Proceedings S.Z.P.G.M.I. Vol: 32(2): pp. 14-18, 2018. PSZMC-676-32-2-2018

Comparative Study of *Uncaria tomentosa* Leaves & Bark Extracts on Lung Smooth Muscle Hyper Responsiveness in a Mouse Model

[1]Gulpash Saghir, [1]ShazanaRana, [2]Zain Aamir, [2]Muhammad Usman
[1]Department of Pharmacology, Fatima Jinnah Medical University, Lahore
[2]Department of Pharmacology, Shaikh Zayed Medical Complex, Lahore

ABSTRACT

Introduction: *Uncaria Tomentosa* leaves and bark extracts are seen to have anti-inflammatory effect on bronchial smooth muscles. This is done by decreasing the synthesis of TNF-α, a pro-inflammatory mediator by inhibiting cyclooxygenase 2 and factor kappa β. This study has opened a new horizon for the treatment of asthma with lesser side effects and greater tolerance. **Aims & Objectives:** The goal of this study is to compare the effect of U*ncaria tomentosa* leaves and bark extracts on lung smooth muscle hyper responsiveness in a mouse model. **Place and duration of study:** Department of Pharmacology, UHS, Lahore, Pakistan. **Material & Methods:** 24 healthy male BALB/c mice were taken, and divided into 4 groups, I, II, III and IV by simple balloting method. Group I (negative control) was sensitized and challenged with 1ml of PBS. Whereas groups II (positive control), III and IV were sensitized once at day zero with 1mg OVA I.P and after two weeks interval challenged intranasally with 5 mg of OVA in 1ml of PBS daily for one week. Following which Groups III and IV were treated with *Uncaria tomentosa* leaves and bark extracts 200µg/animal/day orally for 7 days respectively. **Results:** Treatments with UT extracts in groups III & IV significantly decreased total leucocyte count due to declining eosinophil and neutrophil levels and TNF-α expression versus diseased control group ($P < 0.05$-0.001). **Conclusion:** Ethanolic extracts of leaves and bark of *Uncaria tomentosa* at dose of 200µg/day significantly decreased the total leukocytes i-e, eosinophils, and neutrophils, in the blood. Moreover, the mRNA expression level of TNF-α was also significantly reduced in lung smooth muscle hyper responsiveness in the mouse model. However, the reduction of inflammatory cells and pro-inflammatory mediators was slightly more with treatment with UT bark than UT leaves.

Key words: *Uncaria tomentosa* (UT), Phosphate Buffer Saline (PBS), Ovalbumin (OVA), hyper responsiveness, IL-6, TNF-α, mRNA, COX-1, COX-2, AHR.

INTRODUCTION

Asthma is the most common respiratory disorder described as a reversible chronic disease of airway inflammation. Large number of inflammatory cells e.g, eosinophils, neutrophils, lymphocytes, T-helper 2 cells and mast cells participate in pathogenesis of asthma. Several pro-inflammatory mediators like cytokines and interleukins are released from inflammatory cells which induce mucus production and eosinophilia in lung parenchyma, the typical finding of asthma[1]. TNF-α is also a pro inflammatory cytokine and produced by different cells such as mast cells, macrophages, neutrophils, eosinophils, and epithelial cells when there is allergic pulmonary inflammation. TNF-α synthesis is mediated by nuclear factor kappa β and cyclooxygenase 2. TNF-α along with other mediators e.g, IL-1b, IL-6, IL-8 causes inflammation in the body e.g, joints, lungs etc[2]. The wider acceptance of botanical medicines has opened new horizon for the treatment of multiple diseases with lesser side effects and great tolerance. *Uncaria tomentosa* also known as Cat's claw is frequently used in medicine for arthritis, gastritis, bursitis and even for some sorts of cancers. It has been shown by previous studies that leading mechanism of cat's

claw anti-inflammatory effects are due to immune modulation by reducing TNF-α production. *Uncaria tomentosa* also possess activity against COX 1 and COX 2 enzymes. The aim of this study was to compare the effects of both extracts of *Uncaria tomentosa* on respiratory muscles of asthma induced mice[3].

MATERIAL AND METHODS

24 healthy male BALB/c mice were taken, and divided into 4 groups, I, II, III and IV. Grouping of animals was done in the way they were divided in four groups having six mice in each group. Animals were kept in the experimental research laboratory at a controlled room temperature (22-24^{0}C), humidity (45-65%) and natural light and dark cycle. All mice were fed on standard diet and water ad libitum. Airway sensitization and inflammation was induced as follows;

1. Group I (Negative Control) was sensitized (intraperitoneally) and challenged (intranasally) with phosphate buffer saline solution.

2. Group II (Positive Control), III and IV were sensitized on day 0 by intraperitoneal injection of 1mg of OVA in 50 mg Al $(OH)^3$ (adjuvant) in a volume of 1ml PBS.

3. Intranasal challenge: After two weeks of sensitization, groups II, III and IV were challenged intranasally with 5 mg of OVA in 1ml of PBS daily for 1 week.

4. Treatment with UT: Groups III and IV were treated with **UT leaves** and **UT bark extract** at doses of 200µg/animal/day orally for 7 days respectively.

Preparation of UT bark and Leaves Ethanolic Extract

100 grams of *Uncaria tomentosa* bark and 100 grams of ground leaves (separately) were extracted in 1L of absolute ethanol for 24 h at 37°C then the extract was centrifuged for 15 min at 4000 rpm. Supernatants were evaporated by vacuum centrifuge at low temperature for 1hr. Dry extract was re suspended in ethanol (1L) and stored at -20°C till further use.

Euthanization

Twenty four hours after the last challenge with OVA and respective treatments all mice were sacrificed by giving light ether anesthesia.

Determination of Inflammatory Cells in Blood

Blood sample was taken by cardiac puncture at the time of dissection in EDTA containing tube. Giemsa Wright stained blood smears were seen for inflammatory cells like, lymphocytes, neutrophils and eosinophils, under light microscope.

Determination of mRNA Expression Levels of Pro-inflammatory Cytokine TNF- α

Total RNA from the lung tissue was extracted by using TRIzol method[4] and nano drop spectrophotometer was used to quantify the total RNA in each sample. RNA was reverse transcribed using kit manufacturer's protocol (Thermo Fisher Scientific, USA). Amplification of the cDNA was performed using polymerase chain reaction. Sequence of TNF-α primer was picked from previous publications[4,5]. Thermal cycler was programmed for 35 cycles of denaturation, annealing, and extension. The following cycle profile was used: 95 ºC for 10 sec, 58-62ºC for 20 sec, and 72ºC for 30 sec. The annealing temperature was set as 60 ºC for TNF-α. PCR product was visualized using 2% agarose gel electrophoresis. Image J software was used for densitometry and semi-quantification.

Statistical analysis

All the data were analyzed using Graph Pad Prism v.5 software. One way ANOVA was applied to observe the difference among all groups. Post hoc Tukey's test or student t-test was applied to analyze the comparison between the groups. The data was presented as Mean ± standard deviation (SD) and P-value $\leq$ 0.05 was considered as statistically significant.

RESULTS

Both UT extracts almost normalized the TLC in blood

Total leucocyte count (TLC) numbers of eosinophils, and neutrophils in the blood of diseased group were found significantly elevated ($P<0.001$) as compared to the control group.

Fig-1 & 2 show the treatments with UT extracts decreased ($P < 0.001$) the counts of eosinophils, and neutrophils count ($P<0.05$) in groups III & IV.

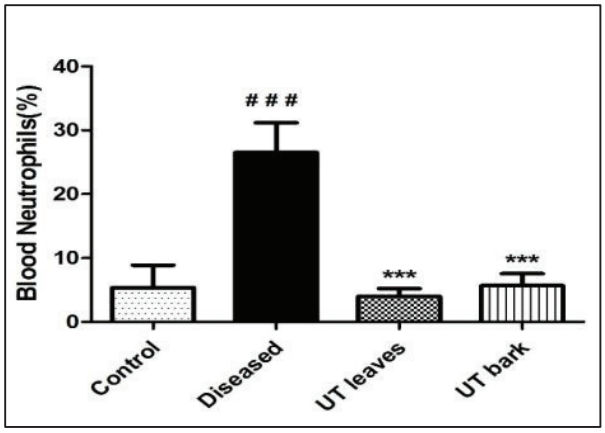

Fig-1: TLC count in blood in all groups

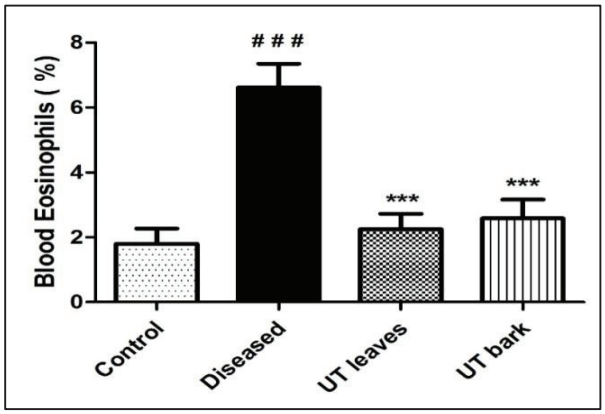

Fig-2: Eosinophil count in blood in all groups

UT extracts decreased TNF-α expression levels in lung tissue

We found TNF-α expression levels significantly raisedin diseased group as compared to control group($P<0.01$).Treatments with UT extracts significantly lessened the TNF-α expression levels in groups III & IV as compared to diseased group ($P<0.01$).

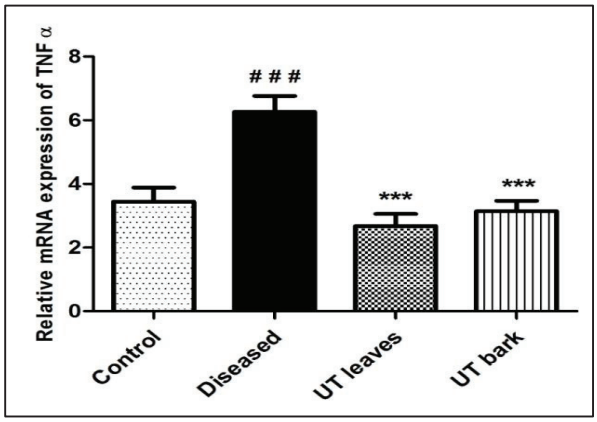

Fig-3: Relative mRNA expression to TNF α in all groups.

DISCUSSION

Asthma has high mortality and it badly affects the quality of life.[6] Allergic inflammatory changes are evident by rise in leukocytes count especially eosinophils infiltrating the lung airways, hyper responsiveness of airways to stimuli and increased mucus secretion.[7] Many pharmacological treatments are available for asthma management, *Uncaria tomentosa*, belongs to the family *Rubiaceae*. Its extracts are broadly consumed in Peruvian medicine for the treatment, cure and management of gastritis, arthritis, and as an anti-inflammatory agent[8]. The effects of *Uncaria tomentosa* extracts are produced due to a mixture of quinovic acid and glycosides aside from pentacyclic or tetracyclic of oxindole alkaloids. The anti-inflammatory effect of *Uncaria tomentosa* is caused by inhibiting the initiation of the transcriptional factor NF-κB, It prevents the expressions of inducible genes that are linked with inflammatory processes[6]. *Uncaria tomentosa* has long been used for medical purposes. It has been seen that *Uncaria tomentosa* has anti-inflammatory effect in arthritis by inhibition of NF-κB and other inflammatory mediators[9].

In this study, we observed the anti-inflammatory effect of ethanolic extracts of *Uncaria tomentosa* leaves and bark in OVA induced allergic airway inflammation in BALB/c mice. There was a significant increase in eosinophils and neutrophils in blood of group II (diseased) as compared to group I (normal). The mRNA expression level of TNF-α in group II was also raised as compared to group I. Increased levels of these inflammatory markers in blood and tissue showed successful induction of allergic airway inflammation in diseased group. Administrations of ethanolic extract of *Uncaria tomentosa*, leaves and bark during airway OVA challenge phase dramatically reduced the levels of these elevated parameters in groups III and IV compared to group II.

Inflammatory cells and their mediators exhibit vital pathogenic role in precipitation of AHR (airway hyper responsiveness) by extensive release of cytotoxic products and pro- inflammatory factors which cause pathological modifications in lung tissues.[10] Our results showed that there was an increase in inflammatory cells, eosinophils, and neutrophils, in blood of OVA exposed mice that were decreased following administration of extracts of *Uncaria tomentosa* leaves and bark. This finding

is consistent with previous studies that found an increase of inflammatory cells after the exposure of ovalbumin and reduced to normal after the treatment with anti-inflammatory agents (black seed oil).[11]

Cytokines perform an indispensable function in pathogenesis of allergic airway inflammation.[12] Allergen contact in the bronchial tree persuades a Th-2 prevailing response by initiating cells of inflammation and so intensifying the concentrations of TNF-α, and some other cytokines example IL-4, IL-5.[7] Our results revealed a raised mRNA expression of TNF-α in diseased group that was remarkably reduced to normal range in the groups that were treated with ethanolic extracts of *Uncaria tomentosa* leaves and bark, respectively. Thus these results are in accordance with the results of some other studies in which TNF-α was raised in allergic airway inflammation.[13]

In this study, we also compared the anti-inflammatory effects of ethanolic extract of UT leaves with UT bark and found that both extracts are equally effective anti-inflammatory agents when compared with one another. Regarding the effect on inflammatory cells in blood, UT leaves and UT bark considerably reduced neutrophils and eosinophils. These findings are in consistent with studies done before in which they showed that extracts of different parts of *Uncaria tomentosa* possessed a significant anti-inflammatory activity in gastritis and arthritis.[8]

Regarding cytokine TNF-α, both extracts reduced the mRNA expression levels of this cytokine to the same level. All these results are in parallel with previous studies which showed that *Uncaria tomentosa* prevents the expression of inducible genes that are linked with inflammation.[14]

CONCLUSION

Ethanolic extracts of leaves and bark of *Uncaria tomentosa* at dose of 200µg/day, significantly decreased the total leukocytes i-e, eosinophils and neutrophils, in the blood. Moreover, the mRNA expression level of TNF-α also significantly reduced. However according to this research the ethanolic extract of *Uncaria tomentosa* bark was slightly more effective then UT leaves extract

REFERENCES

1. Barnes PJ, Shapiro S, Pauwels R. Chronic obstructive pulmonary disease: molecular and cellularmechanisms. European Respiratory Journal. 2003; 22(4):672-88.
2. Barnes PJ. The cytokine network in asthma and chronic obstructive pulmonary disease. The Journal of clinical investigation. 2008; 118(11):3546-56.
3. Valerio LG, Gonzales GF. Toxicological aspects of the South American herbs cat's claw (Uncaria tomentosa) and maca (Lepidium meyenii). Toxicological reviews. 2005; 24(1):11-35.
4. Ashraf MI, Shahzad M, Shabbir A. Oxyresveratrol ameliorates allergic airway inflammation via attenuation of IL-4, IL-5, and IL-13 expression levels. Cytokine. 2015; 76(2):375-81.
5. Rana S, Shahzad M, Shabbir A. Pistacia integerrima ameliorates airway inflammation by attenuation of TNF-alpha, IL-4, and IL-5 expression levels, and pulmonary edema by elevation of AQP1 and AQP5 expression levels in mouse model of ovalbumin-induced allergic asthma. Phytomedicine. 2016; 23(8):838-45.
6. Koleilat A. Asthma the Problem within its Self Asthma Perception and Prevention. EC Paediatrics. 2018; 7:348-9.
7. Bassett D, Hirata F, Gao X, Kannan R, Kerr J, Doyon-Reale N, et al. Reversal of methylprednisolone effects in allergen-exposed female BALB/c mice. Journal of Toxicology and Environmental Health, Part A. 2010; 73(11):711-24.
8. Shaw R, Djukanovic R, Tashkin D, Millar A, Du Bois R, Corris P. The role of small airways in lung disease. Respiratory medicine. 2002; 96(2):67-80.
9. Failla CM, De Luca N, Zaccaria ML, De Domenico E, Avitabile S, Tatangelo L, et al. Mice over-expressing placenta growth factor in the skin exhibit increased vascularization and vessel permeability independently of VEGF-A. Journal of dermatological science. 2018; 90(1):93-6.
10. Mahmood H, Fatima H, Faheem M. Gynecologic Oncology Reports.
11. Raza A, Muhammad S, Xudong Y, Qingzhu S, Fujun Z, Yan H. Suppressive effects of black

seed oil on ovalbumin induced acute lung remodelling in E3 rats. Swiss Med Wkly. 2010; 140:w13128.
12. Nilsson AZ. Targeting The IL-33/ST2 Pathway In Asthma.
13. Yang EJ, Lee J-S, Song BB, Yun C-Y, Kim D-H, Kim IS. Anti-inflammatory effects of ethanolic extract from Lagerstroemia indica on airway inflammation in mice. Journal of ethnopharmacology. 2011; 136(3):422-7.
14. Sandoval M, Okuhama N, Zhang X-J, Condezo L, Lao J, Angeles F, et al. Anti-inflammatory and antioxidant activities of cat's claw (*Uncaria tomentosa* and *Uncaria guianensis*) are independent of their alkaloid content. Phytomedicine. 2002; 9(4):325-37.

The Authors:
Dr. Gulpash Saghir,
Assistant Professor,
Department of Pharmacology,
Fatima Jinnah Medical University, Lahore.

Dr. Shazana Rana,
Assistant Professor,
Department of Pharmacology,
Fatima Jinnah Medical University, Lahore.

Dr. Zain Amir,
M. Phil trainee,
Pharmacology Department,
Shaikh Zayed Medical Complex, Lahore.

Dr. Muhammad Usman,
M. Phil trainee,
Pharmacology Department,
Shaikh Zayed Medical Complex, Lahore.

Corresponding Author:

Dr. Zain Amir,
M. Phil trainee,
Pharmacology Department,
Shaikh Zayed Medical Complex, Lahore.
E-mail:dr.zainaamir@gmail.com