectively. Clinical signs were observed daily during the 28 days of the treatment period. Body weights were measured once a week. On the 29th day, the animals were kept to overnight and blood samples were collected via retro-orbital puncture for biochemical analysis.

Mortality rate and sign of toxicity of CP

The observations after the 28 days study have been made to record the rate of mortality and any other sign of toxicity than the signs observed in acute and subacute toxicity studies for the groups received CP seed oil orally at doses of 300, 2000, and 5000 mg/kg body weight, respectively.

Effect of CP on mean body weight changes in mice

The change in body weight is a very important and conclusive sign of the toxicity caused to the animals by any of the doses. A dose with 10% or more reduction in the body weights declared to be a toxic dose. The animals treated with CP will be observed for their growth pattern and body weight after oral administration of the 2000 mg/kg extract.

Statistical analysis

Data were presented as mean ± SEM. Two way ANOVA followed by Tukey–Kramer post-tests was applied on GraphPad Prism version 5. $p<0.001$ was considered statistically significant.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening of CP seed oil was performed and the observations are summarized in Table 1:

Acute toxicity study

As per the OECD guidelines, to establish the safety and efficacy of a new drug, toxicological studies are crucial in animals like mice, rat, guinea pig, dog, rabbit, and monkey under various conditions of the drug employed. Toxicological studies aid in deciding whether a new drug should be adopted for clinical use or not. OECD guidelines – 401, 423, and 425 intercepts the use of drugs clinically without its clinical trial and the related toxicity studies. Depending on the duration of drug exposure to animals, toxicological studies are of three types: Acute, subacute, and chronic toxicological studies.

The present study has been undertaken to estimate the toxic effects of CP in Swiss albino mice (female) for a period of 14 days using OECD 423 (acute toxic class method) as per the method discussed above in acute toxicity study. Their results and observations were recorded accordingly [13,14].

Mortality rate and sign of toxicity of CP

Mortality rate and sign of toxicity of CP were carried out conforming to OECD guidelines – 423 as per the method discussed above. No symptoms of toxicity, morbidity, or mortality were noted in animals during the 14 days period following single oral administration at a selected dose level of CP (2000 mg/kg). The CP oil was safe until 2000 mg/kg body weight and its lethal dose would be greater than that of the test doses. The results were summarized in Tables 2 and 3:

Effect of CP subacute toxicity study

The repeated dosing oral toxicity study been conducted for 28 days. The effect of CP on cage-side observations was carried out as per the method discussed in subacute toxicity in the study. After oral administration of vehicle and extract, animals were observed continuously during the first 30 min after dosing and observed periodically (with special attention given during the first 4 h) for the next 24 h and then daily thereafter, for 28 days. All observations were systematically recorded, with individual records being maintained for each animal. Observations include changes in skin and fur, eyes, mucous membrane, and behavioral pattern. Attention was given for monitoring of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma, and mortality. Further individual body weights of animals were recorded before the

Table 1: Observations of the preliminary phytochemical screening

Test	Observation
Test for alkaloids	
a. Mayer's test	Negative
b. Dragendorff's test	Negative
c. Wagner's test	Negative
Test for carbohydrates	
a. Molisch's test	Negative
b. Benedict's test	Negative
c. Fehling's test	Negative
Test for proteins	
a. Millon's test	Negative
b. Biuret test	Negative
Test for saponins glycoside	
Foam test	Negative
Test for tannins	Negative
Test for flavonoids	
Shinoda test	Negative
Test for steroids	
Liebermann–Burchard reaction	Positive
Test for terpenoids	Negative
Test for glycosides	Negative

administration of drug on 1st day of the study and thereafter on the 7th and 14th day of the experiment. Changes in the weight of individual animals were calculated. The results were summarized in Table 4:

Effect of CP on mean body weight changes in mice

A dose which produces 10% or more reduction in the body weights was considered to be a toxic dose. The effect of CP on body weight changes in mice was observed as per the method discussed above. The animals treated with CP showed a normal growth pattern and body weight after oral administration of 2000 mg/kg extract.

It indicates that the administration of the extract does not affect the normal growth of the animals. The results were summarized in Fig. 1.

Table 2: Observations of acute toxicity study

S. No.	Parameters	Observations			
		2 h	12 h	24 h	72 h
1	Behavioral profile				
	a. Alertness	Normal	Normal	Normal	Normal
	b. Restlessness	Normal	Normal	Normal	Normal
	c. Irritability	Normal	Normal	Normal	Normal
	d. Fearfulness	Normal	Normal	Normal	Normal
2	Autonomic profile				
	a. Defecation	Normal	Normal	Normal	Normal
	b. Urination	Normal	Normal	Normal	Normal
3	Neurologic profile				
	a. Locomotion	Normal	Normal	Normal	Normal
	b. Reactivity	Normal	Normal	Normal	Normal
	c. Touch response	Normal	Normal	Normal	Normal
	d. Pain response	Normal	Normal	Normal	Normal
4	Physical profile				
	a. Texture of fur	Normal	Normal	Normal	Normal
	b. Nasal secretions	Normal	Normal	Normal	Normal
	c. Ear secretions	Normal	Normal	Normal	Normal
	d. Color and texture of feces	Normal	Normal	Normal	Normal
5	Lethality	Absent	Absent	Absent	Absent

Table 3: Mortality rate and sign of toxicity of *Celastrus paniculatus*

Groups	Dose (mg/kg)	Sign of toxicity (ST/NB)	Mortality (D/S)
Group I	5 mg/kg	0/3	0/3
Group II	50 mg/kg	0/3	0/3
Group III	300 mg/kg	0/3	0/3
Group IV	2000 mg/kg	0/3	0/3

The values are expressed as number of animals (n=4), where ST: Sign of toxicity, NB: Normal behavior, D: Animals died, S: Animals survived

No significant changes were observed in body weight when compared with control. The values are expressed as mean ± SD, n=4. The statistical analysis was carried out using multiple t-test followed by Tukey's multiple comparison test.

DISCUSSION

Plant origin drugs are known to play a vital role in the management of various chronic diseases and alternative sources to allopathic pharmaceutical drugs in recent times. The herbal products ensure safety in contrast to the synthetics that are regarded as unsafe to humans and environment. However, the use of these products should be based on the scientific origin; or else they may be futile and unreliable. Furthermore, the irrational use of this phytotherapy may cause serious toxicity in humans. Unfortunately, a large proportion of the population of humans underestimates the toxicity of natural products and does not realize that these agents could be as toxic or more than synthetic drugs.

Toxicity testing is salient in the screening of newly developed drugs before it is marketed for human use. The guiding principles of toxicity testing are to check the effect of the test substances on laboratory animals and its direct toxic effect on humans and furthermore the exposure of laboratory animals to high doses to evaluate its possible hazard on humans that are exposed to a much lower dose.

Toxicity testing employs an array of test in different species of animals with long-term administration of drug, regular monitoring of physiological, biochemical abnormalities, and detailed post mortem examination toward the end of the trial to detect gross or histological abnormalities.

In acute toxicity studies, a single dose of the drug is given in large quantities on a particular animal species to determine the immediate toxic effect. It is used to determine LD_{50} of drugs or chemicals and natural products.

As per the OECD guidelines, to establish the safety and efficacy of a new drug, toxicological studies are crucial in animals such as mice, rat, guinea pig, dog, rabbit, and monkey under various conditions of the drug employed. Toxicological studies aid in deciding whether a new drug should be adopted for clinical use or not. OECD 401, 423, and 425 intercepts the use of drug clinically without its clinical trial and the related toxicity studies. Depending on the duration of drug exposure to animals, toxicological studies are of three types: Acute, subacute, and chronic toxicological studies.

The acute toxicity study of CP was carried out as per OECD – 423 guidelines. On the basis of literature reviews, it has been reported that CP seems to be safe at a dose level of 2000 mg/kg, and the LD_{50} is considered to exceed 2000 mg/kg [13,14]. A single dose of CP seed oil when administered orally and observed for a period of 14 days did not

Table 4: Effect of *Celastrus paniculatus* subacute toxicity study at bedside

Parameters	Control	*Celastrus paniculatus* at 5 mg/kg	*Celastrus paniculatus* at 50 mg/kg	*Celastrus paniculatus* at 300 mg/kg	CP at 2000 mg/k g
Skin and fur	Normal	Normal	Normal	Normal	Normal
Eye lacrimation	Normal	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal	Normal
Diarrhea	Nil	Nil	Nil	Nil	Nil
Lethargy	Nil	Nil	Nil	Nil	Nil
Respiration	Normal	Normal	Normal	Normal	Normal
Tremors	Nil	Nil	Nil	Nil	Nil
Convulsions	Nil	Nil	Nil	Nil	Nil
Coma	Nil	Nil	Nil	Nil	Nil
Locomotor activity	Nil	Nil	Nil	Nil	Nil
Excitement	Nil	Nil	Nil	Nil	Nil
Other symptoms	Nil	Nil	Nil	Nil	Nil
Morbidity	Nil	Nil	Nil	Nil	Nil
Mortality	Nil	Nil	Nil	Nil	Nil

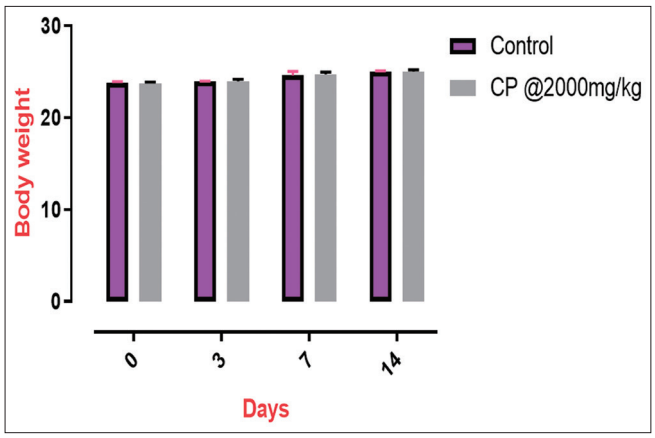

Fig. 1: Effect of *Celastrus paniculatus* on mean body weight changes in mice

exhibit any mortality or toxic symptoms (Table 2). Acute oral toxicity studies aid in evaluating the intrinsic toxicity of the substances and effect on the living tissues [15].

Cage side observations such as behavior, breathing, skin fur, water consumption, and food intake were found to be normal. All treated animals were in live condition after the administration of CP. Mortality was absent in both treatment and control groups (Table 2). This indicates that there were no disturbances in carbohydrate, protein, or fat metabolism. All the treated mice did not exhibit any significant weight loss throughout the experiment, suggesting that the animals were free from wasting syndrome. They presented normal growth pattern and body weight which is similar to the normal control. Decrease or increase in the body weights was associated with toxic effects of chemicals and drugs accompanied with the accumulation of fats and physiological adaptation responses to the plant extracts rather than to the toxic effects of chemicals or drugs that lead to decrease appetite and, hence, lower caloric intake by the animal. Thus, the treatment with CP did not affect the normal health status of animals. It is suggested that CP is considered safe or practically non-toxic. Any pharmaceutical drug or compound with the oral LD_{50} higher than 1000 mg/kg could be considered safe and low toxic. This suggests that CP is practically non-toxic in a single dose level of 2000 mg/kg body weight [13,14]. These results further open the scope for further research on the effects of the plant at the genetic level [16].

CONCLUSION

The acute toxicity study of CP was carried out as per OECD – 423 guidelines. On the basis of literature reviews, it has been reported that CP seems to be safe at a dose level of 2000 mg/kg, cage side observations indicated that there were no signs of toxicity or changes in physical appearances such as skin, fur, eyes, mucous membrane, behavioral pattern, salivation, and sleep of the treated as well as the control animals were found to be normal. Tremors, lethargy, diarrhea, coma, and lethality did not occur in any of the animal at the end of 14 and 28 days of the observation period.

AUTHORS' CONTRIBUTIONS

Conceptualization: Bharat Mishra, Data Collection: Elezabeth John, Krupamol Joy, and Bharat Mishra Formal Analysis: Badmanaban R, Aleesha R, and Bharat Mishra Funding Acquisition: Bharat Mishra and Elezabeth John, Methodology: Badmanaban R, Aleesha R, and Bharat Mishra Project Administration: Bharat Mishra and Elezabeth Visualization: Bharat Mishra and Elezabeth Writing – Original Draft: Bharat Mishra, Elezabeth, and Krupamol Joy Writing – Review and Editing: Bharat Mishra, Elezabeth, and Krupamol Joy.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

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