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IN VIVO ANALGESIC AND ANTIINFLAMMATORY EFFECTS OF *TECTONA GRANDIS* LINN. STEM BARK EXTRACTS

MOHAMMAD ASIF*
Department of Pharmacy, Guru Ram Das (PG) Institute of Management and Technology, Rajpur, Dehradun 248009, Uttrakhand, India

Tectona grandis Linn. is a widespread hard wood plant used for both therapeutic and commercial purposes. It is native to South and Southeast Asia. The present study was carried out to investigate analgesic and antiinflammatory activities of T. grandis (Family Verbenaceae) stem bark extracts. We also determined the preliminary phytochemical screening and acute toxicity of the stem bark extract. Stem bark was extracted with ethanol (TGEE) and water (TGAE). Analgesic and antiinflammatory activities of these extracts were assessed in Wistar rats with hot plate test and carrageenan induced paw oedema model. At the doses used (200, 300 and 500 mg/kg), TGEE and TGAE showed significant and dose-dependent analgesic and antiinflammatory effects. The phytochemical analysis revealed the presence of flavonoids, alkaloids tannins, anthraquinones, saponins, carbohydrates and proteins. None of the extracts had acute toxicity activity up to 2000 mg/kg dose level. The TGEE and TGAE exhibited significant analgesic and antiinflammatory activities of T. grandis stem bark due to the presence of various phytoconstituents such as flavonoids, alkaloids, tannins, anthraquinones and saponins.

Keywords: *Tectona grandis*, *Verbenaceae*, Stem bark extracts, Acute toxicity, Analgesic, Antiinflammatory

INTRODUCTION

Herbs are major component of traditional, ayurvedic, Unani, homeopathic and naturopathic medicines (Barnes, Anderson and Phillipson 2002). There is a belief that natural remedies are superior to man-made drugs because they are always associated with natural and biological entities like protein, lipids and carbohydrates (Retnam and Martin 2006). The World Health Organization (WHO) has estimated that at least 80% of the world's populations rely on traditional system of medicine for their primary health needs. These systems are largely plant based due to the growing awareness about side effects and complications of chemical and synthetic medicines (Nisbet and Moore 1997; Artuso 1997; Banerji 2000; Harvey 2000; Purohit and Vyas 2004).

Tectona grandis Linn., commonly known as teak or sagon (Family Verbenaceae) is a tropical hard wood tree, 30–40 m tall, deciduous and native to the South and Southeast Asia, and has been used for both therapeutic and commercial purposes. The excellent properties and versatile nature of teak wood and its eminent suitability for an array of uses is well documented (Mukherjee 2003). The potential for growing and managing teak in different ecological zones and under different situations is being increasingly recognised, leading to intensive domestication and cultivation of the *T. grandis* species in

*Corresponding author: Mohammad Asif, e-mail: mohd.mpharm@gmail.com

other countries or regions beyond its natural habitat. It grows in a large range of ecological zones in the world. Teak is the world's most cultivated high-grade tropical heartwood. The area of teak plantations, about 94%, are in tropical Asia, with India (44%) and Indonesia (31%) contributing the bulk of the resource. Other countries contribute with 17% in total (e.g. Thailand, Myanmar, Bangladesh and Sri Lanka). About 4.5% plantations are in tropical Africa and the rest are in tropical America, mostly in Costa Rica and Trinidad and Tobago (Bhat 1995; Bhat 1998; Ball, Pandey and Hirai 1999; Bhat, Priya and Rugmini 2001; Bhat and Ma 2004; Diallo *et al.* 2008). Other species of this genus are *T. phillippinensis* and *T. hamiltoniana.*

According to ayurveda or Unani system of medicine, *T. grandis* wood is acrid, cooling and useful in treatment of headache, constipation, biliousness, burning sensation and pain, liver-related troubles, worms, cough, microbial, fungal, piles, leucoderma and dysentery infections. The flowers are acrid, bitter and dry, and useful in treatment of bronchitis and urinary discharges. The bitter taste is due to antinutritional factors such as alkaloids, saponins and flavanoids (P'ei and Chen 1982). Roots are useful in the treatment of urinary system-related troubles, and oil from the flower promotes hair growth and is also useful in the treatment of scabies (Agharkar 1991; Varier 1997; Ragasa *et al.* 2008).

T. grandis has been used in the traditional system of Indian medicine for a number of ailments including asthma, headache, leucoderma, dysentery, bronchitis and inflammation. Several classes of phytochemicals like alkaloids, glycosides, saponins, steroids, flavonoids, proteins and carbohydrates have been reported in *T. grandis* (Gupta and Singh 2004). Secondary metabolites isolated from this plant are flavone, squalene, a mixture of lupeal, B-amyrin, chlorophyllide A and hydrocarbons. Lapachol and its derivatives, methyl quinizarin and squalene from heart wood were found to have cytotoxic (Pathak *et al.* 1988), antiulcer, wound healing and anaemia activities in experimental animals (Goel *et al.* 1987; Majumdar *et al.* 2007).

This work was part of the scientific validation of the ethno pharmacological claim about the analgesic and antiinflammatory properties of stem bark extracts (Bhat 2000). To the best of our knowledge, there are no reports of stem bark extract of *T. grandis* as analgesic and antiinflammatory. Hence, we evaluated the analgesic and antiinflammatory activities of ethanolic and aqueous extracts of *T. grandis* in albino Wistar rats using hot-plate and carageenan-induced paw oedema methods. The effects were compared to indomethacin.

METHODS

Sample Collection of Plant Material

The barks of *T. grandis* were collected from the rural areas of Dehradun, Uttrakhand, India. The plant was identified and authenticated by Dr. Prashant Chaddha, scientist at the Botanical Survey of India (BSI) Forest Research Institute, Dehradun, Uttrakhand, India.

Processing of Sample

The fresh stem barks of *T. grandis* were shade dried at temperature of 25°C–35°C for 12–15 days. The dried material was preserved in air tight containers until further use. The dried

barks were powdered in a grinder and weighed before extraction to calculate the yield of extract.

Preparation of the Extracts

The alcoholic and aqueous extracts of dried powder (500 gm) of the stem barks were prepared by using Soxhlet and simple maceration methods, respectively. The alcoholic extract was concentrated to dryness under reduced pressure and controlled temperature (48°C–50°C) with a rota vapor. The extract was dried in order to produce a dark brown solid extract (Asif and Kumar 2009). The dark brown extract was then subjected to various qualitative phytochemical investigations for the identification of the different phytochemical components. These extract were used for further biological investigation.

Animals

Male and female Wistar rats (200–300 g) were obtained from the Animal House of Guru Ram Das (PG), Institute of Management and Technology, Dehradun, two weeks prior to the experiment for acclimatisation. The rats were maintained under standard animal housing conditions [25±5°C, 40%–70% relative humidity (RH), 12 h light/dark cycle] and had access to food and water *ad libitum* (5 rats per group for each test). They were fasted for 12 h before test. All pharmacological activities were carried out as per Committee for the Purpose of Control and Supervision of Experiments on Animals (Regn, No. 1145/a/07/CPCSEA) norms after obtaining the approval from the Institutional Animal Ethical Committee (IAEC) of Guru Ram Das (PG) Institute of Management and Technology, Dehradun, India.

Experimental Design

Forty albino rats of either sex were taken and divided into 8 groups, each consisting of 5 rats each. Drugs were administered to all the groups (normal, control, tests and standard) through p.o. route, 30 min prior to administration of 1% carrageenan (0.1 mL i.p.) in the sub plantar region of right hind paw. Sodium carboxyl methyl cellulose (CMC) did not produce evident changes in activity response.

Group I (control group): 0.5% sodium CMC in distilled water at 10 mL/kg body weight.
Group II (standard group) for analgesic activity: Paracetamol (50 mg/kg) suspension in 0.5% sodium CMC served as standard drug at 10 mL/kg body weight.
Group II (standard group) for antiinflammatory activity: Indomethacin (10 mg/kg) suspension in 0.5% sodium CMC served as standard drug at 10 mL/kg body weight.
Group III, IV and V (test groups): TGEE (test drugs) suspension in 0.5% sodium CMC (100, 300, 500 mg/kg) at 10 mL/kg.
Group VI, VII and VIII (test groups): TGAE (test drugs) suspension in 0.5% sodium CMC (100, 300, 500 mg/kg) 10 mL/kg and 0.1 mL of 1% carrageenan in 0.9% NaCl was administered into the sub plantar surface of right hind paw of the animals (Winter, Risley and Nuss 1962).

Analgesic Effect in the Hot Plate Test

Wistar rats randomly assigned to 8 groups received through p.o. the control vehicle [distilled 0.5% CMC (0.5 mL/kg)] for the first group and different doses of TGEE and TGAE (100, 300 and 500 mg/kg) for the 6 following groups. The second group served as positive control and received through p.o. the paracetamol (50 mg/kg). Analgesic activity was measured 1 h after administration of extracts and standards drugs (Woolfe and MacDonald 1944). Each rat was placed on Eddy's hot plate at 55±0.5°C and the pharmacological activity was estimated by measuring the latency period preceding the animal reaction of licking its hind paw or jumping.

Antiinflammatory Activity on Carrageenan-induced Paw Oedema

The two TGEE and TGAE extracts (Adzu *et al.* 2003; Costa-Lotufo *et al.* 2004; Puchchakayala *et al.* 2008; Vadivu and Lakshmi 2008) presenting the best analgesic potential were tested for antiinflammatory activity on carrageenan-induced paw oedema, according to Winter, Risley and Nuss (1962). The animals were divided into 9 groups of 5 rats. The negative control group received distilled water (0.5 mL/kg, p.o.), the positive control group received the NSAID indomethacin (10 mg/kg, p.o.) and the test groups received the extracts at the doses of 100, 300 and 500 mg/kg, p.o. The test was conducted using a plethysmometer. Carrageenan 1% (0.1 mL) was injected subcutaneously in the plantar surface of the rat's right hind paw 1 h after oral administration of drugs to induce a progressive swelling of the paw. The paw volume, up to the tibiotarsal articulation, was measured at 0 h (before carrageenan injection) and at 0.5, 1, 2, 3 and 4 h later.

Phytochemical Screening

The extracts were then subjected to preliminary phytochemical screening using standard procedures (Shah and Qadry 1995; Kokate 2001). The phytochemical screening was carried out using phytochemical tests or reagents like Mayer's reagent (potassium mercuric iodide), Hager's reagent (saturated picric acid sol.), Wagner's reagent (potassium iodate) and Deagendroff's reagent (potassium bismuth iodide) for alkaloids; Molisch's test, Benedict's test and Fehling's test for carbohydrates; Legal's test and Baljet test for cardiac glycosides; foam test for saponins glycosides; Salkowski's test for steroids; dilute $FeCl_3$ sol. (5%) test and lead acetate test for phenolic compounds and tannins; Millon's test, Biuret test and ninhydrin test for proteins and amino acids; oil stain test for fixed oil; Salkowski test and Libermann-Burchard test for sterols and steroids; lead acetate test and Mg and HCl tests for flavonoids.

Acute Toxicity Test Studies

Acute toxicity study was carried out for TGEE and TGAE extracts using Acute Toxic Class Method as described in Organization of Economic Cooperation and Development (OECD) guidelines no. 423. Acute toxicity studies were carried out on Wistar rats according to standard procedures. Both TGEE and TGAE at doses of 50, 100, 300, 1000 and 2000 mg/kg body weight were administered to separate groups of mice (n=5) after overnight fasting. Subsequent to administration of drug extract, the animals were observed closely for the first 3 h for any toxic manifestations such as increased locomotor activity, salivation, clonic

convulsion, coma and death. Subsequent observations were made at regular intervals for 24 h. The animals were observed further for a week (Ecobichon 1997).

Statistical Evaluation

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. All values were expressed as mean ± standard error mean (SEM).

RESULTS

Analgesic and Antiinflammatory Activities

The TGEE and TGAE of stem bark of *T. grandis* given by oral route in rat showed significant dose-dependent analgesic and antiinflammatory activities (p<0.001) at doses of 100, 300 and 500 mg/kg. Both TGEE and TGAE bark extracts at 500 mg/kg exhibited significant activity within 15 min which lasted up to 120 min while the higher dose of the same extract showed significant activity after 30 min which gradually decreased after 60 min (Table 1).

Table 1: Analgesic activity of the ethanolic and aqueous extracts of stem bark of *T. grandis*.

Groups	Dose (mg/kg)	Reaction time (s)				
		0 min	15 min	30 min	60 min	120 min
Control	–	4.9±0.21	4.9±0.14	4.9±0.21	5.0±0.14	5.1±0.08
Standard	50	4.7±0.11	5.3±0.10	6.0±0.18^a	6.5±0.16^c	6.7±0.11^c
Ethanol	100	4.9±0.09	4.9±0.16	5.3±0.12	5.8±0.08^b	6.0±0.20^b
Ethanol	300	4.8±0.20	5.2±0.22	5.6±0.15	6.0±0.15^b	6.2±0.15^c
Ethanol	500	4.7±0.12	5.2±0.16	5.7±0.14	6.2±0.22^c	6.3±0.18^c
Aqueous	100	4.8±0.14	5.0±0.11	5.4±0.07	5.9±0.21^b	6.0±0.14^b
Aqueous	300	4.9±0.08	5.2±0.21	5.6±0.09	6.1±0.15^c	6.4±0.17^c
Aqueous	500	4.7±0.14	5.0±0.09	6.0±0.12^a	6.5±0.20^c	6.8±0.11^c

Notes: Values expressed as mean±SEM, n=5 in each group p^a<0.05, p^b<0.01, p^c<0.001, compared with control. p<0.05 indicates significant and p^a<0.001 is extremely significant when compared with control.

Aqueous and ethanol extracts of *T. grandis* had an antiinflammatory effect at 100, 300 and 500 mg/kg, respectively (Table 2). The TGAE had higher antiinflammatory potential than indomethacin, while the activity of the TGEE was approximately similar with that of indomethacin. The TGAE was more active than the standard product, paracetamol (for analgesic) and indomethacin (for antiinflammation) (p<0.001) but this activity was less important for the TGEE, at the dose of 500 mg/kg.

Extraction Yield

Extraction yield values for both TGEE and TGAE extracts were found to be 44.45 g (8.8%) and 43.50 g (8.7%), respectively.

Phytochemical screening

Table 3 shows the results of preliminary phytochemical screening that revealed the presence of alkaloids, saponins, flavonoids, glycosides, tannins, steroids, carbohydrates, proteins and amino acids in the ethanolic as well as aqueous extracts.

Table 2: Comparative studies of antiinflammatory activity of ethanolic and aqueous stem bark of *T. grandis*.

Group	Dose (mg/kg)	Inflammation volume (mL)				
		30 min	1 hr	2 hr	3 hr	4 hr
Control	–	0.64± 0.03	0.88± 0.05	1.0± 0.02	1.22± 0.04	1.35± 0.03
Standard	10	0.51± 0.042	0.60± 0.03^{c}	0.64± 0.04^{c}	0.86± 0.03^{c}	0.90± 0.05^{c}
Ethanol	100	0.58± 0.03	0.72± 0.06	0.79± 0.04^{a}	1.05± 0.03^{a}	1.1± 0.32^{a}
Ethanol	300	0.56± 0.05	0.66± 0.03^{a}	0.74± 0.02^{b}	1.0± 0.04^{b}	1.04± 0.06^{b}
Ethanol	500	0.52± 0.04	0.63± 0.04^{b}	0.69± 0.03^{c}	0.95± 0.02^{c}	1.0± 0.06^{c}
Aqueous	100	0.54 ± 0.03	0.70± 0.02	0.75± 0.06 ^{b}	0.99± 0.03 ^{b}	1.06± 0.05^{b}
Aqueous	300	0.53± 0.03	0.68± 0.03^{b}	0.73± 0.03^{b}	0.9± 0.03^{c}	0.96± 0.05^{c}
Aqueous	500	0.51± 0.02	0.60± 0.04^{b}	0.63± 0.05^{c}	0.84± 0.02^{c}	0.88± 0.03^{c}

Notes: Values expressed as mean±SEM, n=5 in each group $p^{a}<0.05$, $p^{b}<0.01$, $p^{c}<0.001$, compared with control. $p<0.05$ indicates significant and $p^{a}<0.001$ is extremely significant when compared with control.

Acute toxicity activity

Acute toxicity studies did not reveal any toxic symptom or death in any of the animal up to the dose of 2000 mg/kg body weight of the both stem bark extract of TGEE and TGAE.

DISCUSSION

NSAIDs such as paracetamol used in this study are known to inhibit cyclooxygenase enzymes I and II which are implicated in the production of inflammation mediating agent prostaglandin (PGE2) from arachidonic acid (Parmar and Ghosh 1980; Dhara *et al.* 2000).

Table 3: Preliminary phytochemical screening of *T. grandis*.

Name of compounds	Test performed	Observation	Ethanol extract	Aqueous extract
Alkaloids	a. Mayer's	Cream ppt.	+	+
	b. Hager's	Yellow ppt.	+	+
	c. Wanger's	Reddish brown	+	+
	d. Dragendroff's	Brown colour	+	+
Carbohydrates	a. Molisch	Purple ring	+	+
	b. Fehling solution	Brick red ppt.	+	+
Cardiac glycosides	a. Legal's	Pink ppt.	+	+
	b. Balget's	Orange colour	+	+
Saponin glycosides	Foam	Foam formation	+	+
Steroids	Salkowski's	Deep red sol.	+	+
Tannins	Lead acetate	White ppt.	+	+
	Gelatin (1%)	White ppt.	+	+
Proteins and amino acids	a. Biuret	Violet colour	+	+
	b. Ninhydrin	Purple colour	+	+
Flavonoids	FCl_3	Yellow colour	+	+

Note: +: present; ppt: precipitate; sol: solution

The pattern of antiinflammatory and analgesic activities exhibited by these extracts were similar to that of paracetamol in which suggests that the plant's activity may be mediated by cyclooxygenase I and II inhibition. The observation that both extracts increased pain threshold of animals could be due to inhibition of sensitisation of pain receptors by prostaglandins at the inflammation site (Barar 2006).

The development of oedema induced by carrageenan corresponded to the events in the acute phase of inflammation mediated by histamine, bradykinin and prostaglandins produced under an effect of cyclooxygenase. Carrageenan induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The first phase is due to release of histamine and serotonin. The second phase is caused by the release of bradykinin, proteases, prostaglandins and lysosomes (Castro *et al.* 1968). Prostaglandins play a major role in development of second phase of reaction that is measured at 3 hrs. These mediators take part in the inflammatory response and are able to stimulate nociceptors and thus induce pain (Di Rosa 1972; Flower and Vane 1974; Dray 1995; Vane and Botting 1995; McGaw, Jager and Van Staden 1997; Serhan and Savill 2005). Carrageenan-induced oedema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non steroidal antiinflammatory agents that is inhibition of cyclooxygenase in prostaglandin synthesis (Phillipson and Anderson 1998). Based on these reports it may be concluded that the inhibitory effect of the TGEE and TGAE of *T. grandis* (100, 300 and 500 mg/kg) on carrageenan-induced inflammation in rats could be due to inhibition of the cyclooxygenase in prostaglandin synthesis.

Phytochemically, the TGEE and TGAE of stem bark of *T. grandis* showed the presence of phytochemicals like alkaloids, saponins, flavonoids, glycosides, tannins, steroids, carbohydrates, proteins and amino acids. The antiinflammatory and analgesic activities of many plants have been attributed to their saponin (Owoyele *et al.* 2005), flavonoids and steroids contents (Viana *et al.* 1997; Just *et al.* 1998; Adedapo *et al.* 2008). Indeed, most of these compounds are known for their analgesic or antiinflammatory activity (Viana *et al.* 1997; Just *et al.* 1998). We will have to determine which of these compounds are actually present in the most active fraction and to test if their presence explains all the plant activity or if we have to look for other compounds.

These findings should encourage the development of new antiinflammatory drugs in the future. More studies are required to find out more specific biochemical, pharmacological and molecular aspects of the targeted molecules. Further works on the isolation of bioactive phytoconstituents of *T. grandis* should be carried out to provide the exact mechanism of inhibition of inflammation and pain.

CONCLUSION

These experimental results have established a pharmacological evidence for the folklore claim of the drug to be used as an analgesic and antiinflammatory agent. These studies also provide new scientific information about the analgesic and antiinflammatory activities of stem extract of *T. grandis*. These activities may be attributed to the various phytochemical constituents present in the extracts.

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