Original article

# Effect of *Torilis leptophylla* on blood total leukocyte count and differential leukocyte count in asthmatic mice

**Sheikh Maria Qammar[1], Saba Batool[2], Muhammad Umair Samee[3], Iram Imran[4], Bushra Shaheen[5], Javaria Fatima[6]**

[1]Assistant Professor, Pharmacology Department, Rashid Latif Medical College, Lahore, Pakistan, [2]Assistant Professor, Pharmacology Department, Nishtar Medical University, Multan, Pakistan, [3]Associate Professor, Surgery Department, Central Park Medical College, Lahore, Pakistan, [4]Associate Professor, Department of Pharmacology, Amna Inayat Medical College Sheikhupura, Pakistan, [5]Assistant Professor, Department of Pharmacology, Nishtar Medical University, Multan, Pakistan, [6]Demonstrator, Pharmacology Department, Fatima Jinnah Medical University, Lahore, Pakistan

***Correspondence to:*** Sheikh Maria Qammar, Email: mariaqammar@hotmail.com

## ABSTRACT

Background: Chronic inflammation, mucus hypersecretion and airway blockage are the characteristics of asthma. Current treatments of asthma are effective but cause adverse effects on their long term use. This research was designed to explore anti-inflammatory effect of *Torilis leptophylla (T. leptophylla)* on allergic inflammation of airways by estimation of blood total leukocyte count (TLC) and differential leukocyte count (DLC) to compare its effect with a standard drug in an animal model.

Materials and methods: This experimental study was carried out in Pharmacology Department, University of Health Sciences, Lahore. A total of 48 healthy, adult, male BALB/c mice were randomly divided into six groups. Group I (control), group II (diseased); groups III, IV and V were given 100, 200, 400 mg/kg *T. leptophylla* methanolic extract (TLM) respectively, and Group VI treated with standard drug monteleukast. Airway inflammation was induced in all groups with ovalbumin except control group. On day 28, cardiac puncture was done to collect the blood and TLC and DLC (lymphocytes, neutrophils, eosinophils and monocytes) were measured in blood. All the data was interpreted as mean ± SD. SPSS 20 was used to carry out statistical analysis.

Results: *T. leptophylla* extract resulted in significant (p-value ≤ 0.05) decrease of TLC and DLC in blood. *T. leptophylla* cause 59-67% decrease in TLC, 67-80% decrease in lymphocytes, 69 - 72% decrease in neutrophils, and 45-51.7% decrease in eosinophils

Conclusion: In current study *T. leptophylla* successfully treated inflammation in asthmatic mice by reducing proportion of inflammatory cells.

Keywords:

Bronchial inflammation, Ovalbumin, *Torilis leptophylla*, Monteleukast, Anti- inflammatory

## INTRODUCTION

Asthma is a disease characterized by frequent attacks of shortness of breath and wheezing varying in severity and frequency in different individuals. Airways inflammation is the cause of these symptoms in asthmatic patients. It is principal cause of death in developing countries, affecting 4.3% of the world population.[1] Asthma and other allergic diseases are associated with Th2 mediated immune responses. Increase number of Th2 cells cause discharge of cytokines, including interleukin (IL)-4, IL-5, IL-9 and IL-13. These cytokines cause enhanced eosinophilic inflammation and IgE generation by mast cells. Inflammatory mediators like histamine and cysteinyl leukotrienes release is increased as a result of IgE production that results in bronchospasm, edema and increased mucous secretion.[2] Proteases and reactive oxygen species (ROS) produced by eosinophils, neutrophils and macrophages play an important role in inflammation in asthmatic air passage.[3] Eosinophils mediate immune responses by secreting cationic proteins, lipid mediators, and cytokines/chemokines.[4] Neutrophils produce oxidative radicals, lipid mediators and peroxidases that play a vital role in pathogenesis of asthma.[5] Cytokines (IL-4, IL-5 and IL-9) that release from Th2 lymphocytes contribute to enhanced eosinophilic inflammation and mucus secretion in asthma.[6,7] In asthma, several proteases and chemokines are released that invites neutrophils, monocytes and T cells resulting in enhanced inflammation.[8] Current treatments of asthma are β 2-agonists, anticholinergics, cysteinyl leukotrienes receptor 1 blockers, theophylines, corticosteroids, immunomodulators, anti-histamines and anti IgE antibody.[9,10] Their long term use may cause potential adverse effects. Herbal remedies have an integral role in therapeutics in case of asthma. Extract

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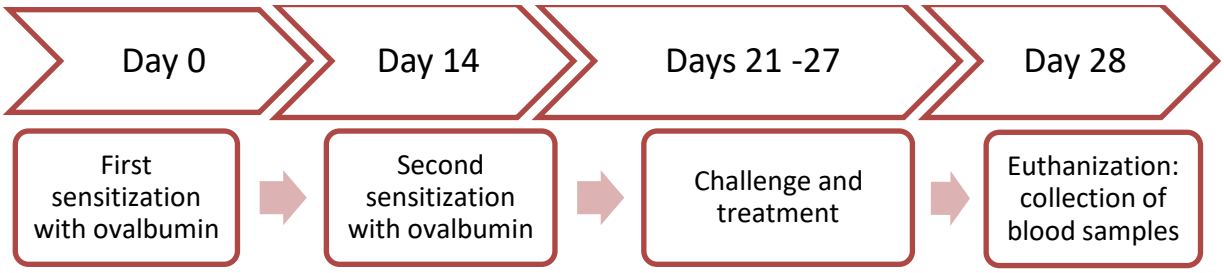


Figure 1: Flow chart showing plan of experiment

of the root of *Ephedra sinica* discovered over 4000 years ago in China was the first known effective herbal remedy for asthma. Anticholinergic alkaloids in herbal cigarettes have also been found effective in asthma. Sodium cromoglycate, mast cell stabilizer, have been extracted as khellin from the root of the Egyptian plant.[11] *Torilis leptophylla (T. leptophylla)*, from Apiaceae family, is known worldwide as "Bristle fruit Hedge Parsley". It is distributed in Europe, Africa, Central and South Asia. It is common weed in Hazara, Peshawar and Taxila and called "charikanger".[12] Its leaves have been used to cure gastrointestinal illnesses in folk medicine.[13] Qualitative analysis of crude methanol extract of *T. leptophylla* illustrated presence of tannins, terpenoids, coumarins.[13] Antioxidant role of *T. leptophylla* has been reported due to its free radical scavenging and cytoprotective activity and its total phenolic content in different fractions was found, ranging from 49.9 ± 4.1 to 121.9 ± 3.1 mg gallic acid equivalent/g dry weight.[13] Its anti-inflammatory effect on allergic inflammation of air passages has not been investigated. Current study is therefore planned to explore the anti-inflammatory effect of *T. leptophylla* on allergic inflammation of airways induced by ovalbumin (OVA) in mouse model as a safe alternative treatment of asthmatic airways. Induction of allergic airway inflammation in BALB/c mice by sensitization and challenge with OVA and assessment of role of *T. leptophylla* as anti-inflammatory agent in allergic inflammation of airways in mouse by determining presence of inflammatory cells in blood constitutes the objectives of this study.

## MATERIALS AND METHODS

Animals were divided into groups by simple random technique. Forty eight healthy, male BALB/c mice of weight 25-30g were obtained Experimental Research Laboratory, UHS, Lahore. Temperature maintained at 24 ± 2ºC, standard commercial pellet diet and water freely available. Twelve hour light and dark cycle was sustained with relative humidity 40-70%. The seven day period was allowed so that animals acclimatize to the laboratory environment. Ethical Review Board for Biomedical Research, University of Health Sciences guidelines were followed strictly.

The fresh flowering shoots of *T. leptophylla* were obtained from the vicinity of National Agricultural Research Centre (NARC), Islamabad. After washing the plant with simple water, it was dried in shade and 300g of it was crushed and soaked in concentrated solution of methanol for 2 days.[13] Rotary evaporator was used to get the concentrated form of extract. The concentrate was freeze dried by lyophilizer and saved at -6 °C.

Airway allergy in mice was induced by sensitization followed airway challenge in all groups.[14] Group 1 (control) was challenged with phosphate buffer saline (PBS). Sensitization was carried out by administering intra peritoneal injection of 0.1 ml PBS containing 20 µg of OVA emulsified in 2 mg aluminium hydroxide on day 0 and 14. For intranasal challenge, solution containing 1 mg of OVA dissolved in 1 ml of PBS was prepared and given intra-nasally to experimental animals of groups II, III, IV, V and VI. Challenge was given once daily for one week from day 21 to day 27.[14] Animals with increased number of inflammatory cells in blood as compared to control group were considered asthmatic.

The animals were divided into six groups. Each group consisted of eight animals. Control group was sensitized and challenged with PBS. Diseased group was sensitized and challenged with OVA. In groups III, IV, V and VI disease was induced by sensitization and OVA challenge. Group III was treated with TLM dose 100 mg/kg body weight. Group IV was treated with TLM dose 200 mg/kg bodyweight. Group V was treated with TLM dose 400 mg/kg bodyweight. Group VI was treated with standard drug monteleukast 6mg/kg body weight. Cardiac puncture was done under light anesthesia and the whole blood was collected in EDTA

Table 1: Blood cell count in ovalbumin induced mice after treatment with *Torilis leptophylla* methanolic extract

| Groups | Total Leukocyte Count ( $^{x10^3}$/µl) | Lymphocytes (%) | Neutrophils (%) | Monocytes (%) |
|---|---|---|---|---|
| Control | 2.53 ± 0.37 | 56.38 ± 5.78 | 29.95 ± 8.08 | 5.26 ± 0.78 |
| Diseased | 9.73 ± 0.79[b] | 87.50 ± 7.3[b] | 85.775 ± 8.51[b] | 16.51 ± 1.59[b] |
| Group III | 3.91 ± 0.78[a] | 80.25 ±9.35 | 71.5 ± 7.84[a] | 5.78 ± 0.54[a] |
| Group IV | 3.71 ± 0.68[a] | 71.38 ±4.41[a] | 69.625 ± 7.13[a] | 5.70 ± 1.21[a] |
| Group V | 3.20 ± 0.44[a] | 68.16 ± 3.72[a] | 35.25 ± 7.63[a] | 5.58 ± 1.27[a] |
| Group VI | 2.66 ± 0.72[a] | 67.00 ± 5.45[a] | 24.85 ± 6.68[a] | 4.58 ± 0.76[a] |

p-value < 0.05 was considered significant and expressed by a and b where a depicts a significant difference of group III, IV, V and VI with diseased and b shows a significant difference of diseased with control.

tubes. TLC and DLC of whole blood were measured on same day by using automated hematology analyzer[15].

The data was expressed as Mean ± SD. Statistical analysis was performed using Statistical Package for Social Sciences (IBM SPSS) version 20. One way analysis of variance (ANOVA) was applied to observe the mean differences among control and experimental groups. Post hoc Tukey's test was used for comparisons. A p-value of ≤0.05 was considered statistically significant.

## RESULTS

### Blood TLC in ovalbumin induced mice after treatment with TLM

All treatment groups exhibited significant decrease in TLC from group II ($p < 0.05$). High dose of TLM and montelukast decreased TLC to the normal level with insignificant statistical difference with group-I (Table 1).

### Blood lymphocyte count in ovalbumin induced mice after treatment with TLM

*T. leptophylla* in medium and high doses i.e. 200 mg/kg and 400 mg/kg significantly decreased number of lymphocytes to 71.38 ± 4.41 and 68.16 ± 3.72 respectively as compared to group II $p < 0.05$ (Table 1).

### Blood neutrophils count in ovalbumin induced mice after treatment with TLM

Group III, IV and V demonstrated significant decrease in neutrophils count in blood of mice as compared to diseased group, $p<0.05$ (Table 1).

### Blood Eosinophils count in Ovalbumin Induced Mice after treatment with TLM

Eosinophils count is found significantly low in groups III, IV and V as compared to diseased, from 0.29 ± 0.032% (group II) to 0.16 ± 0.014%, 0.13 ± 0.026% and 0.14 ± 0.030% of group III, IV and V respectively. Group VI (montelukast 6 mg/kg) reduced number of eosinophils to 0.08 ± 0.018% (Table 1)

### Blood monocytes count in ovalbumin induced mice after treatment with TLM

Number of monocytes significantly reduced in treatment groups: group III, IV V and VI in contrast to diseased group, with insignificant difference among each other. (Table 1)

## DISCUSSION

Current study was planned to determine the role of *T. leptophylla* on allergic inflammation of air passages inflammation in mouse keeping in view the medicines from natural product like ephedrine, sodium chromoglycate and anti cholinergics have proved beneficial role in the management of asthma. Asthma is a disease with chronic inflammation in airways. Asthma is correlated with Th2 response, IgE mediated mast cell activation and other inflammatory factors, including eosinophils, basophils, cytokines and chemokines[16]. In current study BALB/c strain of mice was used because it displays Th2 responses more readily than other strains[17]. Other animal models that can be utilized to induce allergic airway inflammation are rats, mouse and guinea pigs.[18] OVA was used as an allergen to induce allergic inflammation in airways of experimental animals in our study. Ovalbumin is the primary protein in egg white. Hypersensitivity to chicken egg is the second most common allergic reaction to foods. In addition to OVA other allergens that can be used to induce allergic airway inflammation are extracts of cockroach and house dust mite.[19] In present study BALB/c mice were induced by sensitization and challenge with OVA. Sensitization and challenge with OVA significantly induced allergic inflammation that was observed by increase in TLC in blood by 74%. Induction of asthma with ovalbumin also has significantly increased number of lymphocytes in blood by 35.6%, neutrophils 65.1%, eosinophils 82.76% and monocytes 68.14%. Montelukast is a selective CysLT1 antagonist. Binding of cysteinyl leukotrienes to leukotriene receptors has been correlated with the pathophysiology of asthma. Montelukast was used as standard drug in our study because of favorable safety

profile, well-tolerated, 16 effective in reduction in asthma related symptoms, lung function parameters, improved quality of life and reduction in number of asthma exacerbations. It has also a bronchoprotective effect. It does not act on other lung receptors such as the prostanoid, cholinergic, or beta-adrenergic receptors and thus has minimum adverse effects.[20] In current study, montelukast (6 mg/kg) was used as standard drug. According to current study montelukast exhibited suppression of blood TLC by 73%. Progression and remission of airways inflammation in this study were determined by comparing number of inflammatory cells i.e., TLC and DLC among treatment groups with diseased model. The present results clearly demonstrated that *T. leptophylla* significantly reduced TLC in blood. *T. leptophylla* demonstrated 59.8% decrease in TLC in blood with low dose of TLM, 61.9% decrease with medium dose of TLM, 67.2% decrease with high dose of TLM and 73% decrease with montelukast. DLC in current study showed significant decrease in number of inflammatory cells in blood. *T. leptophylla* exhibited significant reduction in neutrophil count in blood by 18.9% with medium dose and 59% with high dose. Reduction in neutrophil count in blood by montelukast in our study was 71%. Current study presented significant increase in number of eosinophils in diseased group verifying allergic inflammation of airways. *T. leptophylla* and montelukast treated inflammation significantly by reducing eosinophilic count in blood by 45%, 55%, 51.7% and 72.5% in groups III, IV, V and VI. Better reduction of TLC and DLC in blood were due to anti-inflammatory effect of *T. leptophylla* as it is found rich in flavonoids, saponins, terpenoids and proved to have exceptional antioxidant and radical scavenging quality.[13]

## CONCLUSION

Allergic inflammation of airways was successfully treated with *T. leptophylla* extract. *T. leptophylla* auspiciously reduced the count of total WBCS, lymphocytes, neutrophils, eosinophils and monocytes in blood *T. leptophylla* reduced number of inflammatory cells with results comparable to that of montelukast. Further studies should be advocated to identify the TLC and DLC in airways.

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