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Antiinflammatory activity of methanolic and petroleum ether extracts of *Tectona grandis*

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ABSTRACT

In the present investigation the methanolic and petroleum ether extracts of *Tectona grandis* seeds were evaluated for anti-inflammatory activity using carrageenan induced foot pad edema model. Three different doses of each extracts i.e. 100, 200 and 400 mg/kg were used in this regard. Results of this study revealed that both the extracts showed significant and dose dependant anti-inflammatory activity by reducing the foot pad edema induced by carrageenan which proved the anti-inflammatory potential of *Tectona grandis* seeds.

Keywords: *Tectona grandis*, anti-inflammatory, carrageenan, foot pad edema

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INTRODUCTION

Pain and inflammatory process involves a series of events that can be elicited by numerous stimuli such as infectious agents, ischaemia, antigen-antibody interaction and thermal or physical injury (Insel, 1990) [1]. It is usually associated with pain as a secondary process resulting from the release of analgesic mediators (Huuskaar and Hole, 1987) [2]. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics or non-narcotics (NSAIDs) and present well known side and toxic effects[3,4,5]. On the contrary herbal medicines with good absorption, less toxicity and easy availability have been used since long[6]. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper and effective drugs[7,8]. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drug[9].

Tectona grandis Linn. commonly known as teak, a member of the family Verbenaceae has been scientifically documented to possess many pharmacological activities such as antibacterial, antifungal, antipyretic, antilithiatic, antioxidant, antitumour, wound healing, antiulcerogenic, anti-diabetic, tocolytic, antiangiogenic and hair growth stimulator activities. Some of the major phytoconstituents reported from *T. grandis* are anthraquinones (tectoquinone; 2-methylantraquinone), naphthoquinone (lapachol, juglone), triterpenes (betulin aldehyde), apocarotenoids, gallic acid, rutin, quercetin, ellagic acid derived from various parts of the plant. Others include commonly occurring compounds such as cellulose, holocellulose, lignin and mineral substances (Nayeem and Karvekar, 2010) [10-14]. Its seeds are traditionally documented for antiinflammatory activity which is not yet been explored in any scientific journals. The present investigation was carried out to evaluate the antiinflammatory activities of two extracts i.e. methanolic (MTG) and petroleum ether (PTG) extracts of *Tectona grandis* seeds using carrangenan induced foot pad edema in rats.

MATERIALS AND METHODS

Plant material

Commercially available dry seeds of *Tectona grandis* Linn. were purchased in the bulk quantity from local market and authenticated by the botanist from the Agriculture College, Pune.

Preparation of extract

The methanolic and petroleum ether extracts of *Tectona grandis* were prepared using appropriate methods.

Chemicals and drugs

Indomethacine and carrageenan were purchased either from approved vendors or from the local market as applicable.

Preparation of drug solution

Accurately weighed quantities of both the powdered extracts were dissolved in distilled water to prepare the appropriate stock solution of the drug from which the different doses were administered by selecting the appropriate concentration of the stock solution.

Animals

Wistar albino mice (18 – 22 g) and rats (120 – 150 g) were used. They were maintained at $25 \pm 2^\circ \text{C}$ and relative humidity of 45 to 55% and under standard environmental conditions (12 hr. light 12 hr. dark cycle). All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of AISSMS College of Pharmacy, Pune, constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), approval no. (CPSEA/IAEC/PC-02/05-2K11). All the experiments were carried out between 9:00- 16:00 hours and ethical guidelines were strictly followed during all the experiments. The respective animals were shifted from animal house to the laboratory 1 hour prior to the start of the experiment to avoid any error in the results due to change in location and environment.

Preliminary acute toxicity test

Healthy adult male wistar albino mice (18- 22g) were subjected to acute toxicity studies as per guidelines (AOT 423) suggested by the OECD-2000 (OECD Guidelines). The rats were continuously monitored immediately from dosing up to next 4 hours for behavioral, neurological and autonomic profiles. The animals were observed after 24 hours and everyday for next 14 days for any sign of toxicity or mortality [15].

Preliminary Phytochemical Analysis of extracts

The both extracts were tested for the presence of various chemical constituents in it (Khandelwal, 2006; Kokate, 1997) [16].

Method for Anti inflammatory activity[17]

48 rats were divided into 8 groups, each containing 6 rats. Group I served as Carrageenan control and received vehicle i.e. distilled water p.o., Groups II–VII received MTG (100, 200 and 400 mg/kg, p.o.) and PTG (100, 200 and 400 mg/kg, p.o.) respectively and group VIII served as standard group and received reference standard Indomethacine (10 mg/kg). These treatments were given for the period of 10 days. On the 10th day, 1 hour after the dosing,

all animals were injected with 0.1 ml of 1% Carrageenan into sub plantar region of hind paw and paw volume was measured at 3rd, 6th and 24th hour using digital plethysmometer.

RESULTS

Preliminary Phytochemical screening of the *Tectona grandis* Linn extract

Preliminary phytochemical analysis of methanolic extract of *Tectona grandis* showed the presence of glycosides, flavonoids, tannins, steroids while petroleum ether extract showed the presence of carbohydrates, saponins, glycosides, flavonoids, tannins, steroids.

Acute oral toxicity study (AOT 425)

Oral administration of methanolic and petroleum ether extract of *Tectona grandis* up to the dose of 2000 mg/kg to the respective rats did not show any serious adverse effects or mortality observed continuously for 04 hours and everyday for next 14 days. From this data and pilot study performed at laboratory, three different doses 100, 200 and 400 mg/kg were selected for further study.

Anti inflammatory activity of Methanolic extract of *Tectona Grandis* (PTG)

The MTG 100 mg/kg treatment showed significant ($p < 0.05$) decrease in inflammation at 3rd and 6th hour when compared with vehicle treated control group. The MTG 200 mg/kg was effective at all three time intervals but was more significant ($p < 0.01$) at 3rd than 6th and 24 hour ($p < 0.05$) whereas MTG 400 mg/kg and indomethacine 10 mg/kg treatment was more significant ($p < 0.01$) at 3rd and 6th hour than 24th hour ($p < 0.05$) (See Figure 1).

Anti inflammatory activity of Petroleum ether extract of *Tectona Grandis* (PTG)

The PTG 200 and 400 mg /kg showed dose dependent reduction in inflammation compared to vehicle treated control rats at 6th hour only. Rest all other doses at all other intervals were ineffective. The reference standard indomethacine showed significant ($p < 0.01$) decrease in inflammation at 3, 6 hour and ($p < 0.05$) 24 hour respectively.

DISCUSSION

Medicinal plants are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches with lesser side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilise eco-friendly and bio friendly plant-based products for the prevention and cure of different human diseases. Considering the adverse effects of synthetic drugs, the world's population is looking for natural remedies which are comparatively safer and equally effect[18,19]. It is documented that 80% of the world's population has faith in traditional medicine, particularly plant based drugs for their primary healthcare[20].

Tectona grandis has been claimed to be useful in treatment of pain and inflammation and the current literature survey revealed that no systematic approach has been made towards documentation of this claim. The validation of usefulness of *Tectona grandis* to combat inflammation as major determinant in variety of serious pathological states such as Alzheimer's disease, cancer, irritable bowel syndrome and hepatitis[21] have made it worthwhile to investigate anti-inflammatory effect of *Tectona grandis*. In this investigation the anti-inflammatory activities of *Tectona grandis* extracts were studied using foot pad edema model which is one of the most widely used primary test to screen new anti-inflammatory agents and measure the ability of a compound to reduce local edema induced in the rat foot pad by injection of an irritant agent. This edema depends on the participation of kinins and polymorphonuclear leukocytes with their proinflammatory factors including prostaglandins [22]. The development of edema in the paw of the rat after the injection of carrageenan has been described as a biphasic event. The initial phase, observed around 1 hour, is attributed to the release of histamine and serotonin; the second, accelerating phase of swelling is mainly due to the release of prostaglandin-like substances [21]. In the present study all the doses of MTG significantly decreased the inflammation at 3rd and 6th and 24th hours after treatment which was statistically equipotent to that of reference standard indomethacine. On the other hand, PTG was found to be effective at 6th hour only. The study indicates the potential of these extracts as anti-inflammatory drugs. The activity may be attributed to the inhibition of the COX-2 enzyme or inhibition of the activation of transcription factors [21].

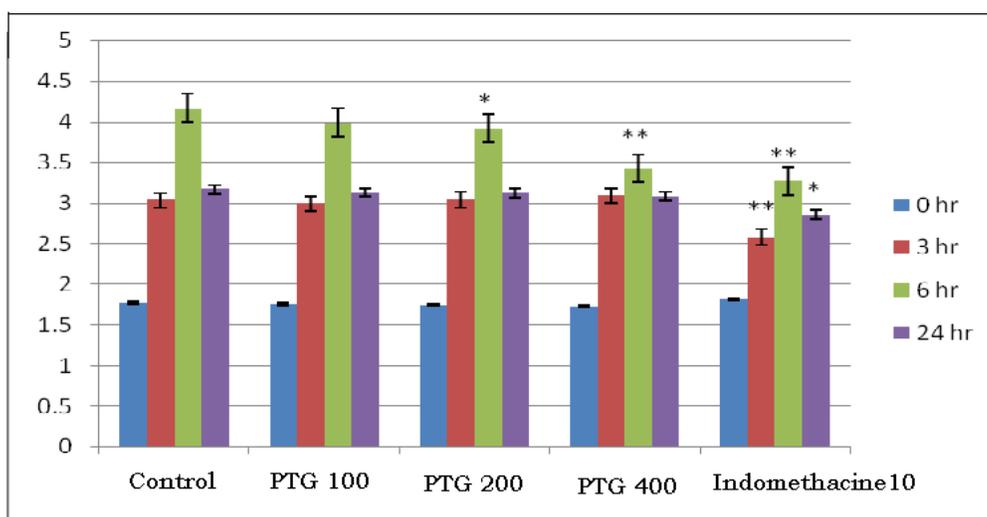


Figure 1. Effect of Various drug treatments on Inflammation (foot pad edema) in rats.

Results are expressed as Mean \pm SEM (n=6). The data was analysed using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- t test*. * $P < 0.05$, ** $P < 0.01$

CONCLUSION

The study validated the methanolic and pet ether extracts of *Tectona grandis* towards management pain and inflammation. The exact role of individual phytoconstituents needs to be illustrated using suitable bio-analytical techniques to extrapolate exact mechanism of this action.

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