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Research Article

ACUTE TOXICITY STUDIES AND EVALUATION OF ANALGESIC PROPERTY OF *TECTONA GRANDIS* METHANOLIC SEED EXTRACT IN SWISS ALBINO MICE

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ABSTRACT

Objective: *Tectona grandis* which is well known as teak possesses a wide range of applications in Indian and African folklore medicine. All the plant parts are having diverse bioactivities, especially the seeds, having hair growth-promoting activity, anti-inflammatory, and antimicrobial activity. Xanthones, proteins, amino acids, and fatty acids have been isolated from the oil and seeds of *T. grandis*.

Methods: The present study was to explore the antinociceptive properties of methanolic seed extract of *T. grandis*. Dried seeds of the plant were defatted with non-polar solvents such as petroleum ether and extracted with methanol. Acetic acid-induced writhing test, tail flick test, and tail immersion test were employed for the extract using Swiss albino mice.

Results: From the results, it is clear that the methanolic teak seed extract is safe at 1000 mg/kg and having a potent analgesic property (at a dose of 200–250 mg/kg body weight) by inhibiting pain response time.

Conclusion: It can be concluded that the methanolic seed extract of *T. grandis* is analgesic in nature. It is considered as safe and the activity may be due to the presence of various bioactive chemicals such as flavonoids, xanthones, and glycosides.

Keywords: *Tectona grandis*, Acute toxicity, Analgesic, Acetic acid-induced writhing, Tail flick, Tail immersion.

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INTRODUCTION

Tectona grandis Linn. is a large deciduous tree 30–35 m tall. It is a well-known tree for its typical strong trunk, having good commercial value in timber industry [1]. The whole plant is having medicinal importance reported to cure several ailments according to Indian traditional system of medicines. The ample literature reveals that the plant is used in the treatment of diabetes, pains, skin allergies such as scabies, respiratory tract infections, and urinary tract infections, as diuretic, sedative, and laxative [2-5]. Various plant parts of *T. grandis* are having ethnopharmacological importance. Diversified phytoconstituents such as tannins, glycosides, sterols, and terpenoids were reported for *T. grandis* [6], which may be culpable for its wide range of pharmacological activities including antibacterial [7], antifungal [8], antioxidant [9], analgesic, anti-inflammatory [10], antidiabetic, diuretic [11,12], antipyretic [13], wound healing [14], antiulcer [15], anticancer [16], and hair growth-promoting activities [17]. The seeds are reported to show antimicrobial [18], antioxidant, and anti-alopecic activity. However, there is no evidence of the analgesic properties of *T. grandis*.

Pain and inflammation are the incessant problems over the sphere. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the primary choice for pain and inflammation. Due to the ulcers, hemorrhage, liver damage, and renal damage constraints of NSAIDs, there is always a need to develop safer analgesics [19]. In our current study, we are evaluating the analgesic activity of methanolic seed extract of *T. grandis*.

METHODS

Plant material

T. grandis fruits were collected in the month of February 2017 in Hyderabad and were identified by Dr. N. Sivaraj, Senior Scientist (Eco Botany), National Bureau of Plant Genetic Resources, Rajendranagar, Hyderabad, seed specimens have been deposited. Extraction of *T. grandis* seeds: 5 kg of the fresh seeds were shade dried at temperature 25–30°C for 7 days. The dried seeds were powdered in a grinder. The alcoholic extract of the seeds was prepared by subjecting to repetitive maceration for 2 weeks with methanol after defatting with n-hexane. The extract was evaporated to dryness with rotary evaporator and lyophilized to get powder. The percentage of yield was calculated as 6% and the final methanolic extract was used for the studies.

Phytochemical screening

Various qualitative phytochemical tests were performed to confirm the presence of various secondary metabolites using standard protocols.

Experimental animals

Swiss albino mice (25–35 g) were used for the pharmacological screening from Sainath Agencies, Musheerabad, Hyderabad. Animals were randomly divided into groups before the commencement of the experiment; the mice were acclimatized in polypropylene cages for a period of 10 days under temperature (26±2°C), relative humidity (45–55%), and dark/light cycle for a period of 12 h. Rodent pellet diet was supplied and water *ad libitum*. The Institutional Animal Ethics Committee has approved (CPCSEA/IAES/JLS/006/01/17/005) the study protocol before the commencement of the experiment.

Acute toxicity studies

Acute toxicity studies have been performed according to the Organisation for Economic Co-operation and Development guidelines 423 in two phases. After grouping animals into six, a single dose of 10, 50, 100, 500, and 1000 mg/kg (dissolved in water) followed by normal saline to the control was given. The mice were observed for mortality and considerable bodily changes for a period of 14 days. In the second phase, a group of six having a single dose of 1000, 1250, 1500, and 2000 mg/kg was administered and observed for behavioral and physiological variations such as body weight, food-water intake, excretion, and recorded [20].

Analgesic activity of methanolic seed extract in albino mice

Acetic acid induced-writhing test

The experiment was performed by the procedure described by Koster and Anderson [21,22]. Swiss albino mice of both sexes were used for this experiment and divided into three groups of six animals each. The control group was administered with normal saline solution, the test groups were administered with 200 mg/kg of methanolic seed extract dissolved in water. The standard group was administered with a dose of 25 mg/kg standard diclofenac sodium prepared in water. Food was restricted during experiments. Writhing's was induced 30 min after the last dose by intraperitoneal injection of 10 ml/kg of 0.6% acetic acid in distilled water. The number of writhing that is a number of abdominal contractions, trunk twist responses, and extension of hind limbs was recorded immediately for a period of 10 min. The percentage inhibition of writhing's for all the groups was calculated using the formula:

$$\frac{\text{Average of control group} - \text{Average of test}}{\text{Average of standard}}$$

Tail flick test

This experiment was done by measuring the reaction latencies of mice [23]. The basal reaction time of mice to the heat was recorded by locating the tip of the tail on a radiant analgesiometer. The tail flick response was taken as the endpoint. The animals were divided into three groups (control, standard, and test) each containing six animals. The control group received the normal saline solution, the standard group received a dose of 2 mg/kg morphine sulfate subcutaneously, and the test group received a dose of 250 mg/kg *T. grandis* methanolic seed extract. The latent period of the tail flick response was determined at 30, 60, and 90 min after the administration of the samples. The cutoff period time was set as 15 s to avoid the skin damage. The percentage elongation of the time was calculated for the analgesic activity.

Tail immersion method

The tail immersion method is also similar to the tail flick method [24]. The animals were divided into three groups (control, standard, and test) each containing six animals. The control group received the normal saline solution, the standard group received a dose of 2 mg/kg morphine sulfate subcutaneously, and the test group received a dose of 250 mg/kg *T. grandis* methanolic seed extract. The basal reaction time of mice to the heat was recorded by locating the tip (around 4–5 cm) of the tail in hot water (55°C±2). The time taken to withdraw its tail from the hot water was taken as the endpoint. The latent period of the tail removal response was determined at 30, 60, and 90 min after the administration of the samples. The cut-off period time was set as 15 s to avoid the skin damage. The percentage elongation of the time was calculated for the analgesic activity.

Table 1: Preliminary phytochemical screening of methanolic seed extract of *Tectona grandis*

Phytochemicals	Present or Absent
Alkaloids	–
Glycosides	+
Flavonoids	+
Terpenoids	+
Steroids	+
Tannins	+
Proteins	+
Carbohydrates	+
Amino acids	+

+: Indicates the presence and, –: Indicates the absence of phytochemicals

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

Preliminary phytochemical analysis of methanolic seed extract was done using standard reagents and protocols. The results (Table 1) revealed the presence of various phytochemicals such as glycosides, flavonoids, terpenoids, tannins, proteins, amino acids, carbohydrates, and steroids.

Acetic acid-induced writhing test

The acetic acid-induced writhing test can be used for screening peripheral and central analgesic properties of the drugs. The pain induced by the noxious substances like intraperitoneally administered acetic acid may be due to the release of some endogenous pain nerve stimulants [25] or pain mediators or by the release of prostaglandins which will sensitize the nociceptors [26]. The peripheral analgesic property can be examined by the writhing responses such as abdominal constrictions, trunk twisting, and extension of hind limbs induced by acetic acid. NSAIDs interfere in the transduction mechanism of primary afferent nociceptors by inhibiting cyclooxygenase in peripheral tissues [27]. Our results (Table 2) showed that *T. grandis* methanolic seed extract significantly ($p<0.001$) reduced the number of writhing responses in acetic acid-induced mice when compared with the control and standard diclofenac sodium. Interestingly, the extract at a dose of 200 mg/kg had considerable percentage inhibition than the standard that is diclofenac sodium.

Tail flick method

Tail flick method was carried in the mice and the results are depicted in Table 3. The control group showed no difference in the responses before and after the treatment with normal saline solution. Animals that received the test sample (250 mg/kg) showed statistically significant latency time (7.4±0.4) compared to the standard drug (7.7±0.3).

Tail immersion method

Tail immersion method was also carried and the results are illustrated in Table 4. The control group that received normal saline solution did not show any difference in the tail withdrawal time before and after the treatment. The test group (250 mg/kg) showed statistically significant latency time (7.3±0.2) compared to the standard drug (7.8±0.3).

The analgesic activity of the extract may be by inhibiting prostaglandin synthesis and/or by involving in the peripheral pain mechanism. Primary qualitative analysis by different chemical tests for the methanolic seed extract of *T. grandis* showed the presence of various phytochemicals including tannins, flavonoids, glycosides, and saponins which may be responsible for the activity. However, further investigation on its phytochemistry is needed to identify the specific phytochemical components which are responsible for the activity.

Table 2: Effect of methanolic seed extract of *Tectona grandis* on acetic acid-induced writhing behavior in mice

S. No.	Group	Total writhing responses			Total
		EHL	ABC	TT	
1.	Control	45±0.15	28±0.11	31±0.12	104±0.76
2.	Test	42±1.23	09±1.3***	10±1.36***	61±0.18**
3.	Standard	22±0.12**	11±0.65**	13±1.74**	46±1.39***

EHL: Extension of hind limbs, ABC: Abdominal constrictions, TT: Trunk twisting. Values are expressed in mean±standard error of the mean. Data compared against positive control group. One-way ANOVA, **p<0.01 and ***p<0.001 were considered statistically significant when compared to control using Tukey–Kramer multiple comparison test

Table 3: Protective effect of methanolic seed extract of *Tectona grandis* on tail withdrawal reflexes induced by tail flick method in mice

Drugs	Before treatment	After treatment	% Inhibition
Control	5.73±0.2	5.7±0.3	0
Test	5.7±0.1	7.4±0.4*	74.02
Standard	5.6±0.3	7.7±0.3*	75.67

Values are expressed in mean±standard error of the mean. Data compared against positive control group. One-way ANOVA, *p<0.05 was considered statistically significant when compared to control using Tukey–Kramer multiple comparison test

Table 4: Protective effect of methanolic seed extract of *Tectona grandis* on tail withdrawal reflexes induced by tail immersion method in mice

Drugs	Before treatment	After treatment	% Inhibition
Control	5.4±0.2	5.4±0.2	0
Test	5.5±0.3	7.3±0.2*	70.51*
Standard	5.5±0.2	7.8±0.3	75.34

Values are expressed in mean±standard error of the mean. Data compared against positive control group. One-way ANOVA, *p<0.05 was considered statistically significant when compared to control using Tukey–Kramer multiple comparison test

CONCLUSION

To curb the results, the current investigation affirms the analgesic potency of methanolic seed extract of *T. grandis* in Swiss albino mice. The activity may be by peripheral and/or by a central mechanism. Further investigation is indispensable to understand and validate the results and to isolate bioactive components from the seeds. It is also essential to develop an advanced model for a better understanding of the literal mechanism involved in the analgesic activity.

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AUTHORS' CONTRIBUTIONS

All the authors contributed equally.

CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

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