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Review Article

Evaluation of the Anti-Inflammatory Activity of Methanolic and n-Hexane Root Extracts of Terminalia paniculata Roth in Formalin-Induced Paw Oedema Rats

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Abstract

Inflammation is a complex biological response associated with various pathological disorders. The present study was designed to evaluate the anti-inflammatory potential of methanolic extract (METP) and n-hexane extract (HETP) obtained from the roots of *Terminalia paniculata* Roth using a formalin-induced paw oedema model in rats. The roots were collected, authenticated, and extracted successively using methanol and n-hexane solvents. Acute toxicity studies were conducted according to OECD guideline 425 and both extracts were found to be safe up to 2000 mg/kg body weight. Anti-inflammatory activity was assessed in female Wistar rats by measuring paw oedema volume at different time intervals following formalin administration. Diclofenac sodium (10 mg/kg) served as the standard drug. Both extracts exhibited significant and dose-dependent inhibition of paw oedema compared to the control group. At the 5th hour, METP at doses of 200, 400, and 600 mg/kg showed 69.53%, 77.34%, and 82.03% inhibition, respectively, while HETP at doses of 200, 400, and 600 mg/kg produced 62.50%, 71.09%, and 76.56% inhibition, respectively. The highest activity was observed with METP at 600 mg/kg, which was comparable to diclofenac sodium (84.38%). These findings suggest that *Terminalia paniculata* root extracts possess significant anti-inflammatory activity and may serve as a promising natural source for the development of anti-inflammatory agents.

Keywords: Terminalia paniculata, Anti-inflammatory activity, Formalin-induced paw oedema, Methanolic extract, Diclofenac sodium

Introduction

Inflammation is a protective physiological response initiated by the body against tissue injury, infection, or exposure to harmful stimuli. Although inflammation plays a vital role in host defense, prolonged or excessive inflammatory responses contribute to the development of numerous chronic diseases, including rheumatoid arthritis, cardiovascular disorders, diabetes, and cancer. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage inflammatory conditions; however, their long-term use is associated with adverse effects such as gastrointestinal irritation, renal dysfunction, and cardiovascular complications. Consequently, there is growing interest in

identifying safer and more effective anti-inflammatory agents from natural sources.

Medicinal plants have been extensively utilized in traditional systems of medicine for the treatment of inflammatory disorders. Terminalia paniculata Roth, belonging to the family Combretaceae, is a medicinal tree distributed widely throughout India. Various parts of the plant have been reported to possess antioxidant, antimicrobial, hepatoprotective, and anti-inflammatory properties. The pharmacological activities of the plant have been attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins, phenolic compounds, triterpenoids, and glycosides.

Despite its traditional use, limited scientific evidence is available regarding the anti-inflammatory potential of the roots of Terminalia paniculata. Therefore, the present study was undertaken to investigate the anti-inflammatory activity of methanolic and n-hexane root extracts of Terminalia paniculata using the formalin-induced paw oedema model in rats. The study also aimed to compare the efficacy of different doses of both extracts with the standard anti-inflammatory drug diclofenac sodium.

Materials and method

Collection and Authentification of Terminalia paniculata Roth

The roots of Terminalia paniculata Roth most widely found in the India. The plant was collected from Ananthagiri forest region, Vishakhapatnam District, Andhra Pradesh, India. The plant species were authenticated by Taxonomist, Department of Botany, Sabarmati University India. The voucher specimen numbers are 934 and 2231 for Terminalia paniculata Roth respectively.

Extraction

The fresh roots were collected, cleaned, shade dried at room temperature for a week and milled using mixer grinder. 100 gms of the coarse powder was continuously extracted with 400 ml of methanol for 18 hrs at 60°C using Soxhlet apparatus. The powder was successively extracted with n-Hexane. All extracts were filtered through a Whatman no.1 filter paper. Later, the extracts was concentrated using rotary flash evaporator (50^{o}C) to 30ml volume. And finally 30 ml extracts were concentrated to constant weight in vacuum oven at 30 to 50˚C. The evaporated residues with constant weight were stored prior to analysis in dark at 4°C. Methanolic residue of Terminalia paniculata was given name as METP and n-Hexane residue of Terminalia paniculata was given name as HETP.

Experimental Animals

Albino wistar rats weighing 180-230g were selected for the study. Animals were maintained under controlled condition of temperature at 27o ± 2o C and 12-h light-dark cycles and relative humidity of 50 ± 15%). All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Govt. of India. (Regd no. 769/2011/CPCSEA) They were housed in polypropylene cages and had a free access to standard pellets and water ad libitum.

Acute Toxicity Studies (LD50)

Animals

Female Albino rats weighing 180-230g were used for the study. They were nulliparous and non-pregnant. These were acclimatized to laboratory condition for one week prior to start of dosing.

Preparation of Dose

METP, HETP were dissolved in distilled water, to prepare a dose of 2000mg/kg p.o. The dose was selected according to the OECD guideline no. 425.

Procedure

The procedure was divided into two phases. Phase I (observation made on day one) and Phase II (observed the animals for next 14 days of drug administration). Two sets of healthy female rats (each set of 3 rats) were used for this experiment. First set of animals were divided into three groups, each of one in a group. Animals were fasted overnight with water ad libitum. Animals received a single dose of 2000 mg/kg, p.o. was selected for the test, as the test item was a source from herb. After administration of extract, food was withheld for 3-4 hrs. If the animal dies, conduct the main test to determine the LD50. If the animal survives, dose four additional animals sequentially so that a total of five animals are tested. However, if three animals die, the limit test is terminated and the main test is performed. The LD50 is greater than 2000 mg/kg p.o, if three or more animals survive. If an animal unexpectedly dies late in the study, and there are other survivors, it is appropriate to stop dosing and observe all animals to see if other animals will also die during a similar observation period. Late deaths should be counted the same as other deaths. The same procedure was repeated with another set of animals to nullify the errors.

In vivo Anti-Inflammatory Activity

Acute Anti-inflammatory Activity (Formalin-induced Paw Oedema in Rats)

Female Albino rats weighing 180-230g were used for the study. Rats were divided into eight groups of six in each group. Acute inflammation was induce by injecting formalin (0.1 ml of 1% suspension in 0.9% saline) in sub-plantar region and paw volume was measured 0, 1, 2, 3, 4 and 5 hour, with the help of Plethysmometer.

All the treatment compounds were administered 30 minutes, prior to formalin. Acute inflammation was induced in right hind paw. A mark was put on the leg second at the leg at the malleolus region to facilitate the dipping of the leg to the same level at the second and subsequent times.

The initial reading was taken at 0 hour, i.e., immediately after injecting formalin and the procedure was repeated at 1, 2, 3, 4 and 5 hour after formalin injection. The difference between 0 hour reading and one of the subsequent readings provides the actual oedema volume at the time. The mean paw volume at different times was calculated and compared with the control and the percentage inhibition was then calculated by using the formula;

Percent inhibition = 100 * (1 – Tt/Tc)

Where, Tt and Tc are the average increase in paw thickness of drug treated and control group respectively.

Group-I: Distilled water was supplied and served as control.

Group-II: Animals received a dose of 10 mg/kg of Diclofenac sodium i.p. and served as standard

Group-III: Animals received a dose of 200 mg/kg of METP p.o.

Group-IV: Animals received a dose of 400 mg/kg of METP p.o.

Group-V: Animals received a dose of 600 mg/kg of METP p.o.

Group-VI: Animals received a dose of 200 mg/kg of, HETP p.o.

Group-VII: Animals received a dose of 400 mg/kg of, HETP p.o.

Group-VIII: Animals received a dose of 600 mg/kg of HETP p.o.

Results and discussion

Administration of formalin produced a marked increase in paw oedema volume in the control group, indicating successful induction of acute inflammation. Treatment with diclofenac sodium significantly reduced paw swelling throughout the experimental period and exhibited 84.38% inhibition at the 5th hour, confirming the validity of the model.

Anti-inflammatory Activity for Terminalia paniculata

Formalin-induced paw Oedema in Rats

All the test compounds (METP and HETP) were tested with the diclofenac sodium as a standard drug in the dose of 10 mg/kg for the anti-inflammatory activity. Presently diclofenac showed significant 84.38 % inhibition of inflammation at 5th hour (0.20 ± 0.018) when compared with control (1.28 ± 0.042) respectively. The test compounds showed maximum percentage of inhibition of oedema at 5th hour significantly in respective dose level i.e., at 200, 400 and 600mg/kg the test compound METP and HETP showed 69.53%, 77.34 %, 82.03% and 62.50%, 71.09%, 76.56%.

Table 1: Effect of METP and HETP on Formalin-induced paw Oedema in Rats (Acute model)

Groups	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	% Inhibition
Group I (Control)	0.18 ± 0.021	0.78 ± 0.064	1.09 ± 0.037	1.14 ± 0.063	1.19 ± 0.038	1.28 ± 0.042	–
Group II (Standard)	0.19 ± 0.035	0.34 ± 0.028***	0.57 ± 0.046***	0.32 ± 0.024***	0.23 ± 0.047***	0.20 ± 0.018***	84.38
Group III	0.16 ± 0.025	0.62 ± 0.043**	0.89 ± 0.037**	1.02 ± 0.041***	0.79 ± 0.056***	0.39 ± 0.023***	69.53
Group IV	0.17 ± 0.027	0.56 ± 0.040**	0.78 ± 0.054***	0.53 ± 0.028***	0.40 ± 0.037***	0.29 ± 0.021***	77.34
Group V	0.18 ± 0.039	0.45 ± 0.033***	0.69 ± 0.047***	0.43 ± 0.026***	0.31 ± 0.034***	0.23 ± 0.028***	82.03
Group VII	0.17 ± 0.020	0.61 ± 0.038**	0.81 ± 0.043***	0.67 ± 0.029***	0.54 ± 0.032***	0.37 ± 0.022***	71.09
Group VIII	0.18 ± 0.034	0.52 ± 0.043***	0.76 ± 0.038***	0.53 ± 0.036***	0.39 ± 0.025***	0.30 ± 0.019***	76.56

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant.

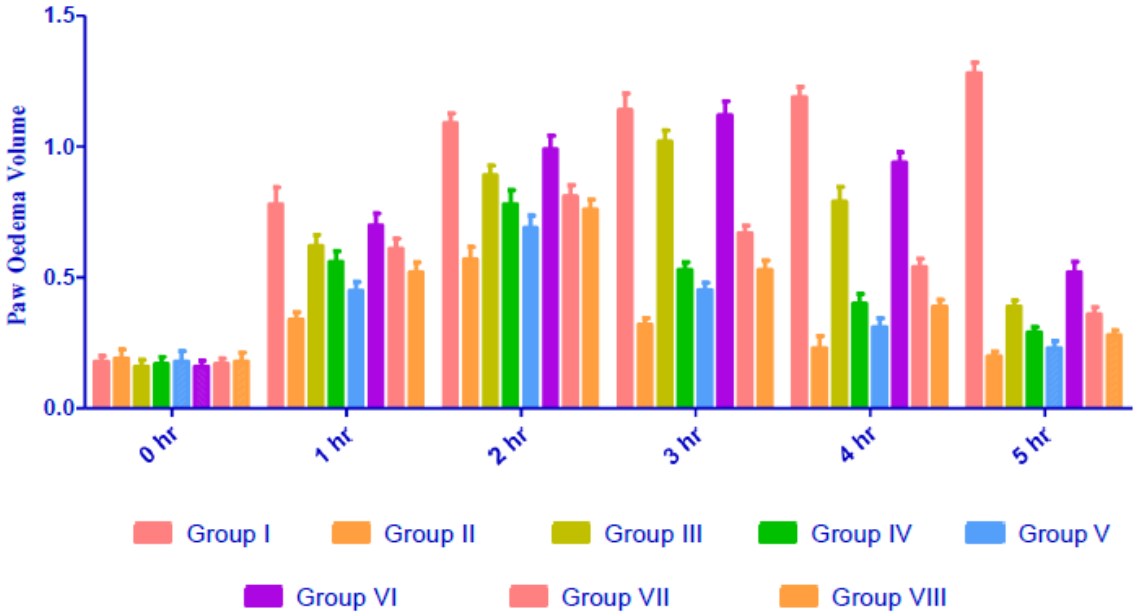

Figure 1: Effect of METP and HETP on Formalin-induced paw Oedema in Rats

Both methanolic (METP) and n-hexane (HETP) extracts of Terminalia paniculata significantly suppressed formalin-induced inflammation in a dose-dependent manner. Among the tested doses, METP at 600 mg/kg demonstrated the highest anti-inflammatory activity (82.03%), closely approaching the effect of diclofenac sodium. HETP also exhibited significant inhibition of oedema, with the maximum activity observed at 600 mg/kg (76.56%). The superior activity of METP may be attributed to the higher extraction of polar phytoconstituents such as flavonoids, tannins, and phenolic compounds, which are known to inhibit inflammatory mediators including prostaglandins, histamine, serotonin, and cytokines. The results indicate that Terminalia paniculata roots possess potent anti-inflammatory properties and support their traditional medicinal use.

Conclusion

The present study demonstrated that methanolic and n-hexane root extracts of Terminalia paniculata possess significant anti-inflammatory activity against formalin-induced paw oedema in rats. The activity was dose-dependent, with the methanolic extract exhibiting greater efficacy than the n-hexane extract. At a dose of 600 mg/kg, the methanolic extract produced anti-inflammatory effects comparable to diclofenac sodium. The observed activity may be attributed to the presence of bioactive phytochemicals capable of suppressing inflammatory mediators. These findings provide scientific evidence supporting the traditional use of Terminalia paniculata in inflammatory conditions and suggest its potential as a source of novel anti-inflammatory agents. Further studies are warranted to isolate the active constituents and elucidate their mechanisms of action.

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