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Original Article

Sub-Acute Anti-Inflammatory Activity of *Leucas indica* Ethanolic Leaf Extract in Wistar Rats Using the Cotton Pellet Granuloma Model

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ABSTRACT

This study investigated the sub-acute anti-inflammatory potential of Leucas indica leaves using the cotton pellet granuloma model in Wistar albino rats. Fifty rats were randomly assigned to five groups (n = 10 per group). Sterile cotton pellets (~20 ± 1 mg) were prepared and autoclaved before subcutaneous implantation at the nape of the neck following sedation with pentobarbitone sodium (30 mg/kg, orally) and skin disinfection with 70% ethanol. Treatment regimens included: a control group receiving 3 mL of 1% gum acacia (orally), a standard group administered Indomethacin (10 mg/kg, orally), and three test groups receiving leaves of *Leucas indica* ethanolic extract at 75, 150, and 300 mg/kg, three times daily for seven days. On the eighth day, the animals were humanely sacrificed, and the implanted pellets were removed, dried, and weighed to assess inflammatory response. Key parameters, including granuloma formation, transudative weight, and percentage inhibition, were analysed using ANOVA with Dunnett's multiple comparison test ($p < 0.05$ considered significant). Standard drug (Indomethacin) produced a highly significant reduction ($p<0.01$) in all three parameters (WW, DW, TW), confirming the model's validity and the drug's strong anti-inflammatory effect. EELLI at 75 mg/kg showed no statistically significant reduction, indicating weak or no anti-inflammatory activity at this dose. EELLI at 150 mg/kg and 300 mg/kg showed a significant reduction in: Wet weight (WW): Suggests decreased exudation and inflammation. Dry weight (DW): Indicates reduced granuloma formation (proliferative phase). Transudative weight (TW): Reflects reduced fluid accumulation. The ethanolic extract of *Leucas indica* (EELLI) exhibited significant sub-acute anti-inflammatory activity at 150 mg/kg and 300 mg/kg, as evidenced by reduced granuloma weight and fluid accumulation. The effect, though moderate compared to Indomethacin, supports the plant's traditional use and warrants further investigation.

Keywords: Cotton pellet granuloma, Leucas indica, Sub-acute anti-inflammatory activity

INTRODUCTION

Understanding the differences in neutrophil function and cellular characteristics is crucial, as these may influence the progression of inflammatory diseases. However, the transition of neutrophil subsets across different stages of inflammation—acute, subacute, and chronic—remains unclear. Investigating these changes is particularly important in light of recent discoveries related to trained immunity within the innate immune system[1].

Inflammation

Although inflammation is a natural defence mechanism, it can also contribute to the onset and progression of various diseases. Treatment typically involves pharmacological interventions, which differ in effectiveness and are often associated with toxicity and adverse effects. Inflammatory responses are driven by the activation of multiple mediators, resulting in symptoms and tissue damage. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used; however, their long-term use is often limited by significant side effects. Consequently, there is increasing interest among researchers and healthcare professionals in exploring the Indian System of Medicine (ISM) as a potential alternative approach for managing inflammation[2].

MATERIAL AND METHODS

Prior to initiating the study, approval was obtained from the Institutional Ethics Committee (Ref. No. YU/IAEC/3/10, dated 06/05/2010). All experimental procedures were conducted in accordance with the guidelines established by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), New Delhi, India.

Wistar albino rats were randomly divided into five groups, with ten rats in each group (n = 10). Sterile cotton pellets weighing 20 ± 1 mg were prepared from absorbent cotton wool and sterilized in a hot-air oven at 120°C for two hours. Anaesthesia was induced using pentobarbitone sodium at a dose of 30 mg/kg, administered orally. Following shaving and disinfection of the nape of the neck with 70% ethanol, a sterile cotton pellet was implanted subcutaneously into each rat.

The treatment protocol was as follows: the control group received 1% gum acacia at a dose of 3 mL/kg orally; the standard group was administered indomethacin at a dose of 10 mg/kg orally; and the three test groups received the ethanolic extract of *Leucas indica* leaves (EELI) at doses of 75, 150, and 300 mg/kg, respectively based on acute toxicity study[3]. The extract was given orally three times daily at six-hour intervals for seven consecutive days.

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On day 8, the animals were euthanized using a high dose of pentobarbital sodium. The implanted cotton pellets were surgically removed, dried at 60°C for 18 hours, cooled, and then weighed. The mean dry weights of the pellets were recorded for each group. Inflammatory parameters—including transudative weight, granuloma formation, and percentage inhibition of granuloma—were calculated and statistically analysed using appropriate methods. An increase in granuloma dry weight serves as an indicator for evaluating the proliferative phase of inflammation[4-6].

Statistics

Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. A p-value < 0.05 was considered statistically significant. The ethanolic extract of *Leucas indica* demonstrated significant sub-acute anti-inflammatory activity in Wistar albino rats, as evaluated using the cotton pellet-induced granuloma model. At a dose of 150 mg/kg, the extract significantly reduced wet weight ($p < 0.05$), dry weight ($p < 0.01$), and transudative weight ($p < 0.01$) compared to the control group. A similar and more pronounced effect was observed at 300 mg/kg, with significant reductions in wet weight ($p < 0.01$), dry weight ($p < 0.01$), and transudative weight ($p < 0.01$). These results indicate that the extract effectively suppressed sub-acute inflammation, as reflected by the significant mean percentage inhibition of granuloma formation.

RESULTS

In this study, vehicle control rats received 1% gum acacia (3 mL/kg, p.o.), serving as the baseline group. Their wet weight (WW), dry weight (DW), and transudate weight (TW) were recorded as 204.5 mg, 57.03 mg, and 151.2 mg respectively. The standard group, administered Indomethacin at 10 mg/kg orally, showed significant reductions in all inflammatory parameters—WW decreased to 114.7 mg, DW to 44.82 mg, and TW to 76.49 mg, with $p < 0.01$. This confirmed the reliability of the inflammation model and validated Indomethacin's potent anti-inflammatory activity (Table 1).

At a dose of 75 mg/kg, the ethanolic extract of *Leucas indica* leaves (EELI) did not produce statistically significant changes in WW (194.9 mg), DW (53.99 mg), or TW (149.7 mg) ($p > 0.05$), indicating negligible anti-inflammatory activity at this dose. However, EELI at 150 mg/kg resulted in significant reductions: WW dropped to 186.9 mg ($p < 0.05$), while both DW (50.02 mg) and TW (83.79 mg) were highly significantly reduced ($p < 0.01$), suggesting moderate anti-inflammatory effects. Notably, the 300 mg/kg dose of EELI showed highly significant inhibition of inflammation across all parameters—WW at 144.5 mg, DW at 45.55 mg, and TW at 77.43 mg ($p < 0.01$), demonstrating a strong anti-inflammatory effect, nearly comparable to the standard drug Indomethacin (Figure 1).

Table 1

Groups / Drugs / Dose	WW (mg)	DW (mg)	TW (mg)
Vehicle Control (1% Gum acacia) 3ml/kg, p.o	204.5±3.31	57.03±0.88	151.2±2.24
Standard (Indomethacin) 10mg/kg, p.o	114.7±2.89 (p<0.01)	44.82±1.10 (p<0.01)	76.49±2.18 (p<0.01)
EELI 75mg/kg, p.o	194.9±2.68 (p>0.05)	53.99±1.82 (p>0.05)	149.7±2.62 (p>0.05)
EELI 150mg/kg, p.o	186.9±7.29 (p<0.05)	50.02±2.55 (p<0.01)	83.79±2.37 (p<0.01)
EELI 300mg/kg, p.o	144.5±6.31 (p<0.01)	45.55±1.13 (p<0.01)	77.43±1.87 (p<0.01)

The observation is mean ± S.E.M. p> 0.05- Not Significant, p<0.05-Significant, p< 0.01- Highly Significant as compared to control (ANOVA followed by Dunnett's multiple comparison test)
WW-Wet weight, DW- Dry weight, TW-Transudative weight
EELI- Ethanolic Extract of the leaves of Leucas indica, p.o- per oral

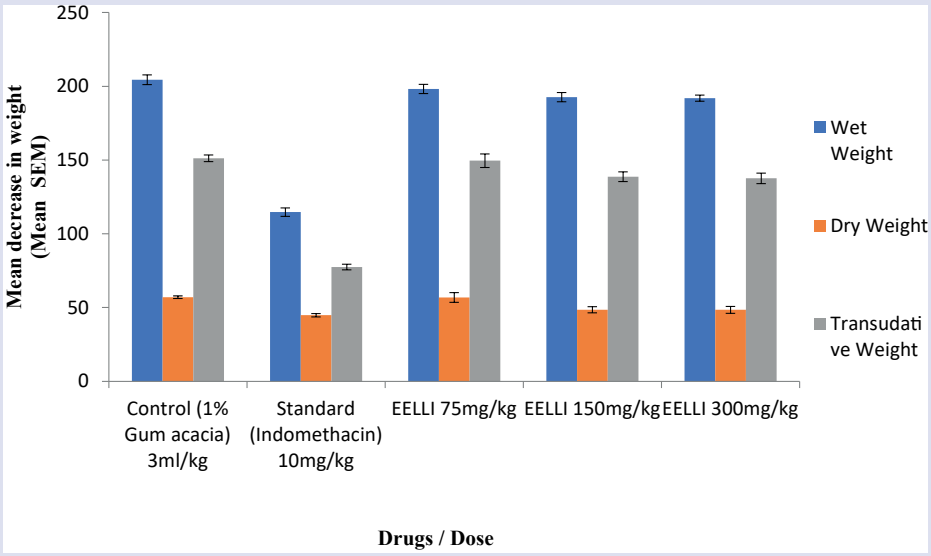

Figure 1. Sub-acute anti-inflammatory activity of EELLI by cotton pellet induced granulome pouch in Wistar albino rats. EELLI- Ethanolic Extract of the leaves of Leucas indica

DISCUSSION

Previous studies have reported that the ethanolic extract of *Leucas indica* leaves possesses both acute and chronic analgesic effects in Wistar albino rats[7]. Oral administration of the ethanolic extract of *Leucas indica* leaves (EELLI) at doses of 150 mg/kg and 300 mg/kg exhibited significant anti-inflammatory activity, with percentage inhibitions of 52.58% ($p < 0.01$) and 36.87% ($p < 0.05$), respectively, when compared to the control group[8]. The present study focused on evaluating the sub-acute anti-inflammatory properties of *Leucas indica* ethanolic leaf extract. When administered at doses of 150 mg/kg and 300 mg/kg body weight, the extract significantly reduced the dry weight, wet weight, and transudative weight in the cotton pellet-induced granuloma model. These findings indicate a robust anti-inflammatory response, with the extract exhibiting superior efficacy compared to both the control group and the standard anti-inflammatory drug, Indomethacin.

CONCLUSION

Leucas indica ethanolic leaf extract demonstrated efficacy comparable to or greater than Indomethacin at higher doses. EELI may contain bioactive compounds that reduce both exudative (fluid) and proliferative (cellular) phases of inflammation

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