

The Journal of Phytopharmacology

(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2320-480X

JPHYTO 2017; 6(3): 183-185

Received: 29-06-2017

Accepted: 21-07-2017

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Acute oral toxicity study of *Tectona grandis* Linn. methanolic seed extract in albino mice

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ABSTRACT

Objective: To evaluate the acute toxicity studies of methanolic seed extract of *Tectona grandis* in albino mice.

Method: The present study has been carried out to evaluate the adverse and hazardous effects of methanolic seed extract of *Tectona grandis* in albino mice through oral route. Acute oral toxicity of methanolic extract was carried out in two phases. In first phase, a single dose of 10, 50, 100, 500 and 1000mg/kg (dissolved in water) body weight in test group, while mice in control group received normal saline was administered. All the animals were individually studied for the mortality, wellness parameters and body weight for 14 days. The experiment was performed under OECD guidelines 423. No mortality or any significant physiological changes in the animals were observed up to 1000mg/kg bodyweight. The experiment was again repeated for second phase with a single doses of 1000, 1250, 1500 and 2000 mg/kg. Mortality was found at 1250 mg/kg. Therefore LD₅₀ of the extract was estimated to be more than 1000mg/kg body weight. Finally, administration of a single dose of 10, 50, 100, 500, 1000 mg/kg of the extract did not showed any significant differences in food and water consumption and other behavioral changes. The extract starts showing toxicity at a dose of 1250 mg/kg body weight.

Conclusion: The analysis of these results with the information of signs, behavior and health monitoring leads to the conclusion that the oral administration of *T. grandis* methanolic seed extract for 15 days does not cause acute toxicity. But, higher doses on long term administering can cause considerable toxicity.

Keywords: *Tectona grandis*, Acute toxicity, Phytochemistry.

INTRODUCTION

Pharmacological evaluation of medicinal plants has become the primary interest in the researchers in the drug discovery globally. The natural products research became the basic area for drug discovery. The huge information about the plant research can give the interest of the researchers worldwide. But the thorough information is only about few plants. There are many more plants to be explored worldwide^[1]. *Tectona grandis* is one of such plant. Toxicology which is traditionally called as the science of knowledge has a very long history as poisons. Later, toxicology included the safety profile of a compound. The term toxicology refers to the detection, prevention and treatment of poisoning and study of its poisonous. After a thorough study of its harmful effects the bio safety level will be established^[2].

Tectona grandis belongs to the family Verbenaceae is indigenous to India and other tropical countries, well known as Teak. The plant is famous for its preeminent strong trunk which is decay resistant^[3]. The plant contains diversified chemical constituents in all of its parts. Leaves, bark, root possess diverse chemical constituents like alkaloids, glycosides, terpenoids, steroids, carbohydrates and proteins^[4]. The leaves contains anthraquinones^[5], lignin derivatives^[6], anthracteone and naphthatectone^[7]. The heart wood contains naphthaquinones^[8]. Bark contains betulinol^[9], lupeol, ursolic acid, eicosanyleicosanoate, betulinic acid and bis(2-ethylhexyl)phthalate^[10]. The root heart wood contains 5-hydroxylapachol which is a cyto toxic agent^[11]. Wood contains various naphthjaquinone derivatives like tectol, dehydrotectol, lapachol, deoxylapachol, 5-hydroxylapachol^[12]. The seed contains fatty acids^[13] amino acids^[14] and xanthenes^[15]. Traditionally, various parts of the plant were used to treat different ailments like anti-inflammatory, laxative, astringent, pain reliever and for anuria^[12]. Especially the seed contains antimicrobial^[16] and anti-inflammatory activity^[17]. The seed oil is used to promote hair growth^[18]. Seeds are not much explored for their phytochemistry and pharmacology. In order to perform a phyto-pharmacological investigation the seeds were used for the current study. As a fundamental screening, the methanolic extract was studied for its oral acute toxicity in albino mice.

MATERIALS AND METHODS

For the present study, the seeds of *T. grandis* were collected from the surrounding areas of the Vanasthalipuram, Hayathnagar (mandal), Rangareddydist, Telangana, India in the month of February 2017. Fresh leaves were authenticated by Dr. N. Sivaraj, Senior Scientist (Eco Botany), National Bureau of Plant Genetic Resources, Rajendranagar, Hyderabad, seed specimens have been deposited at the museum of the college. Fresh seeds were shade dried at room temperature. The seeds were then subjected to size reduction by impact type electric grinder. A coarse powder was obtained maceration starting with petroleum ether and methanol for 7days each. Distillation process was used for the removal of the solvents. The percentage yield of the extract was found to be 12.7%.The extract was stored in airtight container in refrigerator below 10°C.30gms of methanol extract was taken for the future analysis.

Preliminary Phytochemical screening

Preliminary Phytochemical screening was done by using standard procedures for methanolic extract of *Tectona grandis*seed^[19]. The results showed that the seed possess various chemical constituents which are tabulated in Table1.

Experimental animals

Albino Mice (25-35g) were used in the experiments. They were procured from Sainath agencies, musheerabad. The animals were kept in polypropylene cages at animal house for few days, where they acclimatized to standard laboratory conditions for one week. Animals were freely allowed to feed on standard rodent pellet diet (Golden Mohur Lipton India Ltd.) and water *ad libitum*. The temperature (25±2°C), relative humidity (60%±10%) and 12 hr dark/light cycle were maintained throughout the experiment^[20]. The study protocol was approved from the Institutional animal ethics committee (IAEC) before commencement of experimental studies (1292/ac/09/CPCSEA).

Acute toxicity studies

The experiment was performed under OECD guidelines 423. The methanolic seed extract of *T. grandis* was studied for acute toxicity study as reported by Muhammad *et al* with a little modification of two phases^[21]. In first phase, a single dose of 10, 50, 100, 500 and 1000mg/kg (dissolved in water) body weight in test group, while mice in control group received normal saline was administered. All the animals were individually studies for the mortality, wellness parameters and body weight for 14 days. No mortality or any significant physiological changes in the animals were observed in this phase up to 1000mg/kg bodyweight. So, second phase experiment was conducted with a single doses of 1000, 1250, 1500 and 2000 mg/kg. These mice were also observed for toxicity signs for 24hrs followed by 14 days. The behavioural changes and other physiological parameters like body weight, food-water intake, excretion, *etc.* were keenly observed and recorded^[22].

RESULTS AND DISCUSSIONS

The acute toxicity study results were evaluated as per OECD Guidelines 423. The mice were showing no significant abnormal behavioural and physiological symptoms up to 1000 mg/kg body

weight. But the extract was found to be toxic and showing mortality from 1250mg/kg since the mice were exhibiting signs of convulsions, drowsiness, ataxia and death. Hence 1000 mg/kg was selected as safe dose and 1/5 of 1000mg/kg i.e., 200mg/kg of *T. grandis* methanolic seed extract was selected as safer therapeutic dose for the studies and LD₅₀ was considered to be >1000 mg/kg. The parameters were displayed in Table 2.

Traditional herbal medicine has an important input in the healthcare system alternative to the synthetic pharmaceutical products^[23]. Though it is safer than allopathic medicine it is also had its own limitations in toxicity. Before starting a phyto-pharmacological investigation it is necessary to evaluate its toxicity profile. So, our plant *T. grandis* had also been investigated for its acute toxicity and the results showed that the methanolic seed extract was found to be safe at a dose of 1000 mg/kg bodyweight orally. IfLD₅₀ is more than 1000mg/kg it could be considered as safe^[24].

The results also show that the methanolic seed extract at higher dose causes toxicity. It is may be due to various chemical components present in the seeds. So, further phytochemical screening is needed in order to establish a chemical profile. Acute toxicity studies cannot give clear information about the physiological changes in the animals. So, it is also necessary to make appropriate sub-acute and chronic studies to identify the type of damage it is causing in the animals. The phyto-pharmacological profile of the plant seeds is needed to develop an herbal formulation or to explore various new chemical entities for drug discovery.

Table1: Preliminary phytochemical screening

Type of component	Present(+)/absent(-)
Carbohydrates	+
Glycosides	+
Flavonoids	-
Alkaloids	+
Tannins	+
Proteins	+
Triterpenoids	+
Resins	+
Phytosterols	+
Saponins	-

Table 2: Effect of *T. grandis* methanolic seed extract in albino mice

Group	No. of mice	Dose (mg/kg)	Body weight in gm			Outcome
			Day 0	Day 7	Day 14	
1	5	Control	25	27	30	Survival (5/5)
2	5	10	26	28	30	Survival (5/5)
3	5	50	25	27	30	Survival (5/5)
4	5	100	24	25	29	Survival (5/5)
6	5	500	24	25	29	Survival (5/5)
7	5	1000	24	26	28	Survival (5/5)
8	5	1250	25	24	23	Survival (4/5)
9	5	1500	26	25	23	Dead (2/5)
10	5	2000	25	22	20	Dead (4/5)

CONCLUSION

The acute oral toxicity study of the methanolic seed extract of *T. grandis* in albino mice showed that the extract is considered to be safe at a dose of 1000mg/kg bodyweight with for short term usage orally. A detailed experimental analysis on its chronic usage is essential to support safety of this plant.

Acknowledgement

Acknowledgement is given to all the members in the research group of Pharmacognosy, Pharmacology and the management of Vijaya College of Pharmacy for providing the required facilities.

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HOW TO CITE THIS ARTICLE

Dokuparthi SK, Md. Khan A, Anusha A, Bee M, Shaibaz, Shahajeb S, Sunil Kumar K, Suthakaran R. Acute oral toxicity study of *Tectona grandis* Linn. methanolic seed extract in albino mice. *J Phytopharmacol* 2017;6(2):183-185.