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Evaluation of anti-inflammatory and analgesic potential of methanol extract of *Tectona grandis* flowers

Ramachandran S[1*], Rajini kanth B[2], Rajasekaran A[3], Manisenthil Kumar KT[1]

[1]*Department of Pharmacology, KMCH College of Pharmacy, Coimbatore – 641 048, Tamil Nadu, India*
[2]*Department of Pharmacology, Narayana Pharmacy College, Nellore-542 002, Andhra Pradesh, India*
[3]*Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore – 641 048, Tamil Nadu, India*

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ABSTRACT

Objective: To evaluate anti-inflammatory and analgesic activity of methanol extract of *Tectona grandis* (*T. grandis*) flowers (METGF) in animal models to support its traditional use. **Methods:** Acute toxicity study was performed to determine the toxicity level of METGF in mice and rats. Carrageenan (1% w/w) was administered and inflammation was induced in sub-plantar region of rat paw. Analgesia was induced by intraperitoneal injection of 0.6% v/v acetic acid in mice to assess peripheral analgesic action of METGF. Also, hot-plate was used to induce pain in mice to evaluate central analgesic action of METGF. **Results:** Oral administration of METGF in rat and mice did not produce any toxicity at 2000 mg/kg dose level. In carrageenan induced inflammation, administration of METGF (100 and 200 mg/kg) as well as indomethacin (10 mg/kg) reduced rat paw edema significantly at 3, 4 and 5 h. Both the doses of METGF showed significant inhibition in acetic acid induced writhing responses. In thermally induced analgesia models, administration of METG at dose of 100 and 200 mg/kg significantly increased reaction time compared to control animals. The preliminary phytochemical analysis revealed the presence of phenolic compounds and tannins in METGF. **Conclusions:** Present study, for the first time, confirms the anti-inflammatory and analgesic action of METGF and it supports the traditional use of *T. grandis* flowers.

1. Introduction

Plants are the basis for traditional medicine systems and used for thousands of year in countries such as India and China. Numbers of medicinal plants and its derived extracts are used in the treatment of various disorders by Ayurveda, Unani and Siddha systems in India. Scientifically, few of pharmacological properties has been studied to supports its traditional use[1]. Throughout the world many plant species are used for the treatment of inflammation and other diseases. Despite the availability of anti-inflammatory and analgesic agents searching of newer therapeutic agent in this segment from the natural plants is still progressing due to presence of diverse chemical substances that have an better alternative and safer effect on inflammation without or lesser side effects. The World Health Organisation (WHO) estimates that 80% of the world's inhabitants continue to rely on traditional medicines systems and its products[2]. Traditionally used plants possess different therapeutic uses and such properties were well documented[3-9].

Tectona grandis (*T. grandis*) Linn. f. (Verbenaceae), commonly known as teak, has long been used in the treatment of various disorders in India. The flowers of *T. grandis* are acrid, bitter, refrigerant, depurative, diuretic and anti-inflammatory[10]. The current literature survey revealed that there is no scientific documentation on flowers of *T. grandis* for its anti-inflammatory and analgesic activity to support its traditional use. The preliminary phytochemical analysis of methanol extract of *T. grandis* flower (METGF) showed presence of tannins and phenolic compounds which prompted us to evaluate its pharmacological activity[11]. Therefore, present study was aimed to investigate the anti-inflammatory and analgesic efficacy of methanol extract of *T. grandis* flowers in carrageenan induced rat paw edema and thermally induced analgesia animal models.

*Corresponding author: Ramachandran S, Department of Pharmacology, KMCH College of Pharmacy, Kovai Estate, Kalapatti Road, Coimbatore – 641 048, Tamil Nadu, India.
Tel: +914222917282
Fax: +914222369302
E-mail: src28@rediffmail.com

2. Materials and methods

2.1. Plant materials collection and identification

T. grandis Linn. f. (Verbenaceae) flowers were collected from Annaikatti, Coimbatore, Tamil Nadu, India. The specimen was authenticated at Botanical Survey of India (BSI), Coimbatore (BSI/SC/5/23/09-10/Tech.-486) and its certificate was documented in our laboratory. The shade dried, powdered flowers were stored in air-tight container.

2.2. Extraction of plant material

The powdered plant material (60 g) was extracted with petroleum ether (60 ℃-80 ℃), ethyl acetate and methanol using soxhlet apparatus for 72 h. All extracts were concentrated under reduced pressure using rotary-evaporator and stored at 2 ℃- 8 ℃ until the completion of pharmacological studies and yield of METGF alone was 21.62% w/w.

2.3. Drugs and chemicals

Carrageenan and acetic acid were procured from Himedia Laboratories, Mumbai, India. The reference drugs aspirin, indomethacin, pentazocine were commercially purchased.

2.4. Experimental animals

Female and male Wistar albino rats (180-200 g) and male Swiss albino mice (25-30 g) were used to assess anti-inflammatory and analgesic activity of METGF. All animals were kept and maintained under standard laboratory conditions [temperature (22 ± 2) ℃, humidity (45 ± 5) ℃ and 12 h light: 12 h dark cycle]. The animals were fed with standard laboratory diet and allowed to drink water *ad libitum*. The studies were carried out in accordance with institutional animal ethical guidelines for the care of laboratory animals of KMCH College of Pharmacy, Coimbatore, India. The study was initiated after the approval of institutional animal ethical committee (KMCRET/MPharm/41/09).

2.5. Acute toxicity

Female Wistar albino rats (150-180 g) and female Swiss albino mice (25-30 g) were used to assess the toxicity level. Acute oral toxicity study was performed as per Organization for Economic Cooperation and Development guidelines[12]. The METGF at dose of 2 000 mg/kg was administered to 3 female rats and mice in a single dose by gavage using oral needle. If 2 rat and mice died or 2-3 rat and mice are alive out of 3 rat and mice after 24 h, another 3 rat and mice were received METGF 2 000 mg/kg as per guidelines to find the acute toxicity. The rats were fasted over-night and mice were fasted 3 h prior to dosing (food was withheld for 3 h but not water). During the period of fasting, rat and mice was weighed and METGF was administered. After the METGF administration, food was withheld for 2 h. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. Animals are removed, if any humanely killed for animal's welfare reasons or are found dead. The post-mortem examination was carried out in dead animals to determine cause of death.

2.6. Carrageenan induced rat paw edema

Acute inflammation was produced by sub-plantar injection of 0.1 mL of 1% w/v suspension of carrageenan in normal saline, in the right hind paw of the rats, 1 h after oral administration of the doses of METGF, positive and negative control substances to the overnight fasted rats of respective groups. The rats were divided into four groups of six animals in each group. Group 1 served as negative control which received propylene glycol (5 mL/kg); group 2 served as positive control which received indomethacin (10 mg/kg); group 3 and 4 served as extract treatment which received METGF at 100 mg/kg and 200 mg/kg. The paw volume was measured plethysmographically (Inco-Niviqure, Version 60.1, India) at 0, 1, 3, 4 and 5 h after the carrageenan injection. The indomethacin and METGF were freshly suspended in propylene glycol just before oral administration[13,14].

2.7. Analgesic activity

2.7.1. Acetic acid induced writhing response

Male Swiss albino mice were divided into four groups with six animals in each group. Group 1 served as normal control and received propylene glycol (5 mL/kg); group 2 received standard drug treatment with aspirin (100 mg/kg); group 3 and 4 received METGF at doses of 100 mg/kg and 200 mg/kg which served as extract treatment. The acetic acid 0.6% v/v (10 mL/kg, *i.p.*) was injected 1 h after administration of the METGF, positive and negative control substances to the respective groups. After administration of acetic acid, number of writhes (abdominal muscle contraction together with turning of trunk and extension of hind limbs) was counted over a period of 20 min[14,15]. Aspirin and METGF were suspended in propylene glycol before administration and given orally.

2.7.1. Eddy's hot-plate mediated pain reaction

Male Swiss albino mice were divided into four groups of six animals in each group. Group 1 served as control which received propylene glycol (5 mL/kg); group 2 served as reference drug treatment which received pentazocine (5 mg/kg, oral); group 3 and 4 served as METGF treatment

which received drug at a dose of 100 mg/kg and 200 mg/kg respectively. The animals were placed on the hot plate, maintained at (55±1) ℃, and the basal reaction time such as licking of paw or jumping response whichever appeared first was recorded at 0 min. The cut-off period of 15 seconds was established to prevent damage to the paws[14,15]. The reaction time was reinvestigated at 30, 60, 120 and 240 min after the treatment and changes in the reaction time were noted.

2.8. Statistical analysis

All the data were expressed as Mean ± SEM and evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons using prism graphpad version 5.0 and values of $P < 0.05$ were considered as statistically significant.

3. Results

3.1. Acute toxicity

In rat and mice, oral administration of METGF at the dose of 2000 mg/kg does not exhibited any signs of toxicity up to 14 days and no animals died. This indicates that METGF was nontoxic in rat and mice up to an oral dose of 2000 mg/kg of body weight. Therefore, the biological evaluation was carried out using 100 mg/kg and 200 mg/kg dose levels.

3.2. Carrageenan induced rat paw edema

In carrageenan induced rat paw edema, administration of indomethacin 10 mg/kg and METGF 100, 200 mg/kg reduced inflammation significantly ($P < 0.01$ and $P < 0.05$) from 3 h onwards compared to control group (Figure 1). The anti-inflammatory action of both the doses of METGF was persistent up to 5 h. The METGF treatment reduced inflammation in a dose-dependent effect in manner.

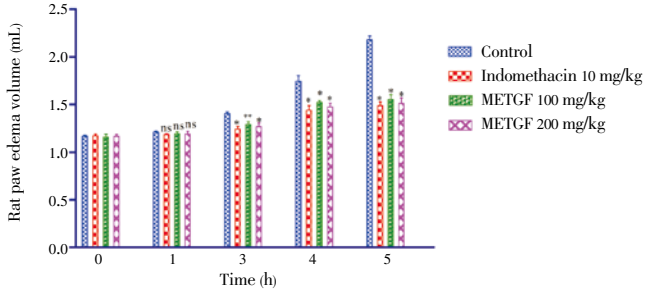

Figure 1. Effect of METGF on carrageenan induced paw edema in rats.
Mean ±SEM (n=6). *$P < 0.01$ METGF 100 and 200 mg/kg, indomethacin 10 mg/kg compared with control group; **$P < 0.05$ METGF 100 mg/kg compared with control group; ns – No significance.

3.3. Acetic acid induced writhing response

In the administration of acetic acid produced writhing responses. METGF pre-treatment at 100, 200 mg/kg and aspirin significantly ($P < 0.001$) inhibited writhing responses compared to control group (Figure 2). Also, administration of METGF 200 mg/kg showed significant ($P < 0.001$) higher inhibition of writhing responses than METGF 100 mg/kg dose.

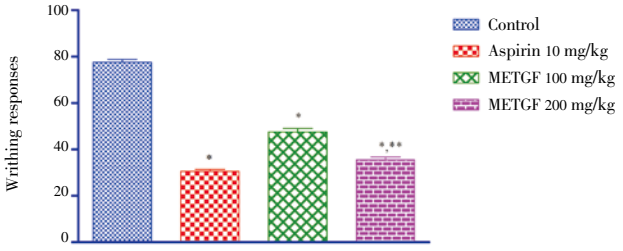

Figure 2. Effect of METGF on acetic acid induced writhing response in mice.
Mean ± SEM (n=6). *$P < 0.001$ METGF 100, 200 mg/kg and aspirin 100 mg/kg compared with control group; **$P < 0.001$ METGF 200 mg/kg compared with METGF 100 mg/kg group.

3.4. Eddy's hot-plate mediated pain reaction

The administration of METGF (100, 200 mg/kg) and pentazocine showed significant ($P < 0.05$ and $P < 0.01$) increase in reaction time compared with control group (Figure 3). The METGF 200 mg/kg produced significantly ($P < 0.001$) higher analgesic efficacy than METGF 100 mg/kg dose.

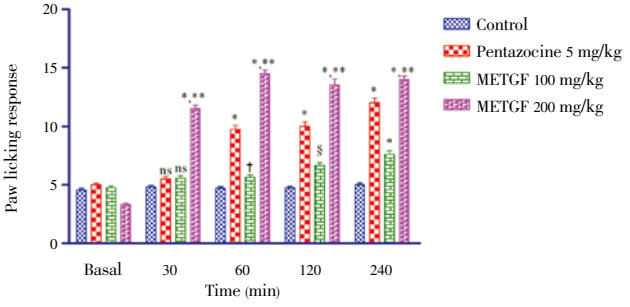

Figure 3. Effect of METGF on thermally induced analgesia (Eddy's hot-plate) in mice.
Mean ±SEM (n=6). *$P < 0.001$ METGF 100, 200 mg/kg and pentazocine 5 mg/kg compared with control group. **$P < 0.001$ METGF 200 mg/kg compared with METGF 100 mg/kg group. †$P < 0.05$ METGF 100 mg/kg compared with control group. §$P < 0.01$ METGF 100 mg/kg compared with control group. ns – No Significance.

4. Discussion

The carrageenan induced inflammation was used as a standard model of screening for anti-inflammatory activity in various experimental compounds. It is commonly used due to absence of apparent systemic effects, antigenic nature of carrageenan and highly reproducible model. An edema induced by carrageenan is a biphasic response. The inflammatory mediators such as histamine, serotonin and kinins are released in the first phase. The prostaglandin

released in the second phase and slow reacting substances peak at 3 h[16-20]. The METGF treated animals showed good anti-inflammatory activity at 3, 4 and 5 h in carrageenan induced inflammation compared with control animals. This action may be due to inhibition of the above inflammatory mediators release by METGF.

We have studied analgesic effect of METGF in two different animal models with the aim to identifying its possible peripheral and central action. In acetic acid induced writhing responses, visceral pain model, abdominal constrictions induced in mice results from an acute inflammatory reaction with production of prostaglandin E2 and F2 in the peritoneal fluid[15]. Therefore, analgesic action of METGF may be due to suppression of the formation of above substances or prevent their action. In central pain model, hot-plate induced analgesia was caused by the direct activation of nociceptors and it will inhibited by centrally acting drugs like morphine and other opioid class of drugs. This antinociceptive action is hypothesised to reflect analgesia at supraspinal level[21-23]. The increase in reaction time after administration of METGF may mediate its action via opioid receptor. In both models, administration of METGF inhibited analgesia in a dose dependent manner and it reflects the peripheral and central analgesic action. The anti-inflammatory and analgesic action may be due to presence of phenolic compounds and tannins in METGF.

The present study findings clearly represent that methanol extract of *T. grandis* flowers possess anti-inflammatory, peripheral and central analgesic action. These findings support the traditional use of *T. grandis* flowers.

Conflict of interest statement

We declare that we have no conflict of interest.

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