DOI: https://doi.org/10.53350/pjmhs2023171196

ORIGINAL ARTICLE

Effect of *Uncaria Tomentosa* leaves extract on Histopathological changes in Murine Model of Asthma

GULPASH SAGHIR[1], MUHAMMAD SAIR[2], IRAM KAMAL[3], SOPHIA ABBASI[4], FAIZA KHAN[5], FAIZA ISHTIAQ[6]
Department of Pharmacology, University of Lahore
Correspondence to Dr. Gulpash Saghir, Email: gul18pharma@gmail.com, Cell: 03208400246.

ABSTRACT

Background: Bronchial asthma is the leading respiratory disease among all chronic inflammatory conditions. Due to various causative factors, it's incidence has been drastically increased over last decades. Despite the availability of potent anti inflammatory & bronchodilator therapies, there is a need to find new pharmacological molecules possessing targeted mechanism and lesser side effects towards it's pathogenesis. In this study, Uncaria tomentosa belonging to family Rubiacae was selected to evaluate anti asthmatic effect owing to it's specific anti inflammatory properties.
Aim: To evaluate the effectiveness of uncaria tomentosa leaves on respiratory smooth muscles in case of bronchospasm and constriction.
Study Design: Experimental comparative type
Place and Duration of Research: University of health sciences. Duration of study is 1 year
Methodology: Ethanolic extract of Uncaria tomentosa leaves was prepared according to the protocol. We took 24 male mice and divided them equally in groups of four. These groups represented control, diseased, UT (Uncaria tomentosa) leaves and MP (Methylprednisolone) treated. Bronchial asthma was induced by Ova sensitization on day 0 and OVA challenge on day 14 followed by treatment with UT leaves extract (200µg/animal/day) and MP (15mg/kg b.w) for next 7 days. Animals were sacrificed and lungs were removed to check histopathological findings.
Results: The animals belonging to diseased group showed all histopathological features suggestive of bronchial asthma as compared to control group. Inflammatory score, epithelial cell hyperplasia and vascular congestion was found to be significantly decreased in both treatment groups.
Conclusion: UT leave extract may possess anti asthmatic activity comparable to Methylprednisolone.
Keywords: Phosphate Buffer Saline PBS, Tumor necrosis factor alpha TNF-α, Methylprednisolone MP, Uncaria tomentosa UT

INTRODUCTION

Asthma is a known to be reversible, chronic disease of airways. Its victims account for more than 300 million people worldwide, and it is expected to still raise further affecting youth over the next 15-25 years[1,24]. Asthma exacerbations are significant health problems and risk for progression to severe disease. Common risk factors include, smoking, viral exposure, race age etc. It is the very common respiratory disorder affecting a numerous people including all ages globally. Its morbidity and mortality have inclined more than the past two decades[2,26] .

Respiratory tract infections due to different viruses (e.g., rhino virus, parainfluenza virus type A, SARS Virus etc.), inhaled atmospheric pollutants are the most common triggers. Many drugs like aspirin by inhibiting COX and shifting the equilibrium of synthesis of leukotrienes causes spasm of bronchial tree is very striking example [3]. The socioeconomic effects are significant, predominantly, when poor control of disease leads to absentees from school, labor and workstations, spontaneous health care visits and hospital admittances[4].

The diagnosis of asthma is built on clinical symptoms, characteristic findings include, cough, wheeze, chest tightness and dyspnea that is usually accompanied by manifestation of airway obstruction changeable in short intervals of time and is reversible with medication[5].

Presence of inflammatory cells, cellular elements and mediators play a role, particularly eosinophils, mast cells, neutrophils, macrophages, lymphocytes and epithelial cells play a major role in its pathogenesis[6].It is a result of very intricate interactions between cells of inflammation and their mediators . Activation of mast cell by allergens and physical stimuli results in release of mediators which cause bronchoconstriction, such as leukotriene D4, histamine and prostaglandins. In addition to bronchoconstriction they also cause microvascular leakage and plasma exudation. Mast cells are activated by IgE, which is formed by activated plasma cells[7].

Received on 20 -11-2022
Accepted on 01-01-2023

There is infiltration of Eosinophils into the airways and they act as primary effector cells by releasing reactive oxygen species and specific granule proteins. Eosinophil derived mediators such as eosinophil peroxidase are supposed to destroy mucosa, epithelial cells and pneumocytes. Increase in quantity of mast cells in smooth muscle of airways is a typical finding of asthma. Respiratory epithelium releases inflammatory mediators and growth factors which correct the damage caused by the inflammatory changes. Intricate networks of cytokines and chemokines, such as growth factors and chemokines, play vital role in orchestrating the inflammatory process[8 ,26].

Long duration and severity of disease leads to thickening and fibrosis of the airway epithelium, permanent decreased caliber of the airways and refractoriness to bronchodilators. Examination of the inflammatory cells, in induced sputum samples demonstrates, although asthma is predominantly characterized by airway eosinophilia, in some patient neutrophilic inflammation preponderates and in others, negligible inflammation is observed: so called 'pauci-granulocytic' asthma[9].

Asthma is an obstructive airway disease with good therapeutic response to corticosteroids and bronchodilators. Glucocorticoids, mainstay of asthma treatment, are not verily specified in their pharmacological actions and also have significant side effects including decreased bone metabolism, decreased growth in children due to early epiphyseal closure of bones and adrenal suppression[10]. In addition to side effects specific to children, glucocorticoids cause many other side effects in all age groups such as increased appetite, obesity, electrolyte imbalance, osteoporosis, diabetes, hypertension, muscle weakness, peptic ulcer, psychological effects and eye diseases[11].

Mostly asthmatic patients respond well to corticosteroids and beta agonists. However, these treatment therapies are not effective at times or cause significant side effects due to the limitations of current therapies, and medications, new treatment modalities for asthma pose a great challenge to the pharmaceutical industries and researchers[12].

Herbal medicine after getting sponsorship of World Health Organization (WHO) is once again gaining popularity. According to WHO in developing countries more than 80% of the world population depends primarily and basically on plant-based

medicine for treatment of ailments (e.g, gastritis, arthritis) and health care needs[13].

Uncaria tomentosa (panja bail), belongs to the family Rubiaceae. The dynamic constituents seem to be polyphenols (flavonoids, porathocynidins) sterols, oxindole alkaloids, glycosides and quinovic acid The extracts of Uncaria tomentosa leaves and also bark, tendrils are frequently used in medicine for treatment and management of inflammation of joints, inflaamation of stomach and its mucosa along with many other inflammatory disorders[14].

Uncaria tomentosa possesses anti inflammatory effect by hindering production of TNF-α , cytokines and special proteins involved in process of transcribing e.g, NF-κB. It also constrains the over expression of precipitating genes allied with inflammation[27]. It also hampers expression of stimulatory genes linked with pathways of inflammation, particularly; decreasing the manifestation of nitric oxide synthase, henceforth reduces production of nitrous oxide[15].

The current investigation was conducted to evaluate anti-inflammatory effect of plant, Uncaria tomentosa leaves excerpt(extract) in the mouse model of allergic airway inflammation and its histopathology after inducing Asthma.

MATERIALS AND METHODOLOGY

This was experimental, comparative study conducted at University of Health Sciences for a period of one year. I have calculated sample size by using Raosoft online sample size calculator. Twenty four healthy male mice, 30-40gms were divided in four groups I, II, III, IV , having 6 mice in each group.

Sampling Technique: Mice were assigned randomly by using Simple random sampling by balloting method.

Preparation & Grouping of Experimental Animals: All Animals were kept in experimental laboratory where research is conducted at a standard and optimum room temperature (22-24ºC), humidity (45-65%) and natural light and dark cycles. Every mouse was fed on good quality and calibrated diet including water ad libitum. Immunization and Challenge of animals concluded as airway inflammation was induced by intraperitoneal (IP) sensitization and challenge of ovalbumin through process of inhalation by nose. Group II, III, 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 solution(phosphate buffer saline). After two weeks of sensitization, every mice exhibited to intranasal challenge of OVA 11(1mg/ml PBS) once daily for 7 days whilst mice of group 1 (control) were sensitized (intraperitoneally) and challenged (intranasally) with phosphate buffer saline solution (PBS)[16,17].

Preparation of UT leaves extract in ethanol: Leaves of UT was collected and dried under shade. Then dried leaves were grinded.100 grams of grinded leaves were extracted in 1L of absolute ethanol for time of 24 h at temperature 37°C. later on, the excerpt(extract) was centrifuged for 15 min at speed of 4000 rpm. Evaporation of Supernatants was done by vacuum centrifuge at low temperature for duration 1hr. Dry extract was resuspeneded in ethanol(1L) and kept at -20°C up untill if needed to be used again[18].

Drugs and Doses: Methylprednisolone (Sigma) 15mg/kg b.w[19]
Ethanolic extract of Uncaria tomentosa leaves 200µg/animal/day[20]

Treatment & Euthanization: After two weeks of sensitization, group III was given Uncaria tomentosa leaves extract orally at dose of 200µg/day for 7 consecutive days. Similarly group IV received methylprednisolone at 15mg/kg body weight intraperitoneally daily for 7 consecutive days. Twenty four hours after the last challenge with OVA and respective treatments all mice were immolated by giving light ether anesthesia[21].

Lung Histopathology: Lungs of each mouse were removed at the time of euthanization and insufflated with 10% formalin solution. Section cut at the thickness of 6µm and then stained with hematoxylin and Eosin (HE) to see inflammation. Histological examination of specimen was done to see morphological changes including, lymphocytes, eosinophilic infiltration and macrophages[22]. According to following semi-quantitative scale inflammatory score was graded: 0, none; 1, mild; 2, moderate; 3, severe.

Statistical analyses: The data was evaluated by using SPSS version 22. One way ANOVA was put on to see the differences in all included groups. Post Hoc Tukey test was also applied to see which group means is different from other. Mean ± Standard Deviation (SD) was given for commonly distributed quantitative variables. A P-value ≤0.05 was taken as statistically significant (valuable).

RESULTS

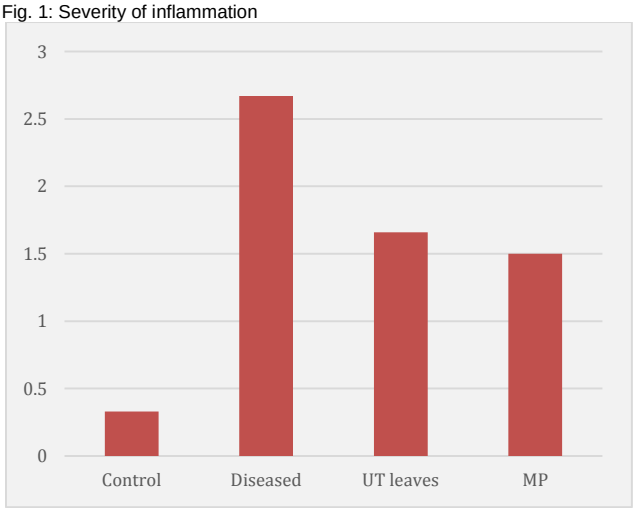
Histopathological Findings

Table 1: Effect on allergic airway inflammation (n=6)

Groups	Group I	Group II	Group III	Group IV
Severity of inflammation	0.33 ± 0.51	2.67 ± 0.47	1.66 ± 0.61	1.50± 0.5
Bronchial epithelial hyperplasia	1.50±0.54	3.41±0.66	1.66±0.40	2.08±0.37
Edema and Vascular Congestion	0.33 ± 0.51	2.66 ± 0.51	1.50 ± 0.54	0.50± 0.54

[a]shows a significant difference with group I. [b]shows a significant difference with group

Fig. 1: Severity of inflammation



Severity of Inflammation: Our findings indicated an increase in severity of airway inflammation in group II(diseased) as compared to group I (control) (2.66 ±0.47 vs 0.33 ± 0.51).UT treated group III significantly decreased the severity of airway inflammation as compared to group II (1.66 ± 0.61vs 2.66 ± 0.47). Similarly, methylprednisolone treated group IV showed a drastic decline in severity of inflammation when compared to group II (1.50± 0.5vs 2.66 ± 0.47).

Fig. 1: Graphical representation of mean ± SD of severity of inflammation in lung tissue in all groups (n=6). *** shows $p < 0.001$ and shows $P < 0.01$ indicates great difference as compared to diseased group while ### shows $p < 0.001$ and depicts significant difference as compared to control group

Bronchial Epithelial Hyperplasia: Our findings indicated an increase in bronchial epithelial hyperplasia in group II (diseased) as compared to group I (control) (3.41±0.66 vs 1.50±0.54). UT treated group III showed a considerable decrease in bronchial epithelial hyperplasia as compared to group II (1.66±0.40 vs 3.41±0.66) Methylprednisolone treated group IV showed a decrease when compared to group II (3.41 ± 0.66 vs 2.08 ± 0.37).

Fig. 2: Bronchial epithelial hyperplasia

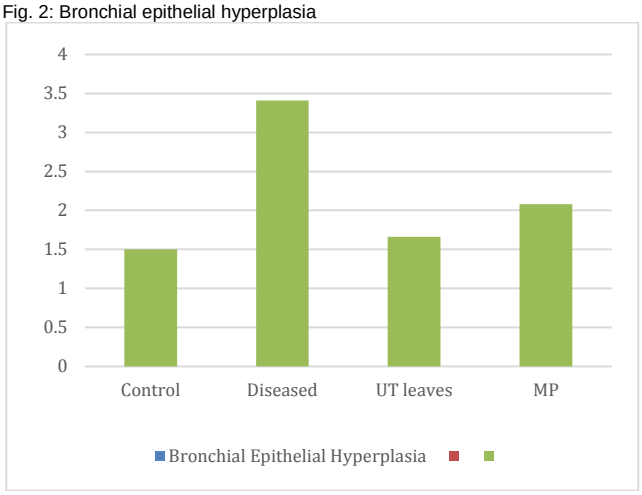


Fig. 2: Graphical representation of mean ± SD of bronchial epithelial hyperplasia in all groups (n=6). *** shows $p < 0.001$ and depicts significant difference as compared to diseased group while shows $p < 0.001$ and shows valuable difference as compared to control group.

Vascular Congestion and Edema: Our findings indicated a significant increase in edema and vascular changes which were manifested by increased in air spaces and congestion of group II (diseased) as compared to group I (control) (2.66 ± 0.51 vs 0.33 ± 0.51). UT leaves treated group III also indicated a significant decrease in edema and vascular changes as compared to group II (1.50 ± 0.54vs2.66±0.51). Methylprednisolone treated group IV showed a significant decrease in edema and vascular changes as compared to group II (0.50± 0.54 vs 2.66 ± 0.51).

Fig. 3: Edema and vascular changes

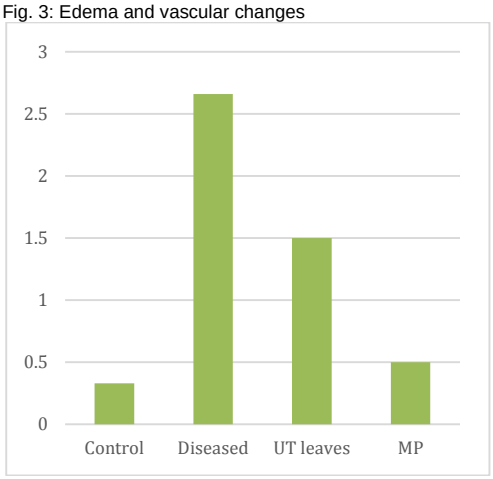


Fig. 3: Graphical representation of mean ± SD of edema and vascular changes in all groups (n=6). ***shows $p < 0.001$ and ** depictsp < 0.01 shows notable difference as compared to diseased group while indicates $p < 0.001$ and shows valuable difference as compared to control group.

Fig 4a: Control group showing normal hisytppathology of epithelium

Fig 4b: Diseased group showing infiltrates of cells

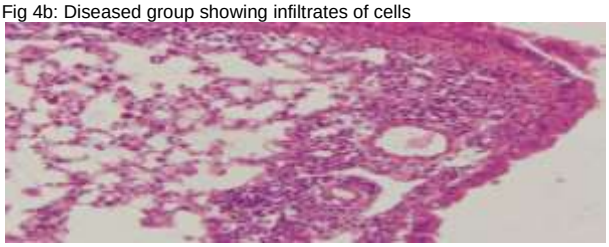


Fig 4c: Treatment group , congestion is decreased

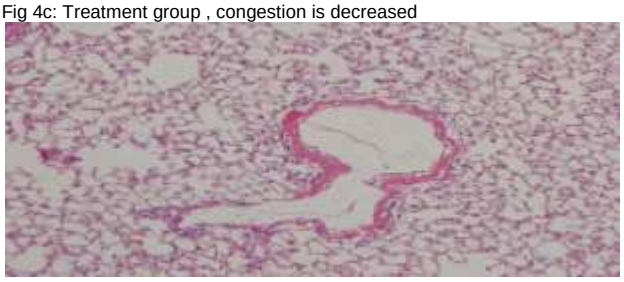
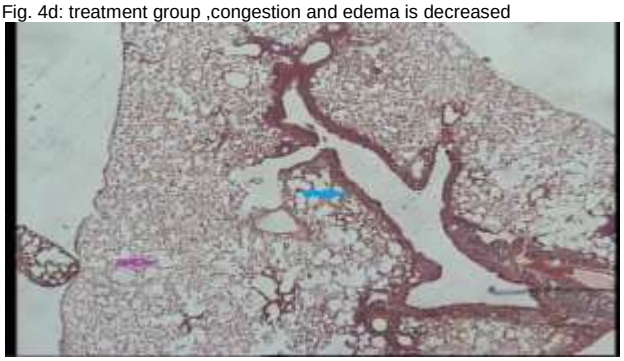


Fig. 4d: treatment group ,congestion and edema is decreased



DISCUSSION

Asthma is a disease of chronic inflammation of respiratory system. Asthma has very soaring number of mortality and it affects the quality and standards of living worst[24,25]. Allergic inciting exchanges are obvious by peaks in total leukocyte count particularly, eosinophils permeating the lung bronchial tree and parenchyma. Stimulion of bronchial tree to excitable and irritable responses induces mucoid secretion[19].*Uncaria tomentosa plant* is from *Rubiaceae ancestry and i*ts extracts are very usually and extensively used in treatment and management of gastritis, many cancers ,joint inflammations, and as an anti-inflammatory agent for other diseases[14]. The effects of *Uncaria tomentosa* excerpts are fabricated due to mixture of quinovic acid and glycosides alongside pentacyclic, tetracyclic of oxindole alkaloids. The effect of *Uncaria tomentosa* in inflammation is caused by interferring in commencement of the gene factor NF-κB, TNF alpha and Interleukins[27] .It also with holds the expressions of absolute genes that are allied with inflammatory processes and pathways[23].

In this following study, we identified the anti-inflammatory effect of ethanolic extracts of *Uncaria tomentosa* leaves in comparison to methylprednisolone in OVA produced inflammation in bronchial tree of mice. There was a drastic increase in eosinophils and total leukocyte count.

Correspondingly, in histopathology of lung, there was noteworthy elevation of inflammatory cells, bronchial epithelial cell hyperplasia, more edema and vascular modifications in diseased group (group II). Histopathology and tissue inflammatory markers all exhibit booming induction of allergic airway inflammation in diseased group. Administration of methylprednisolone, ethanolic extract of *Uncaria tomentosa*, leaves significantly lowered the levels of these elevated parameters as compared to group II.

On histopathological examination of the lung tissue, *Uncaria tomentosa* leaves excerpts showed considerable fall in severity of inflammation, bronchial epithelial cell hyperplasia also alveolar thickening. This is corresponding with other studies that used OVA inducing method of inflammation in respiratory system and histopathological alterations in lung tissue in mice[16,22] while methylprednisolone extract of *Uncaria tomentosa* treated groups showed a significant decrease in magnitude of inflammatory changes on lung histopathology including, severity of inflammation, bronchial epithelial hyperplasia, alveolar thickening[26].

Recommendation: Further human studies are required to evaluate toxic and effective dose of Uncaria Tomentosa

Conflicts of interest: No conflict of interests

Funding by UHS

Ethical Approval: There are no ethical misconducts in this study

CONCLUSION

Outcome of this study illustrates that ethanolic excerpta(extracts)of leaves of plant Uncaria tomentosa following a dose of 200µg/day, erratically declined cells and cytokines that are responsible for inflammatory changes evident by histopathological changes.

Moreover, the changes showed that methylprednisolone showed more anti inflammatory effect compared to uncaria tomentosa, however, uncaria tomentosa may posssess less side effects than methylprednisolone and can be used as alternate in future .I have no conflict of interest and funding source.

REFERENCES:

1. Adcock IM, Caramori G, Chung KF. New targets for drug development in asthma. The Lancet. 2008 Sep 20;372(9643):1073-87.
2. Beasley R, Crane J, Lai CK, Pearce N. Prevalence and etiology of asthma. Journal of allergy and clinical immunology. 2000 Feb 1;105(2):S466-72.
3. Ryhal B. Viral Disease, Air Pollutants, Nanoparticles, and Asthma. *Bronchial Asthma*. 2011;267-283. Published 2011 May 3. doi:10.1007/978-1-4419-6836-4_11
4. Barnes PJ, Jonsson B, Klim JB. The costs of asthma. European Respiratory Journal. 1996 Apr 1;9(4):636-42.
5. Gherasim A, Dao A, Bernstein JA. Confounders of severe asthma: diagnoses to consider when asthma symptoms persist despite optimal therapy. World Allergy Organization Journal. 2018 Dec;11(1):1-1.
6. Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol*. 2006;117(6):1277-1284. doi:10.1016/j.jaci.2006.02.039
7. Arora, P. , Ansari, S. . Role of Various Mediators in Inflammation of Asthmatic Airways. In: Pereira, C. , editor. Asthma - Biological Evidences [Internet]. London: IntechOpen; 2019 [cited 2022 Nov 20]. Available from: https://www.intechopen.com/chapters/66708 doi: 10.5772/intechopen.84357
8. Kan-o K, Matsunaga Y, Fukuyama S, Moriwaki A, Hirai-Kitajima H, Yokomizo T, Aritake K, Urade Y, Nakanishi Y, Inoue H, Matsumoto K. Mast cells contribute to double-stranded RNA-induced augmentation of airway eosinophilia in a murine model of asthma. Respiratory Research. 2013 Dec;14(1):1-9.
9. Baines KJ, Fricker M, McDonald VM, Simpson JL, Wood LG, Wark PA, Macdonald HE, Reid A, Gibson PG. Sputum transcriptomics implicates increased p38 signalling activity in severe asthma. Respirology. 2020 Jul;25(7):709-18.
10. Abbas AT, Abdel-Aziz MM, Zalata KR, Tel-D AA. Effect of dexamethasone and Nigella sativa on peripheral blood eosinophil count, IgG1 and IgG2a, cytokine profiles and lung inflammation in murine model of allergic asthma. The Egyptian journal of immunology. 2005 Jan 1;12(1):95-102.
11. Ericson-Neilsen W, Kaye AD. Steroids: pharmacology, complications, and practice delivery issues. *Ochsner J*. 2014;14(2):203-207.
12. Fajt ML, Wenzel SE. Development of New Therapies for Severe Asthma. *Allergy Asthma Immunol Res*. 2017;9(1):3-14. doi:10.4168/aair.2017.9.1.3
13. Canter PH, Thomas H, Ernst E. Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. TRENDS in Biotechnology. 2005 Apr 1;23(4):180-5.
14. Sandoval-Chacon M, Thompson JH, Zhang XJ, Liu X, Mannick EE, Sadowska-Krowicka H, Charbonnet RM, Clark DA, Miller MJ. Antiinflammatory actions of cat's claw: the role of NF-kB. Alimentary Pharmacology and Therapeutics. 1998;12(12):1279-90.
15. Maroon JC, Bost JW, Maroon A. Natural anti-inflammatory agents for pain relief. Surgical neurology international. 2010;1.
16. El Gazzar M, El Mezayen R, Marecki JC, Nicolls MR, Canastar A, Dreskin SC. Anti-inflammatory effect of thymoquinone in a mouse model of allergic lung inflammation. International immunopharmacology. 2006 Jul 1;6(7):1135-42.
17. Yang EJ, Lee JS, Song BB, Yun CY, Kim DH, Kim IS. Anti-inflammatory effects of ethanolic extract from Lagerstroemia indica on airway inflammation in mice. Journal of ethnopharmacology. 2011 Jul 14;136(3):422-7.
18. Bors M, Sicińska P, Michałowicz J, Wieteska P, Gulewicz K, Bukowska B. Evaluation of the effect of Uncaria tomentosa extracts on the size and shape of human erythrocytes (in vitro). Environmental toxicology and pharmacology. 2012 Mar 1;33(2):127-34.
19. Bassett D, Hirata F, Gao X, Kannan R, Kerr J, Doyon-Reale N, Wilson S, Lieh-Lai M. Reversal of methylprednisolone effects in allergen-exposed female BALB/c mice. Journal of Toxicology and Environmental Health, Part A. 2010 Apr 13;73(11):711-24.
20. Farias I, do Carmo Araújo M, Zimmermann ES, Dalmora SL, Benedetti AL, Alvarez-Silva M, Asbahr AC, Bertol G, Farias J, Schetinger MR. Uncaria tomentosa stimulates the proliferation of myeloid progenitor cells. Journal of Ethnopharmacology. 2011 Sep 1;137(1):856-63.
21. Inam A, Sair M, Ikram S, Majeed S, Nazish GE, Ashraf MI, Zahid M. Carica papaya Leaf Extract modulates mRNA expression of Aquaporins in Mouse Model of Allergic Airway Inflammation. Pakistan Journal of Medical & Health Sciences. 2021 Oct 15: 2512-2515
22. Quan Z, Lee YJ, Yang JH, Lu Y, Li Y, Lee YK, Jin M, Kim JY, Choi JH, Son JK, Chang HW. Ethanol extracts of Saururus chinensis suppress ovalbumin-sensitization airway inflammation. Journal of ethnopharmacology. 2010 Oct 28;132(1):143-9.
23. Allen-Hall L, Arnason JT, Cano P, Lafrenie RM. Uncaria tomentosa acts as a potent TNF-alpha inhibitor through NF-kappaB. *J Ethnopharmacol*. 2010;127(3):685-693. doi:10.1016/j.jep.2009.12.004
24. Lambrecht BN, Hammad H. The immunology of asthma. Nature immunology. 2015 Jan;16(1):45-56.
25. Zein JG, Erzurum SC. Asthma is different in women. Current allergy and asthma reports. 2015 Jun;15(6):1-0
26. Yang IV, Lozupone CA, Schwartz DA. The environment, epigenome, and asthma. Journal of Allergy and Clinical Immunology. 2017 Jul 1;140(1):14-23.
27. Mussbacher M, Salzmann M, Brostjan C, Hoesel B, Schoergenhofer C, Datler H, Hohensinner P, Basílio J, Petzelbauer P, Assinger A, Schmid JA. Cell type-specific roles of NF-κB linking inflammation and thrombosis. Frontiers in immunology. 2019 Feb 4;10:85