

Original Article

ACUTE ORAL TOXICITY OF *BAUHINIA VARIEGATA* AND *MADHUCA LONGIFOLIA* IN MICE

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ABSTRACT

Objective: *Bauhinia variegata* and *Madhuca longifolia*, which belongs to Leguminosae and Sapotaceae, respectively, is traditionally used in India for the cure of many diseases. This study represents the result of acute oral toxicity of *Bauhinia variegata* and *Madhuca longifolia* on swiss albino mice.

Methods: Following acute oral administration of 2500 mg/kg crude extracts of *Bauhinia variegata* and *Madhuca longifolia* to swiss albino mice, no mortalities or evidence of adverse effects were observed.

Results: The results of this study agree with those of *in vitro* experiments, indicating that the use of *Bauhinia variegata* and *Madhuca longifolia* leaves is scientifically validated.

Conclusion: The result was in agreement with that of *in vitro* experiments, whereby the crude extracts of *Bauhinia variegata* and *Madhuca longifolia* did not show toxicity. Based on the outcome of acute toxicity in experimental mice, the crude extracts of both *Bauhinia variegata* and *Madhuca longifolia* could be regarded as safe in experimental mice. Further toxicity study over a longer period of time involving detection of effects on vital organ functions would ensure that the plants are safe for human consumption.

Keywords: Acute oral toxicity, Leguminosae, Sapotaceae, *Bauhinia variegata*, *Madhuca longifolia*

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INTRODUCTION

Herbal medicines have long been used as primary healthcare agents, particularly in Asian countries. In Western countries, rapid changes in the popular use of natural products derived from plant sources for health maintenance and alternative treatment have been observed over the last 20 y [1]. Mountain Ebony (English), Rakta kanchan (Marathi), and Kachnar (Hindi) are all names for *Bauhinia variegata*, which belongs to the Leguminosae family (Caesalpinioideae). It's a medium-sized tree that grows up to 1800 meters in India. It sheds its leaves in the Himalayas on a seasonal basis. Leaves are bent at the apex, connate for about two-thirds of the way up, and broader, with leaflets that are strongly sub-coriaceous ovate, pubescent below when young, and with two leaflets that are highly cordate. Lateral, sessile, 5 stamens, staminodes absent, flat fruits; rough glabrous dehiscent seeds, seeded 10-15. The color of flowers is different [2]. It is grown in both India and China [3]. *Bauhinia variegata* (variegated Bauhinia) Linn is used in the treatment of bronchitis, intestinal worms, fungal infection, hepatitis, dysentery, liver diseases, diarrhoea, leprosy, skin disease, wounds, tumors and bacterial infection [4, 5].

Madhuca longifolia, also known as Mahua, is a member of the Sapotaceae family [6]. *Madhuca* is also known as the Indian butter tree and is derived from the word "Madhu," which means "honey." Mahua is a medium-sized deciduous tree native to India, Nepal, and Sri Lanka [7]. Mahua has many medicinal properties in all of its components. Fruit-cooling, aphrodisiac, tonic, and anti ulcerative properties. Rheumatism, anthelmintic, emollient, and leaf-healing wounds Seeds have diuretic, refrigerant, liquor, hepatoprotective, increased milk production, and antihelmintic properties. Antivenom in snake poisoning, bark-tonsillitis, stomach upset [8]. Flavonoids, triterpenoids, glycosides, saponins, and steroids are among the phytoconstituents found in it [9].

The acute oral toxicity test is the most basic and, in most cases, the first toxicity test performed on a sample. Each experimental animal is given a single, high dose of the test sample and the mortality is monitored; death within the observation period (usually 14 d) is investigated to see if it is natural death or humane killing [10]. The

results of this study backed up the need for a safety study on both *Bauhinia variegata* and *Madhuca longifolia*, which are commonly used in India for primary health care. Such research must be conducted before the continued widespread use of certain species causes long-term and irreversible harm.

MATERIALS AND METHODS

Plant sample collection and identification

The fresh leaves of *Bauhinia variegata* and *Madhuca longifolia* were collected from Shri Ram Murti Smarak (College of Engineering and Technology), Bareilly (U. P.), India in January 2016 and February 2016, respectively. They were identified (specimen number-RU/PS/2016/415) by Prof. A. K. Jaitly, Head, Department of Plant Science, Mahatma Jyotiba Phule Rohilkhand University, Bareilly, Uttar Pradesh. A voucher specimen of the collected sample was deposited in the institutional herbarium for future reference.

Chemicals

Drugs: Piracetam and Colchicine were purchased from Sigma Aldrich.

Chemicals: Chloroform Ethyl Acetate, Ethanol, Petroleum ether and Ethanol were purchased from Central Drug House Laboratory (CDH).

Preparation of extracts

Bauhinia variegata and *Madhuca longifolia* leaves were washed carefully in tap water, dried in the shade and powdered. This powder was packed into a Soxhlet column and extracted for 24 h with petroleum ether (60 °-80 °C). For 24 h, the same marc was extracted with chloroform (50°-60 °C) and then ethanol (68 °-78 °C). On a water bath (50 °C), the extracts were concentrated. The dry powder extract was kept at room temperature after being concentrated. Petroleum extract, chloroform extract, methanol extract, ethanolic extract and water extract of *Bauhinia variegata* yielded 9.50 percent (w/w), 7.65 percent (w/w), 8.95 percent (w/w), 8.50 percent (w/w), and 0.30 percent (w/w), respectively. The yield was 0.83 percent (w/w), 1.73 percent (w/w), 25.5 percent

(w/w), 28.1 percent (w/w) and 25.9 percent (w/w) respectively for petroleum ether extract, chloroform extract, methanol extract, ethanol extract and water *Madhuca longifolia* extract. For the experiment, ethanolic extract of both the plants were used.

Test species

The experiment was performed on healthy mice (five weeks of age, bodyweight 23-28 g), obtained from the Laboratory Animal House, Department of Pharmacy, SRMS (CET), Bareilly, U. P., India. The female mice were confirmed nulliparous and non-pregnant. The mice were assigned to five dosage groups and one control group with 10 mice (five male and five female) for each test group. The experimental procedures involving the animals were approved by the Shri Ram Murti Smarak (CET) Animal Experimental Ethics Committee [Ethical number: (715/PO/Re/S/02/CPCSEA)] before commencing the study.

Procedure of acute oral toxicity

The acute oral toxicity of the crude ethanolic extracts of both *Bauhinia variegata* and *Madhuca longifolia* were evaluated in mice using the procedure described by the OECD (Organization for Economic Co-operation and Development), with some modifications. The mice were housed in suspended, stainless steel, wire-mesh cages in an experimental animal room. Before and after treatment with the extract, the temperature was kept at 22 (3 °C) and the relative humidity was 50-60%. The animal room had a 12 h light/dark cycle and was artificially lit (fluorescent light). Prior to the start of the experiments, the mice were acclimatized to the laboratory conditions for at least five days. The mice were chosen at random for the study and were marked to allow for individual

identification. Except during the fasting period, mice were fed standard mouse diets with an unrestricted supply of drinking water. The mice were fasted for about 12 h before dosing, but they had unlimited access to water. The mice were separated into groups based on sex and dosage levels before and after treatment with the extract. The extracts were suspended (10 percent Tween-80 in distilled water). A 200 mg/ml stock concentration was prepared and the mice were given 0.2 ml of the extract for every 10 g of body weight. The extracts were given to the mice in doses of 500, 1000, 1500, 2000 and 2500 mg/kg. Approximately three to four hours after dosing, the animals were given food. The mice were closely monitored for any signs of toxicity in the first four hours following the treatment period, and then daily for the next 14 d. Mortality, signs of sickness, injury, pain, distress, allergic reactions, changes in outer appearance, behavioral changes (i.e., ataxia, hyperactivity, hypoactivity) and general stimulation or sedation were all monitored twice a day. The observations were meticulously documented, with individual records kept for each mouse.

RESULTS AND DISCUSSION

Extraction yield of *Bauhinia variegata* and *Madhuca longifolia*

The most common method for sample preparation is solvent extraction. Table 1 shows the yields from ethanol extracts of *Bauhinia variegata* and *Madhuca longifolia*. To prevent the presence of water in the extracts, the plant material must be dried prior to extraction. The weight of dried and ground plant materials determines the percentage of crude ethanol extract yield. Because of its polarity and ability to remove compounds like phenolics, flavonoids, and other polar products, ethanol is used as the extraction solvent [11].

Table 1: Yield of ethanol extract of *Bauhinia variegata* and *Madhuca longifolia*

Plants	Samples/extracts	Weight (g)
<i>Bauhinia variegata</i>	Fresh samples	452.50
	Dried and ground plant material	750.90
	Ethanolic extract	77.70
<i>Madhuca longifolia</i>	Fresh samples	408.50
	Dried and ground plant material	35.20
	Ethanolic extract	15.80

Acute oral toxicity assessment of *Bauhinia variegata* and *Madhuca longifolia* crude extracts

The first step in the toxicological study of herbal products is to look at acute toxicity [12]. Overall, animal models have 70-80% predictability for human toxicities [13, 14]. In general, *in vivo* methods will provide the first indications of complex toxicities, as information on certain toxic manifestations cannot be measured using *in vitro* cytotoxicity methods [15]. *In vivo* assays can detect toxic manifestations that affect the entire organism, such as discomfort, anxiety, allergic reactions, changes in outer appearance, behavioral changes, and general stimulation or sedation. However, in most acute toxicity studies, effects on essential functions (cardiovascular, central nervous system and respiratory systems) are not measured.

The current research used acute oral toxicity to assess the safety parameters of *Bauhinia variegata* and *Madhuca longifolia* leaves. After oral administration of crude ethanol extracts of *Bauhinia variegata* and *Madhuca longifolia*, mortality, clinical signs, gross findings and body weights of mice were observed and measured for 14 d. To ensure that all components of the extract were used in this acute oral toxicity analysis, crude ethanol extracts were used. The acute toxicity of crude extracts of *Bauhinia variegata* and *Madhuca longifolia* is shown in table 2. There were no deaths recorded for any of the doses measured for crude ethanol extracts of *Bauhinia variegata* and *Madhuca longifolia*. During the 14-day observation period, no major changes in behavior (i.e., ataxia, hyperactivity, hypoactivity) or appearance were observed in any of the mice.

Table 2: Results of the potential toxic effect of the crude extracts of *Bauhinia variegata* and *Madhuca longifolia* in mice

Plants	Dose (mg/kg)												
	0		500		1000		1500		2000		2500		
	M	F	M	F	M	F	M	F	M	F	M	F	
<i>Bauhinia variegata</i>	0/5 ^a	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
<i>Madhuca longifolia</i>	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

M=Male mice, F=Female mice, ^aNumber of animals dead/Number of animals used

At any dosage, they gained weight with no adverse clinical signs of toxicity. The traditional aim of an acute oral toxicity analysis was to calculate the LD50. The LD50 value is currently the basis for the toxicological classification of chemicals. It is defined as the

statistically derived dose that, when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given time. Laboratory mice and rats are the most common organisms used in traditional LD50 studies. For regulatory

purposes, both sexes are frequently needed [16]. The LD50 values of crude ethanol extracts of *Bauhinia variegata* and *Madhuca longifolia* were 2500 mg/kg because no deaths were observed at any of the doses tested. Both *Bauhinia variegata* and *Madhuca longifolia* did not cause any acute oral toxicity, according to this report. According to OECD-recommended chemical labeling and classification of acute systemic toxicity based on oral LD50 values, both *Bauhinia variegata* and *Madhuca longifolia* crude extracts were classified to class 5 (LD50. 2000 mg/kg), the lowest level of toxicity (no label; unclassified). Oliver pointed out that (i) the LD50 value was not an absolute value, but rather a biological parameter that was inherently variable and could only be defined in terms of precision rather than accuracy [17] and (ii) the LD50 value only applied to mortality and was not indicative of any other clinical manifestation of toxicity.

CONCLUSION

In view of the increasing popular consumption of medicinal plants as an alternative therapy, it is necessary to conduct research to support the therapeutic claims and also to ensure that the plants are indeed safe for human consumption. The present research findings have clearly met the objectives of the study. The result was in agreement with that of *in vitro* experiments, whereby the crude extracts of *Bauhinia variegata* and *Madhuca longifolia* did not show toxicity. Based on the outcome of acute toxicity in experimental mice, the crude extracts of both *Bauhinia variegata* and *Madhuca longifolia* could be regarded as safe in experimental mice. Further toxicity study over a longer period of time involving detection of effects on vital organ functions would ensure that the plants are safe for human consumption.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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