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ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF ROOT OF *TECTONA GRANDIS* IN ALBINO RATS

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ABSTRACT
Following study investigates the inflammation reducing activity of the methanolic *Tectona grandis* roots extract in albino rats. Utilizing a well-established animal model, the research evaluates the extract's efficacy in reducing inflammation induced by carrageenan. Results demonstrate a remarkable decrease in paw edema in dosed groups compared to the control, indicating potent anti-inflammatory properties. Phytochemical analysis reveals the existing phytochemicals such as flavanoids, phenols, quinone and tannins, which are likely responsible for the observed effects. These findings suggest that *Tectona grandis* root extract could be a promising natural remedy for inflammatory conditions. Moreover, supplementary studies are warranted to isolate specific bioactive constituents & elucidate respective mechanisms of action.

Keywords: Anti-inflammatory, *Tectona grandis*, carrageenan, edema.

INTRODUCTION

The complicated process of inflammation is often linked to pain and includes changes to membranes, increased vascular permeability, and increased protein denaturation. The process of applying an external stress or agent like caustics or bases, a concentrated inorganic salt, an organic solvent, heat, or both, causes proteins to lose their secondary and tertiary structures. This is known as protein denaturation. When denatured, the majority of biological proteins stop functioning biologically, one well-established mechanism of inflammation is protein denaturation. Lots of

processes are involved in the mechanisms of inflammation, and arachidonic acid metabolism is one of them. Leukotrienes (LTs) and hydroperoxy-eicosatetraenoic acids (HPETEs), which are significant physiologically active mediators in a range of inflammatory events, can be obtained through the 5-lipoxygenase (5-LOX) pathway or the Cyclooxygenase (COX) pathway. Arachidonic acid is separated from membrane phospholipids by the appropriate activation of neutrophils, and it can then be transformed via the 5-LOX or COX pathways, respectively, into leukotrienes and prostaglandins. Since defunctioning of 5-lipoxygenase and cyclooxygenase tends to decrease in LT and PG production, such a medication may have anti-inflammatory characters as reducing GI side effects[1–3]

All NSAIDs minimize or fully eradicate the erythema, rubor, calor, tumor, dolor brought on by a range of infectious impulses. NSAIDs are drugs with distinct pharmacological characters. But, the exact mechanisms of action of NSAIDs are yet unknown, data indicates that prostaglandin synthesis inhibition is the primary mechanism by which their anti-inflammatory actions are accomplished. This is the way that all NSAIDs work. Each NSAID inhibits COX, an enzyme that mediates pain, inflammation, and fever by converting arachidonic acid to prostaglandins. In this process, PG H2 is transformed into five major PGs, such as vasodilator prostacyclin, which suppresses platelet clumping in the endothelium, also there is TXA2, which accelerates platelet aggregation and blood clot formation. cyclooxygenase-1 and cyclooxygenase-2 are the two widely known COX iso-enzymes. Generally speaking, COX-1 is constitutively expressed, plays a role in platelet-mediated thromboxane production, and protects against stomach acid. COX-2 has been linked to inflammation and is induced by inflammatory mediators in a variety of organs[4–13]

The teak plant, *Tectona grandis*, is a member of the Lamiaceae family. Based on an Indian ancient medical system, several repots promise to cure serious ailments. The entire plant is vital medicinally. According to the survey, the plant is used to cure headaches, bronchial disorders, and urine discharge. It is also used as an anti-diabetic, laxative, sedative, and diuretic. Many pharmacological effects, including anti-bacterial, anti-oxidant, anti-fungal, inflammation reducer, anti-pyretic, pain reducer, anti-diuretic, and anti-hyperglycemic properties, are exhibited by the main ingredient of plants. The root of *Tectona grandis* consist the phytoconstituent lapachol, tectol, tectoquinone, β-sitosterol diterpene, tectograndinol[14–17]

Methods and Materials

Plant Processes

Fresh root of *Tectona grandis* Linn. was collected for present study from Mahadev P.G College Bariyasanpur, Chiraigaon, Varanasi, Uttar Pradesh, and Dr. Vinay Ranjan, Scientist of BSI CRC, Allahabad, 211-002, verified the authenticate of the flora sample, voucher number SIP/2024/055.

Chemicals

Indomethacin was supplied by E.M. Pharmaceuticals Pvt. Ltd, Ankleshwar, Gujarat; Carrageenan and carboxyl methyl cellulose (CMC) was bought from CDH Pvt. Ltd, Daryaganj, New Delhi; and Methanol from Loba chemie Pvt. Ltd, Jehangir villa, Mumbai Maharashtra. all reagents were analytical grade, and all necessary solutions were made fresh[18,19].

Phytochemical Screening

The plant product was screened for various phytochemicals present in it. The crude extracts was put under the testing of 2° metabolites like alkoloids, flavanoids, terpenoids, phenolic groups, quinones, tannins, etc[20].

Preparation of Extract

The Soxhlet method was used to create the Methanolic (alcoholic) extracts of the dried powder (50 gm) of the root of *Tectona grandis*. The Methanolic extract was dried out by intense pressure and temperature control (48–50°C) and a rotating evaporator. To create a solid extract that is dark brown in color, the extract was dried. The individual phytochemical components were subsequently identified by putting the dark brown extract through a series of qualitative phytochemical studies. These extracts were applied to additional biological research[21].

Animals

For this study, either sex albino W. rats weighing around 160g and 210 grams were employed. In experimental purposes, they were housed in the laboratory animal facility of Shambhunath Institute of Pharmacy, Jhalwa, Prayagraj and purchased from Ms. Chakraborty Enterprises in Kolkata, West Bengal, India.

The rats were kept in controlled environments with 12-hrs illuminating surroundings and 12 hrs non-illuminating surrounding, a temp. of 25 ± 3°C, and a moisture content of 50 ± 4%. Prior to the study, all the animals underwent a seven-day acclimatization period. The rats were housed individually in sterilized polypropylene boxes with sterile bedding of rice husk, and they were divided into experimental and control groups at random. They received unrestricted access to water and regular pellets for their base diet. Before beginning the experimental phase, the animals were acclimated to the laboratory environment for 48 hours in order to reduce any potential non-specific stress. The Institutional Animal Ethics Committee gave its approval (Approval no. SIP/IAEC/008/03/24, dated March 18, 2024.) to every study that was carried out.

Acute Toxicity study

The Acute Toxic Class Method, as outlined in O.E.C.D. standards sequence 423, was used to conduct an acute toxicity investigation for TGME extracts. Standard operating protocols were followed when conducting acute toxicity experiments on Wistar rats. TGME was given to five distinct groups of mice (n = 6) at administrations of 50.0mg, 100.0mg, 300.0mg, 1000.0mg, 2000.0mg and 3000.0mg/kg body wt. following an overnight fast[21].

Carrageenan induced paw oedema model

Normal saline was provided freely during a 24 hour fast for albino Wistar rats weighing 150-200g, regardless of gender. Five groups, each with six animals, were formed from them. A 10 ml/kg aqueous 0.5% CMC suspension was given to the first group, which was the normal control gp. And second gp positive control gp was given carrageenin sol. (0.10 ml, 1 % wt./vol.) in left hind paw. The standard group is given oral suspension of Indomethacin (10 mg/kg), the standard medication. The fourth, and fifth groups received suspensions of the methanolic extract orally at dozes of 200 milligram/kg, and 400 milligram/kg. The left hind paw of rats was given 0.10 milliliter of a 1% wt./vol. carrageenin in distilled water, causing paw edema one hour later on the subplantar surface. Plethysmometer was used to measure the paw vol. prior to and half, one, two, three, and four hours following the injection of carrageenan[18].

Experimental Design

five sets of six albino rats each were developed from the thirty rats, irrespective of sex. Standard and test drugs were given by orally (p.o.) and left hind paw's subplantar area given the carrageenan solution 0.1 ml, 1% w/v. There were no noticeable alterations in the activity response when carboxyl methyl cellulose (CMC) was used.

Group. I (Vehicle): Given 0.50% CMC in water at 10.0 milliliter/kg b.w.
Group II (Diseased): Given 0.10 ml (1% wt./vol.) carrageenin sol. in hind paw via subplanter route.
Group III (Standard Control): Given std. drug Indomethacin at dose of 10.0 mg/kg body wt. of rat via oral route and carrageenan sol. 0.1 ml (1% wt./vol.) sub-plantar.
Group IV (Test control 1): Given a single dose of Carrageenin (1% wt./vol.) 0.10 ml in left hind paw (subplantar) and oral dosage of *Tectona grandis* ext. (200 milligram/kg in 0.50 % CMC).
Group V (Test control 2): Given a single dose of Carrageenin (1% wt./vol.) 0.10 ml in left hind paw (subplantar) and oral dosage of *Tectona grandis* ext. (400 milligram/kg in 0.50 % CMC).

Statistical Evaluation

One-way analysis of variance (ANOVA) was used for analyticals. The means ± standard deviation (SD) was used to express each value.

RESULT

Phytochemical Analysis

The yield of the crude *Tectona grandis* root extract was done in two solvents, respectively methanol and distilled water. The percentage yield of extract in both the solvent was found to be:

Table 01: Percentage yield:

Sr. no.	Solvent used	% yield
1.	Methanol	26.01%
2.	Distilled water	18.07%

The presence of following phytoconstituents was found in the crude extract of TG:

Table 02: Result of phytoconstituent analysis of crude powder extract of *T. grandis* root.

S.No.	Test Performed	Methanolic Ext.	Aq. Ext.	Benzene Ext.
01	**Alkaloid**	+	+	-
02	**Flavonoids**	+	+	-
03	**Tannins and Phenols**	+	+	-
04	**Anthraquinones**	++	++	--
05	**Terpenoids**	+	+	-
06	**Amino acids**	-	-	-
07	**Saponins**	+	+	-
08	**Sterols**	+	+	-

Acute toxicity Studies:

Since the *T. grandis* root extract showed no adverse effects and lethality up to 3g/kg per oral in rats, it was determined to be safe for use in other biological investigations. Merely the food intake was raised by 20% at doses of 2g and 3g/kg for four hours, but it thereafter returned to normal.

Anti-inflammatery activity on paw oedema induced by Carrageenan in animals:

In rats with paw oedema generated by carrageenin, the methanol-based extract of *T. grandis* at doses of 200.0 and 400.0 milligram/kg has been shown to considerably reduce paw oedema volume at different time intervals. Indomethacin (10milligram/kg) is implied as std. drug and it gave remarkable reduction in paw oedema in the animals. The data is tabulated in Table 03 and the similar data of comparison of rate of paw oedema at various intervals are graphically shown in the Fig.01 to Fig.05.

Table 03: Study of METG on inflammation:

Groups	Doses (mg./kg.)	Inflamed paw vol. (mL)				
-	-	**30 mins.**	**1 hour**	**2 hrs.**	**3 hrs.**	**4 hrs.**
Diseased gp	-	0.45±0.031	0.47±0.024	0.49±0.019	0.51±0.028	0.53±0.019
Std. gp	10	0.33±0.043	0.36±0.040	0.38±0.035	0.42±0.017	0.44±0.017

Test 1 gp	200	0.38±0.051	0.43±0.030	0.45±0.032	0.48±0.024	0.51±0.035
Test 2 gp	400	0.36±0.042	0.39±0.036	0.42±0.017	0.45±0.037	0.48±0.017

Notes: Values shown as mean ± Std.Dev., no.= 6 in every group

P< 0.050 indicates significant.

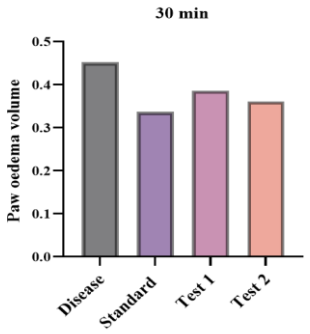

Fig. 01

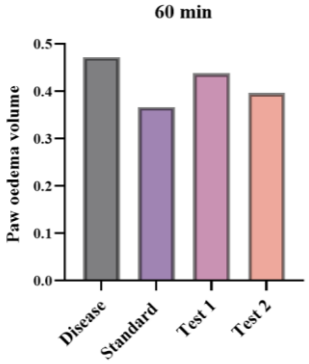

Fig. 02

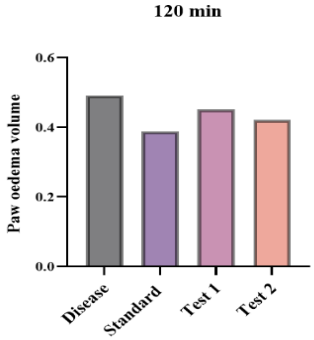

Fig. 03

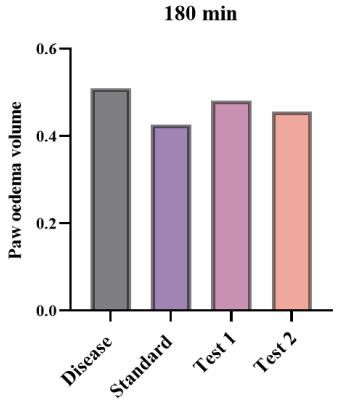

Fig. 04

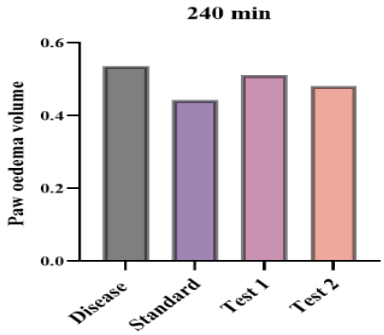

Fig. 05

Figures: Anti-inflammatory effect of METG (200mg/kg and 400mg/kg) on paw vol. in Carrageenin inducing paw edema in rats at time durations of 30, 60, 120, 180 and 240 minutes

DISCUSSION

The important phenomenon in any individual's defense system against any impairment and infection is inflammation; yet, it frequently results in unpleasant or damaging chronic disorders that need to be treated with medications. Because paw oedema generated by Carrageenan is sensitive to oral active anti-inflammatory medications, especially during the acute stage of inflammation, it can be used to test for anti-inflammatory characteristics in natural medicines.[22–24] Because of the great reproducibility of the model, antigenic nature of carrageenan, and lack of

evident systemic effects, it is widely employed.[25] A rat's paw oedema develops in two stages following a carrageenan injection. The histamine and serotonin releases are responsible for the first hour of observation. Prostaglandins, protease, and lysosome release are the causes of the second stage of oedema. Vascular permeability increases as a result, and arterioles and venules enlarge. Oedema develops as a result of plasma proteins and fluid extravasating. After four hours, oral application of TG extract of roots significantly reduced oedema by percent. Thus, the release of inflammation-related mediators may be inhibited by TG root extract. [26]

CONCLUSION

Tectona grandis extracts considerably decreased inflammation in the current investigation. Oxidative stress may be the cause of the inflammation by generating inflammatory mediators. The current study notes that the presence of plant-based constituents such as flavonoids, phenols, quinone and tannins reduces inflammatory mediators and oxidative stress. For the aforementioned phytoconstituents, the activity on inflammation might be consistent.

For determination of the precise mechanism of phytoconstituents causing the aforementioned activities, more research is necessary.

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