

Evaluation of anti-arthritic effect of *Torilis leptophylla* and its comparison with indomethacin

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ABSTRACT

Background: Rheumatoid arthritis is an autoimmune debilitating disease involving multiple joints and organs. Many treatment options are available but all are associated with frequent side effects. Phytochemical screening of *Torilis leptophylla* has shown the presence of anti-inflammatory compounds like flavonoids, phenols and anthraquinones. This study was designed to evaluate its effect on joints inflammation (rheumatoid arthritis). Indomethacin is one of the oldest and most commonly used drugs for arthritis. It was used as a standard drug to compare with indomethacin.

Methods: This experimental study was carried out in Pharmacology Department, University of Health Sciences, Lahore. Thirty six male albino Wistar rats were randomly divided into six groups (group I-control, group II-positive control, group III-10 mg indomethacin, group IV,V,VI--100mg, 200mg, 300mg *Torilis leptophylla* extract (TLE) administered (orally) respectively). Arthritis was induced by sub plantar injection of 0.1 ml Freund's complete adjuvant (FCA) to all groups except the control group. Body weight (weekly) and ankle joint diameter (every 4th day) were measured. At day 29 blood was collected and all animals were killed by overdose of ether. Acid phosphatase and alkaline phosphatase levels were determined by chemistry analyzer (RX MONZA, RANDOX, Republic of Ireland). Statistical analysis was performed using SPSS 20. One way ANOVA and Post hoc -Tukey tests were applied. A p-value of ≤ 0.05 was considered statistically significant.

Results: Treatment with indomethacin caused significant ($p \leq 0.001$) reduction in all the inflammatory parameters. *Torilis leptophylla* extract also significantly ($p \leq 0.05$) reduced all the inflammatory parameters. Anti-inflammatory effect was comparable to indomethacin.

Conclusion: *Torilis leptophylla* has significant anti-arthritic activity as it modified the parameters of joint inflammation and destruction.

Keywords

Rheumatoid Arthritis, Freund's Complete Adjuvant, *Torilis leptophylla*, Indomethacin, Anti-inflammatory

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disorder that primarily affects joints. The disease causes joint destruction and leads to disfigurement, chronic pain and disability.¹ Approximately 1-2% of people are affected by RA around the world. It is more common in middle age and in females. Occurrence of RA in Pakistan is 0.5% and disease is largely polyarticular.² RA mainly involves synovial membranes and causes inflammation mediated by activation of signaling glucocorticoids, non-steroidal anti-inflammatory drugs (NSAIDs) and disease modifying anti rheumatic drugs molecules.³ Numerous treatment choices are present for the management of RA. These comprise

(DMARDs).⁴ Most common treatment for RA is NSAIDs due to their effectiveness in decreasing pain and inflammation. Indomethacin is non-selective inhibitor of COX. It has an additional action of inhibiting phospholipase A and C. Indomethacin is frequently recommended for the treatment of moderate to severe RA, acute gout, ankylosing spondylitis and osteoarthritis.⁵ These treatment modalities are effective but have a number of side effects. For example continued use of NSAIDs may lead to gastrointestinal (GI) ulcers, perforation, bleeding and some cardiovascular threats.⁴ Evaluation and development of cost-effective alternative treatment options is mandatory. *Torilis leptophylla* (*T. leptophylla*) is a plant of Apiaceae family, native term is Chrikanger.⁶ Qualitative phytochemical study of *T. leptophylla* extracts and fractions proved the presence of alkaloids, anthraquinones, terpenoids, and glycosides. These compounds have antioxidant and anti-inflammatory effects. Phenolic content proved significant correlation

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between total phenolic content and 50% effective concentration (EC_{50} value= 189 ± 4 $\mu\text{g/ml}$) for DPPH.⁷ *T. leptophylla* has shown anti-inflammatory, antibacterial and neuroprotective effects.⁸ Limited research work has been carried out regarding effect of *T. leptophylla* on arthritis as per literature review.

MATERIALS AND METHODS

The research work followed an experimental study design. Simple random sampling technique was used to divide the animals in groups. Study was conducted from March to September 2016 in UHS, Lahore. Male Wistar albino rats weighing 140 ± 20 g were randomly divided into 6 groups of six animals each. They were kept in polypropylene cages, at $22 \pm 2^\circ\text{C}$ temperature and 45-60% relative humidity. They were given standard pellet animal feed and water *ad libitum*. Light and dark cycle of twelve hour was maintained. Animals were kept in these conditions for one week to adjust with the environment. The whole plant was washed and air dried for two weeks. Parched plant was crushed into small pieces. 500 g of dried plant material was soaked in 95% methanol. After 48 hours it was filtered and evaporated in rotary evaporator at 37°C . Freeze dried at -44°C using lyophilizer and stored at 4°C . Pharmaceutical grade Indomethacin, Freund's complete adjuvant (FCA) and methanol were used. Arthritis was induced using FCA to all groups except group 1 which was non-arthritic. FCA 0.1 ml was injected into sub plantar surface of left hind paw on day 1. FCA is made of heat killed *Mycobacterium tuberculosis* 1 mg/ml.⁹ Test samples and indomethacin were administered daily by oral route from day 12 till 28th day (Day 1: Induction with FCA, Day 12: Start of treatment with test samples, Day 29: Blood and tissue sampling). Group I (Non-arthritic group) was control group and administered distilled water. The arthritic groups were further divided into Group II (Positive control group, given distilled water), Group III (Indomethacin 10 mg/kg body weight), Group IV (TLE 100 mg/kg body weight), Group V (TLE 200 mg/kg body weight) and Group VI (TLE 300 mg/kg body weight).⁷ Body weight of all the animals in experimental groups was measured at day 0 and then weekly. Digital weighing balance was used.¹⁰ Ankle joint diameter was measured using digital vernier caliper on day 0 before FCA injections and on day 1, 4, 8, 12, 16, 20, 24, and day 28.¹¹ Cardiac puncture was performed to collect blood and stored in serum vacutainers. Blood was permitted to clot for 1 hour and then centrifuged at 4000 rpm for 5 minutes at 10°C . Clear serum was separated by pipette and stored

in plain tubes at -20°C till further processing.¹² Acid phosphatase (ACP) is a lysosomal enzyme its levels are raised in FCA induced arthritis.¹³ Alkaline phosphatase (ALP) is a cytoplasmic cellular enzyme and indicates synovial membrane type II cells secretory activity. Raised levels of these enzymes indicate bone remodeling and damage.¹⁴ Levels of these enzymes were measured to see the effect of TLE on bone remodeling. Serum acid phosphatase (ACP) and alkaline phosphatase (ALP) levels were measured using automated chemistry analyzer.¹⁴ The quantitative data was expressed as Mean \pm SD. Statistical analysis was performed using SPSS 20. One way ANOVA was applied to observe the mean differences among control and experimental groups. Post hoc-Tukey test was used for comparisons. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

After start of treatment, on day 12 an increase in body weight was observed in treatment groups and control group while in group II a decrease in weight was observed (Figure 1). On day 28 body weight of group I was 294 ± 11 g while body weight of group II was 216 ± 4 g which is significantly ($p \leq 0.001$) less than group I. Body weight of group III was 286 ± 2 ($p \leq 0.001$), group IV 268 ± 2 g ($p \leq 0.001$), group V 266 ± 280 g ($p \leq 0.001$) and group VI 278 ± 6 g ($p \leq 0.001$).

Results are represented as Mean \pm S.D. P value ≤ 0.05 is denoted by * and # where: * shows the comparison of group III, group IV, group V and group VI with group II and # represents comparison of group II with group I. Increase in joint diameter was observed in arthritic groups starting from day 4 to 28 that persisted in group II (Figure 2). Group I showed no increase in joint diameter during the study. On day 28 joint diameter in group I was 4.80 ± 0.20 mm, group II 7.0 ± 0.40 mm, group III 4.90 ± 0.09 mm ($p \leq 0.001$), group IV 5.50 ± 0.30 mm ($p \leq 0.001$), group V 5.40 ± 0.23 ($p \leq 0.001$) and in group VI 5.18 ± 0.14 mm ($p \leq 0.001$) (Figure 2).

Results are represented as Mean \pm S.D. P value ≤ 0.05 is denoted by * and # where * shows the comparison of group III, group IV, group V and group VI with group II and # represents comparison of group II with group I.

Treatment with indomethacin and *T. leptophylla* extract reduced the levels of serum ALP significantly as compared to group II (Figure 3). Serum ALP of group III had a mean \pm S.D. of 317.56 ± 21.53 U/L ($p \leq 0.001$). In group IV serum ALP was 515.50 ± 35.30 U/L ($p \leq 0.001$). In group V 456.50 ± 25.30 U/L and in group

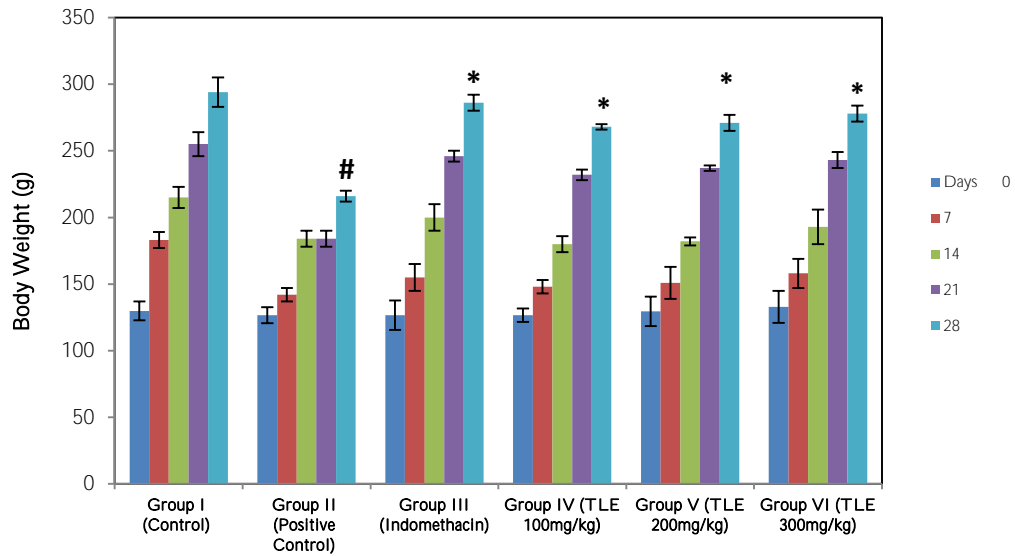


Figure 1. Effect of *Torilis leptophylla* extract (TLE) on body weight in Wistar rats (n=6)

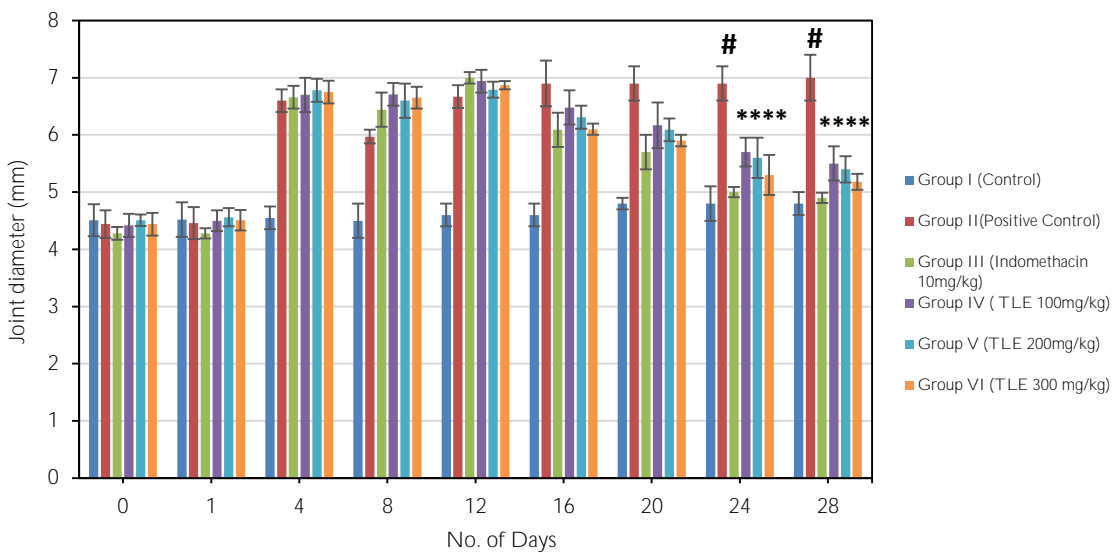


Figure 2. Effect of *Torilis leptophylla* extract (TLE) on joint diameter in Wistar rats (n=6)

VI 409.40 ± 21.23 U/L ($p \leq 0.001$) (Fig.3).

Treatment with indomethacin and *T. leptophylla* extract decreased the levels of serum ACP of groups III, IV, V and VI as compared to the group II (Figure 4). Serum ACP of group III was 6.80 ± 1.20 U/L ($p \leq 0.001$). Group IV 10.80 ± 1.60 U/L, group V 9.70 ± 1.30 U/L and group VI 8.40 ± 1.0 U/L ($p \leq 0.001$) had serum ACP values (Fig. 4).

DISCUSSION

Rheumatoid arthritis (RA) is an autoimmune disease. It is a chronic inflammatory condition that mainly affects

joints. Inflammation caused by RA follows a course of joint destruction leading to chronic pain, deformity and disability.¹

FCA induced arthritis closely resembles human arthritis. Inflammatory response to FCA is reported to follow a biphasic course. Acute phase is localized to injection site and lasts for first 3-4 days. Chronic phase is manifested by multiple joint involvements and appears from 9-12 days post injection.¹⁴ In present study we followed a therapeutic model by starting treatment on day 12.

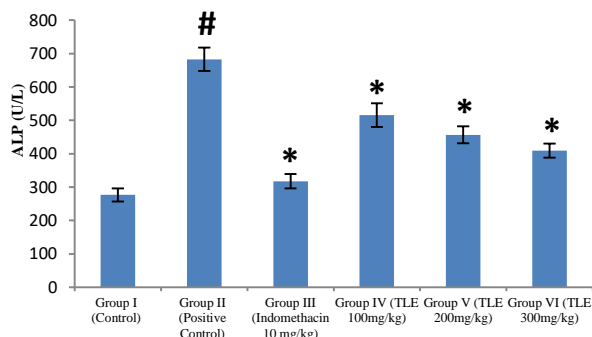


Figure 3. Effect of *Torilis leptophylla* extract (TLE) on serum alkaline phosphatase in Wistar rats (n=6).

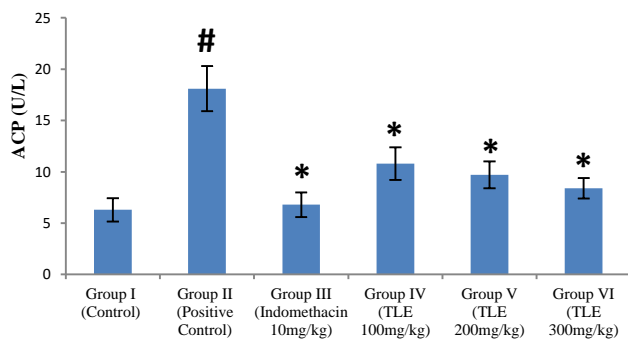


Figure 4. Effect of *Torilis leptophylla* extract (TLE) on serum acid phosphatase in Wistar rats (n=6)

Qualitative phytochemical study of *T. leptophylla* extracts and fractions demonstrated the presence of anthraquinones, terpenoids, alkaloids and cardiac glycosides. Phenolic content proved significant correlation between total phenolic content and 50% effective concentration (EC_{50} value= $189 \pm 4 \mu\text{g/ml}$) for DPPH.⁷ Both of these findings favor its use against oxidative stress.

In this study TLE showed anti-arthritic effect on inflammatory parameters of adjuvant induced arthritis. Body weight of all the animals was measured at the start of study on day 0 and then weekly. Constant decrease in weight gain was observed in group II as compared to the group I. Treatment with plant extract showed significantly less weight loss and the results were comparable to indomethacin. Results clearly indicate a reduction in weight loss in the chronic phase of FCA induced arthritis and supportive role of *T. leptophylla*. This effect may be due to reduced oxidative stress and inflammation due to antioxidants present in extract. Similar results have been documented by Ekambram and coauthors where *Strychnos potatorum* reduced the weight loss by caused by RA.¹⁰

Joint diameter (mm) was also used as an indicator of joint inflammation. In injected paw gradual increase

in joint diameter was observed starting from 4th day. Indomethacin and TLE ameliorated the inflammation after start of treatment on day 12. This finding also indicates the anti-inflammatory properties of the plant. A study carried out by Kadam and Bodhankar indicated the inhibition of 25 % by the *Diplocyclos palmatus* extract. In this study the percentage inhibition by the plant is nearly similar to our study.¹⁵

Increased serum levels of alkaline phosphatase and acid phosphatase have been observed in adjuvant arthritis which may be due to persistent inflammation and reduced cell integrity.¹⁴ Increased levels of serum alkaline phosphatase were observed in group II while indomethacin and plant treatment significantly reduced the ALP levels. Similar results were provided in another study where plant treatment caused reduction in serum ALP levels.⁹

Serum acid phosphatase was also raised in group II as compared to group I. Treatment groups showed a reduction in serum acid phosphatase levels by administration of indomethacin and *T. leptophylla* extract. In group II 66 % rise in acid phosphatase levels had been expressed as compared to the group I. Group III had 62 % decline in serum acid phosphatase as compared to group II. In *T. leptophylla* treated groups 40 %, 47 % and 54 % decrease was observed in groups IV, V and VI respectively. Mishra and coauthors stated 67 % decrease in serum ACP by plant. These results show that indomethacin and plant treatment reduced the bone resorption and remodeling.

CONCLUSION

T. leptophylla extract reduced all the parameters of joint inflammation, in a dose dependent manner. It successfully ameliorated the weight loss associated with RA. A significant decrease was observed in other parameters i.e. joint diameter, serum ALP and serum ACP levels. Anti-inflammatory and anti-arthritic effects of *T. leptophylla* can be attributed to the phenolic and flavonoid contents of the plant which have proven anti-inflammatory and anti-oxidant properties. Molecular basis for the mechanism of action of *T. leptophylla* requires further studies.

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