"EVALUATION OF ESSENTIAL OILS FOR ANTI-ASTHMATIC ACTIVITY BY USING ANIMAL MODELS"

Thesis Submitted to

THE KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH,

BELAGAVI

(KLE DEEMED UNIVERSITY)

[Declared as Deemed-to-be-University u/s 3 & 12 B of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3 (A)]

(Accredited 'A+' Grade by NAAC) (3rd Cycle) [Placed in Category 'A' by MoE (GoI)]

For the award of the degree of

Doctor of Philosophy

In the Faculty of

Pharmacy

By

Ms. Shilpa Sharma **M. Pharm**

(Registration No: KLEU/Ph.D./14-15/DO1214019)

Under the Guidance of

Prof. (Dr.) Vijaykumar P. Rasal **M. Pharm., Ph. D**

Former Professor and Head,
Department of Pharmacology,
Former Principal, KLE College of Pharmacy, Belagavi,
KLE Academy of Higher Education and Research, Belagavi-590010

2024

Undertaking

I, **Shilpa Sharma,** hereby declare that the information and the data mentioned in my thesis entitled **"Evaluation of essential oils for anti-asthmatic activity by using animal models"** belongs to me and is original.

I am aware of definition of plagiarism as detailed below:

- An act or instance of using or closely imitating the language and thoughts of another author without authorization and the representation of that author's work as one's own, as by not crediting the original author.
- A piece of writing or other work reflecting such unauthorized use or imitation.
- The deliberate or reckless representation of another's words, thoughts or ideas as one's own without attribution in connection with submission of academic work, whether graded or otherwise.

I hereby declare that the thesis prepared by me is original-one and does not involve plagiarism anywhere. In case at a later stage it is found that I have indulged in plagiarism, then I am solely responsible for the same and the Institution is at liberty to take any disciplinary action against me including cancellation of dissertation or any other penalties imposed by the University.

Date:

Place: Belagavi

Ms. Shilpa Sharma
Full-time Research Scholar,
KLE College of Pharmacy,
KAHER, Belagavi, Karnataka

PLAGIARISM REPORT

Ref. No. KAHER/AA/23-24/D-377

28th February 2024

Madam,

The soft copy of Ph.D. research thesis of **Ms. Shilpa Sharma, Faculty of Pharmacy, KAHER, Belagavi** has been submitted for anti-plagiarism check at the office of the undersigned through "Turn-it-in" package. The scan has been carried out and the scanned output reveals a match percentage of **8%** which is within the acceptable limit of 10%.

To obtain the comprehensive report of the plagiarism test, research scholar can send a mail to **diracademic@kledeemeduniversity.edu.in** along with the Registration Number, Name of the Scholar, Name of Guide/Co-guide and title of the thesis.

Dr.(Mrs.) Roopa M. Bellad
Director, Academic Affairs

DIRECTOR ACADEMIC AFFAIRS Deemed-to-be University

To,

Ms. Shilpa Sharma
Full-Time Ph.D. Scholar,
2014-15 Batch
KLE College of Pharmacy,
Faculty of Pharmacy, KAHER
Belagavi.

Cc to :

1. The Principal, KLE College of Pharmacy, Belagavi
2. Dr. Vijaykumar P. Rasal, Former Professor, KLE College of Pharmacy, KAHER, Belagavi - Guide

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI

(KLE DEEMED UNIVERSITY)

[Declared as Deemed-to-be-University u/s 3 and 12 B of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3 (A)]
(Accredited 'A+' Grade by NAAC) (3rd Cycle) [Placed in Category 'A' by MoE (GoI)]

COPYRIGHT DECLARATION

We hereby declare that **KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI, KARNATAKA,** *shall have the rights to preserve, use and disseminate this thesis in print or electronic format for academic/research purpose.*

Shilpa Sharma
Full-time Research Scholar,
KLE College of Pharmacy, Belagavi,
KAHER, Belagavi

Prof. (Dr.) Vijaykumar P. Rasal
Former Professor and Head,
Department of Pharmacology,
Former Principal, KLE College of Pharmacy,
Belagavi, Karnataka

Place: Belagavi
Date:

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI

(KLE DEEMED UNIVERSITY)

[Declared as Deemed-to-be-University u/s 3 and 12 B of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3 (A)]
(Accredited 'A+' Grade by NAAC) (3rd Cycle) [Placed in Category 'A' by MoE (GoI)]

DECLARATION

I hereby declare that the thesis entitled ***"Evaluation of essential oils for anti-asthmatic activity by using animal models"*** *is a bonafide and original research carried out by me under the guidance of* ***Dr. Vijaykumar P. Rasal,*** *Former Professor and Head, Department of Pharmacology, Former Principal, KLE College of Pharmacy, Belagavi, Karnataka. The thesis or any part thereof has not formed the basis for the award of any degree/fellowship or similar title to any candidate of any University.*

Place: Belagavi

Date:

Ms. Shilpa Sharma

Full-time Research Scholar,

KLE College of Pharmacy, Belagavi,

KAHER, Belagavi, Karnataka

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI

(KLE DEEMED UNIVERSITY)

[Declared as Deemed-to-be-University u/s 3 and 12 B of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3 (A)]
(Accredited 'A^{+}' Grade by NAAC) (3rd Cycle) [Placed in Category 'A' by MoE (GoI)]

KLE
EMPOWERING PROFESSIONALS

CERTIFICATE

*This is to certify that the thesis entitled "**Evaluation of essential oils for anti-asthmatic activity by using animal models**" is a bonafide and genuine research carried out by **Shilpa Sharma** under the guidance of **Dr. Vijaykumar P. Rasal,** Former Professor and Head, Department of Pharmacology, Former Principal, KLE College of Pharmacy, Belagavi, Karnataka.*

Place: Belagavi

Date:

Dr. Kalpana S. Patil
Dean
Faculty of Pharmacy,
KAHER, Belagavi, Karnataka.

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI

(KLE DEEMED UNIVERSITY)

[Declared as Deemed-to-be-University u/s 3 and 12 B of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3 (A)]

(Accredited 'A^{+}' Grade by NAAC) (3rd Cycle) [Placed in Category 'A' by MoE (GoI)]

CERTIFICATE

*This is to certify that the thesis entitled "**Evaluation of essential oils for anti-asthmatic activity by using animal models"** is a bonafide and genuine research carried out by **Shilpa Sharma** under the guidance of **Dr. Vijaykumar P. Rasal,** Former Professor and Head, Department of Pharmacology, Former Principal, KLE College of Pharmacy, Belagavi, Karnataka.*

Place: Belagavi

Date:

Dr. Sunil S. Jalalpure

Principal,

KLE College of Pharmacy, Belagavi,

KAHER, Belagavi

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI

(KLE DEEMED UNIVERSITY)

[Declared as Deemed-to-be-University u/s 3 and 12 B of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3 (A)]

(Accredited 'A^{+}' Grade by NAAC) (3rd Cycle) [Placed in Category 'A' by MoE (GoI)]

CERTIFICATE

*This is to certify that the thesis entitled "**Evaluation of essential oils for anti-asthmatic activity by using animal models**" is a bonafide record of original research carried out by **Shilpa Sharma** for the award of degree of **Doctor of Philosophy in Faculty of Pharmacy** under my supervision and guidance.*

Place: Belagavi

Date:

Dr. Vijaykumar P. Rasal

Former Professor and Head,

Department of Pharmacology,

Former Principal,

KLE College of Pharmacy, Belagavi,

KAHER, Belagavi, Karnataka

Acknowledgment

One of the most precious things which God has gifted to us is "the expression". I have ventured below my feelings of gratitude from the bottom of my heart to all those who have contributed in completion of my work. With great efforts, invaluable inputs and immense support of all my teachers and colleagues who were pillars of my strength, without which this work wouldn't have been completed.

I would like to express my benevolent thanks to my guide ***Prof. (Dr.) Vijaykumar P. Rasal*** *(former) Principal, KLE College of Pharmacy, who has continually conveyed a spirit of motivation in regard to research. I am grateful to his Guidance, constant encouragement and providing all facilities required for my dissertation work.*

I am immensely thankful to ***Dr. Rajesh K. Joshi****, department of Phytochemistry* ***ICMR-RMRC,*** *without his constant guidance and persistent help this research would not have been materialized and successful. I shall forever remain indebted to him for having inculcated in me a quest for knowledge and for always guiding me in right direction.*

I am highly grateful to Principal ***Prof. (Dr.) Sunil S. Jalalpure*** *for extending his kind support and guidance in right direction. I am Sincerely Thankful to Registrar* ***Dr. M. S. Ganachari*** *for his help. I am Thankful to Vice-Chancellor* ***Dr. Nitin Gangane****. I am also Thankful to former Director of Academic Affairs* ***Dr. Daksha Dixit****, Deputy Director of Academic Affairs* ***Dr. Shwetha Kumbhojkar*** *and* ***Dr. Sheetal Harakuni.***

*I am profusely thankful to **Dr. P. A. Patil**, department of Pharmacology, USM KLE University Sains Malaysia for his invaluable suggestions. I am Thankful to **Kancor, Synthite** and **GR herbals** for providing me Essential Oils.*

*I owe my special Thanks to my friends and Juniors **Dr. Preeti Salve, Dhruvi Panchal, Jyotsana Patil, Dr. Jeswiny Rodrigues, Dr. Pukar Khanal** who have always lent me a helping hand whenever I needed. I am thankful to my senior **Dr. Mahusudhan Telagari** for his inputs. I would also like to thank all the **teaching** and **non-teaching staff** members of Department of Pharmacology, KLE College of Pharmacy.*

*I express my immense gratitude and source of motivation, my family **Mr. Vishal, Mr. Roshan, Dr. Vipul and Mrs. Asha** for their endless encouragement, support through all these years.*

Of course, I can't forget those innocent mice and guinea pigs on which I performed my work without which my dissertation would not have turned into reality.

Most importantly, I Thank the God for continually blessing me and giving me strength and courage to fulfil my endeavors!

Date: ***Shilpa Sharma***

Place:

LIST OF ABBREVIATIONS

OVA	Ovalbumin
PBS	Phosphate buffer solution
Al2(OH)3	Aluminium Hydroxide
AHR	Airway Hyperresponsiveness
PCD	Pre-Convulsion Dyspnoea
Dexa	Dexamethasone
A.galanga	*Alpinia galanga*
A.glauca	*Angelica glauca*
C.aromaticum	*Cinnamomum aromaticum*
C.camphora	*Cinnamomum camphora*
C.domestica	*Curcuma domestica*
M.arvensis	*Mentha arvensis*
M.oleifera	*Moringa oleifera*
O.vulgare	*Origanum vulgare*
Z.officinale	*Zingiber officinale*
Z.armatum	*Zanthoxylum armatum*
EO	Essential Oil
IFN-γ	Interferon- γ
TNF	Tumor Necrosis Factor

IL	Interleukins
LTC4	Leukotriene C4
LTD4	Leukotriene D4
AA	Arachidonic acid
GM-CSF	Granular Macrophage- Colony Stimulating Factor
APC	Antigen Presenting Cell
WHO	World Health Organization
UK	United Kingdom
USA	United States of America
GINA	Global Initiative for Asthma
TLR	Toll like Receptor
BALF	Bronchiolar Lavage Fluid
CNS	Central Nervous System
PDE	Phosphodiesterase Enzyme
VCAM-1	Vascular Cell Adhesive Membrane
IRV	Inspiratory Reserve Volume
ERV	Expiratory Reserve Volume
VT	Tidal Volume
IC	Inspiratory Capacity
VC	Vital Capacity

FRC	Functional Residual Capacity
RV	Residual Volume
IgE	Immunoglobulin E
COPD	Chronic Obstructive Pulmonary Disorder
TLC	Total Leukocyte Count
DLC	Differential Leukocyte Count
AEC	Absolute Eosinophil Count
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
OECD	Organization for Economic Co-operation and Development
LD50	Lethal Dose
H&E	Haematoxylin and Eosin

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Background: The therapeutic management of asthma relies heavily on bronchodilator and anti-inflammatory inhalers, which, although effective, often require long-term use and can be associated with various side effects. Seeking alternatives to mitigate or potentially eliminate these side effects has led to exploration of alternative and complementary medicinal systems, including the use of medicinal plants in folklore therapy. While many plants have been traditionally used for treating various ailments, their diverse ethno-pharmacological applications lack conclusive scientific evidence for safe and rational use. Nevertheless, the phytochemical composition of plants continues to provide a rich source of novel chemical entities for drug discovery. Essential oils derived from plants have long been employed in traditional medicine for respiratory infections and are now being recognized in mainstream medicine for their therapeutic potential in conditions such as bronchitis, sinusitis, and asthma. These essential oils demonstrate anti-inflammatory effects on the respiratory tract, offering promising avenues for both acute and chronic respiratory conditions.

Objective: The present investigation aimed to evaluate the activity of the essential oil of *Mentha arvensis L. Origanum vulgare L, Moringa oleifera, Zanthoxylum armatum, Zingiber officinale, Angelica glauca, Alpinia galangan, Curcuma domestica, Cinnamomum camphora* and *Cinnamomum aromaticum* on exogenously induced bronchoconstriction in experimental animals.

Methodology: The anti-asthmatic effect of these essential oils was studied using histamine aerosol-induced bronchoconstriction in guinea pigs and ovalbumin (OVA) sensitised albino mice.

Results: Treatment with essential oils significantly ($p < 0.001$) increased the time of preconvulsive dyspnoea in histamine-induced guinea pigs. Oral treatment of essential oils significantly ($p < 0.001$) decreased absolute eosinophil count, serum level of IgE and the number of eosinophils, neutrophils in BALF. Histopathological examination of lungs showed that essential oils rescinded bronchial asthma.

Conclusion: The present investigation provides evidence that Essential Oils relaxes bronchial smooth muscles and suppressed immunological response to OVA.

Keywords: GC-MS; essential oils; bronchorelaxation; essential oil composition; immunosuppression; asthma.

1.INTRODUCTION

"An inflammatory condition of airways affecting multiple cells & their elements exerting actions, particularly masts cells, eosinophils, T lymphocytes, macrophages, neutrophils & epithelial cells is referred to as Asthma". "Among individuals who are susceptible, this condition results in recurrent episodes of wheeze, breathlessness, tightness in the chest & cough, especially in the dark or in the early hours".[1]

This disorder affects the sensitivity of the nerve endings in the airways, making them easily motivated. It is caused by inflammation of the lungs' air channels. During an attack, the lining of the passageways swells, narrowing the airways & decreasing the amount of air that can enter & exit the lungs.[2.] Numerous things, including medicines, dust, exercise, cold air, emotions, chemicals, occupational stimulation, histamine, allergies, & genetics, might cause it. The activation of "immunoglobulin-E-mediated mast cell release of interleukins" & other inflammatory agents, is accelerated by these trigger factors. For this reason, it is evident from the illness statistics that the therapy of asthma requires the use of medications that target cytokine inhibitors, neutralising antibodies directed against IgE, histamine, leukotrene blockers, & so forth.[3]

WHO survey results indicate In 2019, asthma claimed the lives of 455,000 people worldwide, affecting over 262 million people. It is the most prevalent chronic illness in kids. Although asthma is present worldwide, the majority of deaths attributable to the condition happen in developing & impoverished nations. According to estimates from the GINA, the prevalence of asthma in children who

already wheeze increases by 50% every ten years worldwide. Over the next 20 years, there is expected to be a significant augment in the global asthmatic population due to the predicted growth in the percentage of the world's population living in cities from 45% to 59% in 2025. According to the phase I of the International Study of Asthma & Allergy in Childhood, asthma is more severe in low- & middle-income nations than it is in high-income nations. India had 10 crore asthma sufferers in 2006. India's asthma prevalence is on the rise. According to the Chest Foundation of India profile, out of 112 countries, 12% of children in some parts of South India have asthma.[4-8]

For therapeutic management of asthma, inhalers of bronchodilators & anti – inflammatory drugs are effectively used, but demand long term usage & is correlated with several side effects. To reduce & probably stop these side effects, medicines of alternative & complementary type are sought.[9] Alternative medicinal system & folklore therapy utilized several medicinal plants for treating several ailments. But diverse ethno pharmacological utilization is yet to be stamped with scientific evidence for its safe & rational use. Also, Phyto constitution of plants frame new sources of chemical entities in new drug discoveries. Some essential oils obtained from greens are utilized in infections of respiratory tract traditionally. Since recent years they are also being used as ethical medicines & in colds.[10-11]

These essential oils are utilized in therapy as inhalers for treatment of acute & chronic bronchitis & acute sinusitis in the ethical discipline. It exerts anti-inflammatory action on trachea & reduces asthma.[12]

Haldi; *Curcuma domestica*, **(**Zingiberaceae) The rhizomes contain pigment curcumin, sesquiterpines, sesquiterpeniods as germacrane, bisabolane guaiane & an essential oil containing pinene, turmerone, sabinene, myrecene, terpinene, limonene, cymene, perillyalcohol, turmerone, dehydroturmerone, alanto lactone, curcumene & cineole,sabinene, cineol, borneol, zingiberene & sesquiterpenes. Analysis of rhizomes also shows moisture, protein, fat, carbohydrates, fiber, & minerals. "Minerals & vitamin composition are calcium, phosphorus, iron, carotene, thiamine & niacin". They are used to treat diabetes, gastric & duodenal ulcers, viral hepatitis, jaundice & for skin infections, antispasmodic, body ache, antibacterial, in arthritis, hepatitis, menstrual disorders, anemia, rheumatism, boils, as blood purifier, conjunctivitis & skin disorders. Its usage is also beneficial in cough, cold, throat infections, bronchitis & in asthma. [13,14]

Dalchini; *Cinnamomum aromaticum*, **(**Lauraceae) Essential oil obtained from the bark & leaves contain, eguenol, cineole, linalool, caryphyllene, humulene, safrole, cinnamaldehyde, cinnamyl acetate, cinassiol, euenol acetate, cumic aldehyde, dipentonides, methyl euenol, camphene, benzaldehyde, geraniol & diaterpenes. The leaves are enriched with minerals like calcium, phosphorus, iron, sodium, potassium & amino acids like thiamine, riboflavin, niacin & vitamins A & C. The root & bark is useful in asthma, bronchitis, anorexia, colic, cough, chronic mastitis, dyspepsia, diminished appetite, fever, gonorrhea, hiccough, inflammation, leprosy, skin disease, sinusitis, tuberculosis, tremors, cephalagia & ophthalmitis or inflammation of eyes. [13,14]

Kulanjan; *Alpnia galangan*, (Zingiberaceae) Rhizomes contain tannin, starch, chlorides, sulphates, phosphates & essential oil contain methyl cinnamate, cineole, pinene & camphor. The seeds contain 7heptadecane, fatty acids methyl ester,

acetoxyeugenol acetate. Rhizomes are used in improving voice, bronchitis, dyspepsia, impotency, nervous debility, inflammation of liver, disease of kidney, respiratory trouble, tuberculosis, in flatulence, colic, vomiting, diarrhea, cancer, cramp pain & back pain. [13,14]

Pudina; *Mentha arvensis Linn*, (Lamiaceae) Major components in the oil are menthol, methone, methyl acetate along with terpenes like pinene, thujene, camphene, carvomenthone, limonine, menthofuran, isomenthol, glutamic acid, aspartic acid, glycine, alanine, lysine, valine, isoleucine, leucine, phenylalanine, linalool & geraniol". The leaves & oils are used in treatment of liver , jaundice, asthma, stomach disorders, headache, rheumatic pains, as expectorants, as antifungal, antibacterial, in nasal & bronchitis catarrh, cough, rhinitis, sore throat, vomiting, dyspepsia & diarrhea, joints pain, carminative & antispasmodic. [13,14]

Timur; *Zanthoxylum armatum*, (Rutaceae) The essential oil from the seeds contains dipentene, linalool, citral, methylcinnamate, pipevine, linalyl acetate, limonene, geraniol, cineole, cymene, terpinene, camphor, carvone, fenchol, thujene, terpineol, caryophyllene, monoterpene & enoic acid. The oils from leaves contain linalyl acetate, sesquiterpenes, hydrocarbons, acids, phenols, cineole, limonene, citronellal & triscosane. "The bark & fruits are useful in asthma, bronchitis, colic, cough, convulsions, cardiac debility, diabetes, diarrhea, dyspepsia, fever, goiter, difficult micturition, eye & ear disease, helminthiasis**,** hepatopathy, leprosy, leukoderma, paralysis, skin disease, stomach disorder, tumors, ulcers & wounds". [13,14]

Adrak; *Zingiber officinale*, (Zingiberaceae) Rhizomes contain curcumin, bergamotene, pamphenen, calamene, pinene, limonene, cineole, citral, citronellal, linalool, zingerol, zingerone, amino acids like asparagine & piperolic acid, ginger

glycolipids, sulfonic acid, geraniol, geranyl acetate, phenylalanine, malonate, serine, valine, leucine, isoleucine, glycine & arginine. It is used in anorexia, asthma, bronchitis, cough, cold, dyspepsia, diarrhea, earache, fainting fever, inflammations, edema, sore throat, urticarial, nausea, tuberculosis of the lungs, cardiac disorders, hiccups, jaundice, kidney stone attack, bladder inflammation, morning sickness, piles, toothache, antiemetic & anti-vertigo effects. [13,14]

Oregano; *Origanum vulgare Linn*, **(**Lamiaceae) Essential oils obtained from oregano primarily constitutes monoterpenoids & monoterpenes, but has a wide variation of concentration of individual compounds based on their location & several other factors. "More than 60 separate compounds are identified with the principal components of carvacrol & thymol in the concentration of 0 over 80% & the lower concentration compounds are cymene, γ terpinene, caryophyllene, spathulenol, germacrene-D, β-fenchyl alcohol & δ-terpineol". It is utilised in treating inflammatory conditions, catarrh, headache, paralysis. The leaves are good for earache, bronchitis, asthma". The plant parts are used for hemicranias & oil is used in rheumatism, skin inflammation, sores, burns & wound healing. [13,14]

Gandrayan; *Angelica glauca*, (Umbelliferae) contains lactones, sesquiterpenes, cadinene, umbelliprenin, terpene & alcohol. Roots are used in wounds & gastric pain. It used in dyspepsia, anorexia, spasm, flatulent, colic's & bronchitis. [13,14]

Kapoor; *Cinnamomum camphora*, (Lauraceae) contains glycerides of lauric, capric & oleic acid. It is useful in hysteria & nervousness, diarrhea, muscular strains, rheumatic condition, inflammation, smallpox, typhoid, whooping cough, hiccup, spasmodic asthma, dysmenorrheal, mania, epilepsy, gout, toothache, chronic bronchitis & uterine pains. It also used in various cardiac depression. [13,14]

Shigru; "*Moringa oleifera*, (Moringaceae) oil contains myristic acid, palmitic acid, oleic acid, stearic acid, behenic acid & lignoceric acid". "The bark contains presence of sterols & terpenes". "Amino acids were extracted from the leaf such as aspartic acid, glutamic acid, serine, glycine, threonine, alanine, valine, leucine, isoleucine, histidine, lysine, arginine, tryptophan, cystine & methionine & also carotene". It is used in anti-diabetic, antispasmodic, antiemetic, antipyretics, anthelmintic, wound, boils, swelling, chronic rheumatism, intermittent fever, throat sores. It is also used in cardiac stimulant in asthma, cough & similar disorders. [13,14]

2.AIM & OBJECTIVES

The study aimed to assess anti-asthmatic activity of essential oils by using animal models.

***IN-VITRO* MODEL**

1. Spasmolytic activity on guinea pig isolated tracheal chain.

***IN-VIVO* MODELS**

1. Ovalbumin induced sensitization in mice
2. Histamine induced bronchospasm in guinea pigs

PARAMETERS TO BE EVALUATED

1. Inflammatory cell count in BALF
2. Absolute eosinophil count (AEC) in blood
3. IgE level in blood serum
4. Histopathology of lungs

- Polyessential oil formulation

3. REVIEW OF LITERATURE

RESPIRATORY SYSTEM[15-20]

The respiratory tract system provides for exchange of gases i.e. intake of O_2 (inspiration) & removal of carbon-di-oxide (expiration) because cells continuously use oxygen for the metabolism reaction that release energy as ATP from nutrients & carbon dioxide get eliminated.

Structure wise, respiratory system is made of two components:

1. The nose, pharynx & correlated structures form the upper part of the respiratory system
2. The larynx, trachea, bronchi & lungs form the lower component.

Inflammation mainly occurs in bronchi & lungs. So, let's discuss the histology of above-mentioned parts.

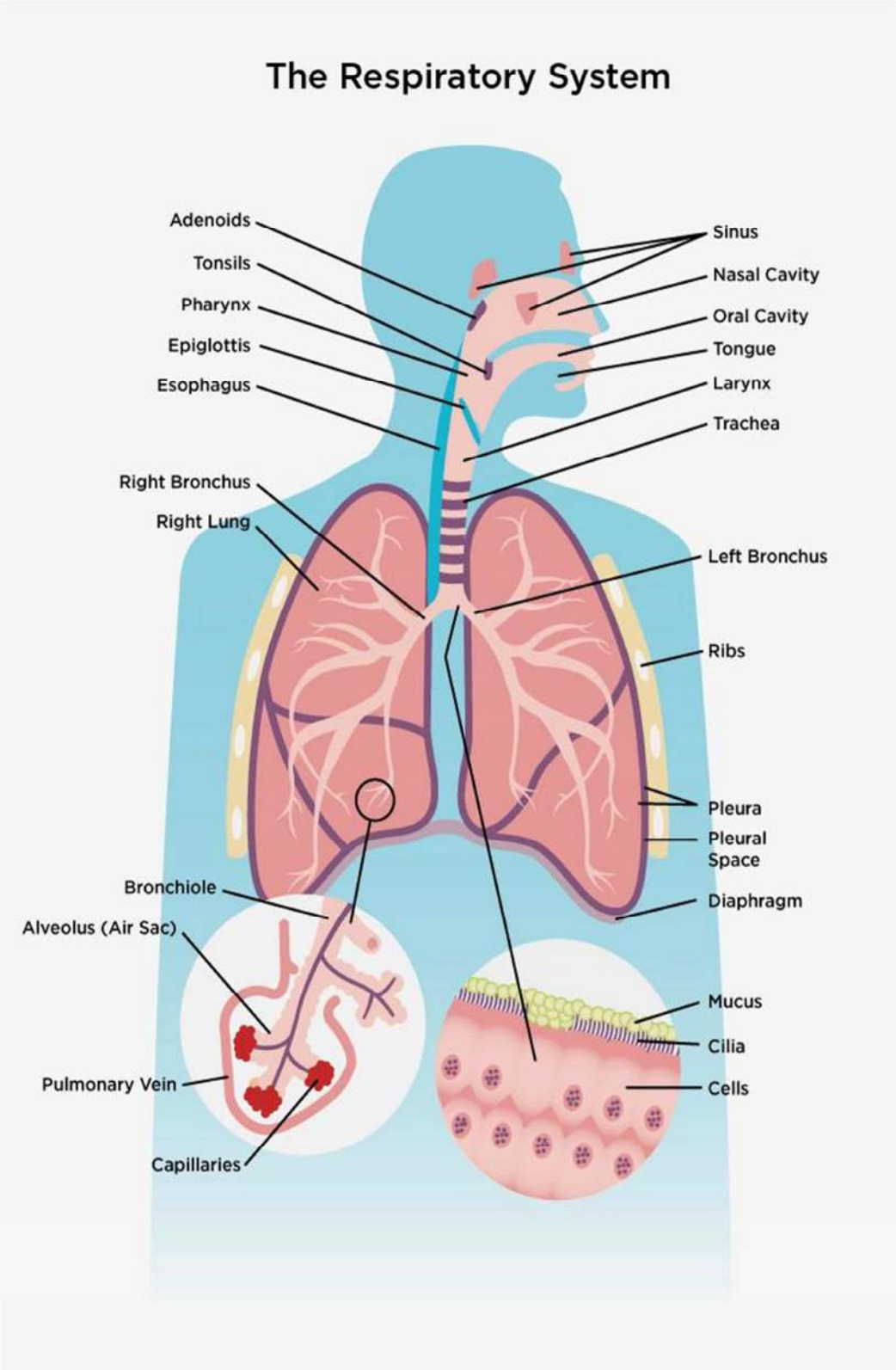


Figure 1. Human Respiratory System

BRONCHI:

"The structure of bronchus starts with trachea dividing as the right & left principal bronchi at the T5 vertebral cadre, running into both lungs, right & left respectively".

Bronchus on the right side is more vertically placed, is short & wide when compared to left. Because of this, any object which is aspirated has a greater tendency in gaining entry & lodging in the right than the primary bronchus. The right main stem of bronchus cuts off into bronchus right upper lobe & bronchus intermedium. The intermedium further branches into the middle lobe bronchus right & lower lobe bronchus right. The bronchus of upper lobes branches into apical, front & back lobe bronchi. "Further the right middle lobe shoots as lateral & medial segmental bronchi, while the lower lobe right bronchus branches into the superior, medial basal, anterior basal, lateral basal, & posterior basal segmental bronchi". The primary bronchi also are made of incomplete cartilaginous rings like the trachea which is lined by pseudostratified ciliated columnar epithelium. The intersection at point of trachea branches into right & left primary bronchi, a structure named carina is found. Carina is an internal ridge framed by a posterior & a kind of interior projection of the last tracheal cartilage. The mucosa of these structure forms a sensitive area of the laryngeal & tracheal structures, which triggers a cough reflex.

Changes in carina such as widening & distortion is an alarming sign as it reflects carcinoma of the lymph nodes in the area where the trachea branches. The ultimate branching named the respiratory bronchiole, divides again to form multiple alveolar ducts. The functional units, namely alveoli begin to appear at the mark of respiratory bronchioles. This multiple division from trachea is similar to an inverted

tree & is usually termed as the bronchial tree. The wall of bronchi composes mucosa, lamina propria, smooth muscle, & submucosa with interspersed cartilage. Bronchi of first generation are almost the same as each in terms of histological appearance, apart from the quantity of hyaline cartilage. The lumen is encircled by the cartilage in the trachea except the further branching of bronchi, which is substituted by reducing amounts of cartilaginous plates.

Bronchial mucosa is constituted by pseudostratified ciliated columnar epithelium mixed with goblet & basal cells. The epithelial cells of respiratory tract is made of basal bodies, referred to as terminal bars, forming a dark bank underneath the cilia which are actually changed centrioles.

- Goblet cells do not have apical cilia but possess mucus granules in their cytoplasm owing for mucin secretion. Goblet cells density progressively reduces from the peripheral areas disappearing as they approach terminal bronchiole level.
- Basal cells are situated proximal to basal lamina farther from bronchi lumen.

Bronchial submucosa is made of glands of mixed compound tubuloacinar type, comprising greatly of cells secreting mucin & serous, composed of mucin, water & electrolytes letting into the lumen of bronchus. [17] "Ciliated cuboidal epithelium, thin non continuous smooth muscle, & submucosal connective tissue is found in the terminal bronchioles". "A sudden transformation of low cuboidal ciliated epithelial cells to squamous respiratory epithelial cells marks the transition to an alveolar duct from a respiratory bronchiole". 2 main types of epithelial cells line the alveoli namely type I & type II. Flat cells made of large cytoplasm form Type I cells which are the prime lining of alveoli. Granular pneumocytes, which are Type II cells are denser

containing inclusion bodies; & are responsible for surfactant secretion & alveoli repairing. "Specialized cells like pulmonary alveolar macrophages, lymphocytes, plasma cells, neuroendocrine cells, & mast cells are found in alveoli". With decreasing cartilage amount, smooth muscle amount increases. As there is no supporting cartilage, the airways closed due to muscle spasms. An asthmatic attack ensues forming a life-threatening condition.

LUNGS[19]

Lungs are conical in shape, respiratory organs situated in the thorax in pairs. Heart & other mediastinal structures separate them from each other, dividing the thoracic cavity into two separate anatomical chambers. Because of which, if either lung collapses due to trauma, the remaining one expands. "A double layered serous membrane encloses & protects the lungs known as pleural membrane & the thoracic cavity wall is lined by a superficial layer called parietal pleura & the visceral pleura is the deeper layer covering the lungs itself". A little space is present between the parietal & visceral pleurae called the pleural cavity containing a little quantity of lubricating fluid secreted by the membranes.

The friction in the mucosal membranes is reduced due to pleural fluid, making them slide comfortably over each one while breathing. The fluid also ensures that the membranes adhere to each one like a water film sticking two glass microscope slides, the phenomenon is termed is surface tension. Both the right & left lungs are capsulated by different pleural cavities. Pleurisy or pleuritis, which is pleural membrane inflammation in its early stages can result in pain because of friction between the pleural layers. Pleural effusion can result if inflammation continues by resulting in excess fluid accumulation in the pleural space. The lungs rest against the

ribs in the front & back extending from diaphragm slightly superior above the clavicles. The base of the lung, which is the inferior broadest portion is concave & snug fits the convexity of diaphragm. Apex is the superior narrow lung portion.

Though the right lung is thick & broad, it is little smaller than the left as on the right part, the diaphragm is higher to accommodate the liver lying inferior to it. Thorax is almost filled by the lungs. The lung apex is present superior to the medial third portion of clavicles, & is the only region which can be palpated. Against the rubs lies the anterior, posterior & lateral surface of lungs. The lung base spreads from the 6^{th} costal cartilage in the anterior region to the tenth thoracic vertebrae's spinous process in the posterior aspect. From 5cm below the lung base, the pleura extends from 6^{th} coastal cartilage anteriorly to 12^{th} rib in the posterior region.

Hence, the pleural cavity is not entirely filled by the lungs in this region. Thoracentesis, a procedure is followed to remove excess fluid in the pleural cavity without any injury to lung tissue by only inserting a needle from the seventh intercostal space anteriorly. The needle flows through the lower rib's superior border to prevent injury to intercostal blood vessels & nerves. The risk of diaphragm penetration arises when passed inferior to the seventh intercostals space.

ALVEOLI[19-20]

Numerous alveoli & alveolar sacs are present circumscribing the alveolar ducts. Alveolus is the shape of cup structure with simple squamous epithelium lined & is guarded by thin elastic basement membrane; two or more alveoli forms the alveolar sac which shares a single entry.

The alveolar walls are made of two varieties of alveolar epithelial cells. **Type I alveolar cells,** are more in number, which is lined by simple squamous epithelial cell in a continuity along the alveolar wall. **Septal cells,** which are **type II** are lesser in quantity which is found interspersed with type I cells. The principal site of gas exchange occurs in type I alveolar cells. The type II ones are either rounded or of cuboid shaped epithelial cells containing microvilli on the free surface, secreting alveolar fluid keeping the cell surface & air moist. A compound mix of phospholipids & lipid proteins, namely the surfactant is also found in the alveolar fluid known as surfactant. It helps to keep the surface tension lower, reducing the alveolar tendency to collapse, thus maintaining its patency. **Alveolar macrophages (dust cells)** are the phagocytic cells, also located in the alveolar wall which remove fine dusty particulate matter & debris from alveolar spaces.

Along with these fibroblasts are also present which produces elastic & reticular fibers. An elastic basement membrane is present under the type I alveolar cells. The alveoli's outer surface a network of capillaries disperses into the lobule's arteriole & venule consisting an endothelial cellular layer & basement membrane. Oxygen & Carbon dioxide gases exchange between the lung space & blood by the process of diffusion via the alveolar & capillary walls, that cumulatively form the **respiratory membrane.**

"The respiratory membrane comprises 4 orders, from the alveolar air space to blood plasma":

1. **"Alveolar wall** constituted by a single layer of alveolar cells of type I & II & alveolar macrophages".
2. **"**Underneath the wall of alveoli is the **epithelial basement membrane".**

3. **"**A **capillary basement membrane** which is usually fused to the epithelial basement membrane".
4. The **capillary endothelium.**

Though there are several layers, the mucosal membrane of the respiratory tract is pretty thin – about 0.5 µm dense, & 1 / 16^{th} the diameter of RBC so as to let fast diffusion of gases. An estimation of about 300 million alveoli to be present in the lungs, giving a greater surface area of 70 m2 (750 ft2) which is just the size of racquet ball court to allow exchange of gases.

ASTHMA[21,22]

"This inflammatory disorder results in recurring episodes of wheezing, breathlessness, tightness of chest & cough, especially during the early morning or night in those individuals who are susceptible." The usual symptoms are bronchoconstriction of reversible nature, increased basal airway tone, lymphocytic activating & accumulating, smooth muscles hypertrophy, fibrosis of submucosal fibrosis, edema of airway wall, excessive mucus secretion & occurrence of non-specific airway hyper responsiveness to particular spasmogens. No therapy exists for asthma, but it is only looked after with appropriate treatment, helping people to lead a normal active life. The airways or breathing passages are greatly sensitive due to variety of stimuli. These extra- sensitive airways become red, swollen & twitchy which undergo spasm.[21]

Asthma can no longer be viewed simply as "reversible airway obstruction" or "irreversible airway obstruction". This condition must be considered as "an inflammatory illness with bronchial hypersensitivity & bronchospasm." This

inflammatory disorder of chronic type affects the respiratory passage & is characterized by enhanced mucosal secretion & hyper responsiveness of airways caused a reduced air flow & highlighted by recurring attacks of wheeze, cough & breathlessness. This disorder has a multifactorial cause correlated with allergic, infectious, genetic, environmental emotional, & nutritional components.

It is an inflammatory condition affecting the air passages of the lungs & affecting the nerve endings sensitivity which get irritated easily. The passage linings get swollen in the process of an attack resulting in the constriction of airways & reduction of air flow to the lungs.[22]

PREVALENCE[4-8,22]

Asthma affects 3.5-20 % of masses in all countries. The evidence in prevalence of asthma in the previous 25 years is bound to vary because of changes in surroundings, as our genetic constitutional makeup variation might take many generations to occur. The occurrence of asthma is greater in urban areas compared to rural areas. It has been reported that in India 40% of 6-7 years children's & 47.8% of 13-14 years children are found to have asthma & rise in prevalence was observed till 2010. WHO survey states that approximately 235 million population globally are presently suffering because of asthmatic condition. Amongst children, it forms the greatest common chronic disease. Majority of asthmatic causalities are seen in under developed & developing nations but asthma exists in all parts of the world. Worldwide, deaths from this condition have reached over 180,000 annually. Global initiative for Asthma (GINA) estimate suggests that asthma prevalence of current wheezing in children raises worldwide by half every ten years. Expecting an exponential increase in the global urban population to 59% from 45%, there is a

likelihood for a significant increase in asthmatic numbers globally in the coming 20 years. "It is put forth that asthma accounts for about 1 in every 250 deaths worldwide & there could be an extra 100 million people affected with asthma by 2025".[4-8] Problems of this condition aggravated in industrial countries & still it is increasing in prevalence & severity worldwide. Some chest specialists consider increase in morbidity in asthma is attributable to non-optimal application of currently available therapy.

ETIOLOGY[23-26]

Significant risk factors in development of asthma are the compliment of genetic pre-disposition factors with exposing to environmental inhaled substances & particulate matter provoking allergy or cause irritation of airways like indoor allergens such as household dust particles, mites, pollution & pet dander. External allergens like pollens & moulds, environmental tobacco smoke, irritants of chemical origin in the work station, air pollution. "Asthmatic condition is complex & is multifactorial involving interplay between genetic factors & environmental stimuli". "Candidate gene/loci studies have suggested linkage of atopy & nonspecific AHR to different chromosomes". Chosen genes amongst these loci which are probably intricate in susceptibility for asthma will be:

- 5q: "interleukin (IL-)-3, IL-4, IL-5, IL-6, IL-9, IL-12, IL-13, transforming growth factor (TGF)- β1, CD14, granulocyte macrophage colony stimulating factor (GM-CSF), catenin, fibroblast growth factor-1, glucocorticoid receptor, & β2 –adrenergic receptor".
- 6p: "HLA complex & tumor necrosis factor (TNF)-α";
- 11q: "FcεR1 & Clara cell secretory protein (CC16)";

- 12q: "interferon (IFN)–γ, IGF1, Glutathione-S-transferase, nitric oxide synthase (NOS1), leukotriene A4 hydrolase, selectin P ligand, & mast cell growth factor".
- 13q: "esterase D";
- 14q: "TCR α/δ complex";
- 6p: "α chain of the IL-4 receptor".

Pathogenesis of asthma mainly leads to the disbalance between TH-1 & TH-2 phenotypes. Type 2 helper T cells are a type of CD4+ helper T-cells & they are significant part in inflammation of bronchus. "T-helper type (Th2) cells has a key part in initiating & maintaining airway inflammation due to allergy & asthma via enhanced secretion of cytokine Th2-type (IL-4, IL-5, & IL- 13)." Th2 cytokines act as mediator for action series in the inflammatory network resulting in developing allergic asthma.

Eosinophils are the predominant inflammatory cells in asthmatic lung tissues.

PATHOGENESIS[23,27-29]

- Inhaled spasmogen like allergens, irritants, medicines (aspirin & B blockers) produce early asthmatic response
- Imbalance between TH-1 & TH-2 phenotypes
- "Th2 helper T *cells* secreting Interleukins which sensitizes inflammation of allergy & B-cells stimulation for production of IgE & allied antibodies".
- IgE antibody sticks to mast cells via Fc receptors.
- The reactions arise as a consequence of granular mediator's release (e.g. histamine & 5 hydroxytryptamine) i.e. mainly produce smooth muscle

contracting bronchi, gut & venules; dilation of capillary & vascular permeability; enhanced mucus secreting.

- Mediators which are newly synthesized (e.g. leukotriene prostaglandins & platelet activating factor) which causes bronchoconstriction; increased mucus secretion & venule permeability.
- Cytokines such as interleukins & tumor necrosis factor are leads to Inflammation, tissue remodeling.[23]

Phosphodiesterase (PDEs) are an enzymatic family catalyzing metabolization of intracellular cyclic nucleotides, c-GMP & c-AMP expressing in diverse cell varieties & in regards to respiratory disorder, to utilize PDE3, PDE4 & mixed PDE3/4 inhibitors providing clinical uphand to asthmatic patient or COPD patients. Currently 11 PDE families & a minimum of 21 isoforms containing many variants of splice characterize into different structure, substrate specificity, selectivity of inhibitors, destruction of tissue & cell, kinase regulation, interaction between protein- protein & distribution at subcellular level. But, PDE 3 & / or 4 when targeted, cyclic AMP metabolizing enzymes & smooth muscles of lungs & inflammatory cells.[27]

Monocyte-derived cyclic AMP-PDE from atopic dermatitis patients. Monocytes working on protein with increased PDE activity improved this population's function. Atopic dermatitis diseases are thought to start here. However, blood leukocytes from mild or severe atopic individuals did not increase soluble PDE4 activity.[28]

In mild asthmatics, peripheral blood monocytes produced more PDE. “Activated macrophages continuously and overtly generate inflammatory mediators like prostaglandins and leukotrienes during inflammation. Cyclooxygenases (COX-1

& COX-2) and lipoxygenases (LOXs) influence prostaglandin and leukotriene production. It is believed that COX-1 is the constitutively expressed isoform and that prostaglandin synthesis mediates basic bodily processes. In many cell types, cytokines, mitogens, endotoxin, and tumour promoters induce COX-2 expression. COX-2 activity produces prostaglandin E2, which is involved in several pathophysiological processes, including pain and inflammation. Arachidonic acid is converted to leukotrienes B4, C4, D4, and E4 by 5-LOX. "Leukotriene B4 forms highly potent vasoactive and leuco tactic mixtures inducing leukocytic entry to inflammation site, neutrophil lysosome release, adhesion molecule expression, and plasma leakage".

TNF-α plays a crucial role in asthma aetiology. Asthmatics secrete TNF into the airway during asthma attacks, and their BALF contains more TNF mRNA and protein than non-symptomatic asthmatics.

Data literature highlights that asthma pathogenesis primarily focuses on genetic factors & lesser for the disbalance of phenotypes TH-1 & TH-2. This balance is delicately held between genetics, respiratory infections, uterine atmosphere, diet, occupational & environmental exposure.[29]

Genetic Factors:

Way back in 1860, familial aggregation of asthma was identified as the mendelian inheritance pattern was not applicable, demonstrating that responsiveness to methacholine has a bimodal distribution in asthmatic families. Around 27% of non-specific bronchial responsiveness has shown inheritance property. It is reported to be 87% in those families with a single asthmatic patent. Hence, data consensus has

concluded about the inheriting pattern either recessive, autosomal, codominant, dominant & polygenic. "Though gene loci & candidate genes in the interior & exterior of loci is correlated with phenotypes of asthma phenotypes (serum IgE levels, atopy, AHR & asthma), asthma related major susceptibility genes is not yet determined".[29]

ASTHMA – CAUSES[30-32]

Environmental triggering factors which are most commonly leading to asthmatic attack are allergens due to exercise, irritants & viral infections.

Irritants such as smoke from cigarettes, change in weather or cold blast, intense smell from painting or cooking, scented products, stress & strongly expressed emotions like crying or laughing hard.

Others like Medicines like beta-blockers & aspirin, sulfides present in food like dried fruit & beverages like wine. Gastro esophageal reflux condition resulting in heartburn will worsen the symptoms of asthma.

SYMPTOMS:[31]

- ***Wheezing****:* An asthmatic attack always starts as wheeze & fast breathing, & with it progressing, it shows visibly active breathing muscles.
- ***Shortness of breath (dyspnea)****:* Major distress in asthmatic patient is due to shortness of breath, though severe dyspnea will not necessarily mean a serious attack or decreased lung function.
- ***Coughing:*** Non productive cough is the first symptom experienced by some individuals.

- ***Chest tightness (pain***): An early indication of asthmatic attack is the tightness of chest minus any miscellaneous symptoms. Tightening of neck muscles along difficulty in talking is seen. In a majority of patients, chest pain occurs which can be severe, though its magnitude might not actually be related to asthmatic attack severity itself.
- Rapid **heart rate** & excessive sweat.
- Cough marks the end of the attack with production of thick mucus. Inflammation generally remains for days to weeks after the initial attack minus any symptoms.

PHYSICAL EXAMINATION[31]

Examination mainly depends upon principle like inspection, palpation, percussion, & auscultation. It helps to recognize lung abnormalities & also reflect underlying lung diseases.

- ***Inspection***- breathing pattern & rate along with lung expansion depth & symmetry are noted.
- ***Palpation***- lung expansion symmetry can be evaluated, usually stamping the results as seen during inspection.
- ***Percussion***- Examiner assesses tissue dullness or relative resonance beneath the chest wall.
- ***Auscultation***- the investigator looks for the intensity & quality of breath sounds & also looks for any extra & adventitious voices likes rales, wheezes & rhonchi.

Crackles are those sounds presenting as discontinuous, usually inspiratory caused when small airways or alveoli open & shut during respiration. This is often cofounded along with interstitial lung disorder, micro atelectasis or liquid filling into alveoli.

Wheezes are usually more promiscus while expiring than inspiring air, which reflect the airway walls oscillation occurring in case of airflow limitation, which may be caused by bronchospasm, edema or airway collapse or any obstruction to lumen by neoplasm or secretions.

Rhonchi is sound produced by the presence of liquid in lumen of airways. A vibratory low – pitched sound is created due to viscous interaction of free liquid & moving air.

Clinical measurements demonstrated (1) reduced maximum expiratory rate & (2) decreased timed expiratory volume. Cumulatively, these together result in dyspnea, or "air hunger."

TYPES OF ASTHMA[31,32,34]

1. Extrinsic
    a) Atopic
    b) Occupational
    c) Non- atopic
2. Intrinsic (Idiosyncratic)
    a) Drug induced
    b) Exercise induced
    3. Mixed

1. Extrinsic asthma

Hypersensitivity reaction of type I initiates this due to allergic exposure or environmental triggers like dust, animal danders, fumes, viruses & chemicals.

a) Atopic asthma:

It gets triggered by antigenic environmental factors like dusts, pollens, animal dander & foods. While airway inflammation in atopic asthmatics is characterized by an increased number of eosinophils, mast cells & T lymphocytes.

b) Occupational asthma:

This is triggered by fumes, organic & chemical dusts, gases & other chemicals. In genetically susceptible individuals, allergens interact with dendritic resulting in stimulation of B- lymphocytes to differentiate in to plasma cell which produce IgE antibodies. Also allergens attach to mast cell &eosinophils, resulting in discharge of different inflammatory mediator such as cysteinyl-leukotrienes (CysLTs), eosinophil major basic protein (EMBP), eosinophils cationic protein (ECP) which causes bronchospasm & airway inflammation.

c) Non-atopic asthma:

Viruses of respiratory infection origin such as rhinoviruses, Para influenza triggers them than the usual bacteria which are usual provokers. A higher number of neutrophils & mast cells are mainly found in non- atopic asthmatic individuals.

2. Intrinsic asthma

It is initiated by various, no immune mechanism involving aspirin ingestion, infection of pulmonary system, cold, irritants which are inhaled, stress & workouts.

Intrinsic asthma can result due to dysbalance in the parasympathetic & sympathetic airway responses. Acetylcholine released by PNS results Broncho constrictors & sympathetic nervous system by stimulating adenyl-cyclase cause bronchodilators.

a) Drug induced asthma

Aspirin triggers asthma in aspirin sensitive individuals by inhibition of arachidonic metabolism regulated by COX pathway without affecting the lipoxygenase route, hence tilting the balance for broncho constrictor leukotrienes elaboration.

b) Exercise induced asthma

It is defined as a constricting of the airway briefly or bronchospasm that's induced by vigorous workout.

3. Mixed asthma

Most individuals do not categorize into either categories & is characterized by mixed features of the above. These individuals get asthma in the earlier life possessing a strong allergy component but those of them developing the disease in the later life have a tendency to remain allergic.

DEVELOPMENT OF ASTHMA

Asthma, especially allergic type progresses in two phases:

1. Immediate phase (sensitizing)

2. Late phase (Delayed response)

1. Immediate phase of asthma

This phase is initiated by mast cells which are IgE sensitized on the mucosa, resulting release or mediators which expands the intracellular tight junctions of mucosa & increases antigenic penetration to the many mast cells present in submucosa. Additionally, vagal receptors present in subepithelial is stimulated directly provoking bronchial constriction via local & central reflexes. Late phase reaction is staged by these inflammatory cells, starting 4 to 8 hours after & remaining for half to full day further.

2. Late phase of asthma

A great number of cytokines are released by epithelial cells after a reaction to infectious agent, drugs, gases & mediators of inflammation. Second wave mediators stimulate late reaction.

SIGNALLING PATHWAYS[33]

Innate immunity activation is decided by Toll-like receptors (TLRs) by recognition of particular patterns of micro-organism components. It was in Drosphilia that initially toll receptors were identified as a necessary receptor for establishing dorso – ventral pattern in embryos which are developing. Hoffmann & co-workers in 1996 showed that flies which were toll mutated exhibited great susceptibility to fungal infection. This data provided knowledge that immune system, in specific the innate immune system provides a skillful method in detection of microbial invasion. Tlr4 gene is responsible for inflammatory response. Whereas in mammals have similar gene called TLRs which contain 10 members (TLR1 to TLR10). It has similar like interleukin receptor therefore it is called Toll Interleukin Receptor (TIR). “TLR

signaling pathways arise from intra cytoplasmic TIR (toll interleukin receptors) domains, that are conserved among all TLRs. TIR domain-containing adaptors, like MyD88, TIRAP & TRIF modulate TLR signaling pathways".

MyD88-

A pathway common to every TLRs is MyD88-dependent one & it is also unique to signaling pathways of TLR3- & TLR4. MyD88 has in the portion of C – terminal a TIR domain & in the part of N – terminal a domain of death. MyD88 & its co-variates with the domain of TIR of TLR s. When stimulated, MyD88 includes IL-1 receptor-associated kinase (IRAK) via interacting to TLRs the death domains of the two molecule. Phosphorylation activates IRAK & after attaches with TRAF6, resulting in activation of separate identity signaling pathway which ultimately activates JNK.

MyD88-independent pathway –

Stimulation of LPS results in transcription factor IRF-3 activation, which further induces of IFN-β. Stat 1 gets activated by IFN – i causing inducing various IFN – inducible genes.

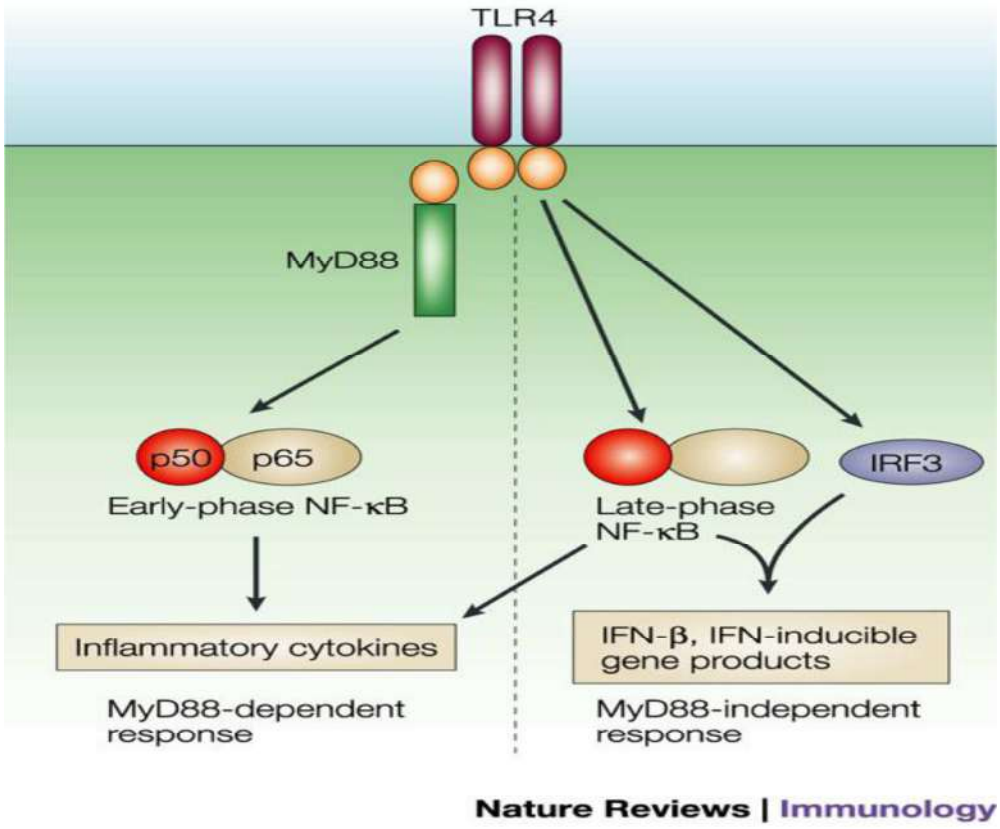


Figure 2. Toll like receptor Signaling Pathway

TIRAP is adaptor protein present in c terminus of TIR. It is responsible for producing of inflammatory cytokine. It is essential in MyD88 dependent pathway.

MANAGEMENT OF ASTHMA[34-41]

Asthmatic condition is a chronic lung disorder symbolized by acute constriction of bronchus episodes resulting in breathlessness, coughing, tightness of chest, wheeze & rapid respirations, that is influenced by several genetic, environmental & developmental factors affecting greater than 300 million population globally, with one in four children in urban population affected.

Therefore, management with various methods is essential. Treatment of asthma is divided into major 2 types:

1. Allopathic medicines
2. Alternative therapies

ALOPATHIC MEDICINES[34-36]

Wide array of drugs & dosage forms are developed for asthmatic treatment in long term symptoms, decrease the quantity & intensity of asthmatic exacerbation & reverse the airflow obstruction. Quick relief by medication includes.

1. β Adrenergic Agonist

Eg. Terbutaline, Albuterol, Metoproterenol, Salmetrol.

"β adrenergic agonist reduces smooth muscle tone by Gs-adenyl cyclase cyclic AMP pathway & another mechanism is to increases conductance of Ca + which sensitive to K+ channel in airway smooth muscle which has showing membrane hyperpolarization & leads to airway relaxation".

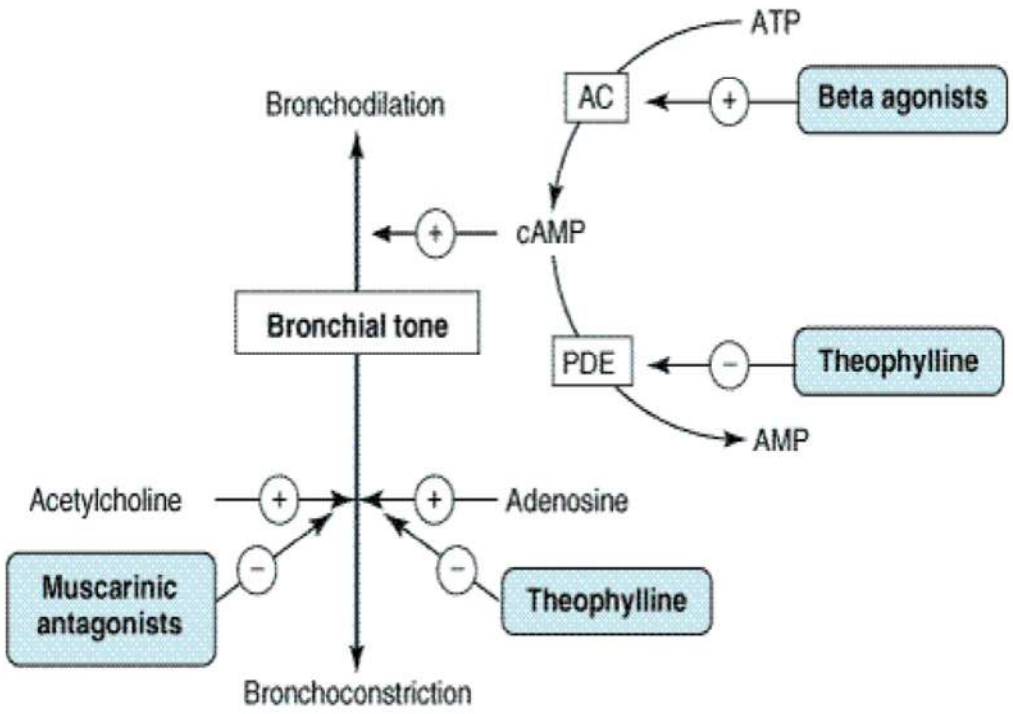


Figure 3. Mechanism action of β adrenergic agonist

2. Glucocorticoids

Eg. Beclomethasone dipropionate, triamicinolone aceonide, Budesonide

Modulation of cytokines, production of chemokines & significantly stops accumulating basophils, eosinophils & other c ells in tissue of lungs showing anti-inflammatory effect. When glucocorticoid/glucocorticoid receptor complexes get directly bound to glucocorticoid elements, it mediates effects of anti – inflammation in promoter location of genes or by interplaying this process with different transcription variables, specifically causing activation of protein 1or nuclear factor-kappa beta.

3. Leukotriene Receptor Antagonists & Leukotriene Synthesis Inhibitors

Eg. Zafirlukast, Montelukast, Zileuton

Leukotrienes forming is dependent on lipo – oxygenation of arachidonic acid by 5 – lip – 0xygenase. Leukotrienes are the important inflammatory mediator therefore leukotrienes inhibitors have inhibits 5 lypoxygenase enzyme activity & block all 5- lipoxygenase products. (Figure 3)

4. **Anti IgE therapy**

Eg. Omalizumab

IgE antibodies are produced by B cell lymphocytes. Fc portion in IgE chains bods with greater affinity towards receptors of mast cells & basophils present in plasma membrane. The allergen reacts with the IgE which is cell bound resulting FcεRI cross linkage & activation of cell. Free IgE present in the serum neutralizes Omalizumab by getting bound to heavy chains of Fc areas in order to form IgE-anti–IgE complexes of high affinity. This helps in prevention of IgE from getting bound to FcεRI, resulting in blockage of cell activation which is allergen induced.

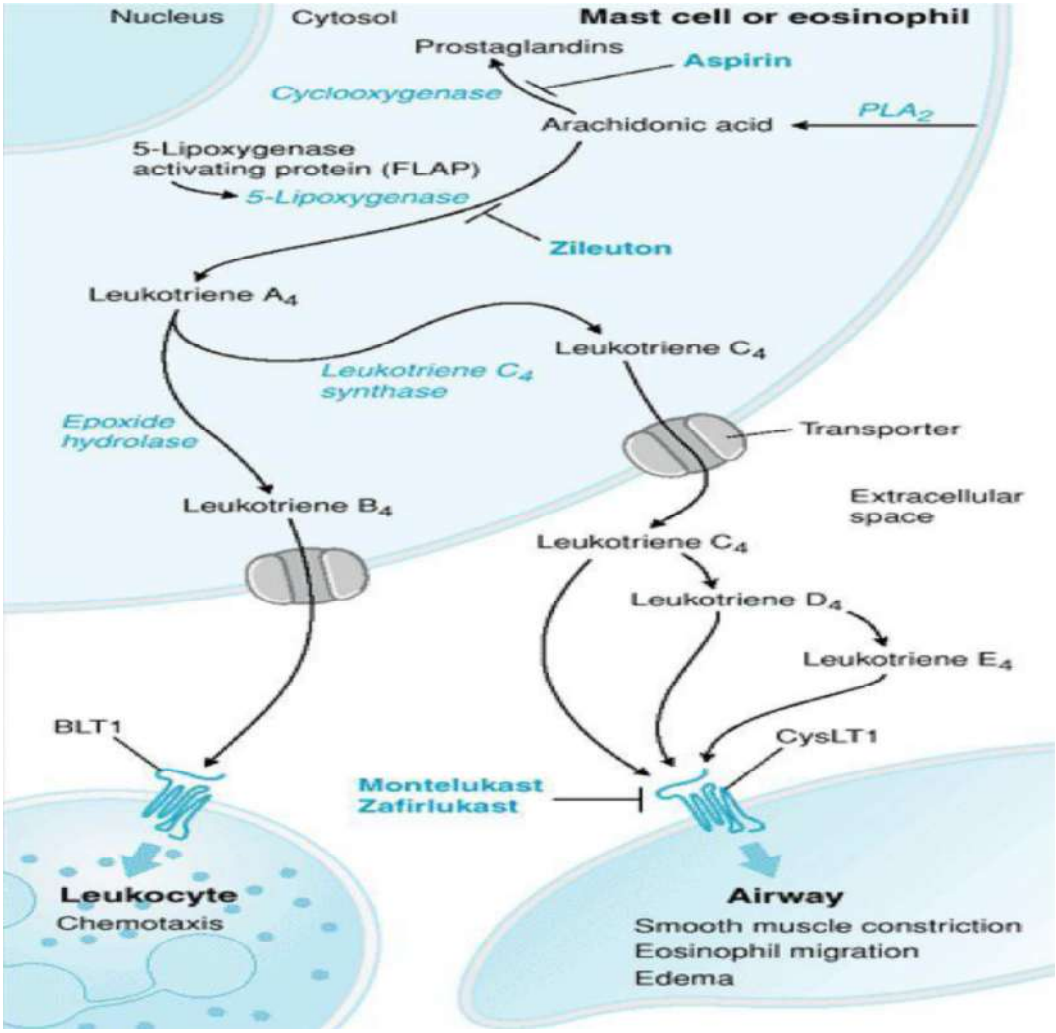


Figure 4. Mechanism action of Leukotriene Receptor Antagonists & Leukotriene Synthesis Inhibitors.

NON- SELECTIVE PHOSPHODIESTERASE INHIBITORS[37-38]

Phosphodiesterase's (PDEs) forms an enzymatic family catalyzing the metabolisation of intracellular cyclic nucleotides, c-GMP & c-AMP which get uttered in diverse cell categories with respect to respiratory disorders. Now it is also identified that utilization of PDE4, PDE3, & mixed PDE3/4 inhibitors will give therapeutic benefits to asthmatic patients or COPD. "The orally active PDE4 inhibitor

Roflumilast-n-oxide has been accepted for therapy of extreme manifestations of COPD as complimentary to conventional treatment".

ALTERNATIVE SYSTEM[39-42]

1. HERBAL THERAPY

Alternative system in treating asthmas has always used herbs for natural therapy. Considering the healing view from nature, asthmatic conditions is a reflection of health in system such as respiratory, nervous & immune. This suggests that is solutions are aimed for long term, then it must treat adrenal, lungs & nervous system. As per Ayurveda, herbal effects liquefy the mucosal bronchial secretion & facilitates cough expectoration. The elements of Vatta, Pitta & Kapha symbolizing air, fire & water are traditionally utilized to cure cough & asthmatic conditions. Bronchioles inflammation is also reduced.

Many different herbs are used to treat asthma like;

Adhatoda (*Adhatoda vasica*), **Bitter Gourd Root** (*Momordica charantia*), **Coltsfoot** (*Tussilago farfara*), **Chinese Skullcap** (*Scutellaria baicalensis*), **Figs (Anjeer)** (*Ficus carica*)**, Garlic** (*Allium cepa*), **Ginkgo Biloba** (*Ginkgo biloba*), **Grindelia** (*Grindelia spp.*), **Indian Gooseberry** (*Emblica officinalis*)**, Linseed** (*Linum usitatissimum*)**, Licqorice** (Glycyrrhiza glabra), **Mustard Oil** (*Brassica spp*)**, Turmeric** (*Curcuma longa*), **Elecampane** (*Inula helenium*), **Skullcap** (*Scutellaria lateriflora*)**, Valerian** (*Valeriana officinalis*), **Kaempferia parviflora** (zingiberaceae). This type of herbs are used in treating asthmatic patients.

NUTRITIONAL THERAPY[40,41]

Antioxidant foods & supplements: Lower consumption of nutrients containing antioxidants such as Vitamins A, C, E & trace element of Selenium might enhance the odds of lung damage. Contrarily, individuals whose diet had higher Selenium such as fish, eggs, chicken, liver & garlic presented with lower asthmatic attack.

Fish oil: Anti – inflammatory effects of cold water oily fish containing Omega-3 fatty acids can benefit in asthma.

Supplements: A disbalance in immune system results in allergies. The possible allergens could be enzymes, fatty acids, minerals & vitamins which might cast a significant role in maintenance of better immunity & sometimes can even relieve allergic reactions.

Enzymes: Immune system function is stimulated by Co-enzyme Q10 which is a strong antioxidant, by moving of energy, enhancing cellular metabolism efficiency & is good for asthma, allergy & low immune resistance. A naturally occuring substance, Co-enzyme Q10 is reported in every asymptomatic tissue in the body, isn't seen in pathological or infected tissue.

Fatty acids: FA prove to be significant for immunity as they decrease allergic response associated inflammation by helping to produce prostaglandins countering inflammation.

Minerals: Nutrients important for sufferer of allergy are calcium & magnesium. They facilitate in relaxing an over reactive nervous system. In treating allergies, sulfur helps either due to environmental of food / drug allergens.

Vitamins: Vitamin A is formed in the body by beta carotene, which helps to heal & sooth mucus membrane which are irritated. The inherent antihistaminic property of Vitamin C marks it a classical therapy for allergy. Tocopherol is also a strong anti - oxidant which facilitates to defend body cells to the action of free radicals. It even cures respiratory disorders & pumps the immunity to defend against infections.

LIFESTYLE THERAPY[40,41]

Lifestyle can prevent an attack of asthma

- Air conditioner usage can reduce the quantity of pollens which are airborne from trees, grasses & weeds which can easily get indoors & hence keep the indoor atmosphere clean.
- Maintenance of optimal humidity
- Reduction of pet dander
- A face mask is helpful if asthma gets aggrevated by cold, air. Face masks are to be worn if asthma gets worsened by dry air or cold.
- Self care of oneself. Conditions linked to asthma must be treated & kept under control. Measures include:

- Heart & lungs to be strengthened by exercising regularly, that facilitates in relieving symptoms of asthmatic attack.
- Consumption of greens & fruits could enhance lung function.
- Regulate GERD

ACCUPUNCTURE

Needles of thin size are placed at certain strategic points of the body in this technique, which is usually painless & is safer. 'Qi', which forms the balance

dictating energy flow balances it, via mediums & meridians in the body. Illness can occur, if this flow of energy gets blackened or is weakened. Every individual is carefully assessed by the acupuncturist to find out the source of blockages. Fine needs are inserted at particular points in the body referred to as acupoints, in stimulating energy flow & correcting imbalance, if any.

ESSENTIAL OILS[43-46]

Essential oils from plant extracts have their usage in folk medicine & preservation of food since ancient times. They are also a storehouse of naturality origin secondary metabolites along with a wide array of biological action. Any product got by distillation in means of hydro, steam or dry types by suitable method minus plant healing. Essential oils are made of combination of natural & volatile materials, symbolized by smell & given by plants with aroma as metabolites of secondary form. exist in liquid state, volatile, are colored rarely, lipid & solvent soluble which is usually of low density than water. These are taken out from several aromatic plants usually found in temperate to warm nations such as Mediterranean & tropical areas. They have a complex composite comprising terpenes along with monoterpenesa & sesquiterpenes. But even, allyl- & propenylphenols (phenyl propanoids) form significant make of certain essential oils.

- Essential oils demonstration anti-inflammatory effect by inhibition of leukotriene production.
- Inflammation, RNS is generated. Nitric oxide & peroxynitrite anion is formed in major amounts by the inducible nitric oxide synthases in activate form macrophages & neutrophils in the immunological & defense actions.

- Protein modulators are ROS & RNS & regulators for expression of key cytokines include ion channels, membrane receptors, & transcription factor, along with nuclear factor-κB.
- They also have a great bearing in free radicals scavenging & anti – inflammatory property.

Mechanism Action of Anti-Inflammatory Effect of Essential Oil[47]

A PUFA, namely Arachionic Acid (AA) gets released after being stimulated by various factors of inflammation via cell membrane to PL A2. The above said acid under the action of COX & LOX enzyme pathways gets metabolized into various eicosanoids like PGs & LTs playing a critical part in inflammation. A transcripory regulator, NF-κB is made of proteins – homo & heteroimers. NF-κB is laid in latent version in the cellular cytoplasm to form complexed with inhibitor protein, IκB. 7 family members of IκB are detected, incuding IκB-α. When NF-κB gets activated, IκB kinases (IKK) phosphorylates IκB-α resulting in IκB, degradation which is dependent on proteasome, allowing a quick translocation of NF-κB to nucleus further binding to DNA. p65: p50 is the important NF-κB dimmer which activates it. Various pro – inflammatory genes like cytokines & inducible enzymes is resulted due to p65: p50 translocation. Figure 4 demonstrates the site of EO or their parts being detected which act as agents of anti-inflammation. A method combining gas liquid chromatography & mass spectrometry to determine various substance present in the compound is Gas chromatography–mass spectrometry (GC-MS). It serves the purpose of detecting the essential oil properties & is used in detection of drugs & identifying not known samples.

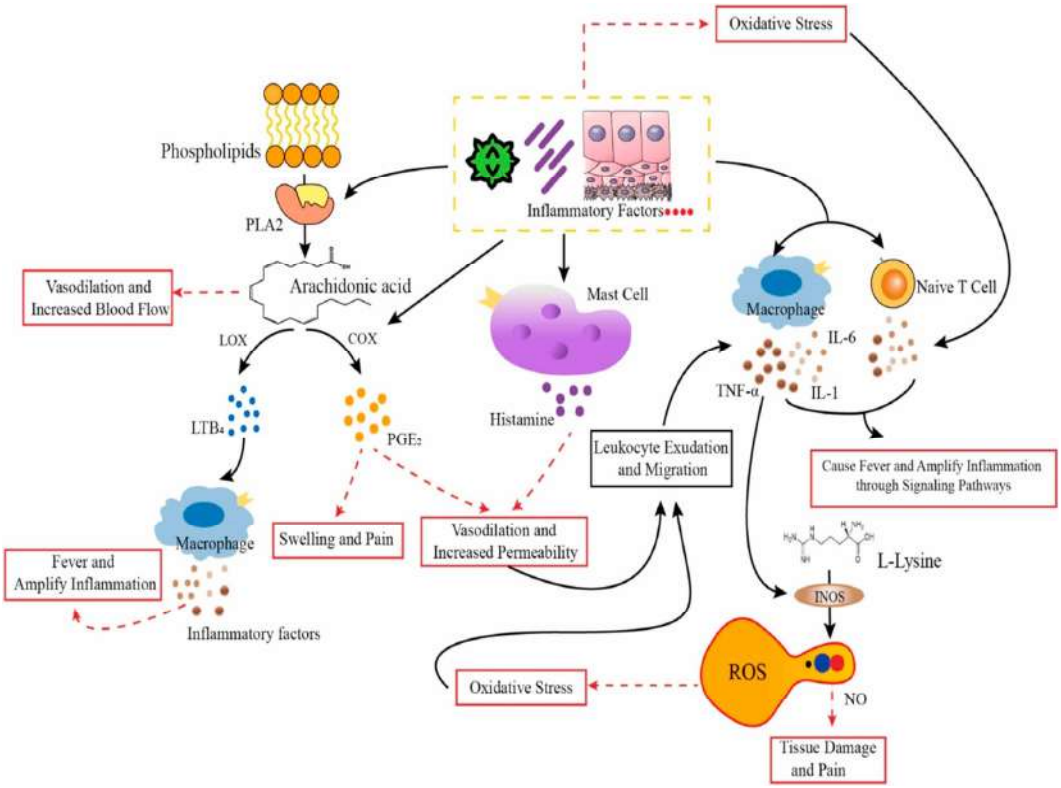


Figure 5: Mechanism action of Essential oils for Anti-inflammatory activity

ANIMAL MODELS- Standard procedure followed as per refrence[48-54]

1. Tracheal chain model-*In Vitro*

2. Ova induced asthma model- *In Vivo*

3. Histamine chamber model- *In Vivo*

Histo-pathological Examination[50-54]

Healthy lungs, structure wise present with alveolar walls which are thin with intra alveolar macrophages sometimes & very scant neutrophils. Contrarily, lungs of an asthmatic individuals demonstrate some damage or injuries such as;

- Infiltration of neutrophils
- Protein rich debris or strands of fibrin deposition in the airspaces
- Thickening of the *walls* of alveoli which may contain abundant neutrophils
- Appearance Hyaline membranes as pink debris on the walls of alveoli
- Goblet cell hyperplasia
- "Th2 Cytokines are also associated with several growth factors, along with vascular endothelial growth factor (VEGF) & transforming growth factor (TGF)". TGF β1 induces the deposition of collagen which leads to pulmonary fibrosis & VEGE enhancing the vascular permeability & increase inflammatory cell infiltration into airways.

PLANT REVIEW[55, 13-14]

GANDRAYAN[56-60]

Botanical name: *Angelica glauca*

Synonyms: "*Angelicanuristanica"*

Family: Apiaceae

Vernacular names: Hindi- Chora, **English**- Smooth angelica, **Sanskrit**- Taskarah, **Gujrati**- Chorak, **Bengali**- Chorak, **Kannada**- Choraka, **Telugu**- Gaddi Davanamu, **Punjabi**- Churaa, **Marathi**- Corak, **Malayalam**- Choraka Pullu

Distribution: This plant considered as endangered plant in Himalayas. This is seen in kashmir at altitude level extending from 8000 to 13000 ft. in Jogi at an altitude of 11000ft. & in Sikkim & Lachen at an height series of 10,000-11,000 ft.

Chemical constituents: It contains sesquiterpenes, cadinene, umbelliprenin, terpene & alcohol.

Traditional uses as medicines: Roots are used in wounds, gastric pain. Aromatic plant in known as carminative, stimulant. It used in dyspepsia, anorexia, spasm, flatulent, colic & bronchitis.[56]

Review studies:

Sarker SD *et al* (2004) many species of this *Angelica glauca* has been used traditionally as expectorant, chronic bronchitis & remedy for colds.[57]

Puri A *et al* (2014) Thus, they examined the synergistic effects of *Angelica glauca* and celastrus paniculatum on scopolamine-induced dementia in rats. They found that scopolamine-induced dementia showed promise memory enhancement due to its antioxidant capability.[58]

Carlos Cavaleiro *et al* (2015) concluded that Antifungal activity of the essential oil of *Angelica* major against Candida, Aspergillus, Cryptococcus,& dermatophyte species.[59]

Muhammad Irshad *et al* (2012) investigated that essential oil *Angelica glauca* plants demonstrated *in vitro* action against "Microsporum canis & Fusarum solani".[60]

KULANJAN[61-69, 55]

Botanical name: *Alpinia galanga*

Synonyms: "*Alpinia galanga* (L.) Sw, *Amomum galanga* (L.) Lour, *Alpinia viridifola* Griff *Maranta galanga* (L), *Longuas galang*a (L) Stuntz, *Longuas vulgare* J Koeing, *Alpinia bifidia* Warb., *Alpinia carnea* Griff, *Zingiber galangal* (L) stokes".

Family: Zingiberaceae

Vernacular names: Hindi, **Sanskrit**, **Bengali** - berakulanjar, kulanjan **Tamil** – perarattai, **Telugu** – paddadumparashtram, **Marathi** – koshtkulayan, **English** - greater galangal, **Malyalam** - arratta, perratta, kul-inji, **Gujarati** – kulinjan, **Kannada** – dumparasmi.

Distribution: It is seen in countries of India, China, Saudi Arabia, Egypt, Indonesia & Sri Lanka. It can grow in woods & brushwood under open sunny positions.

Chemical constituents: Rhizomes contain tannin, starch, chlorides, sulphates, phosphates & an essential oil containing methyl cinnamate, cineole, pinene & camphor. The seeds contain 7- heptadecane, fatty acids methyl ester, acetoxyeugenol acetate.

Traditional uses as medicine: Rhizome are used in improving voice, bronchitis, dyspepsia, impotency, nervous debility, inflammation of liver, disease of kidney, respiratory trouble, tuberculosis, as in depressant of cardiovascular system, in flatulence, colic, vomiting, diarrhea, cancer, cramp pain, back pain.[61]

Review studies:

Rizki Damayanti *et al* (2015) essential oil of *Alpinia galanga* contains β-bisabolene & trans caryophyllene are responsible compounds for slimming aromatherapy effect.[62]

Afzal Unnisa *et al* (2011) performed Anti-inflammatory activity using carrageenan induced paw oedema & acute toxicity studies of the extracts from the rhizomes of *Alpinia galanga* Willd.[63]

Akhtar MS *et al* (2002) investigated Hypoglycemic action of *Alpinia galanga* rhizome & its extracts in rabbits.[64]

Saha S *et al* (2013) reported Central Nervous System stimulant action of *Alpinia galanga* with a preliminary study."[65]

Jirovetz L *et al* (2003) analyzed the essential oils of various part of medicinal plant *Alpinia galanga* from southern India.[66]

Lenardao EJ *et al* (2016) reviewed that essential oil has shown antinociceptive & analgesic effect.[67]

Matsuda H *et al* (2003) extracted rhizomes of Alpinia galanga rhizomes & found that they inhibited B-hexosaminidase release.[68]

DALCHINI[55,69]

Botanical name: *Cinnamomum aromaticum*

Synonyms: *Cinnamomum cassia* Linn., *Cinnamomum verum, Cinnamomum zeylanicum*

Family: Lauraceae

"Vernacular names: Hindi- Dalchini, **English**- Cinnamon, **Punjabi**- Darchin, **Telugu**- Lavanga patta, **Tamil**- Ilayangam, **Bengali**- Daruchini, **Malayalam**- Karuvapatta, **Oriya**-Dalechini, guda twa, **Gujrati**- Taja"

Distribution: It is indigenous to Sri Lanka & china but is now cultivated in Egypt, Europe, Japan & India. In India, is cultivated in South Indian states upto 1500 mt altitudes, in western Ghats up to 200 mt height & in semi-evergreen forests of Eastern Indian states.

Chemical constituents: Essential oil obtain from bark & leaves contain, eguenol, cineole, linalool, caryphyllene, humulene, safrole, cinnamaldehyde, cinnamyl acetate, cinassiol, euenol acetate, cumic aldehyde, dipentonides, methyl euenol, camphene, benzaldehyde, geraniol & diaterpenes. The leaves are enriched with minerals like calcium, phosphorus, iron, sodium, potassium & amino acids like thiamine, riboflavin, niacin & vitamins A & C.

Traditional uses as medicine: The root, bark useful in asthma, bronchitis, anorexia, colic, cough, chronic mastitis, dyspepsia, diminished appetite, fever, gonorrhea, hiccough, inflammation, leprosy, skin disease, sinusitis, tuberculosis, tremors, cephalagia & ophthalmic or inflammation of eyes.

Review studies:

De Cassia de Silveira E Sa R *et al* (2014) They studied that essential oils present as a significant source for exhibiting pharmacological effects when utilized as active components. They discussed about the anti-inflammatory effect produced by phenylpropanoids extracted via essential oils & also about the possible mode of action in anti-inflammatory response evaluated via particular models.[70]

KAPOOR[55, 71]

Botanical name: *Cinnamomum camphora*

Synonyms: *Camphora officinarum*, *Laurus camphora*

Family: Lauraceae

"Vernacular names: Bengali- karpur, **Hindi-** Kapur, **Kannada**- chandara, davala, dhavala, kappara, kapura, **Malayalam**- chutakkarpuram, ghanarasam, himamsu, himavaluka, **Manipuri**- karpura, **Marathi**- kapoora, **Oriya**- ramokorpuro, **Sanskrit**-

candraprabha, chandrahba, gandhadravya, **Telugu**- candramu, candrasanguyamu, **Tamil**- chukantamaram"

Distribution: This plant is originated to Republic of China & Japan, & it is grown in Nilgiri mountains of India.

Chemical constituents: "It treated with chloride of zinc & distilled converted into cymene, when it treated with nitric acid it oxidized & form camphoric acid. It also contains glycerides of lauric, capric & oleic acid."

Traditional uses as medicine: It is useful in hysteria & nervousness, diarrhea, muscular strains, rheumatic condition, inflammation, smallpox, typhoid, whooping cough. It also used in various cardiac depression.[74,75]

Review studies:

Akram J *et al* (2006) investigated that camphor had sexual desire & sexual performance enhancing properties.[72]

Agarwal R *et al* (2012) published chapter in Chemistry of Phyto potentials regarding chemical composition & biological activities of *Cinnamomum camphora* essential oil growing in Uttarakhand possess good antioxidant & antimicrobial effects, which could be serve as a food preservatives & medications.[73]

Guo S *et al* (2016) reported that *C. camphora* essential oils were thought as a natural resource for insect management in 2 store products.[74]

Prabuseenivasan S *et al* (2006) investigated that Cinnamon oil is a great source of anti - bacterial agents.[75]

Silva-Filho SE *et al* (2014) demonstrated anti-inflammatory action in camphor which was associated with leukocytic migration inhibition & anti edematous effect.[76]

Hiramatsu Yasushi *et al* (2001) resulted that *Cinnamomum camphora* oil highly suppressed mites activity & also it having anti-inflammatory activity.[77]

Lee HJ *et al* (2006) concluded that extract of *cinnamomum camphora* on different inflammation phenomenon to evaluate its probable anti –inflammation mechanism in non-toxic conditions. Data suggested anti-inflammatory actions could be because of cytokine modulators, NO & PGE2 production & oxidative stress.[78]

HALDI[55]

Botanical name: *Curcuma domestica*

Synonyms: *Curcuma longa* Linn.

Family: Zingiberaceae

Vernacular names: Gujrati-Halada, **Hindi-** Pitras; **Nepali**- Haldi, **English-** Turmeric, **Kannada**- Arishina, **Malyalam**- Manjai, **Marathi**- Halad, **Oriya**- Haladi, **Sanskrit**- Haridra, **Tamil**- Manjal, **Telugu**- Pasupu, **Bangla**- Haqda.

Distribution: It is extensively cultivated in India. It is commercially grown in A.P., T.N., West Bengal, Maharashtra, Karnataka, Kerala, Orissa.

Chemical constituents: The rhizomes contain pigment curcumin, sesquiterpines, sesquiterpeniods as germacrane, bisabolane guaiane & an essential oil containing pinene, turmerone, sabinene, myrecene, terpinene, limonene, cymene, perillyalcohol, turmerone, dehydroturmerone, alanto lactone, curcumene & cineole, sabinene, cineol, borneol, zingiberene & sesquiterpenes. Tolymethyl carbinol, ketones. Analysis of

rhizomes also shows moisture, protein, fat, carbohydrates, fiber, & minerals. Minerals & vitamin contents are calcium, phosphorus, iron, carotene, thiamine & niacin.

Traditional uses as medicines: It is used in diabetes, gastric & duodenal ulcers, viral hepatitis, jaundice, anti-parasitic for skin infections, antispasmodic, body ache, antibacterial, in arthritis, hepatitis, menstrual disorders, anemia, rheumatism, boils, as blood purifier, conjunctivitis & skin disorders. It can be utilized in cough, cold, throat infections, bronchitis & in asthma.

Review studies:

Ram A *et al* (2003) results demonstrated about the action of curcumin in bettering the features of impaired airways when tested on OVA-sensitized guinea pigs.[79]

Ma C *et al* (2013) this study evaluated the protective action & mechanism of curcumin in mouse models who were ovalbumin induced for allergic asthma. They concluded that curumin therapy accentuated the asthmatic model inflammation by regulation of Treg/Th 17 balance.[80]

Sukandar EY *et al* (2014) study aimed to enable comparison of effectiveness & toxicity of curcuma with glibenclamide in diabetes patients-type II with or without dyslipidemia. They found that it possessed the capability to remain an anti – diabetic agent.[81]

Kuptniratsaikul V *et al* (2009) investigated that *C. domestica* seem to be as efficient & safer as ibuprofen in treating Osteoarthritis of knee.[82]

PUDINA[55]

Botanical name: *Mentha arvensis* Linn

Synonyms: *Mentha spicata, Mentha longifolia, Mentha piperita*

Family: Lamiaceae

Vernacular names: Sanskrit- Pudin, Putani, Putika, Rochani, Vyanjan, **Bangla**- Podina, Puthina, **Gujrati**- Fudino, **Hindi**- Pahadi pudina, **Kannada**- Chetni, **Malyalam**- Putiyina, **Marathi**- Padina, **Punjabi**- Belane, baburi, koshu, **Tamil**- Putina, **English**- Corn mint

Distribution: In India it is cultivated specially in Northern states but now on all over the country & even in kitchen gardens.

Chemical constituents: Major components in the oil are menthol, methone, methyl acetate along with terpenes like pinene, thujene, camphene, carvomenthone, limonine, menthofuran, isomenthol, aspartic acid, glutamic acid, glycine, lycine, alanine, valine, leucine, isoleucine, phenylalanine, linalool, geraniol.

Traditional uses in medicine: Leaf & oils are utilised in managing liver & spleen disease, asthma, jaundice, stomach disorders, headache, rheumatic pains, as expectorants, as antifungal, antibacterial,in nasal & bronchitis catarah, cough, rhinitis, sore throat, vomiting, dyspepsia & diaahorea, joints pain, carminative & antispasmodic.

Review studies:

Malik *et al* (2012) resulted that *Mentha arvensis* oil that provides management action against allergic & inflammatory disease.[83]

Duarte MC *et al* (2005) observed that *Mentha arvensis* oil & ethanolic extracts usually utilized in Brazil was tested for anti-Candida albicans activity.[84]

Wannissorn B *et al* (2005) investigated that *Mentha arvensis* oil showed promising antibacterial action against the microbe tested.[85]

Kishore N *et al* (1993) stated that *Mentha arvensis* oil demonstrated strong action fungitoxicity against dermatophytes.[86]

Jagetia GC *et al* (2002) observed that mint oil extract from *Mentha arvensis* provides protection against the radiation-induced sickness.[87]

Kumar P *et al* (2011) investigated that *Mentha arvensis* oil showed Insecticidal properties against against various stored grain pests & vectors.[88]

Londonkar RL *et al* (2009) investigated that *Mentha arvensis* extract showed defensive action against acid secretion & gastric ulcers in ibuprofen plus pyloric ligation, 0.6 mol/L HCl induced & 90% ethanol-induced ulcer models.[89]

Sharma N *et al* (2001) investigated & reported reversible antifertility property in male mice when administered with ether extract of *Mentha arvensis* leaves without any side effects.[90]

OREGANO[55]

Botanical name: *Origanum vulgare* Linn

Family: Lamiaceae

Synonyms: *Origanum creticum, Origanum officinale, Origanum orientale*

Vernacular names: Gujarati- ajamo, jungli marvo, **Hindi-** ban tulsi, sathra, mirzanjosh, **Kannada**- maruga, **Kashmiri-** marzan-josh, **Konkani-**ovey, **Malayalam**- kattumaruva, **Marathi-** jangali maruaa, ova, **Nepali**- raamtulasi, sajeevan, sathra, **Oriya**- saptala, **Sanskrit-** maruvaka, **Telugu**- mridumaruvamu

Chemical constituents: Monoterpenoids & monoterpenes are primarily found in the oregano essential oil. But the number of specific compounds alter based on geographic location & several other variables. More than 60 separate compounds are segregated, with the principal compounds being thymol & carvacrol varying from 0 to 80% & compounds present in lesser abundance are cymene, γ terpinene, caryophyllene, spathulenol, germacrene-D, β-fenchyl alcohol & δ-terpineol.

Traditional uses as medicine: It is useful in inflammations, catarrh, headache, paralysis. The leaves are beneficial in earache, bronchitis, asthma. The flowers are used for hemicranias & oil is used in rheumatism, skin inflammation, sores, burns, wound healing.[91]

Review studies:

Lambert RJ *et al* (2001) reported that potential use of Oregano oil has revealed the bacteriostatic or bactericidal activity of essential oil.[92]

Haberbeck LU *et* al (2012) examined the thermal & thermo – chemical effect of oregano essential oils, tested by inactivating bacillus coagulant spores in nutrient broth adjusted at 4 brix & 4.2 pH. Their results indicated that oregano oils might be used to make Bacillus coagulans spore susceptible to toxic effect of thermal heat.[93]

SHIGRU

Botanical name: *Moringa oleifera*

Family: Moringaceae

Synonyms: *Moringa pterygosperma*

Vernacular names: **Sanskrit**- Shobhajana, **Hindi**- Sahinjna, Sajna, **English**- Drum stick, **Malyalam-** Muringa, **Kannada-** Guggala, Mochaka, **Telugu**- Mochakamu, **Tamil**- Murungai **Marathi**- Shevga, **Konkani**-Mashinga, **Malayalam**-Muringai

Distribution: 2 species have been reported from India. Often, it is found associated in Sal & Khair forests in sub-Himalayan tracts up to 1200m.

Chemical constituents: Its oil contains myristic acid, palmitic acid, oleic acid, stearic acid, behenic acid, lignoceric acid. The bark contains presence of sterols & terpenes.

Traditional uses as medicine: It is used in anti-diabetic, antispasmodic, antiemetic, antipyretics, anthelmintics, wound, boils, swelling, chronic rheumatism, intermittent fever, throat sores. It is also used in cardiac stimulant in asthma, cough & similar disorders.[94-95]

Review studies:

Mahajan SG *et al* (2014) assessed ovalbumin induction of airway inflammation when treated with B-sitosterol isolated from an n-butanol extract of *Moringa oleifera* plant seeds amongst guinea pigs. It possesses anti-asthmatic action through inhibition of cellular response subsequently releasing Th2 cytokines. Hence, this formulation might possess therapeutic properties in allergic asthma.[96]

Jaiswal D *et al* (2013) their study assessed antioxidant activity of young leaves of Moringa oleifera's water extract in both invitro & in vivo assays. *M. oleifera* leaves demonstrated a significant antioxidant activity in both assays suggesting that consumption of these leaves regularly in the diet will better both diabetic & normal individuals against oxidative damage.[97]

Hannan MD *et al* (2014) this study evaluated the action of *Moringa oleifera* on the neurotrophic & neuroprotective property in the hippocampal neurons. The study results showed that MOE facilitates maturation of axodendritic & even gives protection to neurons demonstrating a upcoming pharmacological significance, about this ethnomedical & nutritionally significant plant for betterment of nervous system.[98]

TIMUR

Botanical name: *Zanthoxylum armatum*

Family: Rutaceae

Synonyms: *Zanthoxylum allatum, Zanthoxylum hostile, Zanthoxylum planispinum*

Vernacular names: Sanskrit- Andhak, Dhiva, **Bangla**- Gaira, Nepali Dhania, Tun, Tambul, **Gujrati**- Tambaru, Tambruphala, **Hindi**- Darmar, Dhaniya, Tej-Phal, **Kannada**- Dhiva, **Malayam**- Tumpuni, **Marathi-** Chirphal, **Tamil**- Tumpunalu, **Telugu**- Gandhalu, **English**- Prickly Ash

Distribution: The plant is found in India on subtropical Himalaya from Jammu to Bhutan upto 2100 mt. altitude, in the Khasi hills between 600- 900 mts & in Andhra Pradesh upto 1350 mts altitude.

Chemical constituents: The essential oil from the seeds contain dipentene, linalool, citral, methylcinnamate, pipevine, linalyl acetate, limonene, geraniol, cineole, cymene, terpinene, camphor, carvone, fenchol, thujene, terpineol, caryophyllene, monoterpene, enoic acid. The oils from leaves contain linalyl acetate, sesquiterpenes, hydrocarbons, acids, phenols, cineole, limonene, citronellal & triscosane.

Traditional uses as medicine: The bark & fruits are useful in bronchitis, asthma, colic, convulsions, cough, cardiac debility, diarrhoea, diabetes, dyspepsia, goiter, difficult micturition, fever, eye & ear disease, hepatopathy, helmenthiasis**,** leukoderma, leprosy, paralysis, stomach disorder, skin disease, tumors, ulcers & wounds.[99]

Review studies:

Mehta DK *et al* (2014) suggested that *Zanthoxylum armatum* as an anthelmintic have been long-established as the seed extracts displayed activity next to the worms.[100]

Guo T *et al* (2011) "found that ethanolic extract of *Zanthoxylum armatum* could significantly decrease acetic acid induced writhing numbers, & suppress formalin induced licking time in different doses. Thus, they observed *Z. armatum* extract possess powerful ant nociceptive activity".[101]

Singh TP *et al* (2011) their review highlighted on in – depth phytochemical makes & therapeutic utilization clubbed into pharmacological properties of various types of *Zanthoxylum armatum*. Various study indicated that it possesses anti-larvicidal, antifungal, hepatoprotective & allelopathic properties.[102]

Gilani SN *et al* (2010) investigated regarding *Zanthoxylum armatum* exhibiting spasmolytic action, mediated probably via Ca++ antagonist effect that gives a pharmacological foundation for therapeutic application in the gastrointestinal, respiratory & cardiovascular disturbances.[103]

Muhammad I *et al* (2012) investigated that essential oil of *Zanthoxylum armatum* leaves for anticonvulsant Antinociceptive & anticonvulsant activities.[104]

ADRAK[55]

Botanical name: *Zingiber officinale*

Family: Zingiberaceae

Vernacular names: Sanskrit- Adrakam, **Bangla**- Ada, **Gujarati**- Adul, Aradu, **Hindi**- Adarak, **Kannada**- Ardaka, Alla, Haisunti, **Malyalam**- Amdrakam, **Marathi**- Adaki, **Tamil**- Inji, **Telugu**- Allamu

Distribution: In India it is cultivated in tropical & central states & is also found naturally in western Ghats & hills or central India.

Chemical constituents: Rhizomes contain curcumene, bergamotene, pamphenen, calamene, pinene, limonene, cineole, citral, citronellol, linalool, zingerol, zingerone, amino acids like aspargine & piperolic acid, gingeroglycolipids, sulfonic acid, geraniol, geranyl acetate, phenylalanine, malonate, serine, valine, leucine, isoleucine, glycine & arginine.

Traditional uses as medicine: It is used in anorexia, asthma, bronchitis, cough, cold, dyspepsia, diarrhea, earache, fainting fever, inflammations, edema, sore throat, urticarial, nausea, tuberculosis of the lungs, cardiac disorders, hiccups, jaundice, kidney stone attack, bladder inflammation, morning sickness, piles, toothache, antiemetic & anti-vertigo effects.[105]

Review studies:

Tewtrakul S *et al* (2007) *Zingiber officinale* has been reported as useful in allergic related disease.[106]

Ahui ML *et al* (2008) Literature has reported that compounds obtained from rhizomes of *zingiber officinale* possess anti- inflammatory property. The foremost evidence of ginger suppressing immune responses mediated by Th2 was this, promising a probable therapeutic use in allergic asthma.[107]

Gomar A *et al* (2014) this study was evaluated the chronic treatment with hydro ethanolic extract of ginger (50, 100 & 200 mg/kg) would result on the passive avoidance learning & memory in rats. [108]

Zafar I *et al* (2001) It is concluded based on the findings of the present research that *Zingiber officinale* possess anthelmintic activity.[109]

Goel Rajkumar *et al* (2002) investigated that *Zingiber officinale* showed Anti-ulcer activity.[110]

Hammer KA *et al* (1999) investigated that *Zingiber officinale* oil showed Anti-microbial activity.[111]

Literature review on few essential oils for asthmatic activity:

Soyingbe *et al* (2015) Eucalyptus grandis essential oil, containing α-pinene, borneol, p-cymene, 1,8-cineole, d-limonene, & α-terpineol, exhibits concentration-dependent anti-inflammatory, anti-cough, & anti-asthma.[112]

Wright *et al* (1997) Apparently menthol's combined action on sensory neurons & smooth muscle may treat upper respiratory tract infections, bronchitis, & asthma.[113]

Juergens *et al* (2004) showed that 1,8-cineole in eucalyptus oil inhibits cytokines to decrease airway mucus hypersecretion, indicating long-term treatment for asthma, sinusitis & COPD exacerbation.[114]

Juergens *et al* (1998) Euclyptol oil (1,8-cineole) inhibits arachidonic acid metabolism in human blood monocytes *ex vivo*, reducing inflammation in bronchial asthma.[115]

Boskabady *et al* (2003) In guinea pig smooth muscles, Carvacrol inhibits phosphodiesterase & Ca antagonism, bronchodilating.[116]

Morice *et al* (1994) found that menthol relieves citric acid-induced coughs in healthy people.[117]

Carvacrol in *Carum copticum* essential oil relaxes guinea pig tracheal chains, according to **Boskabady *et al* (2003).**[118]

In citric acid-induced asthma in conscious guinea pigs, **Laude *et al* (1994)** found that Menthol, camphor, & cineole were antitussive[119]

4.MATERIALS & METHODS

MATERIALS

CHEMICAL REAGENTS:

The various chemicals, drugs & kits used are mentioned in the table below - Table1.

Table 1: Chemicals/Drugs/Kits & their source

CHEMICAL	COMPANY
1. NaCl	Sigma chemicals, USA.
2. KCl	Sigma chemicals, USA.
3. Na_2HPO_4	Himedia pvt ltd., Mumbai.
4. KH_2PO_4	Himedia pvt ltd., Mumbai.
5. Ovalbumin	Sigma chemicals, USA.
6. Alumina ($Al_2(OH)_3$)	Himedia pvt ltd., Mumbai
7. Histamine hydrochloride	Himedia pvt ltd., Mumbai
8. Dexamethasone	Centaur Pharmaceuticals, Goa.
9. Tween 80	Himedia pvt ltd., Mumbai
10. Feotal Bovine Serum	Himedia pvt ltd., Mumbai
11. Trisodium citrate	Rankem, New Delhi.
12. Potassium bicarbonate ($KHCO_3$)	SD fine chem. Ltd. Mumbai.

13. Ammonium chloride (NH_4Cl)	Himedia pvt ltd., Mumbai
14. Disodium EDTA	Nice chemical Pvt Ltd, Cochin.
15. WBC Diluting Fluid, Turk solution	NICE Chemical Pvt Ltd, Cochi.
16. Chloroform	Himedia pvt ltd., Mumbai
17. Diethyl Ether	Himedia pvt ltd., Mumbai
18. Formaldehyde	S.D fine chem. Ltd. Mumbai.

EQUIPEQUIPMENTS USED

Equipment & their descriptions given in the Table 2.

Table 2: Equipment & their source

Sr No.	Equipment	Company
1.	Micro centrifuge	Genei Pvt. Ltd., Bangalore
2.	Microscope	Pilot products, Delhi
3.	Electronic weighing balance	The Bombay Burma Trading Corporation Ltd., Mumbai
4.	Histamine chamber	Inco Pvt Ltd.
5.	Heme cytometer	Rohem Instruments ltd. Nashik India

NEEDLE SELECTION

Table 3: Oral feeding Needles & their description

Sr No.	Purpose	Needle Size
1.	Oral dosing needle for mice	24G, 1/2 “, curved ball ended
2.	Oral dosing needle for guinea pigs	18G, 1 & 1/2 “, curved ball ended

PROCUREMENT OF ESSENTIAL OIL

Essential oil of *Cinnamomum aromaticum*, *Curcuma domestica*, *Mentha arvensis* procured from Kanchor ingredients ltd, Kerala. *Origanum vulgare* Linn, Procured from Synthite Herbals, Mumbai. *Zanthoxylum armatum* & *Angelica glauca* procured from Kshipra Biotech Private Limited, Indore, Madhya Pradesh. *Alpinia galanga*, *Moringa oleifera* & *Cinnamomum camphora* were procured from GR Herbals Pvt. Ltd. 11-B Kankariya Road, Industrial Area, Hatod, Dist- Indore. Along with all certificate of Analysis (COA) & essential oil was stored at 2 to 8^0C.

METHODS

PHYTOCHEMICAL INVESTIGATIONS

Chromatographic assessment of the essential oils

“Essential oil chemical constitution (1% solution of essential oil in equal ratio of n-hexane: dichloromethane) were evaluated using gas chromatograph (GC) (Varian 450 fitted with a fused silica capillary column TG-5 (5% diphenyl-95% dimethylpolysiloxane; Thermo Scientific), 30 m× 0.25 mm i.d., 0.25 μm film thickness) in strict laboratory situations as mentioned earlier”.[120,121]

Temperature in oven was set at 220℃, utilizing nitrogen as carrier. Temperature of the injector & the flame ionization detector (FID) well maintained between 230 & 240 degree Celsius. “Gas chromatography-mass spectrometer (GC-MS) evaluation utilised a Thermo Scientific Trace Ultra GC interfaced with a Thermo Scientific ITQ 1100 mass spectrometer fitted with a BP-1 (100% dimethyl polysiloxane; SGE Analytical Science) fused silica capillary column (30 m × 0.25 mm; 0.25 μm film thickness)”. “Oven temperature level was programmed from 60 to 220°C at 3°C/min, & helium was used as carrier gas at 1.0 mL/min for analysis”. “The injector temperature was set at 230℃, & the injection volume was 0.1 μL in n-hexane, with a split ratio of 1: 50. MS was taken at 70 eV with a mass range of m/z 40-450 & variables utilized were the ones already reported”. The principal composition of the essential oils were determined & confirmed. [122,123]

PHARMACOLOGICAL INVESTIGATION

ANIMAL SELECTION:

“The female Guinea pigs (400-600 g) purchased from Jawaharlal Nehru Medical College, Belagavi, India & kept in standard temperature (22 ± 2°C), relative humidity (55 ± 5%) & light (12 hr light/dark cycles) were used for bronchoconstriction activity”. “Albino mice (18-25 g) females were purchased from Sri Venkateshwara enterprises, Bangalore, India & kept in standard temperature (22 ± 2°C), relative humidity (55 ± 5%) & light (12 hr light/dark cycles) were utilised for bronchial asthma activity”. “They were fed with standard pellet diet & water ad libitum. All the experiments were conducted in prior approvals of ‘Institutional Animal Ethics Committee’ (IAEC), of KLE’s College of Pharmacy, Belagavi (resolution number KLECOP/IAEC/Res. 22-10/10/2015) & all examinations was

conducted in line with 'Committee for the Purpose of Control & Supervision on Experiments on Animals' CPCSEA) guideline, Government of India for animal experiments".

ANIMAL DOSE SELECTION[124-127,63,92]

Since acute toxicity studies of EOs have been reported in earlier literature & IAEC suggested to use the same data with These studies we have taken 1/5 & 1/10 dose as the safer therapeutic dose.

PREPARATION OF DRUG & REAGENTS

Kreb'sSolution[126,63]

Table 4: Below table showing composition of Kreb's solution

Sr. No.	Chemicals	Kreb's solutions (gm/liter)
1.	NaCl	6.9
2.	KCl	0.35
3.	Calcium chloride	0.28
4.	$MgSO_4$. $7H_2O$	0.28
5.	$NaHCO_3$	2.1
6.	KH_2PO_4	0.16
7.	Glucose	2.0

Preparation of test drugs

For *in vitro* model - 100µg/ml of Each Essential oil was prepared in 1% tween 80.

For *in vivo* models - Essential oils were emulsified with water in proportion of 2:2:1(essential oil: water: tween 80).[126-127]

Phosphate Buffered Saline (pH 7.4):[130]

Procedure:

1. 800 ml of ddh 20 measured with the help of graduated cylinder was transferred to Erhlenmyer flask.
2. Magnetic stir bar was added to Erhlenmyer flask; after which the flask is put on a magnetic stir plate. The magnetic stir bar's speed was adjusted so as to keep out oxygen from the solution during rapid mixing.
3. For about 3 – 5 minutes, solute were rested to dissolve.
4. No remaining particles undissolved salts were ensured prior to pH adjusting. If any particles present, it has to be continuos & vigorously stirred.
5. Magnetic stir bar's speed is reduced while mixing the solution gently.
6. pH meter calibration is ensured properly (look & rinse the pH probe with distilled water. Any extra H_2O from tip of probe is removed using a clean paper napkin. The pH probe is then placed in the solution.
7. Then 1 M hydrochloric acid is added drop by drop using a transfer pipette allowing HCl to completely dissolve in the solution.
8. Stirring the solution is ceased.
9. Measure pH with the pH meter.

10. Steps 8 to 10 is repeated till the solution reaches a pH of 7.4.
11. The solution is then put into a neat graduated cylinder with final volume adjusted to 1 liter with distilled water.
12. The solution is taken into another autoclavable container (15 lb/in2)
13. Solution autoclaved at 15 lb/in2 for 20 minutes on liquid cycle.
14. Solution allowed to cool till it attains room temperature.
15. The PBS solution is stored at room temperature.

Alum precipitated Ovalbumin:

Dissolve 50µg of OVA in 0.2ml of PBS which contains 1mg of Al(OH)3 for each animal.[129]

10mM Sodium EDTA

Molecular weight of Sodium EDTA = **372.24**

Dissolve 3.72gm of Sodium EDTA in 100ml of distilled water & make up to 1 litre.

ACK Buffer:

Use: ACK lysing buffer is utilized to break RBCs in a preparation that contains WBCs

Formula

NH_4Cl 8,024 mg/l (53,49g/mol)

$KHCO_3$ 1,001 mg/l (100,12g/mol)

$EDTA.Na_2 \cdot 2H_2O$ 3.722 mg/l (372,24g/mol)

Procedure:

1. After blood is spinned, supernatant is removed.
2. To which ACK lysing buffer in equivalent volume is added to the pellet. The volume should be comparable to that of the pellet.
3. After re suspending gently, tube is swirled for 30-60 seconds.
4. The tube is filled with a medium which will neither contain centrifuge & serum.
5. Then supernatant is removed & pellet retained.
6. Repetition of the process, if required.
7. Then normal cell protocol is followed.

METHODOLOGY[48-54]

In-Vitro: Spasmolytic Activity on Guinea Pig Isolated Tracheal Chain [128,129,131]

Principle – Many investigations found that isolated guinea tracheal preparation had more H1 excitatory receptors than H2 receptors. The guinea pig trachea contracts with Ach, histamine, 5-hydroxy tryptamine, & bradykinin. Both tracheal chain & strip preparations are ideal for screening respiratory smooth muscle spasmogenicity.

Preparation of drug solution

- Test drug was emulsified in 1% tween 80 (100μg/ml).
- Histamine (10 μg/ml) dissolved in distilled water.

Procedure

1. Guinea pig was sacrificed using anesthesia, trachea in toto was dissected out.
2. Trachea was sectioned into separate rings. Rings were tied together with thread.
3. Krebs solution has used for suspending trachea in organ bath solution under the tension 0.5g with carbogen gas at 37±50C.
4. S shaped aerator tube was attached to one corner of tracheal chain while the other was tagged to iso - tonic frontal writing lever to smoked drum.
5. To normalize the tissue it had to be kept in the same position for 45 minutes before adding spasmogen.
6. Following spasmogen histamine was added. 10-15 minutes was required to attain the concentration to maximum level.
7. Test drugs has added & bronchial response recorded up to plateau stage. Tissue has rinsed thoroughly after adding each drug. Spasmogen was added to regulate contractions.
8. The percentage inhibition of spasmogen inducing contractions was be calculated from Dose Response Curve (DRC).

Formula

Height of contraction after exposure to antigen

% inhibition = 100 - [------- X 100]

Height of contraction before exposure to antigen

***In-Vivo* Model: Histamine-induced bronchoconstriction in guinea pigs**[132-133,128-29]

Table 5: Grouping of animals

Group No.	Groups	Treatment
I	Normal	Received PBS (pH 7.4) p.o for 7 days
II	Negative Control	Histamine aerosol (day 0 & day 7^{th})
III	Treated with Dexamethasone(2mg/kg)	Dexamethasone p.o per day (1^{st} to 7^{th}) days + Histamine aerosol (day 0 & day 7^{th})
IV	Treated with essential oils (lower dose-200µl/kg)	Essential oils p.o per day (1^{st} to 7^{th}) days + Histamine aerosol (day 0 & day 7^{th})
V	Treated with essential oils (higher dose-400µl/kg)	Essential oils p.o per day (1^{st} to 7^{th}) days + Histamine aerosol (day0 & day 7^{th})

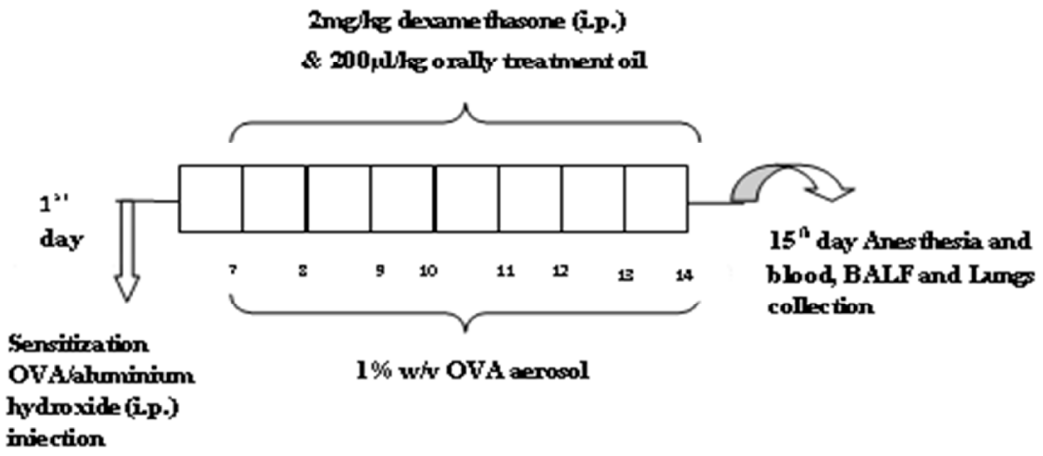
A constant-pressure perplex glass histamine chamber gave the guinea pigs 1% w/v histamine aerosol on days 0 and 7. Each animal was placed in a histamine aerosol chamber after preconvulsive time. Animals in histamine chambers recorded baseline PCT. Therapeutic baseline on PCT Day 0. After PCD as detected, the animals were taken outside to recover. After PCD development, rats were separated into four groups (n=6): group I received histamine aerosols 1% w/v as a negative control, group II received intraperitoneal dexamethasone 2 mg/kg, and groups III and IV received oral MAEO 200 & 400 µl/kg daily for seven days. The PCT onset time was observed on day seven, two hours after the prior dose. PCT time % increase formula: Percentage increased in time of PCT= (1-T1/T2) × 100

Where; T1 = time for PCT onset on day 0, T2 = time for PCT onset on day 7.

***In-Vivo* Model: Ovalbumin induced sensitization in mice**[132-133,128-29]

Table 6: Experimental design

Group No	Groups	Treatment
I	Normal	Received PBS (pH 7.4) p.o for 14th days.
II	Negative Control	Received OVA + $Al_2(OH)_3$ s.c. for 1st & 7^{th} day.
III	(Standard Group) Treated with Dexamethasone (2mg/kg)	Received OVA+ $Al_2(OH)_3$ s.c for 1st & 7^{th} day followed by dexamethasone from day 8^{th} to 14th .
IV	(Treatment Group) Treated with EOs 200µl/kg	Received OVA+ $Al_2(OH)_3$ s.c. for 1st & 7^{th} day followed by essential oil from day 8th to 14^{th}



Four groups of six female mice were created: normal, negative control, standard, & treatment. To sensitise the other groups, except normal mice, 50µg ovalbumin & 1mg aluminium hydroxide were injected intraperitoneally in 200µl PBS on days 1 & 7. Group I received regular saline p.o. & fed for 14 days. Group II got 1%W/V OVA aerosols in PBS for 30 minutes after sensitization. Group III got OVA aerosols & 2 mg/kg dexamethasone intraperitoneally from Day 8^{th} to 14^{th} daily. Group IV got 200µl/kg oral OVA aerosols & EOs for seven days.

SENSITIZATION

Principle: IgE antibody induction results from exposure to immunogenic allergy, combining T cell participation along with B cells & macrophages. Mast cells bind to IgE antibodies via Fc receptors. Fc receptors are present on mast cell surface which possess extreme greater affinity for IgE antibodies. At this level, allergic response is initiated.

Method: Ovalbumin used as a exogenous antigen. On day 1 & 7, positive control group mice were systemically sensitized with a subcutaneous injection of a suspension containing 50µg of ovalbumin (OVA) & 1mg aluminum hydroxide [$Al_2(OH)_3$] in 200µl of phosphate buffer solution (PBS).

CHALLENGE

Principle:

With re- exposure to allergen, mast cell bound IgE antibodies bind to allergen. This results in IgE molecules cross linking to mast cell surface by antigen which are multivalent which is important for consequent reactions. The mast cell granule which

are of basophilic type & large are given out in tissues. This degranulation method releases a plethora of compounds which were pharmacologically active; few were from granule directly such as histamine. other chemical mediators synthesised by the cells.

Method: the treatment drugs viz. dexamethasone (2mg/kg) to 3rd group whereas 200µl/kg to 4th group were given on 8th day to 14th day & Ovalbumin allergen is given through the aerosol form at W/V 1% concentration in PBS solution from 8th day to 14th day.

PRINCIPLE & PROCEDURE OF PARAMETERS EVALUATED:

Blood serum preparation[134]

After allowing blood to coagulate for 20 minutes, & centrifuging at 2000 x g for 15 minutes, the serum was segregated & used for purpose of estimations.

Collection of BALF[131-133]

- Bronchiolar lavaging was done with the PBS solution
- 7ml PBS was flushed in to bronchi by using feeding needle.
- Cells in the Lavage treated with ACK buffer.
- WBC cells counting is done utilizing WBC diluting fluid.
- By using TLC & DLC

Collection & storage of blood[135]

Blood was collected in eppendroff tube containing sodium citrate solution of 4% in 1:16 ratio of sodium citrate to blood & AEC was performed in the lab as per standard procedure.

Isolation & collection of Lungs.[131-134]

- Animal was properly anaesthetized & sacrificed by cervical dislocation.
- Lungs were separated carefully & stored in 10% formal saline solution.

Total leukocyte count[135]

It was performed as per standard procedure**.**

Eosinophils count:[135]

It was performed as per standard procedure**.**

Histopathological examination[138]

1. To obtain information about the histological changes in lungs was observed by performing Histopathology of Lungs in normal, diseased & treated group's animals.
2. Lungs were stuffed in paraffin, sectioned & stained with hematoxylin & eosin[138]
3. Pathological changes in lungs were observed & reported.

STAIN: Hematoxylin & eosin

Principle:

Hemotoxylin:

Haematoxylin generally used in histology analysis, usually used to color cell nuclei & other variables like keratohyalin granules blue. Alum & Iron were used to present structures of nucleus & cytoplasm forming colored complexes the colour of

which depends on the salt utilized. Lakes of aluminium salts appear generally as blue white colored while lakes of ferric salt are bluish black in colour.

The 3 principle "alum haematoxylin solutions utilized are Ehrlich's, Harris's & Mayer's haematoxylin. Haemalum is term used to "haematoxylin" for the solutions because haematein retains as a product of haematoxylin oxidation, & is the mixture which combines with Al ions to result active dye metal complexes". The solutions of Alum haematoxylin will effect nucleus of the cell – it turns the transparent light red stain to blue colour on getting exposed to an alkaline or neutral liquid.

The mordant utilized, Alum or potassium – aluminium – sulfate generally dissociates in basic solution, on combining with hydroxyl ion of water resulting in insoluble aluminium hydroxide.

Alum haemotoxylin – acidic solutions turn red. When stained, alum Haemotoxylin stained sections are usually cleared to basic or neutral solution so as to neutralize acid for forming an insoluble complexe aluminium haematin, which of blue color. Blueing is the term used for this procedure.

If water is not adequately alkaline, or also acidic which is non-satisfactory for haemotoxylin blueing, a tap water substitute comprising of 3.5 g $NaHCO_3$ & 20 g $MgSO_4$. $7H_2O$ in I litre of thymol dissolved water (for inhibiting mould formation) is employed for accelerating thin paraffin sections.

A competent blueing solution is obtained on adding alkali in trace amounts; little amount of strong "ammonium hydroxide or aqueous lithium carbonate" which is added right after before utilizing, is enough for staining with 400 ml. Using cold water

reduces the process of blueing, while warming fastens it. Actually, using water under 10 °C for blueing sections might also result in pink artifact discoloration in tissue.

DNA is not needed for hemalum to stain nuclei. This may be because dye metal complexes bind to "histone-rich arginine nucleoproteins". The mechanism differs from nuclear staining by basic (cationic) dyes like "thionine or toluidine blue". Basic dye stains are prevented by chemical or enzymatic nucleic acid extraction. Such extractions will not stop hemalum nuclei staining.

Eosin:

The effect of fluorescein or bromine forms a fluorescent red dye named Eosin, which is used in staining & for microscopic examination. Those structures which readily stain with eosin are called eosinophilic. It is generally used to counter stain hematoxylin in H&E (hematoxylin & eosin) staining. The staining with eosils of RBC is intense red color. It is acidic in nature which shows basic parts of the cell. For staining purpose, it is conventionally used in 1 – 5 percent concentration of weight by volume, which is either water or ethanol dissolved. Thymol is occasionally added to prevent mold growth in aqeous solutions. 0.5 % acetic acid concentration generally gives the tissue deep red stain.[133-138]

STATISTICAL ANALYSIS

Results are presented as Mean ± Standard Deviation, where n= 6. One way ANOVA was used to determine differences among data followed by Dunnette's multiple comparison test (Graph Pad Prism software, version 5.01).

p value lesser than 0.05 was considered statistically significant.

5. RESULTS

PHYTOCHEMICAL ANALYSIS

Qualitative analysis of *Angelica glauca* essential oil

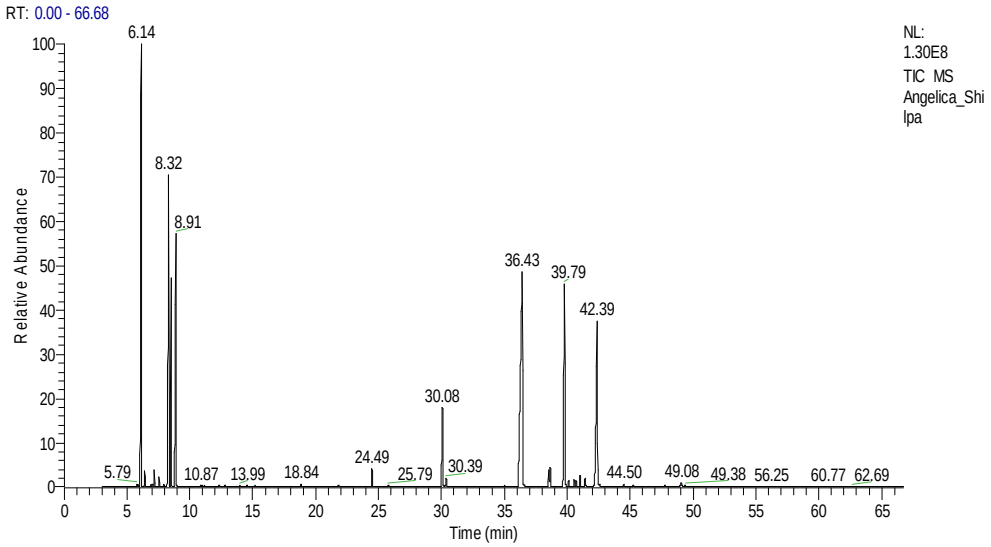


Figure 6: GC-TIC chromatography of *AG* essential oil

Qualitative analysis of *Alpinia galanga* essential oil

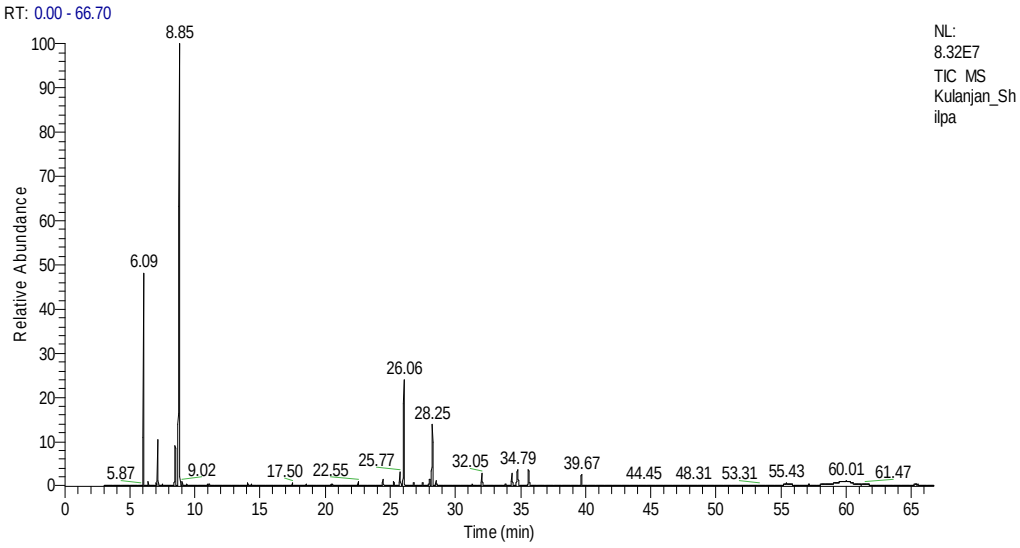


Figure 7: GC-TIC chromatography of *AG* essential oil

Qualitative analysis of *Cinnamomum aromaticum* essential oil

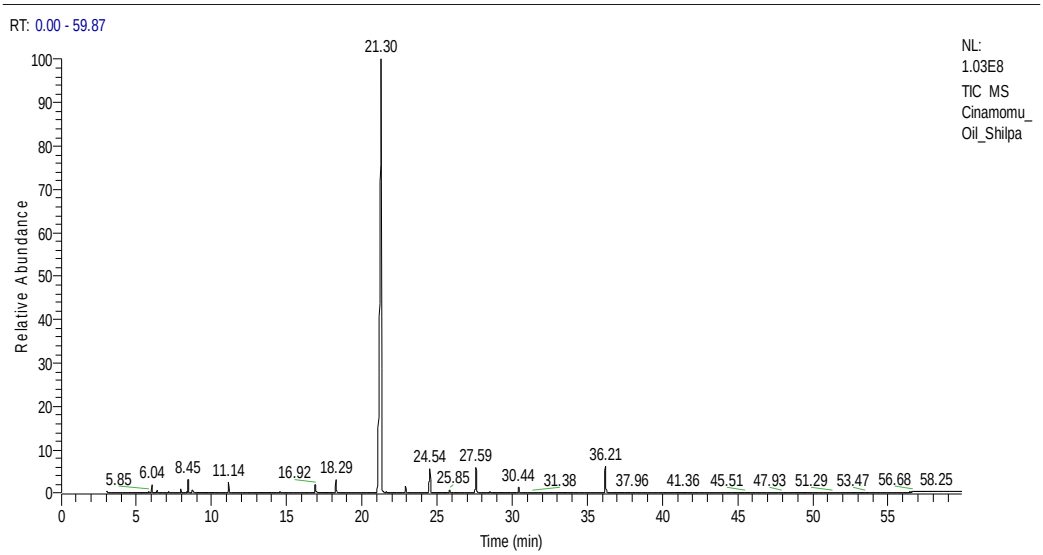


Figure 8: GC-TIC chromatogram of *CA essential* oil

Qualitative analysis of *Cinnamomum camphora* essential oil

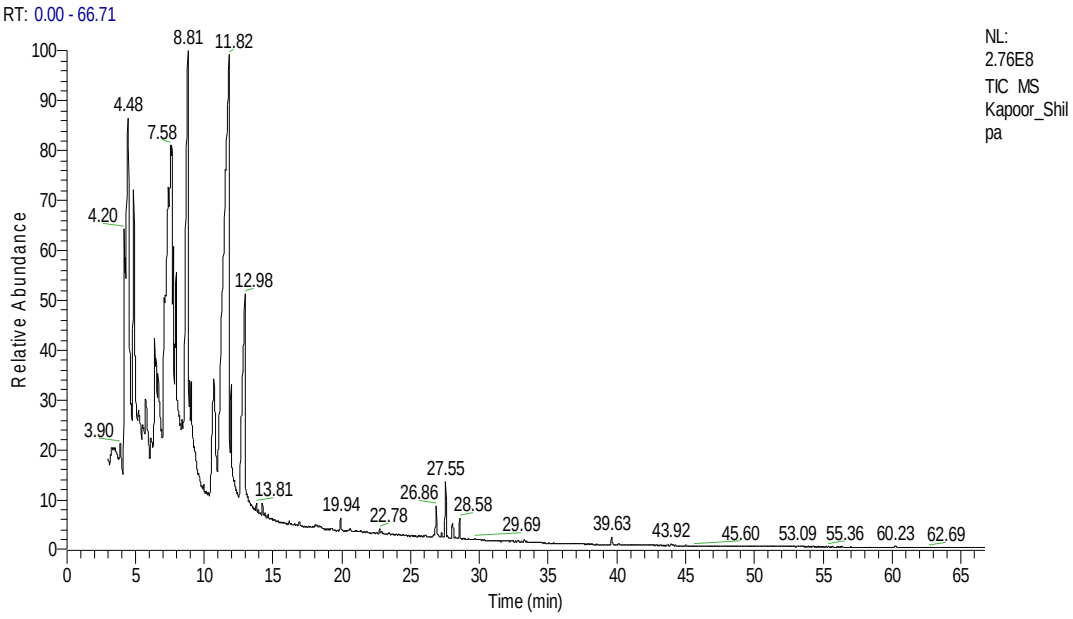


Figure 9: GC-TIC chromatogram of *CC* essential oil

Qualitative analysis of *Curcuma domestica* essential oil

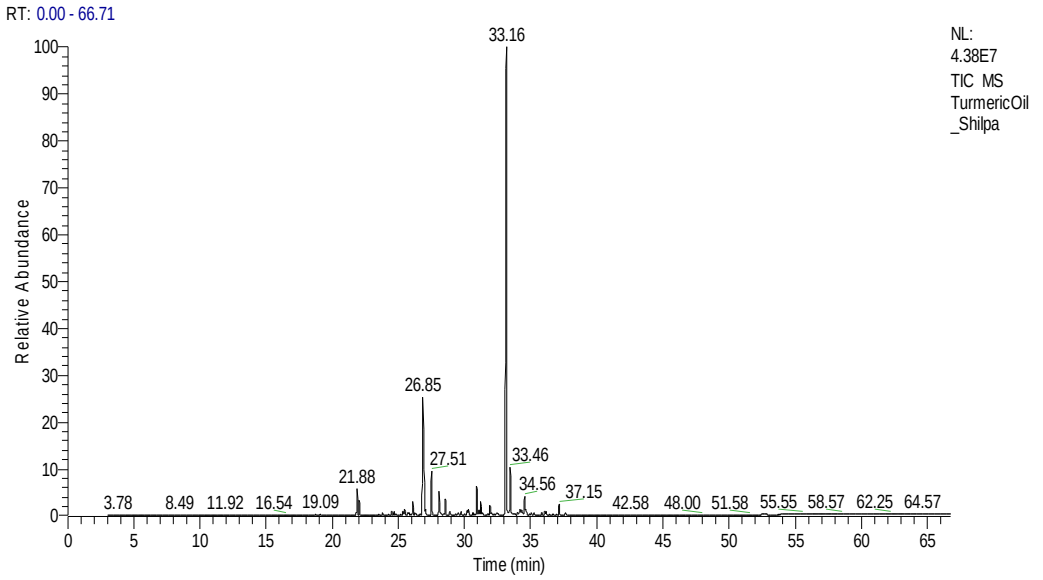


Figure 10: GC-TIC chromatogram of *CD* oil

Qualitative analysis of *Mentha arvensis* essential oil

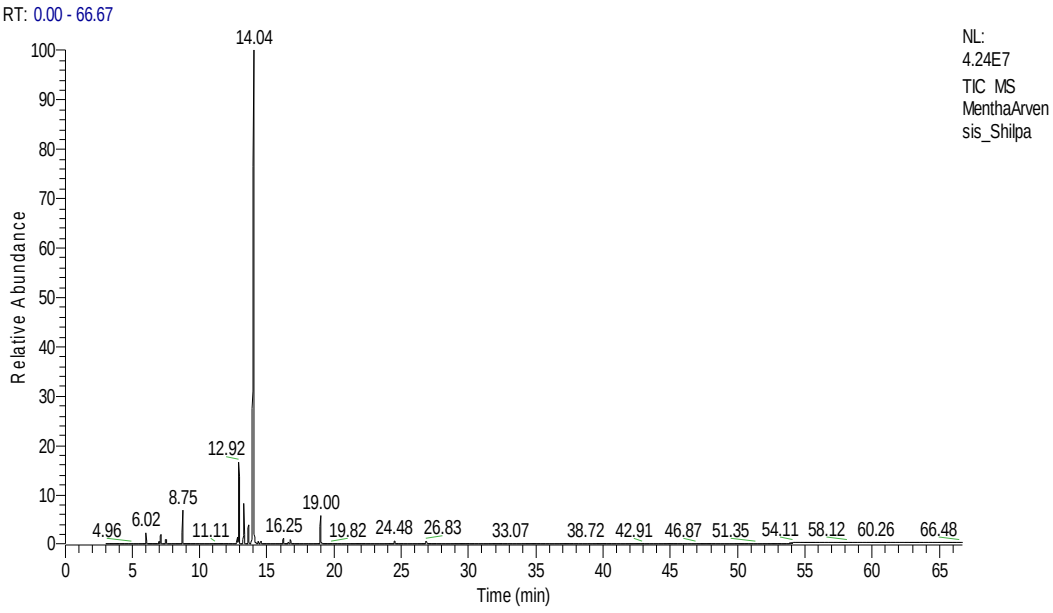


Figure 11: GC-TIC chromatogram of *MA* essential oil

Qualitative analysis of *Moringa oleifera* essential oil

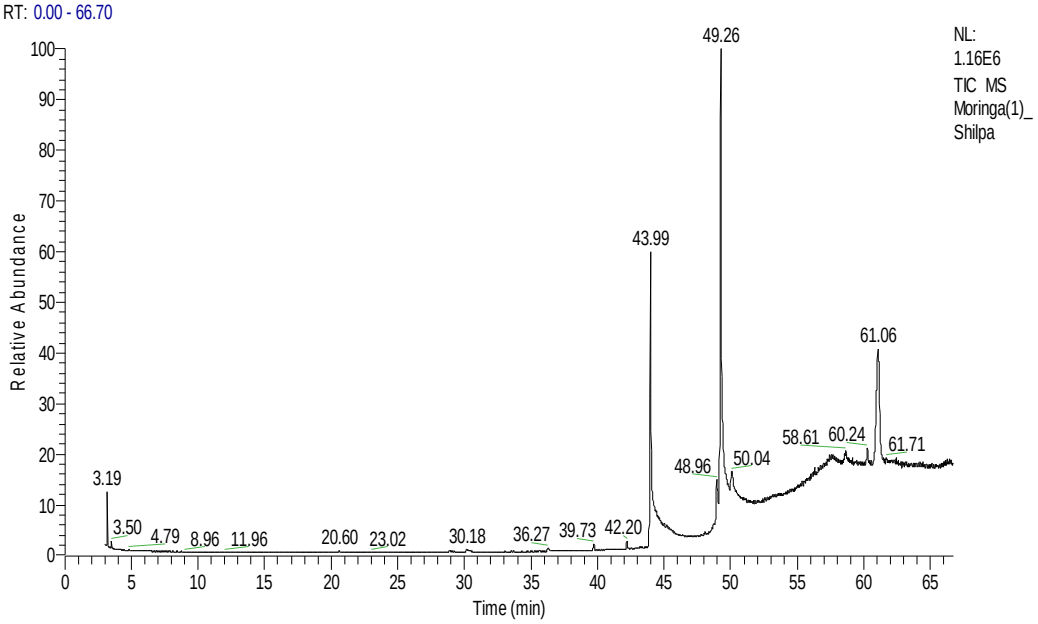


Figure 12: GC-TIC chromatogram of *MO* essential oil

Qualitative analysis of *Origanum vulgare* essential oil

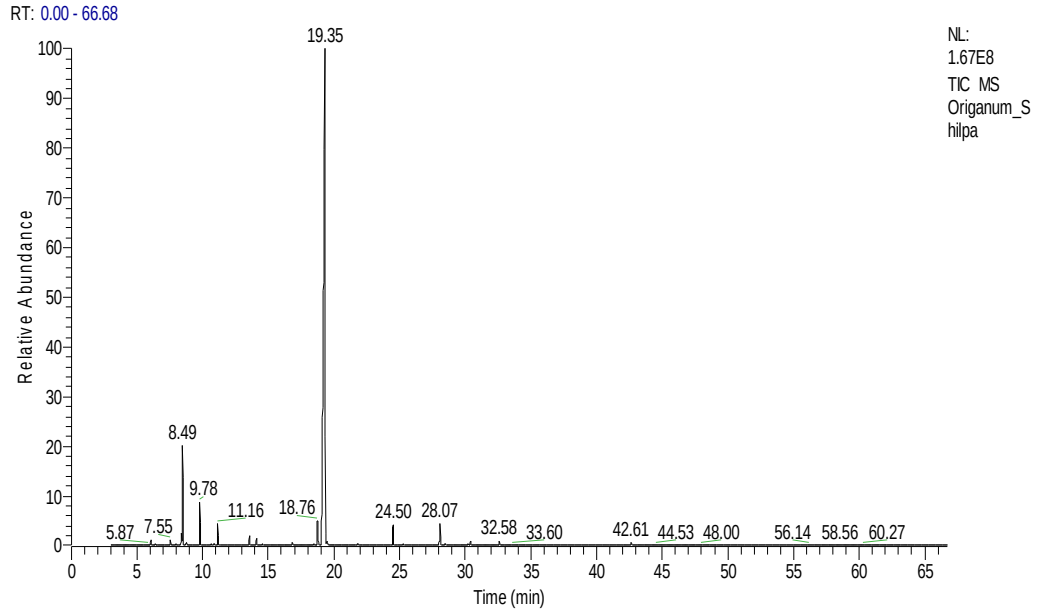


Figure 13: GC-TIC chromatogram of *OV* essential oil

Qualitative analysis of *Zanthoxylum armatum* essential oil

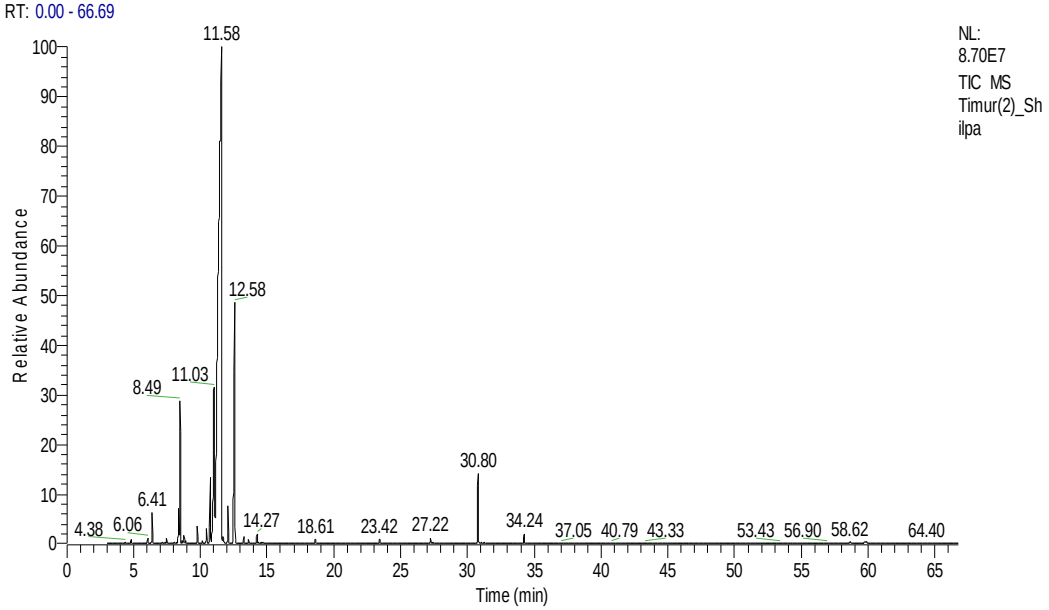


Figure 14: GC-TIC chromatogram of *ZA* essential oil

Qualitative analysis of *Zingiber officinale* essential oil

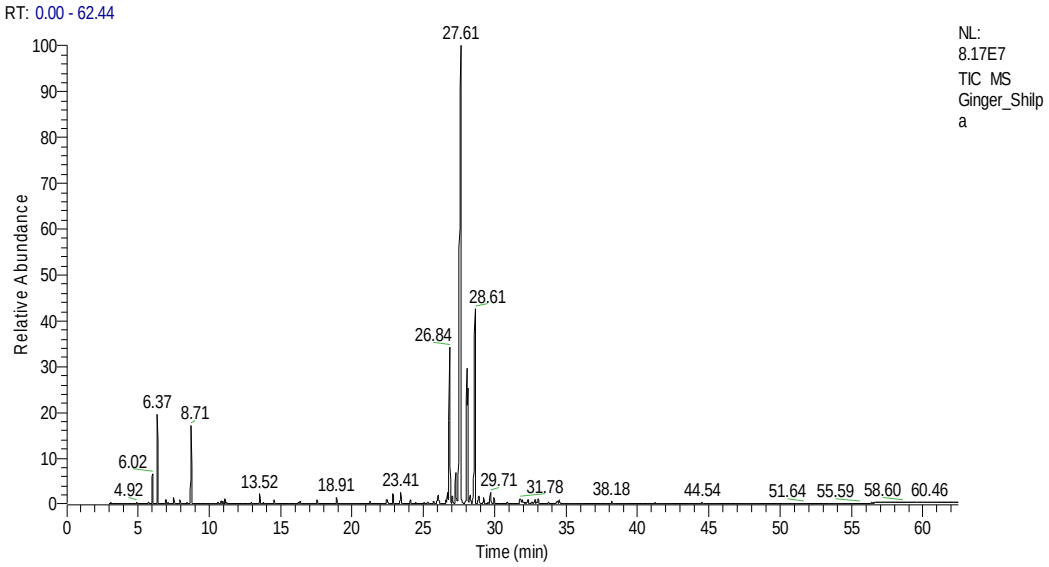


Figure 15: GC-TIC chromatogram of *ZO* essential oil

Spasmolytic tracheal chain activity of Guinea pig using Essential oils

Guinea pig tracheal chain is much more sensitive for dose relative contraction of agonist like histamine (1µg, 2µg, 4µg, 8µg & 16µg of histamine dose given respectively) in isolated tracheal chain preparation. Out of 10 essential oils 2 did not showed decrease in contractions of tracheal smooth muscle in presence of *Zingiber officinale & Alpinia galanga.*

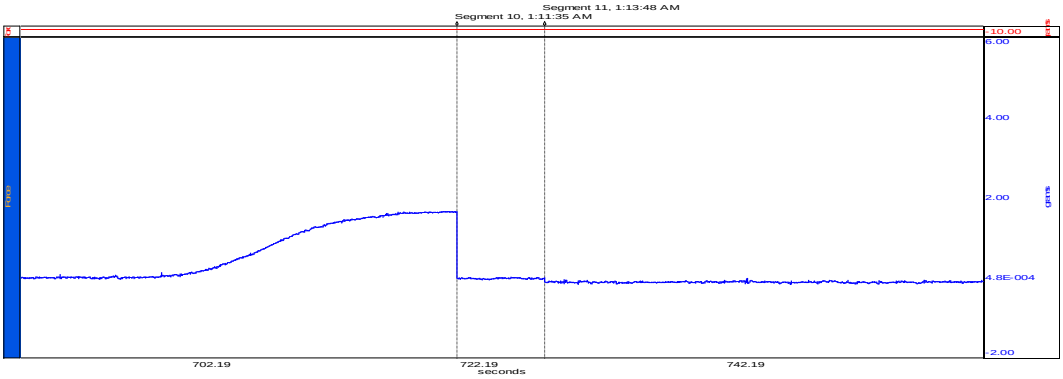


Figure 16: *Curcuma domestica* oil showing relaxation property in guinea pig tracheal chain preparation against spasmogen*

***Spasmogen;** Histamine (10 µg/ml) dissolved in distilled water.

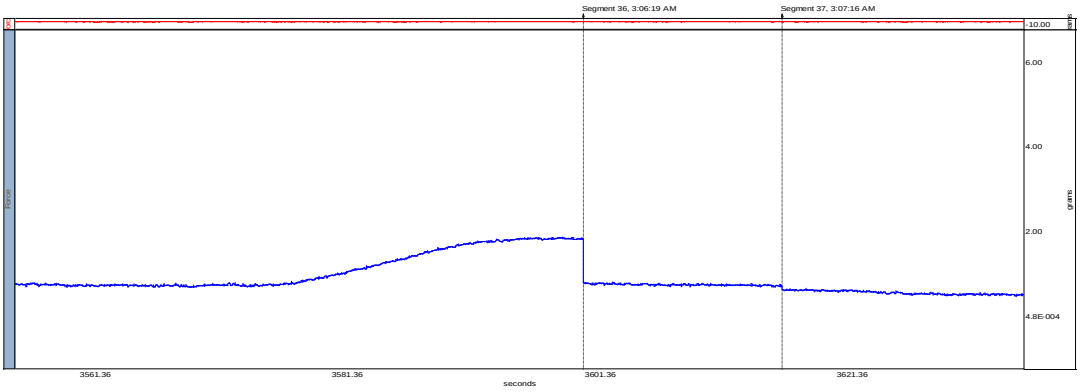


Figure 17: *Moringa oleifera* oil showing relaxation property in guinea pig tracheal chain preparation against spasmogen

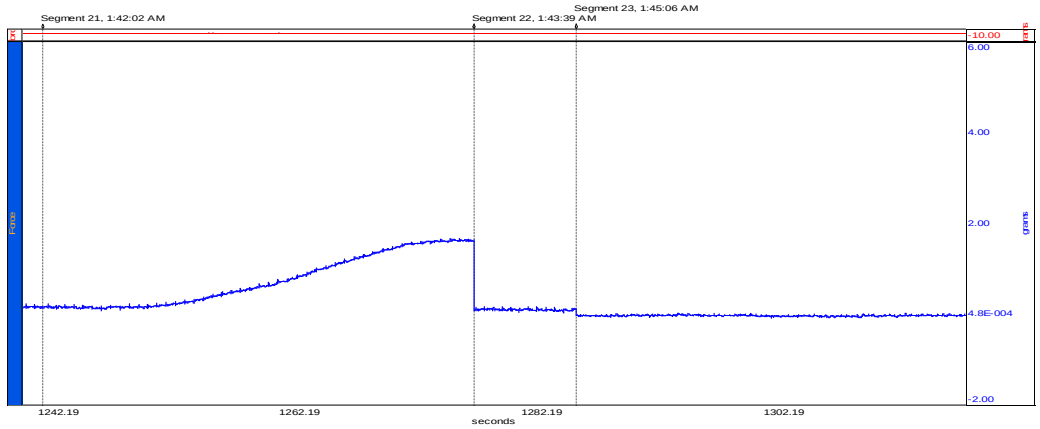


Figure 18: *Cinnamomum camphora* oil showing relaxation property in guinea pig tracheal chain preparation against spasmogen

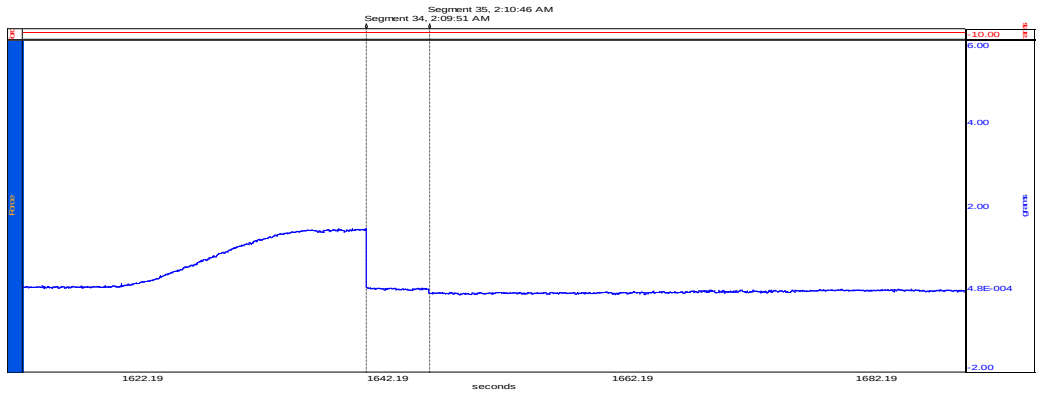


Figure 19: *Mentha arvensis* oil showing relaxation property in guinea pig tracheal chain preparation against spasmogen

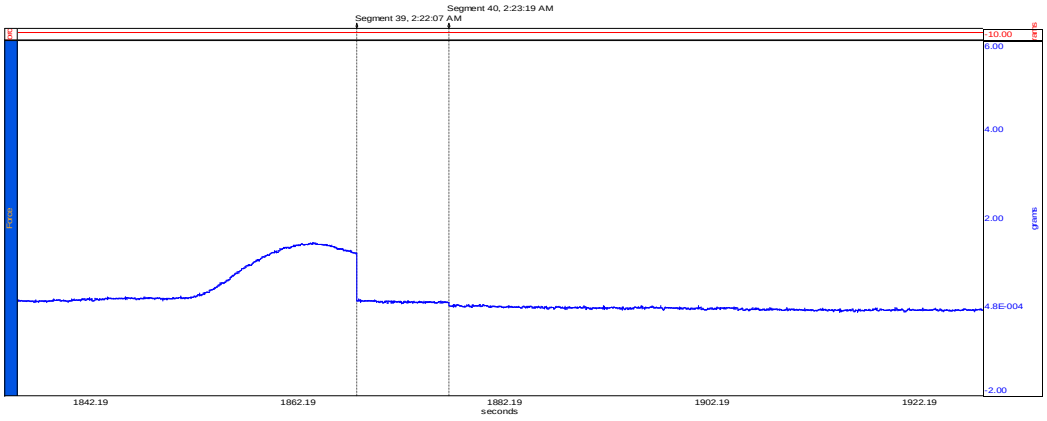


Figure 20: *Zanthoxylum armatum* oil showing relaxation property in guinea pig tracheal chain preparation against spasmogen

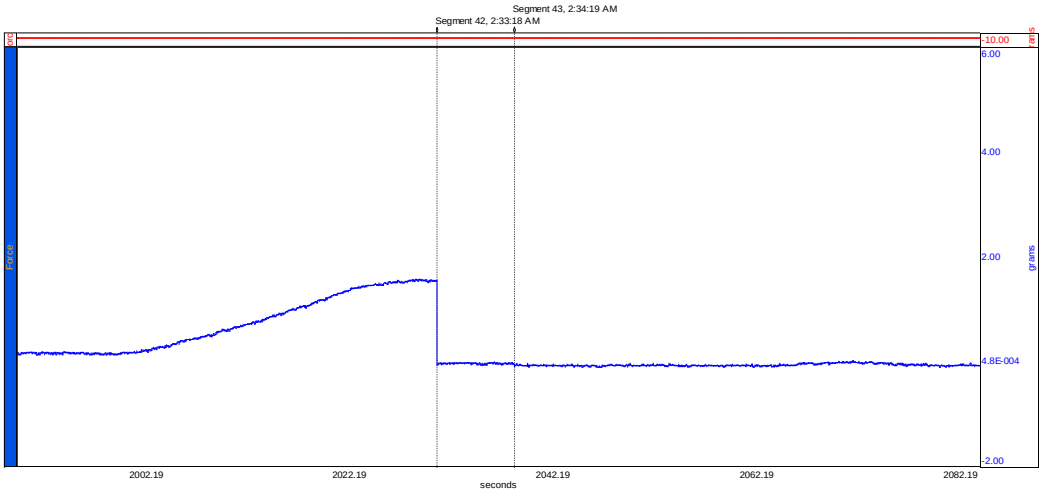


Figure 21: *Angelica glauca* oil showing relaxation property in guinea pig tracheal chain preparation against spasmogen

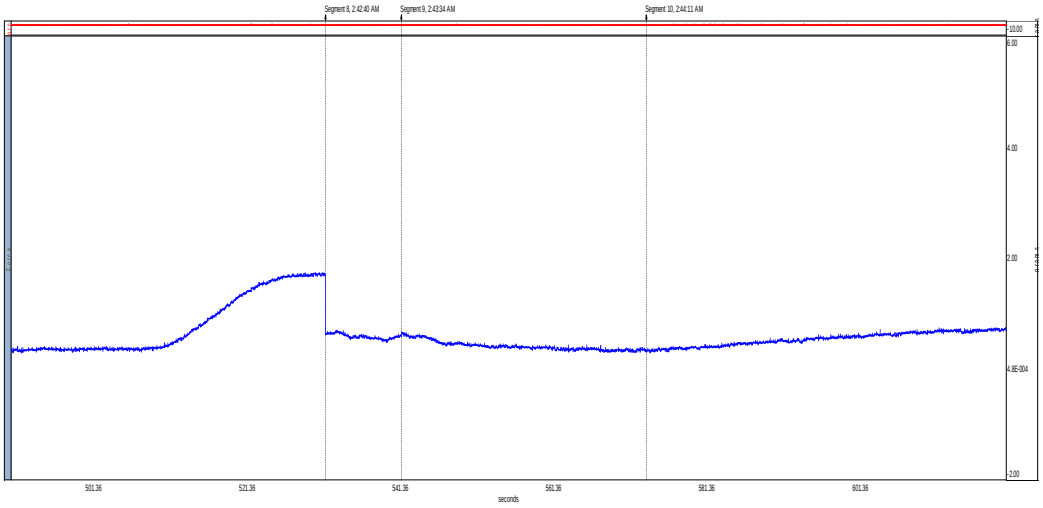


Figure 22: *Origanum vulgare* oil showing relaxation property in guinea pig tracheal chain preparation against spasmogen

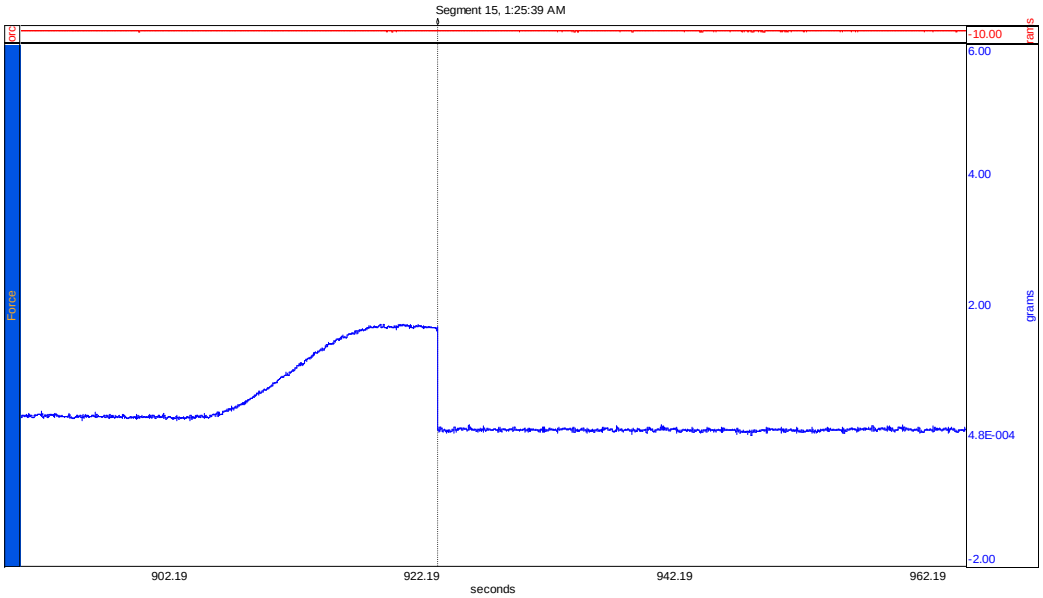


Figure 23: *Cinnamomum aromaticum* oil showing relaxation property in guinea pig tracheal chain preparation against spasmogen

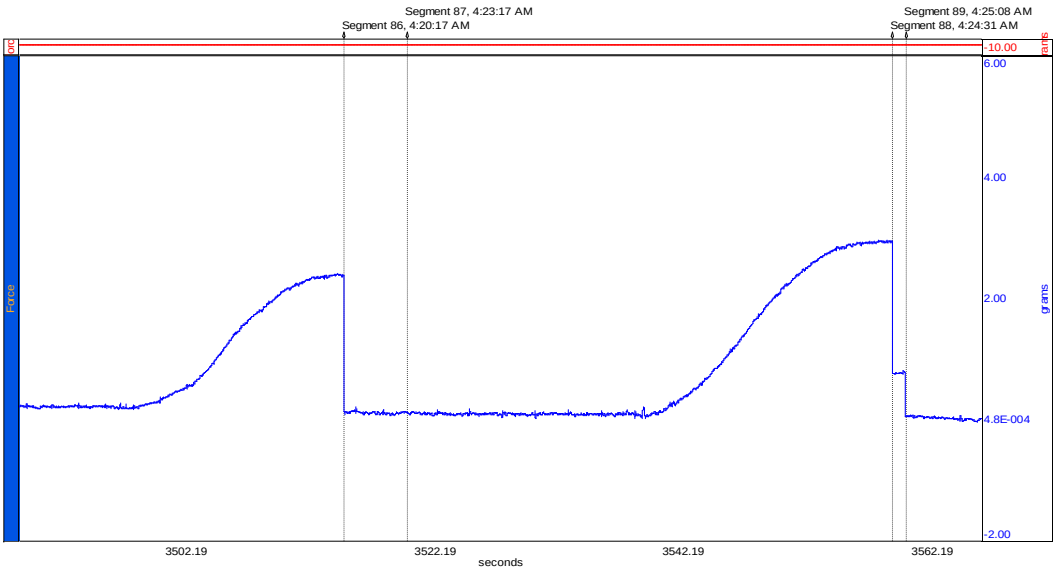


Figure 24: *Alpinia galanga* oil is not showing relaxation property in guinea pig tracheal chain preparation against spasmogen

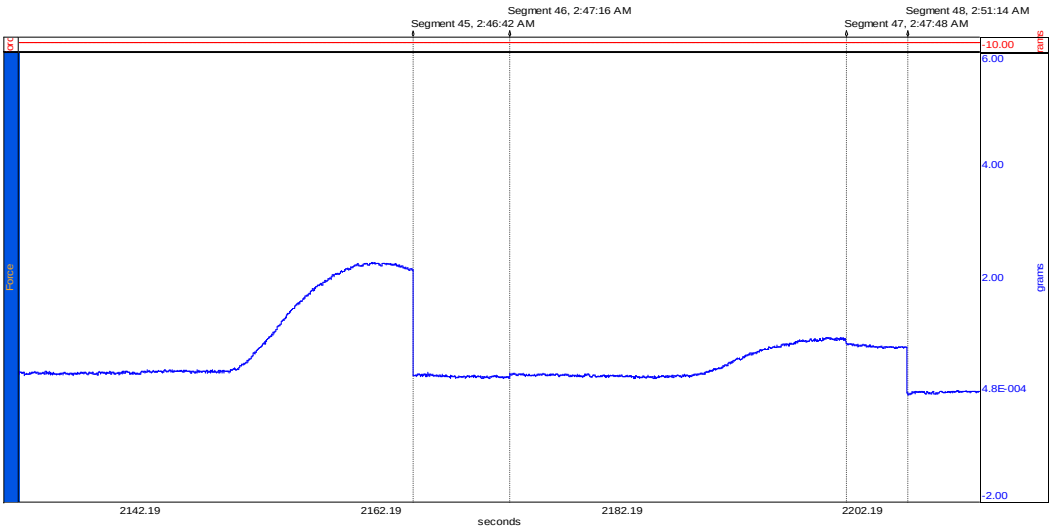


Figure 25: *Zingiber officinale* oil is not showing relaxation property in guinea pig tracheal chain preparation against spasmogen

Effect of Essential oil on Histamine induced bronchospasm in guinea pig

Histamine causes very strong smooth muscle contraction. After introducing histamine aerosol to guinea pig (highly sensitive for histamine) it produces Pre convulsion dyspnea (PCD). The time taken to initiates PCD was observed. There was no significant difference in PCD time in asthmatic control, there was significant ($p<0.001$) increase in PCD time after treatment with dexamethasone (2mg/kg) & significant ($p<0.001$, $p<0.01$) increases the time with the dose of Essential oils 200 µL/kg & 400 µL/kg respectively.

Highly significant ($p<0.001$) % protection showed in dexamethasone treated group (50.53± 3.776) & in Asthma group (6.171± 2.495) whereas in Essential oils treated group showed significant increase % protection at lower dose (200µL/kg) & at higher dose (400µL/kg) given in the table.

Table 7. Effect of Essential oils on Histamine induced bronchospasm in guinea pig

Grouping	% increase in pre convulsive dyspnea time PCD (sec)
Negative Control	6.17± 2.49
Dexamethasone (2mg/kg)	50.53± 3.77***
A. glauca 200µL/kg	53.74 ± 6.36***
A. glauca 400µL/kg	67.17 ± 6.48***
C. aromaticum 200µL/kg	23.98 ± 4.69**
C. aromaticum 400µL/kg	51.67 ± 7.93***
C. camphora 200µL/kg	42.94 ± 4.49***
C. camphora 400µL/kg	51.00 ±0.73***
C. domestica 200µL/kg	60.75 ± 0.30***
C. domestica 400µL/kg	74.35 ± 0.91***
M. arvensis 200µL/kg	37.77± 3.52***
M. arvensis 400µL/kg	63.00 ± 0.73***
M. oleifera 200µL/kg	32.94 ± 8.92***
M. oleifera 400µL/kg	70.00 ± 1.09***
O. vulgare 200µL/kg	27.03± 4.41**
O. vulgare 400µL/kg	60.49 ± 2.22***
Z. armatum 200µL/kg	61.20 ± 0.29***
Z. armatum 400µL/kg	69.48 ± 0.99***

Each values were expressed as Mean±SEM; where n=6 in each group: significant ***P<0.001,**P<0.01 compared with negative control by one way ANOVA followed by Dunnett's multiple comparison test.***P<0.001 when compared with Disease group.

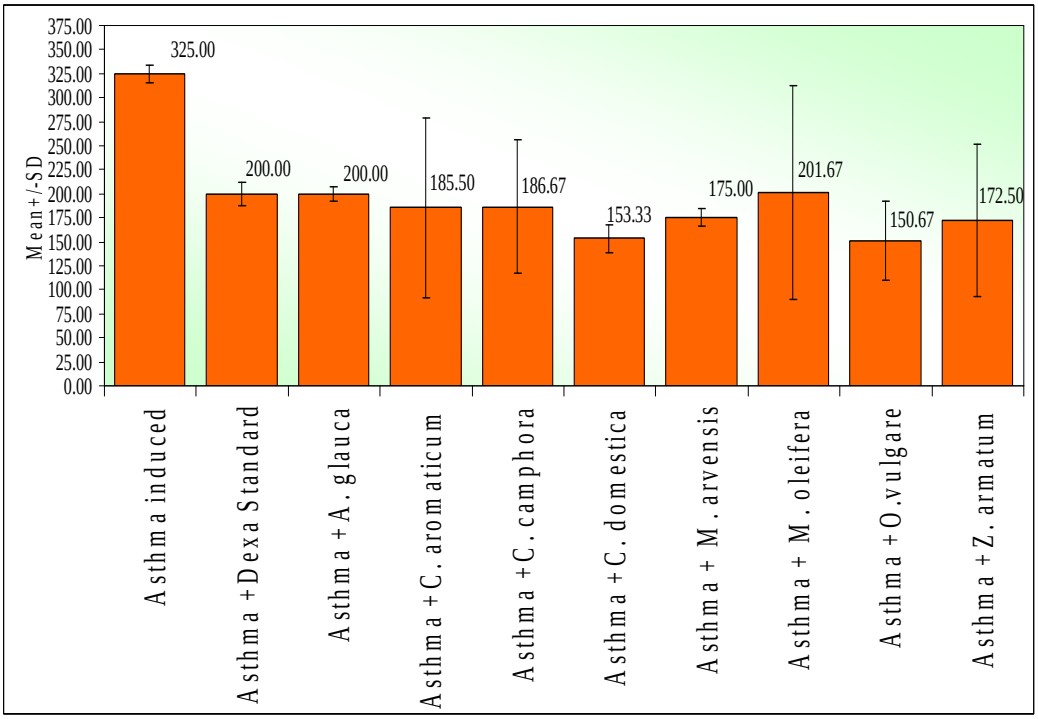
Table 8: Consequence of Essential oil on AEC in blood in control & experimental mice

Groups	Mean	SD	SE	95% CI for mean	
				Lower	Upper
Asthma induced	325.00	9.06	3.70	315.50	334.50
Asthma +Dexa Standard	200.00	12.18	4.97	187.22	212.78
Asthma +*A. glauca*	200.00	7.48	3.06	192.15	207.85
Asthma +*C. aromaticum*	185.50	93.99	38.37	86.86	284.14
Asthma +*C. camphora*	186.67	68.97	28.16	114.29	259.04
Asthma +*C. domestica*	153.33	14.67	5.99	137.94	168.72
Asthma + *M. arvensis*	175.00	9.06	3.70	165.50	184.50
Asthma + *M. oleifera*	201.67	111.39	45.47	84.78	318.56
Asthma +*O.vulgare*	150.67	40.80	16.66	107.85	193.48
Asthma +*Z. armatum*	172.50	79.47	32.44	89.10	255.90
F-value	4.1977				
P-value	0.0004*				

*p<0.05

Asthmatic group showed significant increase in AEC (325±3.69) compared to normal group (125±3.69), while dexamethasone treatment resulted in significant decrease in AEC (200±4.97). Treatment group with 200µl/kg dose of EO had significant decrease in AEC compared to asthma induced group. The data are shown as Mean±SEM, with n = 6 in each group. One-way analysis of variance test revealed a p value 0.0004* difference when compared to Asthma control group.

Figure 26: Comparison of ten groups for AEC in blood by one way ANOVA analysis



Effect of essential oil on absolute eosinophils (cells/mm^3) count in blood of mice values were expressed as mean±standard error of the mean compared with ovalbumin-treated group.

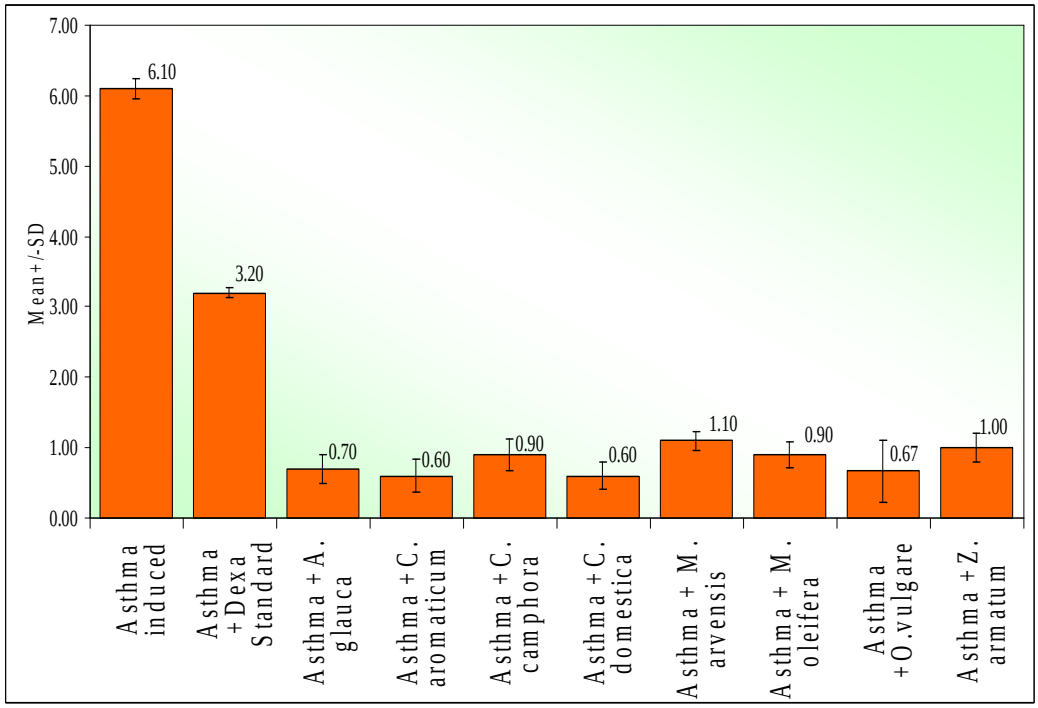
Table 9: Effect of Essential oils on IgE antibody concentration in blood serum in control & experimental mice

Groups	Mean	SD	SE	95% CI for mean	
				Lower	Upper
Asthma induced	6.10	0.14	0.06	5.95	6.25
Asthma +Dexa Standard	3.20	0.07	0.03	3.13	3.27
Asthma +*A. glauca*	0.70	0.20	0.08	0.49	0.91
Asthma +*C. aromaticum*	0.60	0.23	0.09	0.36	0.84
Asthma +*C. camphora*	0.90	0.23	0.09	0.66	1.14
Asthma +*C. domestica*	0.60	0.20	0.08	0.39	0.81
Asthma + *M. arvensis*	1.10	0.13	0.05	0.96	1.24
Asthma + *M. oleifera*	0.90	0.18	0.07	0.71	1.09
Asthma +*O.vulgare*	0.67	0.44	0.18	0.21	1.12
Asthma +*Z. armatum*	1.00	0.20	0.08	0.79	1.21
F-value	381.4940				
P-value	0.0001*				

*p<0.05

Ovalbumin induced model of allergic airway inflammation (inflammatory mediators) activates B-lymphocytes to produce IgE antibodies. There was significant increased serum IgE level in asthmatic group (6.10±0.05) as compared to normal group (0.80±0.03) whereas after treatment with dexamethasone it decreased significantly to (3.20±0.02). Treatment group with 200µl/kg dose of EO treatment groups showed decreased serum IgE level which was highly significant like dexamethasone group as shown in the table. One-way analysis of variance test revealed a p value 0.0001* difference when compared to Asthma control group.

Figure 27: Comparison of ten groups for IgE antibody concentration in blood serum by One way ANOVA analysis



Effect of essential oils immunoglobulin E (IU/L) in blood serum of mice; values were expressed as mean ± standard error of the mean compared with ovalbumin-treated group.

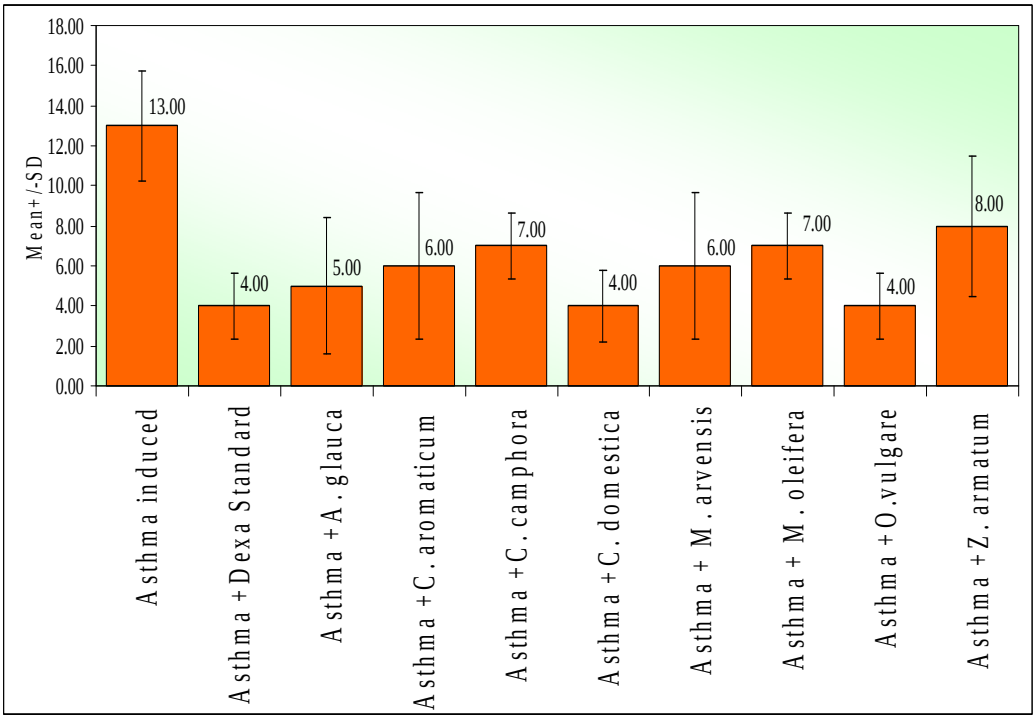
Table 10: Effect of Essential oils on Total Neutrophil Count in BALF in control & experimental mice

Groups	Mean	SD	SE	95% CI for mean	
				Lower	Upper
Asthma induced	13.00	2.76	1.13	10.11	15.89
Asthma +Dexa Standard	4.00	1.67	0.68	2.24	5.76
Asthma +*A. glauca*	5.00	3.41	1.39	1.43	8.57
Asthma +*C. aromaticum*	6.00	3.69	1.51	2.13	9.87
Asthma +*C. camphora*	7.00	1.67	0.68	5.24	8.76
Asthma +*C. domestica*	4.00	1.79	0.73	2.12	5.88
Asthma + *M. arvensis*	6.00	3.69	1.51	2.13	9.87
Asthma + *M. oleifera*	7.00	1.67	0.68	5.24	8.76
Asthma +*O.vulgare*	4.00	1.67	0.68	2.24	5.76
Asthma +*Z. armatum*	8.00	3.52	1.44	4.30	11.70
F-value	6.0474				
P-value	0.0001*				

*p<0.05

Neutrophil count in BALF was also eminent in reply to the inflammation due to the OVA exposure. Asthmatic group showed significant elevation in Neutrophil count (13.00±1.12) whereas dexamethasone group (4.00±0.68) and treatment groups with 200µL/kg of EO showed significant decrease in Neutrophil count as compared with asthma induced group given in this above table. One-way analysis of variance test revealed a p value 0.0001* difference when compared to Asthma control group.

Figure 28: Comparison of ten groups for Total Neutrophil Count in BALF by One way ANOVA analysis



Effect of essential oils on inflammatory cells; eosinophils in bronchoalveolar lavage fluid values were expressed as mean ± standard error of the mean compared with ovalbumin-treated group.

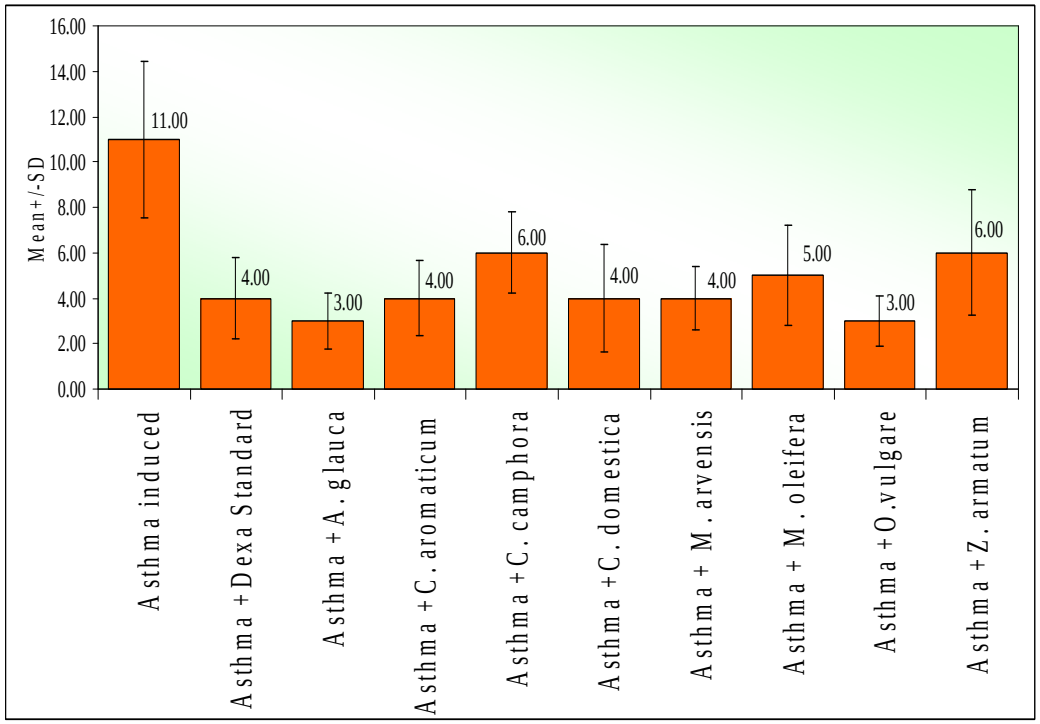
Table 11: Effect of Essential oils on Total Eosinophil Count in BALF in control & experimental mice

Groups	Mean	SD	SE	95% CI for mean	
				Lower	Upper
Asthma induced	11.00	3.46	1.41	7.36	14.64
Asthma +Dexa Standard	4.00	1.79	0.73	2.12	5.88
Asthma +*A. glauca*	3.00	1.26	0.52	1.67	4.33
Asthma +*C. aromaticum*	4.00	1.67	0.68	2.24	5.76
Asthma +*C. camphora*	6.00	1.79	0.73	4.12	7.88
Asthma +*C. domestica*	4.00	2.37	0.97	1.52	6.48
Asthma + *M. arvensis*	4.00	1.41	0.58	2.52	5.48
Asthma + *M. oleifera*	5.00	2.19	0.89	2.70	7.30
Asthma +*O.vulgare*	3.00	1.10	0.45	1.85	4.15
Asthma +*Z. armatum*	6.00	2.76	1.13	3.11	8.89
F-value	7.5758				
P-value	0.0001*				

*p<0.05

Eosinophil count in BALF was also eminent in reply to the inflammation due to the OVA contact. Asthmatic group showed significant elevation in Eosinophil count (11.00±1.41) whereas dexamethasone group (4.00±0.73) & 200µL/kg of EO groups showed significant decrease in eosinophil count as compared with asthmatic control given in this above table. One-way analysis of variance test revealed a p value 0.0001* difference when compared to Asthma control group.

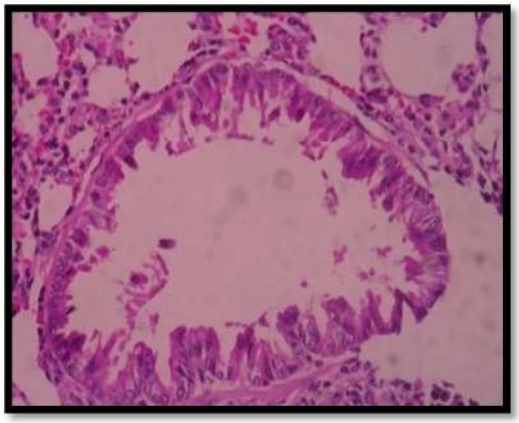
Figure 29: Comparison of ten groups for Total Eosinophil Count in BALF by One way ANOVA analysis



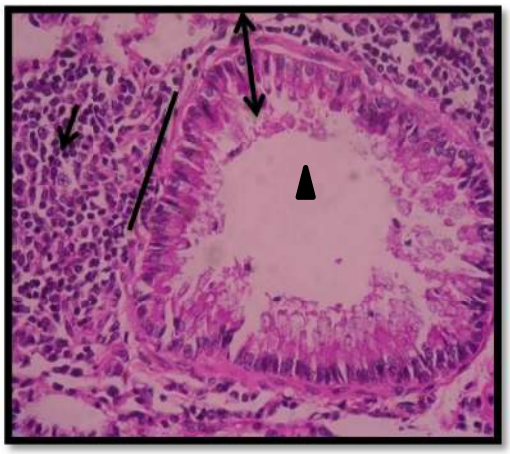
Effect of essential oils on inflammatory cells; neutrophils in broncho alveolar lavage fluid of mice values were expressed as mean ± standard error of the mean compared with ovalbumin-treated group.

Figure 30: Histopathology of mice lungs 40x images

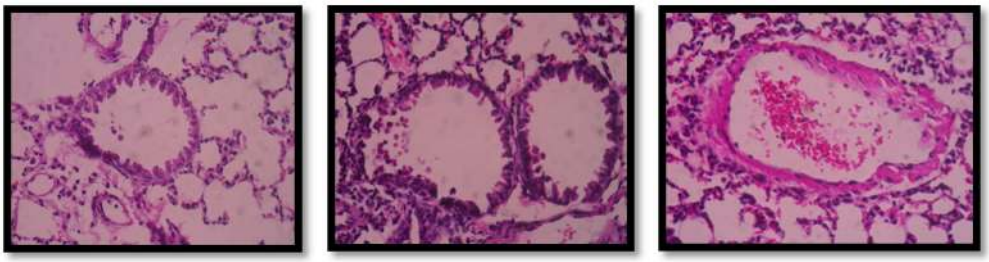
The lungs were dissected out and fixed in 10% formalin for 24hours and then cut section stained with H&E were observed under light microscope to assess the degree of peri bronchial and perivascular inflammation.



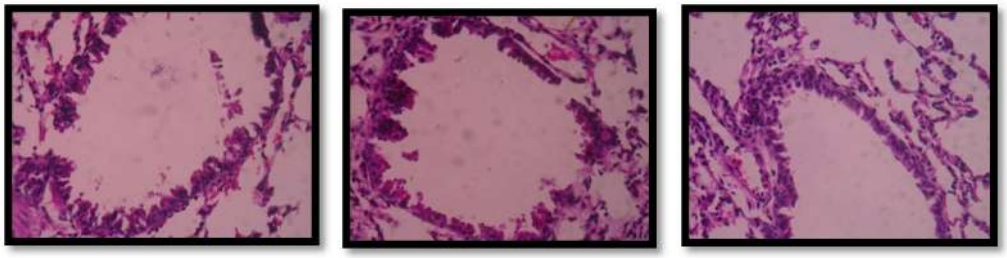
(a) Normal group: Normal bronchial epithelium layers were observed in normal saline group



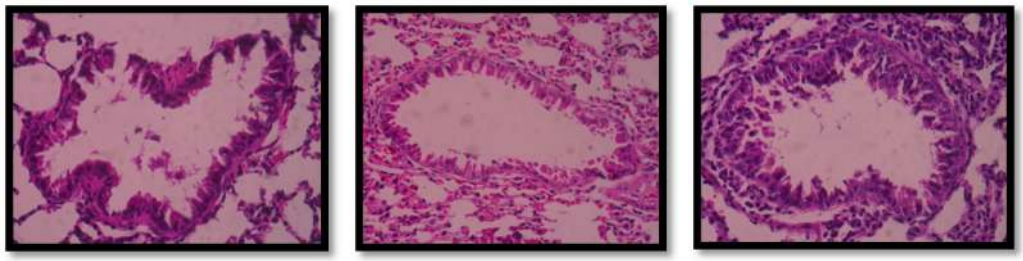
(b) Asthma group (OVA induced) : Histopathology of lung section of mice Ovalbumin treated group (figure b, 40X, H&E stained) showed marked reactions in the lung characterized by the infiltration of inflammatory cells specially in peribronchial and perivessel area. (Narrowing of Airway, Epithelial hyperplasia, inflammatory cells, Peribronchial cells).



(c) Dexamethasone group **(d) *Angelica glauca*** **(e) *Cinnamomum aromaticum***



(f) *Cinnamomum camphora* **(g) *Mentha arvensis*** **(h) *Moringa oleifera***



(i) *Origanum vulgare linn* **(j) *Curcuma domestica*** **(k) *Zanthoxylum armatum***

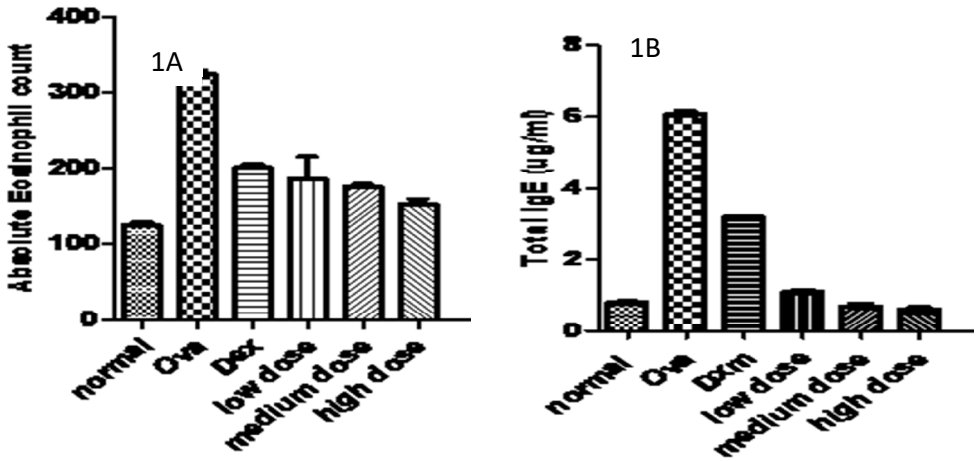
Lung section of mice expressed to Ovalbumin group **(figure b)** stained with H&E showed significant inflammatory changes in peribronchial and perivascular areas, increased bronchial muscle thickening and epithelial hyperplasia.The histopathological changes induced by OVA were markedly suppressed by treatment with essential oils with dose 200 µl/kg **(figure; d to k)** and was almost comparable to normal group **(Figure a)** as well as dexamethasone treated group **(figure c).**

Table 12: Polyessential oil formulation of *A. glauca, C. aromaticum, C. domestica, M. arvensis, O. vulgare.* Ovalbumin induced sensitization in mice with low, medium and high dose

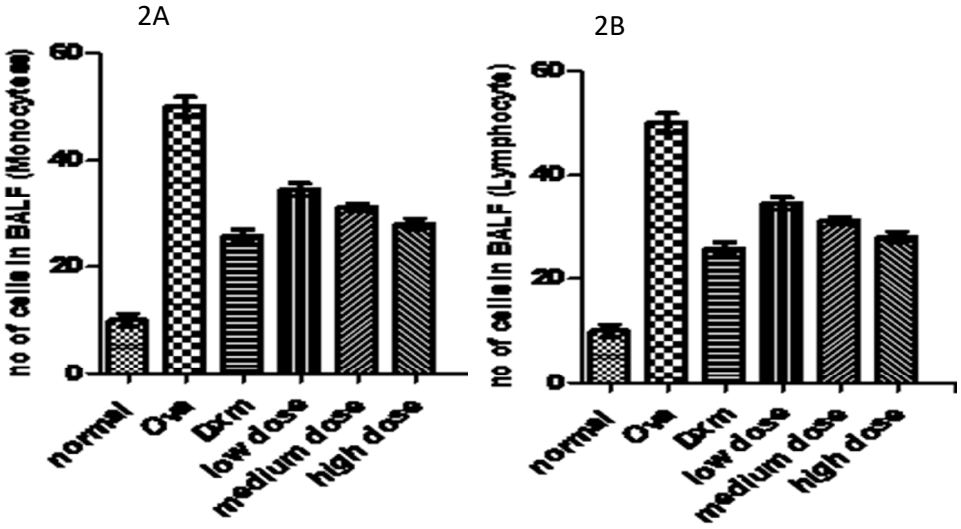
Grouping	Absolute eosinophil count	IgE in blood serum	Monocytes in BALF	Neutrophils in BALF	Lymphocytes in BALF	Eosinophils in BALF
Normal	125±3.677	0.800±0.033	54.00±2.082	3.00±0.4472	10±1.155	2.00±0.2582
Ovalbumin	325±3.697	6.100±0.057	88±2.000	13.00±1.125	50±1.88	11.00±1.414
Dexamethasone (2mg/kg)	200±4.973	3.200±0.028	55±1.155	4.00±0.6831	25.83±1.078	4.000±0.7303
Low dose (125µL/kg)	186±28.16***	1.100±0.054***	64±1.528***	7.000±0.6831***	34.5±1.258***	6.00±0.7303**
Medium dose (250 µL/kg)	175±3.697***	0.700±0.081***	59±1.862***	5.000±1.390***	31±0.7303***	3.167±0.4773***
High dose (500µL/kg)	153±5.987***	0.600±0.081***	55±1.155***	4.000±0.6831***	28±0.9309***	2.833±0.4773***

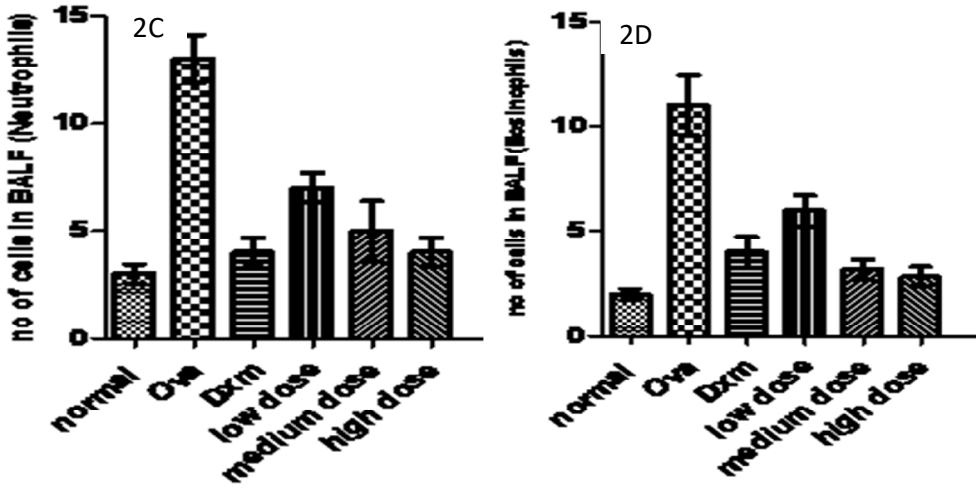
Ovalbumin induced asthmatic group showed significant elevation in all inflammatory mediators (AEC in blood, Serum IgE, monocyte, neutrophils, lymphocytes, eosinophils in BALF). Whereas, standard dexamethasone (2mg/kg) group and Eos treated (125µL/kg, 250µL/kg, 500µL/kg) groups. showed significant decrease in inflammatory cells when compared to asthma induced group. One-way analysis of variance test revealed p value 0.0001* difference when compared to Asthma control group.

Figure 31: Polyessential oil parameters evaluated in mice



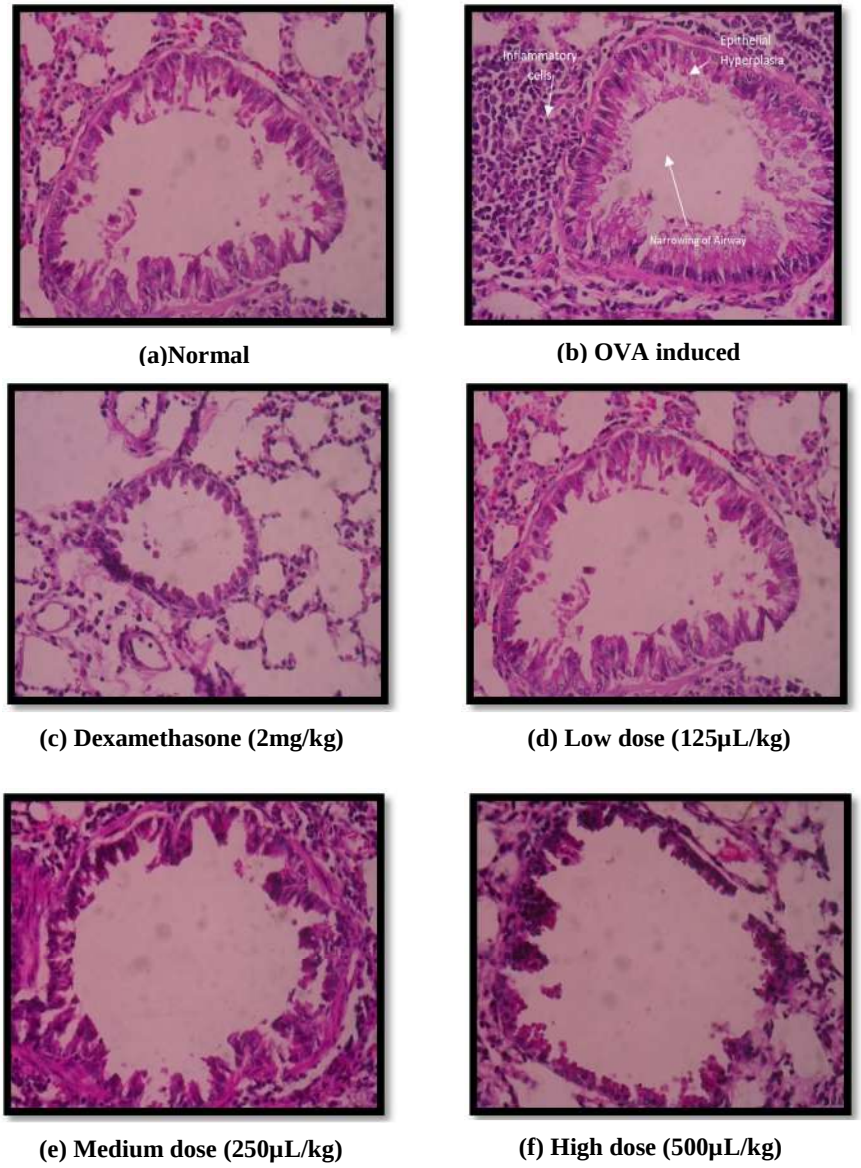
Polyessential oil treatment groups low dose, medium dose and high dose is *p<0.005 compared to ovalbumin-treated group, represented as mean ± standard error of the mean, n±6 per group. Effect of polyessential oil formulation on mice blood serum immunoglobulin E, IU/L (1B); Absolute eosinophil count, cells/cubic millimeter (1A).





Polyessential oil formulation's (low, medium and high dose) effect on monocytes & lymphocytes, two types of inflammatory cells found in the bronchoalveolar lavage fluid of mice, percentage (2A, 2B); Impact of polyessential oil on inflammatory cells: percentage (2C, 2D) of neutrophils in the bronchoalveolar lavage fluid of mice; For every group (n = 6), the mean ± standard error of the mean was employed, with $p < 0.001$ in relation to the ovalbumin group.

Figure 32: Histopathology of mice lungs in Polyessential oil formulation



(a)Normal **(b) OVA induced**

(c) Dexamethasone (2mg/kg) **(d) Low dose (125µL/kg)**

(e) Medium dose (250µL/kg) **(f) High dose (500µL/kg)**

Histopathology of lung section of mice Ovalbumin treated group (figure b, 40X, H&E stained) showed significant inflammatory changes on OVA-induced allergic asthmatic mice Δ Narrowing of Airway, ↕ Epithelial hyperplasia, ↓ inflammatory cells, / Peribronchial cells. The histopathological changes induced by OVA were markedly suppressed by treatment of Polyessential oil formulation low dose 125 µL/kg treated group (figure d); medium dose 250 µL/kg treated group (figure e); high dose 500 µL/kg treated group (figure f) was almost comparable to normal group (figure a) as well as dexamethasone treated group (figure c).

6.DISCUSSION

Essential oils have different pharmacological properties, as proven by several studies. In recent years, life scientists & clinicians have focused more on natural goods as an alternative to traditional treatments. Natural remedies have lower side effects, more patient compliance, & lower costs than orthodox therapy. Mainstream drugs & treatments sometimes have few benefits & many side effects.[139]

Respiratory disease of allergic type which gets triggered due to various spasmogens like ACH, LTs, PGs or an particular allergen exposure reflecting the acute broncho constriction signals is referred to asthma. Inflammatory mediators such as histamine & others results in series of changes in bronchial host tissue by enhancing the secretion of mucus along with constriction of bronchial smooth muscles rapidly.

Firstly, we have performed qualitative analysis of 10 essential oils as we had procured EO's from various sources. GCMS was done in association with RMRC, Belagavi. After that we started pilot study to evaluate the broncho dilating activity of 10 Essential oils by using in vitro model i.e. spasmolytic activity on guinea pig in isolated tracheal chain model. Guinea pig tracheal chain is much more sensitive for dose relative contraction of agonist like histamine (1µg, 2µg, 4µg, 8µg & 16µg of histamine dose given respectively) in isolated tracheal chain preparation. Out of 10 essential oils 2 did not showed decrease in contractions of tracheal smooth muscle in presence of *Zingiber officinale & Alpinia galanga*. Hence, we finally selected 8 essential oils (*A. glauca, C. aromaticum, C. camphora, C. domestica, M. arvensis, M. oleifera, O. vulgare & Z. armatum*) to start our main research objective.

As preclinical study, *In vivo* guinea pigs used to examine EOs' bronchodilating effect had increased PCT latency due to histamine.[140] PCT latency increased significantly ($P < 0.001$) in this investigation, showing EOs' bronchodilating effect against histamine.

The study aimed to evaluate the effects of various essential oils on pre-convulsive dyspnea (PCD) induced by histamine in guinea pigs, a model known for its sensitivity to histamine-induced respiratory distress. The essential oils were administered at two different doses (200µL/kg and 400µL/kg), and dexamethasone was used as a positive control. Dexamethasone (2mg/kg), a known anti-inflammatory and bronchodilatory agent, significantly increased PCD time by 50.53 seconds compared to the negative control group 6.17 seconds. This significant increase suggests its potent protective effect against histamine-induced respiratory distress in the guinea pig model. Various essential oils (*A. glauca, C. aromaticum, C. camphora, C. domestica, M. arvensis, M. oleifera, O. vulgare, Z. armatum*) were tested at both low dose (200µL/kg) and high dose (400µL/kg) doses. ***A. glauca*** significantly increased PCD time at both doses 200µL/kg: **53.74 ± 6.36 seconds** & 400µL/kg: **67.17 ± 6.48 seconds** compared to the negative control, ***C. aromaticum*** significantly increased PCD time at both doses 200µL/kg: **23.98 ± 4.69 seconds** & 400µL/kg: **51.67 ± 7.93 seconds** compared to the negative control, ***C. camphora*** significantly increased PCD time at both doses 200µL/kg: **42.94 ± 4.49 seconds** & 400µL/kg: **51.00 ± 0.73 seconds** compared to the negative control, ***C. domestica*** significantly increased PCD time at both doses 200µL/kg: **60.75 ± 0.30 seconds** & 400µL/kg: **74.35 ± 0.91** seconds compared to the negative control, ***M. arvensis*** significantly increased PCD time at both doses 200µL/kg: **37.77 ± 3.52 seconds** & 400µL/kg: **63.00 ± 0.73 seconds** compared to the negative control, ***M. oleifera*** significantly increased PCD time at both doses

200µL/kg: **32.94 ± 8.92 seconds** & 400µL/kg: **70.00 ± 1.09 seconds** compared to the negative control. ***O. vulgare*** significantly increased PCD time at both doses 200µL/kg: **27.03 ± 4.41 seconds** & 400µL/kg: **60.49 ± 2.22 seconds** compared to the negative control, ***Z. armatum*** significantly increased PCD time at both doses 200µL/kg: **61.20 ± 0.29 seconds** & 400µL/kg: **69.48 ± 0.99 seconds** compared to the negative control. There was a dose-dependent response observed with essential oils. The higher dose (400µL/kg) showed a greater increase in PCD time compared to the lower dose (200µL/kg), indicating a potentially more pronounced protective effect at higher concentrations.

Another *in vivo* model of OVA-induced allergic airway inflammation showed raised blood IgE & eosinophilic infiltration in the lungs, similar to asthma. Eosinophil count increased in untreated animals after OVA exposure. Asthmatic group showed significant increase in **AEC (325±3.69)** compared to normal group **(125±3.69),** while dexamethasone treatment resulted in significant decrease **(200±4.97).** 200µL/kg dose of essential oil had significant decrease in AEC compared to asthmatic control given in the **table 8.** There was significant increased in **Serum IgE** level with **asthmatic group (6.10±0.05)** as compared to **normal group (0.80±0.03)** whereas after treatment with **dexamethasone** it decreased significantly to **(3.20±0.02)** & after 200µL/kg dose of EOs treatment, groups show decreased serum IgE level which was highly significant as like dexamethasone group as shown in the **table 9. Neutrophil count in BALF** was also eminent in reply to the inflammation due to the OVA exposure. **Asthmatic group** showed significant elevation in Neutrophil count **(13.00±1.12)** whereas **dexamethasone group (4.00±0.68)** & dose 200µL/kg of Essential oil groups showed significant decrease in Neutrophil count as compared with asthmatic control given in this above **table 10. Eosinophil count in BALF** was also eminent in reply to

the inflammation due to the OVA contact. Asthmatic group showed significant elevation in Eosinophil count **(11.00±1.41)** whereas dexamethasone group **(4.00±0.73)** & dose 200µL/kg of Essential oil groups showed significant decrease in eosinophil count as compared with asthmatic control given in this above **table 11**. Histopathology of lung section of mice Ovalbumin treated group (figure b, 40X, H&E stained) showed marked reactions in the lung characterized by the infiltration of inflammatory cells specially in peribronchial and perivessel areas. Eosinophilic inflammation of the airways is a common feature of allergic asthma. Structural remodeling of airway wall observed.

Overall, in OVA-induced asthma manifests as neutrophil, macrophagic, eosinophilic, & lymphocyte infiltration into the bronchus lumen & resembles chronic airway inflammation. Due to allergies, infections, or other medical conditions, eosinophils become active. Blood eosinophils decreased considerably during EO therapy.[141–142]

Since asthma is usually linked to IgE, it's allergic.[143] Skin tests & serum IgE specificity levels show a substantial link between asthma & allergic sensitization in many studies. An allergic reaction caused by eosinophilic recruitment & mast cell inflammatory mediator production.[144]

Thus, IgE blocking may prevent & ameliorate allergy symptoms.[145] Essential oils significantly reduced serum IgE in one investigation. Granule-derived basic proteins from asthma eosinophils have been found in BALF.[145]

The vicious cycle of tissue injury & inflammatory cell recruitment shows that chronic inflammation can persist without allergens. Eosinophil in sputum correlates

with persistent airway blockage.[146] The study found that essential oil treatment significantly reduced BALF eosinophils ($P < 0.001$), while dexamethasone treatment had a similar effect.[146] No comparison was performed between essential oils & other bronchodilators. Along with eosinophils, neutrophils are important in the late phase of asthma. There is little study on essential oils' anti-inflammatory & anti-asthmatic qualities. The primary compounds in essential oils are thymol, carvacrol, linalool, camphor, 1,8-cineole, menthol, & others. Our chosen essential oils contain anti-asthmatic chemicals.[147]

Soyingbe *et al* (2015) concluded that *Eucalyptus grandis* essential oil having major chemical constituents α-pinene, borneol, p-cymene, 1,8-cineole, d-limonene & α-terpineol having concentration dependent anti-inflammatory, anti-cough & anti-asthmatic activity.[112]

Wright CE *et al* (1997) reported menthol has dual action on sensory nerves & smooth muscle, this compound might possess therapeutic part in URTI, bronchitis & asthma.[113]

Juergens UR *et al* (2004) found that 1,8-cineole in eucalyptus oil inhibits cytokines to regulate airway mucus hypersecretion, indicating long-term treatment for asthma, sinusitis, & COPD exacerbation.[114] **Juergens *et al* (1998)** found that eucalyptol oil (1,8-cineole) inhibits arachidonic acid metabolism in human blood monocytes, resulting in anti-inflammatory effects in bronchial asthma.[115] **Boskabady MH *et al* (2003)** found that carvacrol inhibits phosphodiesterase & Ca antagonism in guinea pig smooth muscles, bronchodilating them.[116] **Morice AH *et al* (1994)** found that menthol reduces citric acid-induced cough in normal people.[117] **Boskabady MH *et al* (2003)** found that *Carum copticum* essential oil relaxes guinea pig tracheal

chains. Menthol, camphor, & cineole were antitussive in citric acid-induced asthma in conscious guinea pigs.[118] *Nepeta cataria* Linn oil spasmolytic and bronchodilatory, according to **Gilani AH *et al* (2009).** The twenty-seven components of NC oil include 1,8cineol (21.00%), humulene (14.44%), pinene (10.43%), and geranyl acetate (8.21%). In rabbits, Nc. Oil, papaverine, and verpamilin jejunum block calcium channels by inhibiting immediate and enhanced K+(80mM) precontractions and shifting Ca++ CLRCs to the right. [148]

Wang ZW *et al* (2015) concluded that volatile oil of *Angelicae Sinensis* at the amount of 40-160 mg/kg could enhance the respiratory capacity & the asthmatic behaviors in rats, & also upgrade IL-10 levels.[149] **Gong JH *et al* (2015)** presented anti-asthma, anti-cough & expectorant activities in *D. Sophia* seed oil *in-vivo*. The seed oil of *D. Sophia* inhibits frequency or enhances cough's latent period, that even supports the effect of anti-tussiveness. In addition, when *D. Sophia* seed oil fraction on evaluating *in- vivo* bronchial constriction induces heterogenous spray of histamine & ACH in guinea pigs, significantly supporting the effect of anti-asthma on them.[150] **Modupe O *et al* (2009)** evaluated essential oil derived from *Euphorbia hirta Lin's* dried leaves, for potentially treating asthma. The isomers of Phytol, 3,7,11,15-tetramethyl-2-hexadecene-1-ol & 2,6,10-trimethyl tetrad cane identified in the *E. Hirta* leaves has demonstrated that inflammatory conditions can be managed.

Therefore, it could be this mixture which provided relief to asthmatic patients which is an inflammatory disorder of airways of chronic type. The presence of oleic acid even exerts anti – inflammatory activity producing a promising effect in patients affected with asthma.[151] **de Sousa AA *et al* (2010)** explored the anti – spasmodicity of essential oil procured from *Mentha piperita* on rat's tracheal muscles. The authors

intended to describe the anti-spasmodic action of pepper mint oil in isoltated trachea of rats with carbachol contraction, an ingredients exerting spasmodic action through bounding muscrainic receptors. Enhanced degree of NO was seen in specific spasmodic situations, such as allergic rhinitis, ARDS & both phases of asthma – immediate & late.

Their experiments suggested that the Nitric oxide synthase enzyme influences the peppermint oil relaxing action on smooth muscles of trachea along with nitric oxide synthase enzyme involvement.[152] **Farooq A *et al* (2015)** tested Extra Virgin Olive oil (Oleaeuropea) against Milk-Induced Leukocytosis & Eosinophilia under asthma conditions. Eosinophils are important in late asthmatic inflammation. Eosinophils secrete mediators that cause epithelial layer shedding, broncho-constriction, & respiratory inflammation, which lead to allergic conditions. Olive oil orally inhibited milk-induced eosinophilia, while milk intoxicants increased the count. This suggests that olive oil reduces milk-induced leukocytosis via stabilising tissue oxidative stress.[153]

Shirole L *et al* (2015) described the anti-asthmatic, sedative, & spasmolytic effects of *Pistacia Integerrima* (EOPI) Stewart Ex Brandis Indian folk medicine oil. [154]**de Cassia da Silveira *et al* (2013)** conducted a review studying monoterpenes from essential oils & their mode of actions The principal monoterpene, thymoquinone essential oil procured from *Nigella sativa* L. (Ranunculaceae) seeds, which is a herb in folk medicinal usage for treating eczema, asthma & inflammation of bronchus. Cineole or 1,8-cineole or 1,8-cineol (eucalyptol), represents a monoterpene oxide found in essential oils of various plants, such as eucalyptus, commonly utilised in treating respiratory disorder triggered by infection. monoterpene borneol

demonstrated as a stabilizer of mast cell membrane which can be utilised to treat type I allergy. Borneol showed to exhibit inhibitory action on histamine release from mast cells of abdomen by 40.4%. thymol nullifies the neutrophilic release. Carvacol & Linalool found in monoterpenes is utilized for inflammation of chronic type. Their review demonstrated the anti – inflammatory role of monoterpenes which was bioactive on inflammatory experimental models. The reports demonstrated the management ability of this chemical category for treating novel anti – inflammatory problems like asthma, allergy, inflammation, lymphocytes, cytokines & immune disorders. The principal materials of EOs were menthol, menthone & 1,8-cineole etc which of its components is/are responsible for anti-allergic action demands research.[155]

Furthermore, On the basis of *in-vivo* studies i.e. Histamine induced Bronchospasm for Polyessential oil formulation we have selected these essential oils; *Mentha arvensis, Curcuma domestica, Origanum vulgare, Angelica glauca, Cinnamomum aromaticum* have shown significant results. To prepare poly essential oil formulation with tween 80 & water as emulsion. We had selected three dosage low 125 µL/kg, medium 250 µL/kg & high dose 500 µL/kg.we have taken *in-vivo* model for pilot study i.e. ovalbumin induced sensitization in mice & taken normal group, ovalbumin group & dexamethasone group values from previous study as reference.

BAL fluid revealed airway inflammatory cells after OVA challenges. Ovalbumin increased overall inflammatory cell counts too much.

At high dose 500µL/kg showed highly significant decrease in inflammatory cells comparative to standard dexamethasone group. Absolute Eosinophil Count was 153±5.987, IgE in blood serum was 0.600±0.081. Monocytes, neutrophils,

lymphocytes, eosinophils in BALF 55±1.155, 4.000±0.6831, 28±0.9309 & 2.833±0.4773 respectively. In standard dexamethasone (2mg/kg) group the Absolute Eosinophil Count was 200±4.973, IgE in blood serum was 3.200±0.028. Monocytes, neutrophils, lymphocytes, eosinophils in BALF 55±1.155, 4.00±0.6831, 25.83±1.078 & 4.000±0.7303.

Medium dose 250µL/kg significant decrease in inflammatory cells. Absolute Eosinophil Count was 175±3.697, IgE in blood serum was 0.700±0.081. Monocytes, neutrophils, lymphocytes, eosinophils in BALF 59±1.390, 5.000±1.390, 31±0.7303 & 3.167±0.4773.

Low dose also 125µL/kg showed significant decrease in inflammatory cells. Absolute Eosinophil Count was 186±28.16, IgE in blood serum was 1.100±0.054. Monocytes, neutrophils, lymphocytes, eosinophils in BALF 64±1.528, 7.000±0.6831, 34.5±1.258 & 6.00±0.7303.

Further research using *in vivo* models, particularly in guinea pigs and mice, confirmed the anti-inflammatory effects of selected essential oils. These studies highlighted reductions in eosinophilic infiltration in the lungs, decreased serum IgE levels, and modulation of inflammatory mediators crucial in asthma pathogenesis. Notably, essential oils such as those derived from *Mentha arvensis, Curcuma domestica, Origanum vulgare, Angelica glauca,* and *Cinnamomum aromaticum* showed significant antiasthmatic effects in histamine-induced bronchospasm models.

The mechanisms behind these therapeutic effects involve various functional groups present in essential oils, such as **terpenes, esters, alcohols, aldehydes, phenols**, and **aromatic ketones**. Various functional groups in essential oils (EOs)

significantly influence their anti-inflammatory activity. These compounds or functional group act through inhibition of inflammatory mediators leading to reduced inflammation and improved respiratory function.[47]

Terpenoids in essential oils act like natural anti-inflammatory agents by blocking NF-κB and MAPK pathways. Compounds like **alcohols, aldehydes** and **ketones** found in essential oils have groups (-OH, –CHO, –C– – O) that lower inflammation by reducing cytokines or boosting antioxidants. Specific compounds in plants like **β-caryophyllene, limonene**, and **nerolidol** reduce inflammation by blocking processes like neutrophil migration. **β-eudesmol** binds strongly to COX-2 and TNF-α, while **β-caryophyllene** affects receptors like TLR4 and Nrf-2, reducing inflammation and enhancing antioxidant defenses. **Limonene** and **1,8-cineole** targets multiple inflammation pathways. **Cinnamaldehyde** from cinnamon fights inflammation, **turmerone** in turmeric block PGE2 and TNF-α production, which helps to prevent inflammation-related issues. Additionally, some compounds like **β-caryophyllene** and **limonene** exert antioxidant effects and inhibit pro-inflammatory enzymes, further contributing to their antiasthmatic properties.[47]

Essential oils, represent a promising avenue for the management of asthma and other inflammatory respiratory conditions due to their multifaceted pharmacological actions and favourable safety profile compared to conventional therapies. Further research is warranted to elucidate specific mechanisms of action and optimize formulations for clinical use.

7. CONCLUSION

Essential oils demonstrated significant broncho-relaxant effects in both histamine-induced bronchoconstriction in guinea pigs and ovalbumin-induced airway inflammation in mice.

Key findings from the study include:

1. **Histamine-Induced Bronchoconstriction**: Treatment with Eos increased the time of preconvulsive dyspnea in histamine-induced guinea pigs, indicating a broncho-relaxant effect.
2. **Ovalbumin-Induced Airway Inflammation**: Administration of Eos significantly reduced:
    - Absolute blood eosinophil count
    - Serum levels of immunoglobulin E (IgE)
    - Percentage of eosinophils and neutrophils in bronchoalveolar lavage fluid (BALF)
    - Histopathological changes in lung tissues were notably suppressed by Eos.
3. **Comparison with Dexamethasone**: The efficacy of EOs in reducing inflammatory markers (blood eosinophils, IgE levels, BALF eosinophils and neutrophils) was comparable to dexamethasone group.
4. **Histopathological Improvement**: Histopathological examination of lung tissues showed that EOs ameliorated bronchial asthma-related changes, indicating its potential to alleviate structural and inflammatory aspects of asthma pathology.

The poly essential oil formulation composed of *Mentha arvensis, Curcuma domestica, Origanum vulgare, Angelica glauca,* and *Cinnamomum aromaticum,* formulated with Tween 80 and water as an emulsion, demonstrated significant anti-inflammatory effects in an ovalbumin-induced sensitization model of asthma in mice.

Key findings from the study include:

1. **Inflammatory Cell Counts**: Treatment with the poly essential oil formulation at all dosage levels (125 µL/kg, 250 µL/kg, and 500 µL/kg) resulted in a significant decrease in inflammatory cells in the bronchoalveolar lavage (BAL) fluid compared to the ovalbumin group.
2. **Comparison with Dexamethasone**: At the high dose of 500 µL/kg, the poly essential oil formulation showed a highly significant decrease in inflammatory cells compared to the standard dexamethasone treatment (2 mg/kg). This included a notable reduction in Absolute Eosinophil Count and serum IgE levels, as well as decreased levels of monocytes, neutrophils, lymphocytes, and eosinophils in BAL fluid.
3. **Dose-Dependent Effects**: The anti-inflammatory effects were dose-dependent, with higher doses (500 µL/kg) generally resulting in greater reductions in inflammatory markers compared to medium (250 µL/kg) and low doses (125 µL/kg). However, even the low and medium doses showed significant decreases in inflammatory cell counts and associated markers compared to the ovalbumin group.

4. **Therapeutic Potential**: These findings suggest that the poly essential oil formulation has potential as a therapeutic option for managing asthma, potentially offering benefits comparable to or even exceeding those of dexamethasone in terms of reducing inflammation.

Overall, present investigation found that the Polyessential oil formulation dose-dependently inhibits ovalbumin-induced sensitization in mice, likely due to the chemical contents of each oil. This research needs to be confirmed before applying it to humans because poly essential oil formulation has not been studied before. Few research had examined poly herbal anti-asthmatic formulations.[156-158]

Furthermore, research on the EO effect on human cells is needed to understand & advance this topic. Essential oils & natural chemicals offer promise, although their pharmacological characteristics & processes are unknown. Essential oils may improve or replace clinical standard treatments, which are becoming ineffective, inefficient, & more harmful. Future investigations could explore its specific mechanisms of action and potential clinical applications in humans.

8. SUMMARY

Based on the in vivo studies using a histamine-induced bronchospasm model and ovalbumin-induced sensitization in mice, four essential oils (*Mentha arvensis*, *Curcuma domestica*, *Origanum vulgare*, *Angelica glauca*, and *Cinnamomum aromaticum*) were selected for the preparation of a poly essential oil formulation with Tween 80 and water as an emulsion. Three dosage levels (low: 125 µL/kg, medium: 250 µL/kg, high: 500 µL/kg) were tested in the pilot study.

Results from the study indicated that ovalbumin sensitization increased overall inflammatory cell counts in the bronchoalveolar lavage (BAL) fluid. However, treatment with the poly essential oil formulation at all dosage levels led to a significant decrease in inflammatory cells compared to the ovalbumin group.

At the high dose of 500 µL/kg, there was a highly significant decrease in inflammatory cells compared to the standard dexamethasone group (2 mg/kg). Specifically, the Absolute Eosinophil Count and serum IgE levels were significantly reduced compared to the dexamethasone group, and the levels of monocytes, neutrophils, lymphocytes, and eosinophils in BAL fluid were also notably lower.

Similarly, at the medium dose of 250 µL/kg and low dose of 125 µL/kg, there were significant decreases in inflammatory cells compared to the ovalbumin group. Absolute Eosinophil Count and serum IgE levels decreased with decreasing dosage, and the levels of monocytes, neutrophils, lymphocytes, and eosinophils in BAL fluid showed a decreasing trend as well.

In summary, the poly essential oil formulation demonstrated significant anti-inflammatory effects in the ovalbumin-induced sensitization model, with higher doses leading to greater reductions in inflammatory cell counts and associated markers, suggesting its potential as a therapeutic option for asthma management.

9. REFERENCES

1. Akporiaye ET, Aminoff MJ, Benowitz NL, Berkowitz BA, Biaggioni I, Bikle DD, Katzung BG. Basic & Clinical Pharmacology. 11th ed. China: McGraw-Hill Companies; 2009.
2. Kumar V, Abbas AK, Fausto N. Robbins & Cotran Pathologic Basis of Disease. 7th ed. India: Elsevier Inc; 2004.
3. Sagar R, Mohapatra R, Kar B. Pharmacological evaluation of Calendula officinalis L. on bronchial asthma. IJNPND. 2014;4(2):95-103. doi:10.4103/2231-0738.129595.
4. Asthma Facts Sheet N°307 [Internet]. 2023 May [cited 2024 Dec 26]. Available from: http://www.who.int/mediacentre/factsheets/fs307/en/
5. Masoli M, Fabian D, Holt S, Beasley R; Global Initiative for Asthma (GINA) Program. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy. 2004 May;59(5):469-78.
6. Atri Savinenapu GV. Sharp raise in asthma patients. The Hindu. 2006 May 01; Karnataka. Sect. A:3.
7. Chowgule RV, Shetye VM, Parmar JR, Bhosale AM, Khandagale MR, Phalnitkar SV, Gupta PC. Prevalence of respiratory symptoms, bronchial hyperreactivity, & asthma in a megacity. Results of the European Community Respiratory Health Survey in Mumbai. Am J Respir Crit Care Med. 1998 Aug;158(2):547-54. doi: 10.1164/ajrccm.158.2.9708064.
8. Chest Foundation of India-Asthma Health Care Center. [Internet]. 2005 [cited 2014 Oct 17]. Available from: www.chestfoundationofindia.com/inIndia.htm

9. Bryan SA, Leckie MJ, Hansel TT, Barnes PJ. Novel therapy for asthma. Expert Opin Investig Drugs. 2000 Jan;9(1):25-42.
10. Angsten JM. Use of complementary & alternative medicine in the treatment of asthma. Adolesc Med. 2000 Oct;11(3):535-46.
11. Shirole RL, Shirole NL, Kshatriya AA, Kulkarni R, Saraf MN. Investigation into the mechanism of action of essential oil of Pistacia integerrima for its antiasthmatic activity. J Ethnopharmacol. 2014 May 14;153(3):541-51.
12. Inouye S, Takizawa T, Yamaguchi H. Antibacterial activity of essential oils & their major constituents against respiratory tract pathogens by gaseous contact. J Antimicrob Chemother. 2001 May;47(5):565-73.
13. Kapoor LD. CRC Handbook of Ayurvedic Medicinal Plants. India: CRC Press LLC; 1990.
14. Deshpande DJ. Handbook of Herbal Remedies. Jodhpur: Anmol Prints; 2008.
15. Harsh Mohan. Textbook of Pathology. 6th ed. New Delhi: JP Medical Publishers Ltd; 2010. p. 461-66.
16. Marieb E, Hoehn K. Human Anatomy & Physiology. 7th ed. Pearson Education Inc; 2011. p. 1273-88.
17. Tortora GJ, Derrickson B. Principles of Anatomy & Physiology. 13th ed. USA: John Wiley & Sons; 2012. p. 918-50.
18. Klocke RA, Siebens AA, Elliott DH, Beers MF, Burri PH, Heath DA, et al. Human respiratory system. Encyclopedia Britannica. 2024 May 27. Available from:https://www.britannica.com/science/human-respiratory-system. Accessed 2024 Jul 20.

19. Adams L, Alberg AJ, Albert RK, et al. Anatomy of the lungs. In: Murray & Nadel's Textbook of Respiratory Medicine. 5th ed. Philadelphia: Saunders; 2010. p. Chapter 1.
20. Mescher AL. Junqueira's Basic Histology: Text & Atlas. 12th ed. New York: McGraw-Hill; 2009.
21. Soler-Segovia D, de Homdedeu M, Sánchez-Díez S, Romero-Mesones C, Espejo D, Marain F, et al. Immunological effects of diesel particles in a murine model of healthy mice. Toxics. 2024 Aug;12(8):530.
22. Dennie C, et al. Canadian Society of Thoracic Radiology/Canadian Association of Radiologists consensus statement regarding chest imaging in suspected and confirmed COVID-19. Can Assoc Radiol J. 2020;71(4):470-81.
23. Ghaffari J, et al. Serum zinc level and children's asthma: A systematic and meta-analysis review article. Caspian J Intern Med. 2021;12(3):236.
24. Wang Z, Liang M, Li H, Cai L, Yang L. Rice protein exerts anti-inflammatory effect in growing and adult rats via suppressing NF-κB pathway. Int J Mol Sci. 2019 Dec 6;20(24):6164.
25. Kim HH, et al. Functions of flavonoids in three Korean native varieties of Artemisia species. J Biomed Transl Res. 2020;21(2):39-49.
26. Chu KB, Lee HA, Kang HJ, Moon EK, Quan FS. Preliminary Trichinella spiralis infection ameliorates subsequent RSV infection-induced inflammatory response. Cells. 2020;9(5):1314.
27. Altaii HA, Al-Tae FM. Investigation of serum total IgE and eosinophil levels in different allergic diseases together with the study of their correlations with various possible allergens in Mosul City. Immunol Invest. 2022;51(3):567-87.

28. Kawamatawong T. Phosphodiesterase-4 inhibitors for non-COPD respiratory diseases. Front Pharmacol. 2021 Aug 5;12:518345.
29. Boskabady M, et al. Thymoquinone ameliorates lung inflammation and pathological changes observed in lipopolysaccharide-induced lung injury. Evid Based Complement Alternat Med. 2021 Mar 30;2021:6681729.
30. Chopra H, et al. An insight into advances in developing nanotechnology based therapeutics, drug delivery, diagnostics and vaccines: Multidimensional applications in tuberculosis disease management. Pharmaceuticals. 2023;16(4):581.
31. Park SH, et al. Human understanding is expected of the physician: Proposing a model of disease development. Korean J Med. 2024 Apr 1;99(2):84-95.
32. Johnson JO. Autonomic nervous system: Physiology. In: Pharmacology and physiology for anesthesia. Elsevier; 2019. p. 270-81.
33. Collao N, et al. Anti-inflammatory effect of exercise mediated by Toll-like receptor regulation in innate immune cells–A review. Int Rev Immunol. 2020;39(2):39-52.
34. Sharun K, et al. Current therapeutic applications and pharmacokinetic modulations of ivermectin. Vet World. 2019 Aug;12(8):1204-1211.
35. Kraneveld AD, et al. Key role for mast cells in nonatopic asthma. J Immunol. 2002 Aug 15;169(4):2044-53.
36. Barar FSK. Essentials of pharmacotherapeutics. 3rd ed. Delhi: Schand & Company Ltd; 2003. p. 544-52.
37. Katzung BG. Basic clinical pharmacology. 10th ed. New York: McGraw-Hill Companies; 2007. p. 945-62.

38. Brunton LL, Lazo JS, Parkar KL, Undem BJ, Randal A. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New York: McGraw-Hill Medical Publishing Division; 2006. p. 464-85.

39. Sadhana K, Abbulu K. Holistic approach to management of asthma. IJRAP. 2010;1:367-83.

40. Vemula SK, Tirupti PR, Ponugoti RS, Gerrepally S. Asthma: Alternative management approaches. AJSPR. 2011;4:1-8.

41. Ladde S, Shalam. A short review on management of asthma through alternative therapies. IJLBPR. 2012;1.

42. Taur DJ, Patil RY. Some medicinal plants with antiasthmatic potential: A current status. Asian Pac J Trop Biomed. 2011 Oct;1(5):413-8.

43. Raynaud A, et al. Honey-induced macrophage stimulation: AP-1 & NF-κB activation & cytokine production are unrelated to LPS content of honey. Int Immunopharmacol. 2013 Nov;17(3):874-9.

44. Osaili TM, Dhanasekaran DK, Zeb F, Faris MAIE, Naja F, Radwan H, Ismail LC, Hasan H, Hashim M, Obaid RS. A Status Review on Health-Promoting Properties and Global Regulation of Essential Oils. Molecules. 2023 Feb;28(4):1809.

45. de Cassia da Silveira e Sa R, Andrade LN, de Sousa DP. A review on anti-inflammatory activity of monoterpenes. Molecules. 2013 Jan 18;18(1):1227-54.

46. Miguel MG. Antioxidant & anti-inflammatory activities of essential oils: a short review. Molecules. 2010 Dec 15;15(12):9252-87.

47. Qian Zhao, Liyun Zhu, Sunan Wang, Yongsheng Gao, Fei Jin. Molecular mechanism of the anti-inflammatory effects of plant essential oils: A systematic review. J of Ethnopharmacol. 2023 Jan 30:301:115829.

48. Vogel HG,Vogel WH. Drug discovery & evaluation pharmacological assay. Germany: springer-verlag berlin Heidelberg. 1997:514-15.

49. Evans RL, Nials AT, Knowles RG, Kidd EJ, Ford WR, Broadley KJ. A comparison of antiasthma drugs between acute & chronic ovalbumin-challenged guinea-pig models of asthma. Pulm Pharmacol Ther. 2012 Dec;25(6):453-64.

50. Shin IS, Jeon WY, Shin HK, Lee MY. Effects of montelukast on subepithelial/peribronchial fibrosis in a murine model of ovalbumin induced chronic asthma. Int Immunopharmacol. 2013 Nov;17(3):867-73.

51. Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, Kuebler WM; Acute Lung Injury in Animals Study Group. An official American Thoracic Society workshop report: features & measurements of experimental acute lung injury in animals. Am J Respir Cell Mol Biol. 2011 May;44(5):725-38.

52. Gibson Corely KN, Downey G. Principles for valid histopathologic scoring in research. Vet Pathol. 2013;1-9.

53. Porter DW, Hubbs AF, Mercer R, Robinson VA, Ramsey D, McLaurin J, Khan A, Battelli L, Brumbaugh K, Teass A, Castranova V. Progression of lung inflammation & damage in rats after cessation of silica inhalation. Toxicol Sci. 2004 Jun;79(2):370-80.

54. Olmez D, Babayigit A, Erbil G, Karaman O, Bagriyanik A, Yilmaz O, Uzuner N. Histopathologic changes in two mouse models of asthma. J Investig Allergol Clin Immunol. 2009;19(2):132-8.

55. VK Bisht, LS Kandari, JS Negi, AK Bhandari, RC Sundriyal, Traditional use of medicinal plants in district Chamoli Uttarakhand India. J Med Plants Res. 2013;7(15):918-29.

56. Sowndhararajan K, Deepa P, Kim M, Park SJ, Kim S. A Review of the Composition of the Essential Oils & Biological Activities of Angelica Species. Sci Pharm. 2017 Sep 20;85(3):33.

57. Sarker SD, Nahar L. Natural medicine: the genus Angelica. Curr Med Chem. 2004 Jun;11(11):1479-500.

58. Puri A, Srivastava P, Pandey P, Yadav RS, Bhatt PC. Scopolamine induced behavioural & biochemical modifications & protective effect of *Celastrus paniculatous* & *Angelica glauca* in rats. IJNPND. 2014;4(3):158-69.

59. Cavaleiro C, Salgueiro L, Goncalves MJ, Hrimpeng K, Pinto J, Pinto E. Antifungal activity of the essential oil of Angelica major against Candida, Cryptococcus, Aspergillus & dermatophyte species. J Nat Med. 2015 Apr;69(2):241-48.

60. Muhammad Irshad, Shaid Aziz, Habib-ur-Rehman, Hidayat Hussain, GC-MS Analysis & Antifungal Activity of Essential oils of *Angelica glauca, Plectranthus rugosus,* & *Valeriana wallichii*. J Essent Oil Bear Plants. 2012:15(1):15-21.

61. Arambewela L, Wijesinghe A. Sri Lankan Medicinal Plant Monographs & Analysis *Alpinia galanga*. National Science Foundation.2006;1-26.

62. Rizki Damayanti, Irmanida Batubara & Irma Herawati Suparto, Essential Oil of Red Galangal (*Alpinia Galanga* (L) Willd) Rhizomes as Slimming Aromatherapy. Int J Pharm Bio Sci. 2015;6(1):283-89.

63. Afzal Unnisa & Thahera D. Parveen. Anti-inflammatory & acute toxicity studies of the extracts from the rhizomes of *Alpinia galanga* Willd. Der Pharmacia Sinica, 2011;2(2):361-67.

64. Akhtar MS, Khan MA, Malik MT. Hypoglycaemic activity of *Alpinia galanga* rhizome & its extracts in rabbits. Fitoterapia. 2002 Dec;73(7-8):623-28.

65. Saha S, Banerjee S. Central nervous system stimulant actions of *Alpinia galanga* (L.) rhizome: a preliminary study. Indian J Exp Biol. 2013 Oct;51(10):828-32.

66. Jirovetz L, Buchbauer G, Shafi MP, Leela NK. Analysis of the essential oils of the leaves, stems, rhizomes & roots of the medicinal plant *Alpinia galanga* from southern India. Acta Pharm. 2003 Jun;53(2):73-81.

67. Eder J. Lenardao, Lucielli S, Raquel G. Jacob, Francine N. Victoriaa & Debora M. Antinociceptive Effect of Essential Oils & Their Constituents: an Update Review. J Braz Chem Soc. 2016 Mar;27(3):435-74.

68. Matsuda H, Morikawa T, Managi H, Yoshikawa M. Antiallergic principles from *Alpinia galanga:* structural requirements of phenylpropanoids for inhibition of degranulation & release of TNF-alpha & IL-4 in RBL-2H3 cells. Bioorg Med Chem Lett. 2003 Oct 6;13(19):3197-202.

69. de Cassia da Silveira E Sa R, Andrade LN, Dos Reis Barreto de Oliveira R, de Sousa DP. A review on anti-inflammatory activity of phenylpropanoids found in essential oils. Molecules. 2014 Jan 27;19(2):1459-80.

70. Chen W, Vermaak I, Viljoen A. Camphor-a fumigant during the Black Death & a coveted fragrant wood in ancient Egypt & Babylon-a review. Molecules. 2013 May 10;18(5):5434-54.

71. Rafie Hamidpour, Soheila Hamidpour, Mohsen Hamidpour, Mina Shahlari. Camphor (*Cinnamomum camphora*), a traditional remedy with the history of treating several diseases. IJCRI. 2013;4(2):86–9.

72. Akram Jamshidzadeha, Javad Sajedianfardb, Ali Akbar Nekooeianc, Fateme Tavakolia, Gholam-Hossein Omranid. Effects of Camphor oil on Sexual Behaviors in Male Rats. Iran J Pharm Sci. 2006;2(4):209-14.

73. Agarwal R, Pant A, Prakash O. Chemical Composition & Biological Activities of Essential Oils of *Cinnamomum Tamala*, *Cinnamomum Zeylenicum* & *Cinnamomum Camphora* Growing in Uttarakhand. Chemistry of Phytopotentials: Health, Energy & Environmental Perspectives. 2012:87-92.

74. Guo S, Geng Z, Zhang W, Liang J, Wang C, Deng Z, Du S. The Chemical Composition of Essential Oils from *Cinnamomum camphora* & Their Insecticidal Activity against the Stored Product Pests. Int J Mol Sci. 2016 Nov 4;17(11):1836.

75. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. In vitro antibacterial activity of some plant essential oils. BMC Complement Altern Med. 2006 Nov 30;6:39.

76. Silva-Filho SE, et al. Effect of Camphor on the Behavior of Leukocytes In vitro & In vivo in Acute Inflammatory Response. Trop J Pharm Res. 2014 Dec;13(12):2031-37.

77. Hiramatsu Y, Miyazaki Y. Effect of volatile matter from wood chips on the activity of house dust mites & on the sensory evaluation of humans. J Wood Sci. 2001;47:13-7.

78. Lee HJ, Hyun EA, Yoon WJ, Kim BH, Rhee MH, Kang HK, Cho JY, Yoo ES. In vitro anti-inflammatory & anti-oxidative effects of *Cinnamomum camphora* extracts. J Ethnopharmacol. 2006 Jan 16;103(2):208-16.

79. Ram A, Das M, Ghosh B. Curcumin attenuates allergen-induced airway hyperresponsiveness in sensitized guinea pigs. Biol Pharm Bull. 2003 Jul;26(7):1021-4.

80. Ma C, Ma Z, Fu Q, Ma S. Curcumin attenuates allergic airway inflammation by regulation of CD4+CD25+ regulatory T cells (Tregs)/Th17 balance in ovalbumin-sensitized mice. Fitoterapia. 2013 Jun;87:57-64.

81. Sukandar EY, Sudjana P, Adnyana KI, Setiawan AS, Yuniarni U. Recent study of turmeric in combination with garlic as Antidiabetic agent. Procedia Chem. 2014;13:44-56.

82. Kuptniratsaikul V, Thanakhumtorn S, Chinswangwatanakul P, Wattanamongkonsil L, Thamlikitkul V. Efficacy & safety of *Curcuma domestica* extracts in patients with knee osteoarthritis. J Altern Complement Med. 2009 Aug;15(8):891-7.

83. Malik F, Hussain S, Sadiq A, Ghazala P, Wajid A, Shafat S, Channa RA, Mahmood R, Riaz H, Ismail M, Raja FY. Phytochemical analysis, anti-allergic & anti-inflammatory activity of *Mentha arvensis* in animals. Afr J Pharm Pharmacol. 2012 Mar;6(9):613-19.

84. Duarte MC, Figueira GM, Sartoratto A, Rehder VL, Delarmelina C. Anti-Candida activity of Brazilian medicinal plants. J Ethnopharmacol. 2005 Feb 28;97(2):305-11.

85. Wannissorn B, Jarikasem S, Siriwangchai T, Thubthimthed S. Antibacterial properties of essential oils from Thai medicinal plants. Fitoterapia. 2005 Mar;76(2):233-6.

86. Kishore N, Mishra AK, Chansouria JP. Fungitoxicity of essential oils against dermatophytes. Mycoses. 1993 May-Jun;36(5-6):211-5.

87. Jagetia GC, Baliga MS. Influence of the leaf extract of *Mentha arvensis* Linn. (mint) on the survival of mice exposed to different doses of gamma radiation. Strahlenther Onkol. 2002 Feb;178(2):91-8.

88. Kumar P, Mishra S, Malik A, Satya S. Insecticidal properties of Mentha species: A review. Ind Crops Prod. 2011 July;34(1):802-17.

89. Londonkar RL, Poddar PV. Studies on activity of various extracts of *Mentha arvensis* Linn against drug-induced gastric ulcer in mammals. World J Gastrointest Oncol. 2009 Oct 15;1(1):82-8.

90. Sharma N, Jocob D. Antifertility investigation & toxicological screening of the petroleum ether extract of the leaves of *Mentha arvensis* L. in male albino mice. J Ethnopharmacol. 2001 Apr;75(1):5-12.

91. Sharma PK, Lal B. Ethnobotanical notes on some medicinal & aromatic plants of Himachal Pradesh. Indian J Tradit Knowl. 2005;4(4):424-8.

92. Lambert RJ, Skandamis PN, Coote PJ, Nychas GJ. A study of the minimum inhibitory concentration & mode of action of oregano essential oil, thymol & carvacrol. J Appl Microbiol. 2001 Sep;91(3):453-62.

93. Haberbeck LU, Riehl CAS, Salomao BCM, Aragao GMF. Bacillus coagulans spore inactivation through the application of oregano essential oil & heat. LWT Food Sci Technol. 2012 Apr;46(1):267-73.

94. Stohs SJ, Hartman MJ. Review of the Safety & Efficacy of *Moringa oleifera*. Phytother Res. 2015 Jun;29(6):796-804.

95. Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. *Moringa oleifera* Seeds & Oil: Characteristics & Uses for Human Health. Int J Mol Sci. 2016 Dec;17(12):2141.

96. Mahajan SG, Banerjee A, Chauhan BF, Padh H, Nivsarkar M, Mehta AA. Inhibitory effect of n-butanol fraction of Moringa oleifera Lam. seeds on ovalbumin-induced airway inflammation in a guinea pig model of asthma. Int J Toxicol. 2009 Nov-Dec;28(6):519-27.

97. Jaiswal D, Rai PK, Mehta S, Chatterji S, Shukla S, Rai DK, Sharma G, Sharma B, Khair S, Watal G. Role of Moringa oleifera in regulation of diabetes-induced oxidative stress. Asian Pac J Trop Med. 2013 Jun;6(6):426-32.

98. Hannan MA, Kang JY, Mohibbullah M, Hong YK, Lee H, Choi JS, Choi IS, Moon IS. Moringa oleifera with promising neuronal survival & neurite outgrowth promoting potentials. J Ethnopharmacol. 2014 Feb;152(1):142-50.

99. Kala CP, Farooquee NA, Dhar U. Traditional Uses & Conservation of Timur (*Zanthoxylum armatum* DC.) through Social Institutions in Uttaranchal Himalaya India. Conserv Soc. 2005;3(1):224-30.

100. Mehta DK, Das R, Bhandari A. In-vitro anthelmentic activity of seeds of *Zanthoxylum armatum* against pheretima posthuma. IJGP. 2014 Oct 6:26-8.

101. Guo T, Deng YX, Xie H, Yao CY, Cai CC, Pan SL, Wang YL. Antinociceptive & anti-inflammatory activities of ethyl acetate fraction from *Zanthoxylum armatum* in mice. Fitoterapia. 2011 Apr;82(3):347-51.

102. Singh TP, Singh OM. Phytochemical & pharmacological profile of *Zanthoxylum armatum* - An overview. IJNPR. 2011;2:275-85.

103. Gilani SN, Khan AU, Gilani AH. Pharmacological basis for the medicinal use of *Zanthoxylum armatum* in gut, airways & cardiovascular disorders. Phytother Res. 2010 Apr;24(4):553-8.

104. Muhammad I, Naveed M, Barkatullah, Haroon K, Jahan F, Ashraf N. Antinociceptive & anticonvulsant activities of essential oils of *Zanthoxylum armatum.* Phytopharmacology. 2012;3(1):191-98.

105. Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological & toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. Food Chem Toxicol. 2008 Feb;46(2):409-20.

106. Tewtrakul S, Subhadhirasakul S. Anti-allergic activity of some selected plants in the Zingiberaceae family. J Ethnopharmacol. 2007 Feb;109(3):535-38.

107. Ahui ML, Champy P, Ramadan A, Pham Van L, Araujo L, Brou André K, Diem S, Damotte D, Kati-Coulibaly S, Offoumou MA, Dy M, Thieblemont N, Herbelin A. Ginger prevents Th2-mediated immune responses in a mouse model of airway inflammation. Int Immunopharmacol. 2008 Dec;8(12):1626-32.

108. Gomar A, Hosseini A, Mirazi N. Memory enhancement by administration of zinger extract on morphine-induced memory impairment in male rats. J Acute Dis. 2014 Sep;20:212-17.

109. Zafar I, Qazi KN, Khan MN, Akhtar MS, Faisal NW. In Vitro Anthelmintic Activity of *Allium sativum, Zingiber officinale, Curcurbita mexicana* & *Ficus religiosa*. Int J Agri Biol. 2001;3(4):454-57.

110. Goel R, Raj Kumar R, Sairam K. Anti-ulcer drugs from indigenous sources with emphasis on *Musa sapientum, tamra bhasma, Asparagus racemosus & Zingiber officinale*. Indian J Pharmacol. 2002;34:100-10.

111. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils & other plant extracts. J Appl Microbiol. 1999 Jun;86(6):985-90.

112. Soyingbe SO. Anti-asthmatic & anti-cough activities of the essential oil of *Eucalyptus Grandis* W. Hill Ex Maiden. 2015.

113. Wright CE, Laude EA, Grattan TJ, Morice AH. Capsaicin & neurokinin A-induced bronchoconstriction in the anaesthetised guinea-pig: evidence for a direct action of menthol on isolated bronchial smooth muscle. Br J Pharmacol. 1997 Aug;121(8):1645-50.

114. Juergens UR, Engelen T, Racke K, Stober M, Gillissen A, Vetter H. Inhibitory activity of 1,8-cineol (eucalyptol) on cytokine production in cultured human lymphocytes & monocytes. Pulm Pharmacol Ther. 2004;17(5):281-7.

115. Juergens UR, Stober M, Schmidt-Schilling L, Kleuver T, Vetter H. Antiinflammatory effects of eucalyptol (1.8-cineole) in bronchial asthma: inhibition of arachidonic acid metabolism in human blood monocytes ex vivo. Eur J Med Res. 1998 Sep;3(9):407-12.

116. Boskabady MH, Jandaghi P. Relaxant effects of carvacrol on guinea pig tracheal chains & its possible mechanisms. Pharmazie. 2003 Sep;58(9):661-3.

117. Morice AH, Marshall AE, Higgins KS, Grattan TJ. Effect of inhaled menthol on citric acid induced cough in normal subjects. Thorax. 1994 Oct;49(10):1024-6.

118. Boskabady MH, Ramazani M, Tabei T. Relaxant effects of different fractions of essential oil from *Carum copticum* on guinea pig tracheal chains. Phytother Res. 2003 Dec;17(10):1145-9.

119. Laude EA, Morice AH, Grattan TJ. The antitussive effects of menthol, camphor & cineole in conscious guinea-pigs. Pulm Pharmacol. 1994 Jun;7(3):179-84.

120. Joshi RK, Pai SR. Reinvestigation of carvotanacetone after 100 years along with minor terpenoid constituents of *Blumea malcolmii* Hook. F. essential oil. Nat Prod Res. 2016 Oct;30(20):2368-71.

121. Joshi RK. Chemical composition of the essential oil of *Baccharoides lilacina* from India. Nat Prod Commun. 2013 Mar;8(3):401-2.

122. Joshi RK. Essential oil of flowers of *Anaphalis contorta*, an aromatic & medicinal plant from India. Nat Prod Commun. 2013 Feb;8(2):225-6.

123. Joshi RK. GC/MS analysis of the essential oil of Leucas indica from India. Nat Prod Commun. 2014 Nov;9(11):1607-8.

124. Gardner Z, Mugufin M. American Herbal Products Association Botanical safety Handbook. 2nd ed. U.S: CRC Press; 2013. p. 251.

125. Lisbachin M, Gardner Z, Mugufin M. American Herbal Products Association Botanical safety Handbook. 2nd ed. U.S: CRC Press; 2013. p. 110.

126. Aggarwal ML, Chacko KM, Kuruvilla BT. Systematic & comprehensive investigation of the toxicity of curcuminoid essential oil complex: A bioavailable turmeric formulation. Mol Med Rep. 2016 Jan;13(1):592-604.

127. Lis-Balchin M. Aromatherapy Science: A Guide for Healthcare Professionals. Pharmaceutical Press; 2006.

128. Kulkarni SK. Handbook of Experimental Pharmacology. 1st ed. MK Jain Vallabh Prakashan, Delhi; 1987. p. 23.

129. Ghosh MN. Fundamental of Experimental Pharmacology. 5th ed. Hilton & Company, Kolkata; 2011. p. 25.

130. India. Ministry of Health & Family welfare. The Indian Pharmacopoeia commission. Indian Pharmacopoeia. Buffer Solutions. 2007;1:241-45.

131. Priyashree S, Jha S, Pattanayak SP. Bronchodilatory & mast cell stabilising activity of *Cressa cretica* L. evaluation through in vivo & in vitro experimental models. Asian Pac J Trop Med. 2012 Mar;5(3):180-6.

132. Rathore C, Dutt KR, Sahu S, Deb L, Gandhi RA. Antiasthmatic activity of the methanolic extract of *Physalis angulata* Linn. J Med Plants Res. 2011;5:5351-55.

133. Sagar R, Sahoo HB, Kar B, Mishra NK, Mohapatra R, Sarangi SP. Pharmacological evaluation of *Calendula officinalis* L. on bronchial asthma in various experimental animals. Int J Nutr Pharmacol Neurol Dis. 2014;4:95-103.

134. Anticoagulant Sodium Citrate: Vaccines Blood & Biologics. 2014;4-6. Available from: http://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/NewDrugApplicationsNDAs/ucm082845.htm

135. Pal GK, Pravati P. Text book of Practical Physiology. 3rd ed. Hyderabad: Universities Pvt Ltd; 2011. p. 24-84.

136. Immunoperoxidase Assay for Determination of IgE in Rat Samples. Available from: geneway biotech inc proien & antibody solutions (cited 2013 Nov 1).

137. Jin H, Luo Q, Zheng Y, Nurahmat M, Wu J, Li B, Lv Y, Wang G, Duan X, Dong J. CD4+CD25+Foxp3+ T cells contribute to the antiasthmatic effects of *Astragalus membranaceus* extract in a rat model of asthma. Int Immunopharmacol. 2013 Jan;15(1):42-9.

138. Ah F, Ka J, Rose J, Zeller R. Hematoxylin & eosin staining of tissue & cell sections. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21356829.

139. Anastasiou C, Buchbauer G. Essential Oils as Immunomodulators: Some Examples. Open Chem. 2017;15(1):352-370.

140. Saha S, Banerjee S. Central nervous system stimulant actions of *Alpinia galanga* (L.) rhizome: a preliminary study. Indian J Exp Biol. 2013 Oct;51(10):828-32.

141. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation & remodeling. Am J Respir Crit Care Med. 2000 May;161(5):1720-45. doi: 10.1164/ajrccm.161.5.9903102.

142. Wu J, Zhang Y, Liu Q. All-trans retinoic acid attenuates airway inflammation by inhibiting Th2 & Th17 response in experimental allergic asthma. BMC Immunol. 2013;14:28.

143. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels & skin-test reactivity to allergens. N Engl J Med. 1989 Feb;320(5):271-7.

144. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) & the development of asthma in childhood. A prospective study. N Engl J Med. 1990 Aug;323(8):502-7.

145. Platts-Mills TA. The role of immunoglobulin E in allergy & asthma. Am J Respir Crit Care Med. 2001 Oct 15;164(8 Pt 2).

146. Bloemen K, Verstraelen S, Van Den Heuvel R, Witters H, Nelissen I, Schoeters G. The allergic cascade: review of the most important molecules in the asthmatic lung. Immunol Lett. 2007 Oct;113(1):6-18.

147. Gibson PG, Simpson JL, Saltos N. Heterogeneity of airway inflammation in persistent asthma: evidence of neutrophilic inflammation & increased sputum interleukin-8. Chest. 2001 May;119(5):1329-36.

148. Gilani AH, Shah AJ, Zubair A, Khalid S, Kiani J, Ahmed A, Rasheed M, Ahmad VU. Chemical composition & mechanisms underlying the spasmolytic & bronchodilatory properties of the essential oil of *Nepeta cataria* L. J Ethnopharmacol. 2009 Jan;121(3):405-11.

149. Wang ZW, Li RK, Lin XY, Ren Y, Liu XF, Cheng XL, Wang RQ. Effect of Volatile Oil of Radix *Angelicae Sinensis* on experimental asthma in rats. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2015 Mar;31(2):107-10.

150. Gong JH, Zhang YL, He JL, Zheng XK, Feng WS, Wang XL, Kuang HX, Li CG, Cao YG. Extractions of oil from *Descurainia sophia* seed using supercritical CO2, chemical compositions by GC-MS & evaluation of the anti-tussive, expectorant & anti-asthmatic activities. Molecules. 2015 Jul;20(7):13296-312.

151. Modupe O, Wesley O, Edith O, Elizabeth O. Analysis of the essential oil from the dried leaves of *Euphorbia Hirta* Linn. A potential medication for asthma. Afr J Biotechnol. 2009;8(24):7042-50.

152. de Sousa AA, Soares PM, de Almeida AN, Maia AR, de Souza EP, Assreuy AM. Antispasmodic effect of *Mentha piperita* essential oil on tracheal smooth muscle of rats. J Ethnopharmacol. 2010 Jul;130(2):433-6.

153. Farooq A, Shaik R, Bilal A. Evaluation of Antiasthmatic effect of extravirgin oil against milk induced Leukocytosis & Eosinophilia. Adv Biores. 2015;6(1):15-8.

154. Shirole RL, Shirole NL, Saraf MN. In vitro relaxant & spasmolytic effects of essential oil of *Pistacia integerrima* Stewart ex Brandis Galls. J Ethnopharmacol. 2015 Jun;168:61-5.

155. de Cassia da Silveira e Sa R, Andrade LN, de Sousa DP. A review on anti-inflammatory activity of monoterpenes. Molecules. 2013 Jan;18(1):1227-54.

156. Patil SS, Burande MD. Antiasthmatic Evaluation of Polyherbal Formulations in Laboratory Animals. Res J Pharm Biol Chem Sci. 2011;2:1003.

157. Saleem U, Usman M, Anwar F, Akhtar MF, Ahmad B. Pharmacological & toxicological evaluation of two anti-asthmatic polyherbal formulations. An Acad Bras Cienc. 2020 Oct;92(3).

158. Parmar S, Gangwal A, Sheth N. Evaluation of antiasthmatic activity of a polyherbal formulation containing four plant extracts. J Chem Pharm Res. 2010;2(1):40-44.

ANNEXURES

ANNEXURE I

ETHICAL CLEARANCE LETTER

KLE University's
College of Pharmacy
(Recognized by PCI, AICTE & Accredited by NBA & 'A' grade By NAAC)
A constituent Unit of KLE Academy of Higher Education and Research
[Under section 3UGC Act, 1956 vide Govt. of India Notification No. F.9-19/2000-U.3 (A)]
Nehru Nagar, Belagavi – 590 010, Karnataka, India
Phone: 0831-2471399 **Fax:** 0831-2472387 **Web:** http://www.klepharm.edu **E-mail:** principal@klepharm.edu

Ref: No. KLEUCOP/_____ Date: 10/10/15

CERTIFICATE

This is to certify that the research project, " **Evaluation of essential oils for antiasthmatic activity using animals",** Submitted by **Ms. Shilpa Sharma** has been approved in the Institutional Animal Ethics Committee meeting held on 10th October 2015, resolution No.KLECOP/IAEC/Res.22-10/10/2015 and was permitted to use 48 Guinea pigs and 54 female mice Rats/ Mice/ Rabbits/Guinea pig.

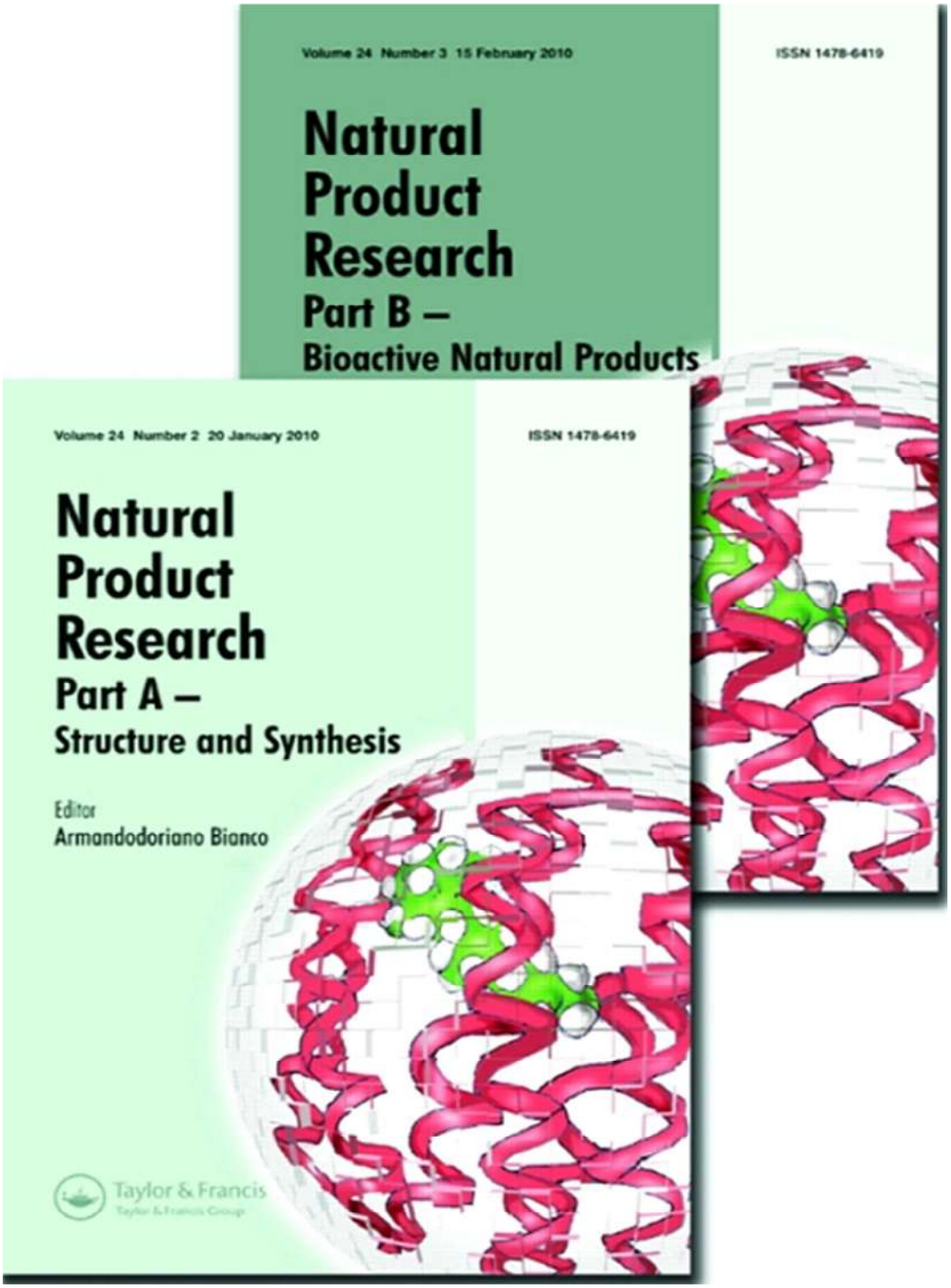
You are hereby informed to strictly adhere to the protocol submitted for approval. Further you are required to keep the account of animals used for the project in specified Performa, **Form D.**

MEMBER SECRETARY
Institutional Animal Ethical Committee,
KLES's College of Pharmacy,
BELGAUM - 590010

10/10/2015
CPCSEA Nominee
Institutional Animal Ethics Committee
KLES's College of Pharmacy,
BELGAUM.

ANNEXURE II

PUBLICATIONS



Taylor & Francis

Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

Mentha arvensis essential oil suppressed airway changes induced by histamine and ovalbumin in experimental animals

Shilpa Sharma, Vijaykumar P. Rasal, Paragouda A. Patil & Rajesh K. Joshi

To cite this article: Shilpa Sharma, Vijaykumar P. Rasal, Paragouda A. Patil & Rajesh K. Joshi (2018) Mentha arvensis essential oil suppressed airway changes induced by histamine and ovalbumin in experimental animals, Natural Product Research, 32:4, 468-472, DOI: 10.1080/14786419.2017.1311891

To link to this article: https://doi.org/10.1080/14786419.2017.1311891

NATURAL PRODUCT RESEARCH, 2018
VOL. 32, NO. 4, 468–472
https://doi.org/10.1080/14786419.2017.1311891

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Check for updates

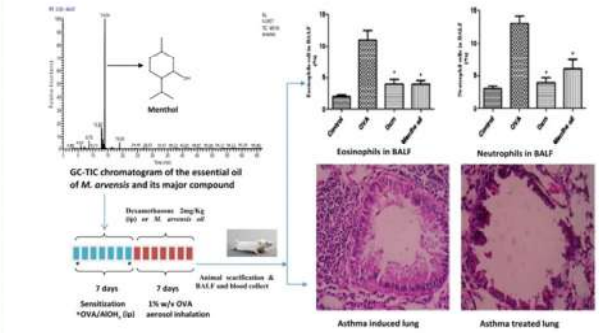
Mentha arvensis essential oil suppressed airway changes induced by histamine and ovalbumin in experimental animals

Shilpa Sharma[a], Vijaykumar P. Rasal[a], Paragouda A. Patil[b] and Rajesh K. Joshi[c]

[a]Department of Pharmacology, KLE University, College of Pharmacy, Belagavi, India; [b]Department of Pharmacology, USM KLE International Medical Programme, Belagavi, India; [c]Department of Phytochemistry, Regional Medical Research Centre, Indian Council of Medical Research, Belagavi, India

ABSTRACT

The present investigation aimed to evaluate the activity of the essential oil of *Mentha arvensis* L. on exogenously induced bronchoconstriction in experimental animals. The anti-asthmatic effect of *M. arvensis* essential oil (MAEO) was studied using histamine aerosol-induced bronchoconstriction in guinea pigs and ovalbumin (OVA) sensitised albino mice. Treatment with *M. arvensis* oil significantly ($p < 0.001$) increased the time of preconvulsive dyspnoea in histamine-induced guinea pigs. Oral treatment of MAEO significantly ($p < 0.001$) decreased absolute eosinophil count, serum level of IgE and the number of eosinophils, neutrophils in BALF. Histopathological examination of lungs showed that essential oil rescinded bronchial asthma. The present investigation provides evidence that MAEO relaxes bronchial smooth muscles and suppressed immunological response to OVA.



ARTICLE HISTORY
Received 7 January 2017
Accepted 15 March 2017

KEYWORDS
Mentha arvensis L.; immunosuppression; bronchorelaxation; essential oil composition; GC-MS

1. Introduction

Bronchial asthma, a common chronic inflammatory airway disease affects as many as 334 million people of all ages worldwide. It is a substantial health care burden and affects quality of life, due to its physical, psychological and social effects (GAN 2014). Currently available

CONTACT Rajesh K. Joshi joshirk_natprod@yahoo.com
Supplemental data for this article can be accessed at http://dx.doi.org/10.1080/14786419.2017.1311891.

treatments for asthma such as beta-2 agonist, anticholinergics, methylxanthines, mast cell stabilizers, leukotriene antagonist, glucocorticoids, IgE antibody like omalizumab etc. on long term basis, are associated with several adverse effects like muscle tremors, restlessness, hyperglycaemia, tachycardia, flushing, convulsions, mood changes, adrenal crisis etc. (Katzung et al. 2009). Essential oils of plants have been traditionally used for respiratory tract infections, and are used nowadays for cold (Inouye et al. 2001).

In *Ayurvedic* medicine *M. arvensis* is advocated for the treatment of asthma, nasal catarrh, bronchitis, cough, liver and spleen disease (Kapoor 1990). Moreover, the essential oil constituents viz., l-menthol, menthone and 1,8-cineole (chewing gum constituents) exhibited anti-allergic effects (Arakawa & Osawa 2000). Marked variation (30.0–93.7%) of menthol content and other chemical constituents in the essential oil of *M. arvensis* from different region has been reported (Pino et al. 1996; Alankar 2009; Joshi 2014a). The aim of present study was to evaluate the anti-asthmatic activity of commercially available essential oil of MAEO using *in vivo* models.

2. Results and discussion

2.1. Analysis of the essential oil

The identified compounds are listed in Table S1. The major constituent of the MAEO was menthol (72.6%). The other minor constituents were menthone (8.5%), limonene (3.3%) and menthyl acetate (2.4%).

2.2. Effect of MAEO on histamine-induced bronchoconstriction in guinea pigs

The MAEO significantly ($p < 0.001$) and dose dependently increased the latency of preconvulsive time (PCT) in histamine-challenged animals with the values of 37.77 ± 3.52% and 63.00 ± 0.73% at the dose of 200 and 400 µl/kg, respectively. The increase of PCT at a dose of 400 µL/kg was comparable to that of dexamethasone (Table S2).

2.3. Effect of the MAEO on OVA-sensitised mice

2.3.1. Absolute eosinophil count in blood and serum IgE level in OVA-sensitised mice

MAEO treated group at the dose of 200 µl/kg body weight significantly ($p < 0.001$) decreased inflammatory cells in blood (Figure 1A). On the day 15, after final OVA sensitisation, significantly increased serum level of IgE was observed in untreated mice. The treatment with MAEO significantly ($p < 0.001$) decreased the level of serum IgE at the dose of 200 µl/kg (Figure 1B).

2.3.2. Inflammatory cells from BALF

In animals, sensitised with OVA and treated with MAEO (200 µL/kg), the inflammatory cells were significantly ($p < 0.001$) decreased as compared to that of untreated group (Figure 1C, D).

2.4. Histopathological changes in lung tissues

Lung sections of mice exposed to OVA stained with haematoxylin and eosin showed significant inflammatory changes in peribronchial and perivascular areas, increased bronchial

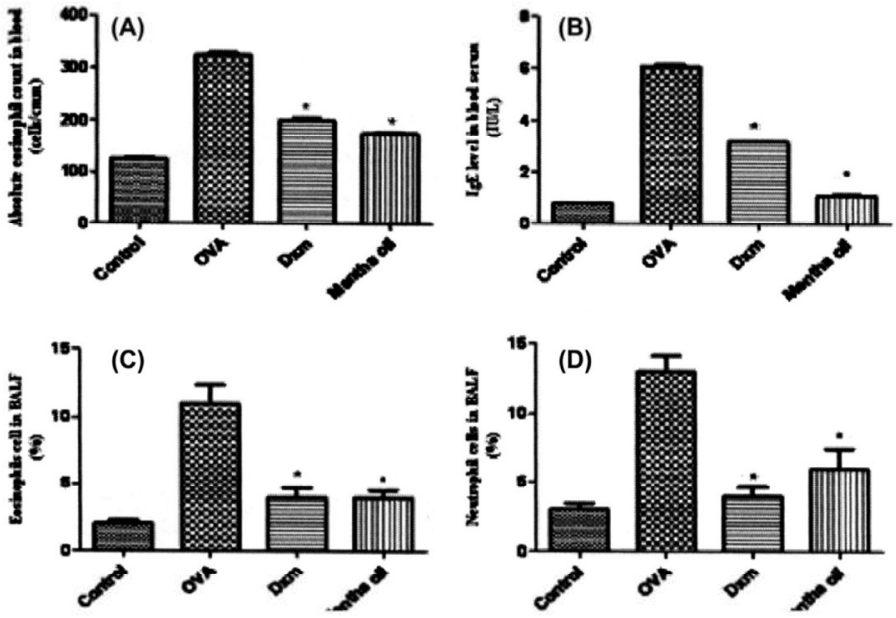


Figure 1. Effect of MAEO on: (A) blood eosinophils count in mice; (B) serum IgE in mice; (C) BALF eosinophils in mice; (D) BALF neutrophils in mice. Values are expressed as mean ± standard error of the mean, where $n = 6$ in each group, $^*p < 0.001$ compared with ovalbumin treated group.

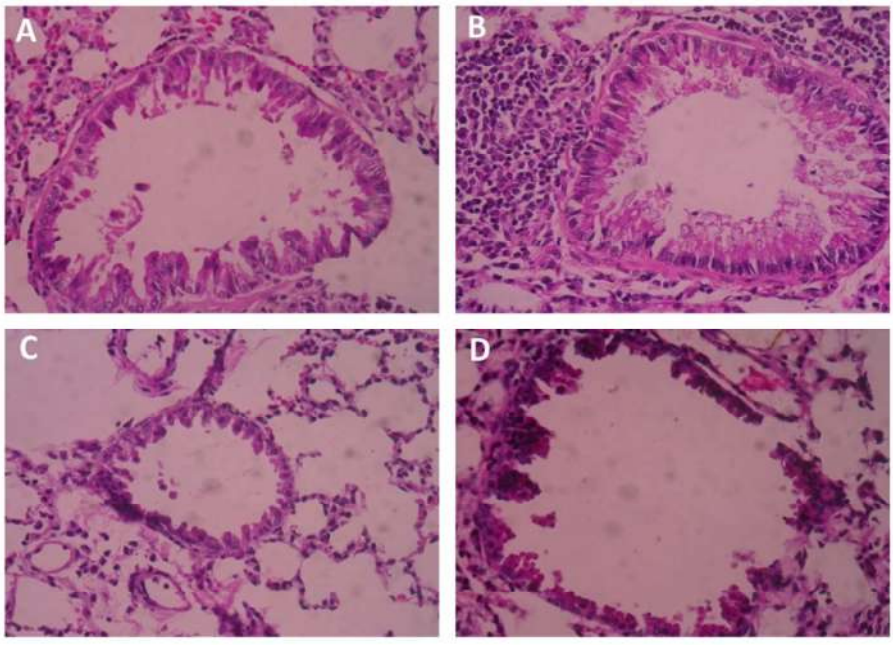


Figure 2. Microphotography of mice lung section stained with haematoxylin-eosin (40×). Normal control (A); Ovalbumin treated (B); Dexamethasone treated(C); MAEO (200 µL/kg) treated (D).

muscles thickening and epithelial hyperplasia (Figure 2B). The histological changes induced by OVA were markedly suppressed by treatment with MAEO (200 µL/kg) (Figure 2D) and was almost comparable to normal (Figure 2A) as well as dexamethasone (Figure 2C) treated groups.

Asthma is an allergic respiratory disease commonly characterised by increased airway reactivity to different spasmogens. Bronchial asthma is triggered by the release of inflammatory mediators like histamine, acetylcholine, leukotrienes, prostaglandins, or specific exposure to allergens, leading to acute bronchoconstriction (Nelson 2003). Bronchodilator effect of MAEO was evident by significantly increased latency for PCT in guinea pigs. Eosinophil suppressant effect of MAEO suggests its anti-allergic activity and its efficacy in ameliorating allergen-induced asthma. IgE is invariably associated with the pathogenesis of asthma and its inhibition by MAEO indicates its efficacy in asthma and other allergic condition (Platts-Mills 2001). Moreover, eosinophil granule derived basic proteins have been implicated with asthma (Bloemen et al. 2007). Suppression of neutrophils in BALF and inflammatory changes in lung tissue indicate anti-inflammatory activity of MAEO. Thus, anti-allergic, anti-inflammatory, immunosuppressant and bronchodilator activity of MAEO together appear to contribute for its efficacy in bronchial asthma. The major compounds of MAEO were menthol, menthone and 1,8-cineole, but which of its constituents is/are responsible for anti-allergic activity need to be explored.

In conclusion, the essential oil used in the present study contains more than 70% menthol and oxygenated monoterpenes along with other chemicals. It is difficult to predict the contribution of chemicals other than menthol for the observed anti-asthmatic effect of MAEO. The present investigation provides evidence that MAEO relaxes bronchial smooth muscles and suppresses immunological response to OVA. The traditional use of MAEO for bronchial asthma could be attributed to its bronchodilator and immunosuppressant properties as observed in present study.

Acknowledgements

The authors are grateful to Director-in-Charge Regional Medical Research Centre (ICMR), Belagavi for encouraging the study. The authors are indebted to Kancor Pvt. Ltd, Mumbai, India for providing essential oil of *M. arvensis*.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Alankar S. 2009. A review on peppermint oil. Asian J Pharm Clin Res. 2:27–33.

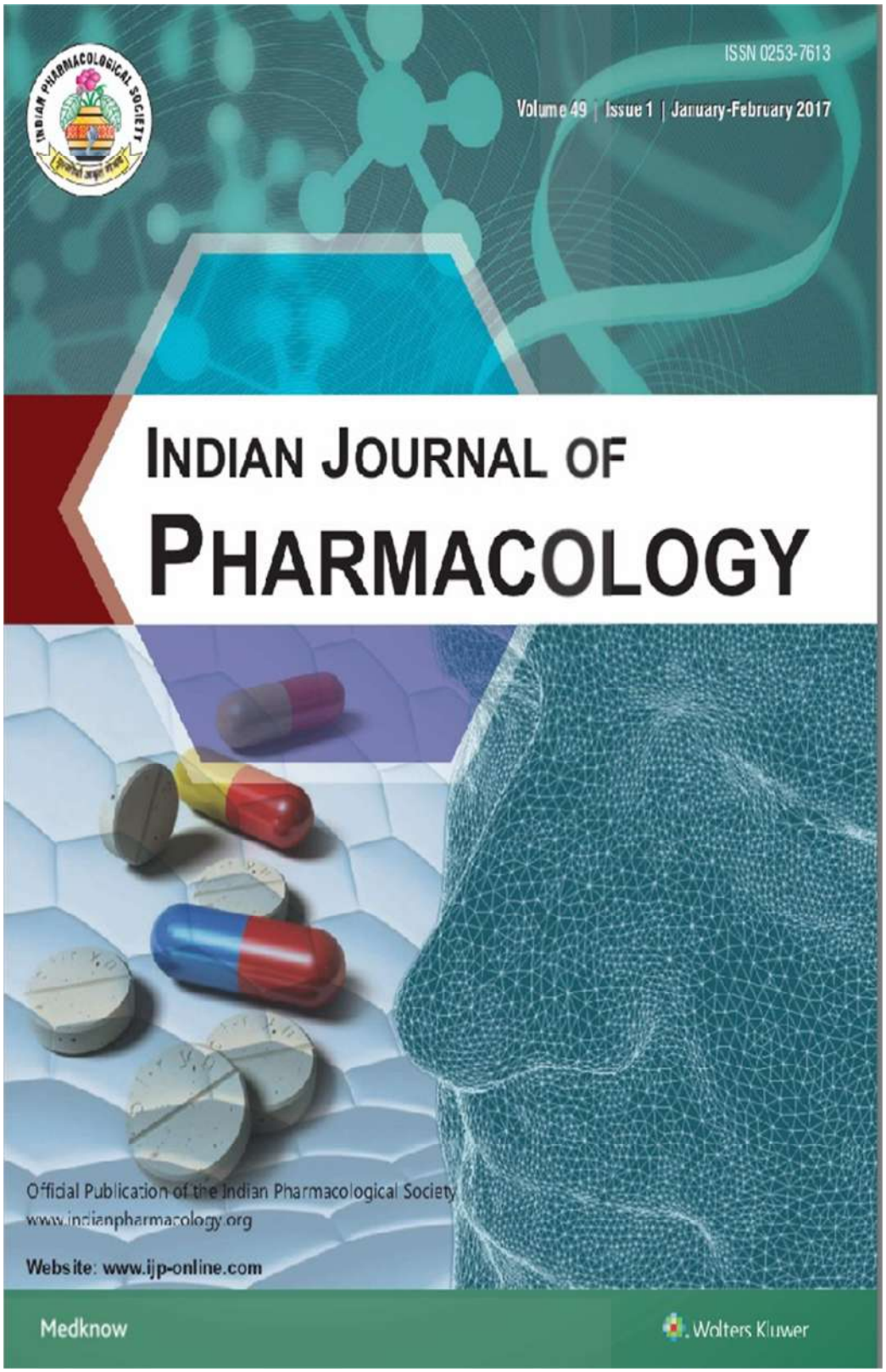
Arakawa T, Osawa K. 2000. Pharmacological study and application to food of mint flavor-antibacterial and antiallergic principles. Aroma Res. 1:20–23.

Bloemen K, Verstraelen S, Van Den Heuvel R, Witters H, Nelissen I, Schoeters G. 2007. The allergic cascade: Review of the most important molecules in the asthmatic lung. Immunol Lett. 113:6–18.

GAN. 2014. The Global Asthma Report 2014. Auckland: Global Asthma Network.

Inouye S, Takizawa T, Yamaguchi H. 2001. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J Antimicrob Chemother. 47:565–573.

Joshi RK. 2014a. Aroma profile of *Mentha arvensis* Linn. growing in Belgaum region of Karnataka. India J Essent Oil Bear Pl. 17:1397–1399.
Kapoor LD. 1990. CRC handbook of ayurvedic medicinal plants. Florida: CRC Press.
Katzung BG, Masters SB, Trevor AJ. 2009. Basic and clinical pharmacology. New York, NY: McGraw-Hill Companies.
Nelson HS. 2003. Prospects for antihistamines in the treatment of asthma. J Allergy Clin Immunol. 112:S96–S100.
Pino JA, Rosado A, Fuentes V. 1996. Chemical composition of the essential oil of *Mentha arvensis* L. var. *piperascens* Maling. from Cuba. J Essent Oil Res. 8:685–686.
Platts-Mills TAE. 2001. The role of immunoglobulin E in allergy and asthma. Am J Respir Crit Care Med. 164:S1–S5.


ISSN 0253-7613
Volume 49 | Issue 1 | January-February 2017
INDIAN JOURNAL OF
PHARMACOLOGY
Official Publication of the Indian Pharmacological Society
www.indianpharmacology.org
Website: www.ijp-online.com
Medknow
Wolters Kluwer

In vivo Evaluation of Antiasthmatic Activity of the Essential Oil of Zanthoxylum armatum

Article *in* Indian Journal of Pharmaceutical Sciences · January 2018

DOI: 10.4172/pharmaceutical-sciences.1000369

CITATIONS
10

READS
270

4 authors, including:

Dr. R. K. Joshi
ICMR-National Institute of Traditional Medicine (Formerly Regional Medical Research...
152 PUBLICATIONS **1,777** CITATIONS
SEE PROFILE

Paragouda Algouda Patil
Jawaharlal Nehru Medical College
101 PUBLICATIONS **840** CITATIONS
SEE PROFILE

www.ijpsonline.com

In vivo Evaluation of Antiasthmatic Activity of the Essential Oil of *Zanthoxylum armatum*

S. SHARMA, V. P. RASAL, R. K. JOSHI[1]* AND P. A. PATIL[2]

March-April 2018 Indian Journal of Pharmaceutical Sciences 383

www.ijpsonline.com

Department of Pharmacology and Toxicology, KLE University, College of Pharmacy, [1]Department of Phytochemistry, Regional Medical Research Centre, Indian Council of Medical Research, [2]Department of Pharmacology, USM KLE University Sains Malaysia, Nehru Nagar, Belagavi-590 010, India

Sharma, *et al.*: Antiasthmatic Activity of *Zanthoxylum armatum* Oil

***Zanthoxylum armatum* DC. is used in traditional medicines as a therapy of many diseases including bronchial asthma. The antiasthmatic effect of *Zanthoxylum armatum* essential oil was studied using histamine aerosol-induced bronchoconstriction in guinea pigs and ovalbumin-sensitized albino mice. Absolute eosinophils count in blood, total immunoglobulin E, eosinophils and neutrophils in bronchoalveolar lavage fluid and histopathology of lung tissue were investigated. Treatment with *Zanthoxylum armatum* essential oil significantly increased the time of preconvulsive dyspnoea in histamine-induced guinea pigs. Oral treatment of *Zanthoxylum armatum* oil showed significant decrease in absolute eosinophils count, serum level of immunoglobulin E and the number of eosinophils, neutrophils in bronchoalveolar lavage fluid. Histopathological examination of lungs showed that essential oil rescinded bronchial asthma. Results obtained in the present investigation provided evidence that essential oil of *Zanthoxylum armatum* caused bronchorelaxation and showed antiasthmatic properties. The traditional use of *Zanthoxylum armatum* essential oil against asthma could be attributed to the bronchorelaxation and antiasthmatic activity.**

Key words: *Zanthoxylum armatum*, bronchorelaxation, antiasthmatic activity, essential oil

Asthma is a chronic disease involving the airways inflammation of the lungs. These airways or bronchial tubes allow air to come in and out of the lungs. Asthma can be classified as allergic asthma, caused by exposure to an allergen and non-allergic asthma caused by stress, exercise, illnesses like a cold or flu or exposure to extreme cold weather, irritants in the air or by some medications[1]. Sometimes asthma is called bronchial asthma or reactive airway disease. Asthma is characterized by inflammation of the bronchial tubes with increased production of sticky secretions inside the tubes. Common asthma symptoms include, coughing especially at night, wheezing, shortness of breath, chest tightness, pain or pressure[2]. The burden of asthma is immense, with more than 300 million individuals currently suffering from asthma worldwide, about a tenth of those living in India. The prevalence of asthma has been estimated to range 3-38 % in children and 2-12 % in adults, being the commonest chronic disorder among children[3]. Although currently available treatments for asthma include β-2 agonists, anticholinergics, methylxanthines, mast cell stabilizers, leukotriene antagonists, glucocorticoids, antiimmunoglobulin E (antiIgE) antibody like omalizumab, which are administered for long duration. Moreover, these treatments are associated with several adverse effects, like muscle tremors, restlessness, hypotension, hyperglycaemia, tachycardia, flushing, convulsions, mood changes and adrenal crisis[4]. To minimize and possibly prevent these side effects alternative and complementary medicine is being sought. Some essential oils obtained from plants have been traditionally used in respiratory tract infections. Essential oils inhalation therapy has been used to treat bronchitis as it has antiinflammatory effect on trachea[5].

Zanthoxylum armatum DC. (Rutaceae) is an important medicinal plant, which is commonly known as Indian prickly ash, Nepal pepper or toothache tree, *Tejphal* (*Hindi*), *Tejowati* (*Sanskrit*), and *Mukthrubi* (*Manipuri* and *Nepal*)[6]. In traditional medicine *Z. armatum* is used in treatment of asthma, bronchitis, colic, cough, convulsions, cardiac debility, diabetes, diarrhoea, dyspepsia, fever, goitre, difficult micturition, eye and ear disease, helminthiasis, hepatopathy, leprosy, leucoderma, paralysis, skin disease, stomach disorder, tumours, ulcers and wounds[7,8]. The *Bhotiya* tribes of India use this plant to treat cough and colds[9]. The major essential oil constituents such as 3-borneol, iso-bornyl acetate, dihydrocarveol[10], linalool, α-limonene diepoxide, α-pinene, myrcene, D-limonene[11], have been reported as the major constituent of the *Z. armatum* essential oil. Various biological

***Address for correspondence**
E-mail: joshirk_natprod@yahoo.com

Accepted 15 February 2018
Revised 07 July 2017
Received 04 June 2016
Indian J Pharm Sci 2018;80(2):383-390

activities *viz.* antioxidant activity[12], anticonvulsive, antinociceptive activity[13] and antifungal, antispasmodic, antibacterial[11] have been reported from essential oil of *Z. armatum*. The essential oil of *Z. armatum* has not been explored for its effect related to medicinal use as asthma disorder. Therefore, the aim of present study was to evaluate antiasthmatic activity of the essential oil of *Z. armatum* using *in vivo* models.

The following reagents were commercially purchased and used: ovalbumin (OVA, Sigma-Aldrich, USA), aluminium hydroxide, histamine dihydrochloride, Tween 80 (HiMedia, Mumbai, India), dexamethasone (Centaur Pharmaceuticals, Goa), Turk solution (Nice Chemical Pvt Ltd., Cochi), ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) and *Z. armatum* oil (Kshipra Biotech Private Limited, Indore, India).

Female guinea pigs (400-600 g) purchased from J. N. Medical College, Belagavi, India and housed in standard conditions of temperature (22±2°), relative humidity (55±5 %) and light (12 h light/dark cycles) were used for bronchoconstriction activity. They were fed with vegetables, fruits, grass and water *ad libitum*. Female albino mice (18-25 g) were purchased from Sri Venkateshwara enterprises, Bengaluru, India and housed in standard conditions of temperature (22±2°), relative humidity (55±5 %) and light (12 h light/dark cycles) were used for bronchial asthma activity. They were fed with standard pellet diet and provided water *ad libitum*. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol (KLECOP/IEAC/Res.22-10/10/2015). All experiments were conducted in strict compliance with the ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals. As per the IAEC advice, since *Z. armatum* is used in traditional medicine determination of toxicity of *Z. armatum* oil was not performed. However, as per literature available, the acute toxicity of rat and mice was determined to be safe therapeutic at a dose of 2000 mg/kg (limit dose)[13,14].

The chemical composition of the essential oil (1 % solution of essential oil in equal ratio of n-hexane:dichloromethane) was analysed using a gas chromatograph (GC; Varian 450 fitted with a fused silica capillary column TG-5, 5 % diphenyl-95 % dimethyl polysiloxane; Thermo Scientific, 30 m× 0.25 mm i.d. 0.25 µm film thickness) under the experimental conditions reported earlier[15-17]. The oven temperature was programmed from 60 to 220° at 3°/min, using nitrogen as carrier gas. The injector and the flame ionization detector temperature were set at 230 and 240°, respectively. Gas chromatography-mass spectrometer (GC-MS) analysis was employed a Thermo Scientific Trace Ultra GC interfaced with a Thermo Scientific ITQ 1100 mass spectrometer fitted with a BP-1 (100 % dimethyl polysiloxane; SGE Analytical Science) fused silica capillary column (30 m×0.25 mm; 0.25 µm film thickness). The oven temperature range was programmed from 60 to 220° at 3°/min, and helium was used as carrier gas at 1.0 ml/min for analysis. The injector temperature was set at 230°, and the injection volume was 0.1 µl in n-hexane, with a split ratio of 1:50. MS was taken at 70 eV with a mass range of m/z 40-450 and other parameters used were those reported earlier[18-20]. The major constituent of the essential oil of *Z. armatum* was identified and confirm (co-injection of commercial sample from Sigma-Aldrich, India (≥98 % purity).

Experimental bronchial asthma was induced in guinea pigs by exposing them to 0.1 % w/v histamine aerosol under constant pressure in an aerosol chamber (24×14×24 cm^3) made of perplex glass. Each animal was placed in a chamber with histamine aerosol and pre-convulsive time (PCT; time of histamine aerosol exposure to guinea pig for initiating dyspnoea leading to the appearance of convulsions) was noted. On day 0 animals were kept in the histamine chamber and PCT was recorded as a baseline value. Day 0, PCT was taken as before treatment value. As per pre-convulsion dyspnoea (PCD) was recorded, the animals were removed from the chamber and exposed to fresh air for recovery. After development of PCD, the animals were divided into 4 groups (n= 6 in each group): group-I negative control, received histamine aerosols 0.1 % w/v; group-II received intraperitoneal injection of dexamethasone 2 mg/kg once daily for 7 d, group-III and IV received orally *Z. armatum* oil at the dose of 200 and 400 µl/kg once daily for 7 d. On the d 7, 2 h after the last dose, the time for the onset of PCT was recorded[21]. The percent increase in the time of PCT was calculated using following formula. Percent increase in PCT = $(1–T_1/T_2)\times100$, where, T_1= time for PCT onset on day 0, T_2= time for PCT onset on day 7.

Female mice were divided into four groups (n= 6 in each group), normal, negative control, standard and treatment group. Except normal mice other groups were sensitized by intraperitoneal injection of 50 µg OVA and 1 mg aluminium hydroxide in 200 µl

phosphate buffer saline (PBS) on day 1 and day 7. Group-I was given normal saline and feed for 14 d. After sensitization, from 8 to 14 d group-II was given OVA aerosols (1 % w/v) in PBS for 30 min. Group-III was given dexamethasone (2 mg/kg) intraperitoneal injection daily for 7 d along with OVA aerosols. *Z. armatum* oil was given orally to group-IV at a dose of 200 µl/kg for 7 d along with OVA aerosols. On day 15, 24 h after the final allergen lung lavage was performed for preparation of bronchoalveolar lavage fluid (BALF), trachea was aspirated (three time) with PBS until 2 ml of BALF was taken. The suspension of BALF was centrifuged and the supernatant collected and stored at –80°. Blood was collected in blood collecting tube containing disodium EDTA and absolute eosinophils were determined by direct microscopic counting with a haemocytometer. Blood serum was collected to estimate IgE level; lungs were collected for histopathological examination[22]. The accumulation of inflammatory cells in BALF was examined to evaluate airway inflammation. Briefly, 24 h after the final inhalation of antigen (day 15), animals were sacrificed by over dose diethyl ether inhalation, the left bronchus was tied for histological examination. Then, the right air lumen was washed four times with 0.5 ml PBS containing. BALF from each animal was pooled in a plastic tube, cooled in ice, and centrifuged (5000 g) at 4° for 10 min. Cell pellets were re-suspended in the same buffer (1 ml). A portion of the cell suspension was mixed with Turk solution and nucleated cells were counted in a haemocytometer.

Twenty-four hours after the last OVA challenge, mice were anesthetized with diethyl ether and blood was drawn. Differential cell counts were performed after staining with a modified Giemsa stain and cells with red cytoplasmic granules were counted as eosinophil's to calculate absolute eosinophil's count. The serum level of OVA-specific IgE was measured using an ELISA kit with commercially available reagents, according to the manufacturer's instructions. The process of measurement was same as cytokine analysis. The detection limit was 0.1 ng/ml for IgE.

The lungs were harvested after dissection and fixed in 10 % buffered formalin for 24 h, dehydrated, embedded in paraffin, sectioned into thin slices, stained with haematoxylin-eosin and observed by light microscopy, The degree of peribronchial and perivascular inflammation was observed.

Results were expressed as mean±SEM where n= 6. Differences among data were determined using one-way ANOVA followed by Dunnet's multiple comparison test (GraphPad Prism software, version 5.01). $P \leq 0.05$ was considered statistically significant.

The GC-MS analysis of essential oil of *Z. armatum* revealed that the main compound was identified as linalool (75.7 %). The essential oil of *Z. armatum* was significantly and dose-dependently increased the latent period of PCD. Percent increase in PCT in histamine-induced bronchoconstriction at the dose of 200 and 400 µl/kg body weight was found to be 61.20±0.29 and 69.48±0.99 %, respectively. Hence, the increase of PCT at a dose of 400 µl/kg was observed maximum protection compared to standard drug (dexamethasone)-treated group (Table 1).

Control group showed maximum increase in eosinophil count 24 h after the final exposure of OVA blood was drown in blood collecting tube containing disodium EDTA. *Z. armatum* oil-treated group at the dose of 200 µl/kg significantly inhibited the increased inflammatory cell (absolute eosinophils count) in blood (fig. 1A).

Antigen-specific Th2 responses are known to induce antigen-specific IgE antibody production. Repeated OVA inhalation significantly increased the number of inflammatory cells in blood serum. On the day 15, immediately after the final OVA challenge, significantly increased the total serum level of IgE. The treatment with essential oil of *Z. armatum* significantly inhibited the increased level of serum IgE at a dose of 200 µl/kg body weight (fig. 1B).

After challenging with OVA, the levels of inflammatory cells including eosinophil's and neutrophils were

TABLE 1: EFFECT OF *Z. ARMATUM* OIL ON HISTAMINE-INDUCED BRONCHOCONSTRICTION IN GUINEA PIGS

Grouping	Before treatment	After treatment	% Increase in pre-convulsive dyspnoea time PCD
Negative control group	108	120	6.17±2.49
Dexamethasone	90	180	50.53±3.77*
Z. armatum 200 µl/kg	249	642	61.20±0.29*
Z. armatum 400 µl/kg	83	271	69.48 ±0.99*

Each value was expressed as mean±SEM; where n= 6 in each group: *p≤0.05 as compared with control by one-way analysis of variance, followed by Dunnett's test

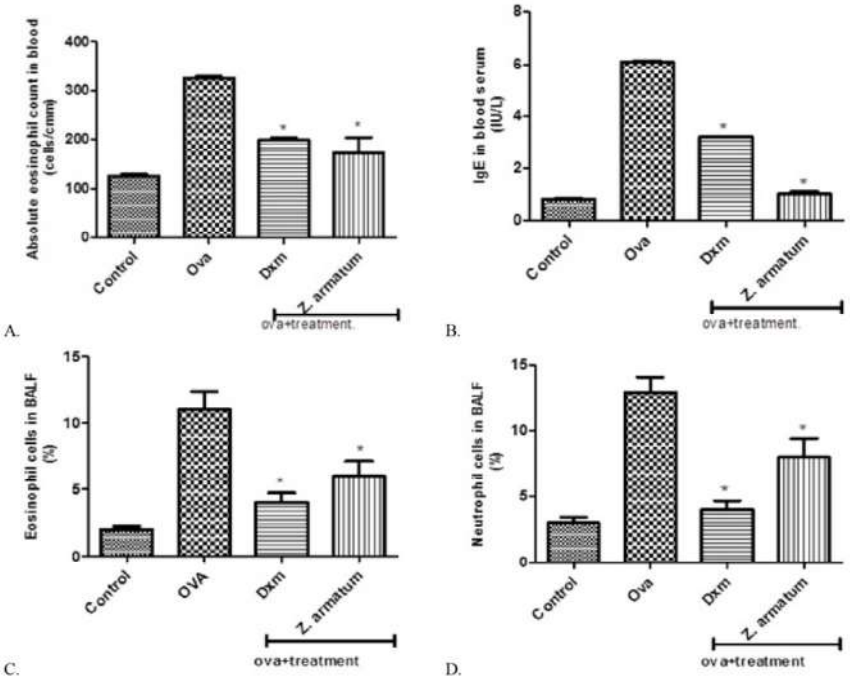


Fig. 1: Effect of essential oil on absolute eosinophils count in blood of mice
Cells/cubic mm (A); effect of *Z. armatum* essential oil on Immunoglobulin E in blood serum of mice, IU/l (B); effect of *Z. armatum* oil on inflammatory cells: eosinophils in bronchoalveolar lavage fluid of mice, % (C); effect of *Z. armatum* oil on inflammatory cells: neutrophils in bronchoalveolar lavage fluid of mice, % (D); values were expressed as mean±SEM, where n= 6 in each group, *p≤0.05 compared with ovalbumin-treated group

significantly increased in OVA-treated group as compared with normal group. But, sensitization of OVA along with the essential oil treatment, the levels of inflammatory cells were significantly decreased as compared with asthma-induced OVA group. The accumulation of inflammatory cells in BALF was significantly inhibited by 200 µl/kg body weight of essential oil (fig. 1C and 1D).

The microscopic images of tissue sections of each group stained with haematoxylin and eosin. The histopathological examination of lungs of mice exposed to OVA showed significant inflammatory alteration in peribronchial area and also increased bronchial muscles thickening, epithelial hyperplasia (fig. 2). In contrast, *Z. armatum* oil treatment at a dose of 200 µl/kg body weight showed significant changes (fig. 2) as compared to normal (fig. 2) and dexamethasone-treated groups (fig. 2).

Asthma is an allergic and respiratory disease commonly characterized by increased airway reactivity to different spasmogens. An initial attack of asthma was triggered by the release of inflammatory mediators like histamine, acetylcholine, leukotriene, prostaglandins or specific exposure of allergens, which reflected the signals of acute bronchoconstriction[23,24]. Histamine and other inflammatory mediators causes a host of changes in bronchial tissue by increasing the mucous secretion and simultaneous rapid constriction of bronchial smooth muscle, which narrows the bronchial tube and reduce the amount of air passes through them. Bronchodilating effect of *Z. armatum* oil was evaluated by observing its effects to increases the latent period of PCT in guinea pigs. The study revealed that the time of occurrence of PCT was significantly increased that suggests bronchodilating activity of *Z. armatum* oil against spasmogens.

Ovalbumin-induced model of allergic airway inflammation demonstrates that IgE levels in blood and eosinophilic infiltration in the lungs are markedly increased in asthmatic condition. Eosinophil count

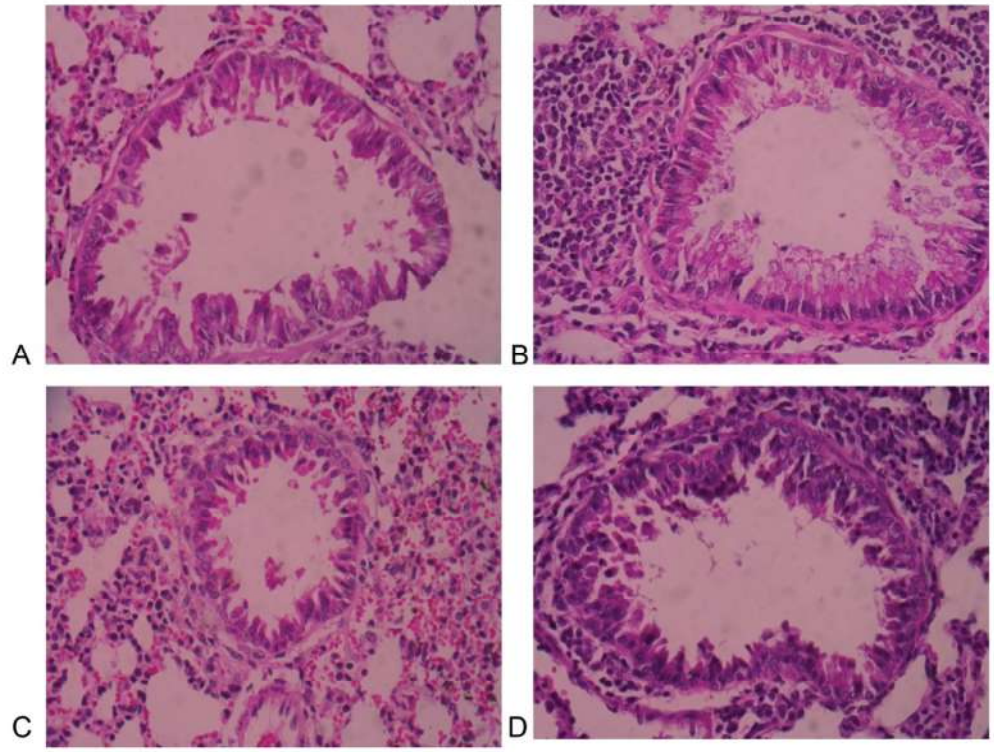


Fig. 2: Histopathology (40 x) of lungs
(A) Normal control; (B) ovalbumin; (C) dexamethasone; (D) *Zanthoxylum armatum* oil (200 µl/kg) treated

is elevated in response to the inflammation due to the OVA exposure. The pathogenesis of asthma is associated with increased infiltration of inflammatory cells and excessive mucus secretion into airway. OVA-induced asthma is recognized as a disease that results from chronic airway inflammation characteristically associated with the infiltration of lymphocytes, eosinophil's, macrophages and neutrophils into the bronchial lumen.

An absolute eosinophil count is a blood test that measures the number of white blood cells called eosinophils. Eosinophil's become active at a time of certain allergic diseases, infections, and other medical conditions. Treatment of the essential oil of *Z. armatum* significantly reduced the total absolute eosinophils in the blood. Asthma is almost always associated with some type of IgE-related reaction and therefore has an allergic basis. Numerous epidemiologic studies have shown a highly significant relationship between asthma and sensitization to various allergens as demonstrated by skin tests or the presence of specific IgE in the serum. IgE initiates the allergic response by causing mast cells to release inflammatory mediators and by recruiting eosinophils. Thus, blocking the effects of IgE is a promising strategy for preventing or ameliorating allergic symptoms[25]. This study showed significantly reduction in serum IgE level by *Z. armatum* oil-treated group. Eosinophils mediator secretion in asthma has been confirmed by BAL fluid analysis, which shows increased concentrations of granule-derived basic proteins[26].

Once established, the repetitive cycle of tissue damage and inflammatory cell recruitment becomes chronic. Even in the absence of sustained allergen, the chronic inflammation persists. Eosinophil level in sputum is associated with the degree of chronic airway obstruction[26]. In the present studies eosinophil cells in BALF significantly lower in *Z. armatum* oil treated groups as compared to the group treated by standard drug dexamethasone. Beside eosinophils, neutrophils also have an important role in the late-phase asthmatic reaction. Neutrophil products can cause airway

narrowing, increased mucus secretion and increased antigen-presenting cells responsiveness. In this studies essential oil of *Z. armatum*-treated group reduced the elevated neutrophils in BALF. The elevated numbers of the inflammatory cells reflects the sign of asthma. The results of this study showed that the treatment of *Z. armatum* oil (200 µl/kg) with OVA- sensitized mice significantly reduced the levels of eosinophils, neutrophils, and total inflammatory cells in the BALF as compared with OVA-sensitized mice. In this study linalool, a monoterpene was identified as the major constituent of *Z. armatum* essential oil and a number of linalool and its acetate-producing species are used in traditional medicine systems to relieve symptoms and cure a variety of ailments, both acute and chronic. Linalool was evaluated for its psychopharmacological activity in mice, revealing marked dose-dependent sedative effects on the central nervous system[27,28] as well as protection against pentylenetetrazol, picrotoxin and transcorneal electroshock-induced convulsions, hypnotic and hypothermic properties[29,30].

Moreover, linalool modulates glutamate activation expression *in vitro* to competitive antagonism of L-[3H] glutamate binding and *in vivo* model to delayed subcutaneous N-methyl-D-aspartames-induced convulsions and blockade of intra cerebroventricular quinolinic acid-induced convulsions[31-33]. Furthermore, presence of linalool in essential oil exhibited potential antiinflammatory activity in *in vivo* models[27]. Hence, obtained results of essential oil of *Z. armatum* provides evidence as bronchodilator and antiasthmatic properties in histamine and OVA-induced allergens in guinea pigs and mice. In conclusion, the essential oils obtained from the plants have been used traditionally for the treatment of respiratory tract infections. The present investigation provides evidence that essential oil of *Z. armatum* has bronchorelaxation and antiasthmatic properties. The traditional uses of *Z. armatum* against asthma could be attributed to their antiasthmatic activity as observed in present study.

Acknowledgements:

The authors are grateful to Director-in-Charge Regional Medical Research Centre (ICMR), Belagavi for encouraging the study. The authors are indebted to Kshipra Biotech Private Limited, Indore, India for providing essential oil of *Z. armatum*.

Conflicts of interest:

There are no conflicts of interest.

Financial support and sponsorship:

Nil.

REFERENCES

1. http://www.aaaai.org/conditions-and-treatments/asthma (Accessed on June 1, 2016).
2. http://www.webmd.com/asthma (Accessed on June 1, 2016).
3. Koul PA, Patel D. Indian guidelines for asthma: Adherence is the key. Lung India 2015;32:S1-S2.
4. Boushey HA. Drugs Used in Asthma. In: Katzung BG, Masters SB, Trevor AJ, editors. Basic and Clinical Pharmacology. 12th ed. China: McGraw-Hill Companies; 2009. p. 339.
5. Inouye S, Yamaguchi H, Takizawa T. Screening of the antibacterial effects of a variety of essential oils on respiratory tract pathogens, using a modified dilution assay method. J Infec Chemother 2001;7:251-4.
6. Singh TP, Singh OM. Phytochemical and pharmacological profile of *Zanthoxylum armatum* DC. Indian J Nat Prod Res. 2011;2:275-85.
7. Deshpande DJ. Handook of herbal remedies. Jodhpur: Anmol prints, 2008.
8. Kapoor LD. CRC Handbook of ayurvedic medicinal plants. India: CRC press, LLC; 1990.
9. Kala CP, Farooquee NA, Dhar U. Traditional uses and conservation of Timur (*Zanthoxylum armatum* DC.) through social institutions in Uttaranchal Himalaya, India. Conservat Soc 2005;3:224-30.
10. Waheed A, Mahmud S, Akhtar M, Nazir T. Studies on the components of essential oil of *Zanthoxylum armatum* by GC-MS. Am J Anal Chem 2011;2:258-61.
11. Barkatullah, Ibrar M, Muhammad N, Rehman I, Rehman M, Khan A. Chemical composition and biological screening of essential oils of *Zanthoxylum armatum* DC leaves. J Clin Toxicol 2013;3:1-6.
12. Negi JS, Bisht VK, Bhandari AK, Bisht R, Negi SK. Major constituents, antioxidant and antibacterial activities of *Zanthoxylum armatum* DC essential oil. Iranian J Pharmacol Ther 2012;11:68-72.
13. Ibrar M, Muhammad N, Barkatullah, Khan H, Jahan F, Ashraf N. Antinociceptive and anticonvulsant activities of essential oils of *Zanthoxylum armatum*. Phytopharmacology 2012;3:191-8.
14. Verma N, Khosa RL. Hepatoprotective activity of leaves of *Zanthoxylum armatum* DC in CCl4 induced hepatotoxicity in rats. Indian J Biochem Biophys 2010;47:124-7.
15. Joshi RK. Chemical composition of the essential oil of Baccharoides lilacina from India. Nat Prod Commun 2013;8:401-2.
16. Joshi RK. Essential oil of flowers of *Anaphalis contorta*, an aromatic and medicinal plant from India. Nat Prod Commun 2013;8:225-6.
17. Joshi RK. Chemical composition of the essential oil of *Croton bonplandianus* from India. Nat Prod Commun 2014;9:269-70.
18. Joshi RK. Essential oil of Senecio bombayensis from Western Ghats region of India. Chem Nat Compd 2014;50:382-83.
19. Joshi RK. GC/MS analysis of the essential oil of *Leucas indica* from India. Nat Prod Commun 2014;9:1607-08.
20. Joshi RK. Chemical composition *In vitro* antimicrobial and antioxidant activities of the essential oils of *Ocimum gratissimum*, *O. sanctum* and their major constituents. Indian J Pharm Sci 2013;75:457-62.

21. Parmar NS. Screening methods in pharmacology. New Delhi: Narosa Publishing House; 2006. p. 200.
22. Bates JHT, Rincon M, Irvin CG. Animal models of asthma. Am J Physiol Lung Cell Mol Physiol 2009;L401-L10.
23. Nelson HS. Prospects for antihistamines in the treatment of asthma. J Allergy Clin Immunol 2003;112:96-100.
24. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma from bronchoconstriction to airway inflammation and remodelling. Am J Respir Crit Care Med 2000;1720-45.
25. Platts-Mills TAE. The role of immunoglobulin E in allergy and asthma. Am J Respir Crit Care Med 2001;164:1-5.
26. Bloemen K, Verstraelen S, Heuvel RVD, Witters H, Nelissen I, Schoeters G. The allergic cascade: Review of the most important molecules in the asthmatic lung. Immunol Lett 2007;113:6-18.
27. Peana AT, Aquila PS, Panin F, Serra G, Pippia P, Moretti MD. Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. Phytomedicine 2002;9:721-6.
28. Jirovetz L, Jager W, Buchbauer G, Nikiforov A, Raverdino V. Investigations of animal blood samples after fragrance drug inhalation by gas chromatography/mass spectrometry with chemical ionization and selected ion monitoring. Biol Mass Spectrom 1991;20:801-03.
29. Buchbauer G, Jirovetz L, Jager W, Dietrich H, Plank C. Aromatherapy: evidence for sedative effects of the essential oil of lavender after inhalation. Z Naturforsch 1991;46:1067-72.
30. Elisabetsky E, Coelho De Souza GP, Dos Santos MAC, Siqueira IR, Amador TA, Nunes DS. Sedative properties of linalool. Fitoterapia 1995; 66(5):407-14.
31. Elisabetsky E, Brum LF, Souza DO. Anticonvulsant properties of linalool in glutamate-related seizure models. Phytomedicine 1999;6:107-13.
32. Silva Brum LF, Elisabetsky E, Souza D. Effects of linalool on [3H] MK801 and [3H] muscimol binding in mouse cortical membranes. Phytother Res 2001;15:422-5.
33. Brum LFS, Emanuelli T, Souza DO, Elisabetisky E. Effects of linalool on glutamate release and uptake in mouse cortical synaptosomes. Neurochem Res 2001;26:191-4.

Research Article

Access this article online

Quick Response Code:

Website: www.ijp-online.com

DOI: 10.4103/0253-7613.201019

Effect of *Angelica glauca* essential oil on allergic airway changes induced by histamine and ovalbumin in experimental animals

Shilpa Sharma, Vijaykumar P. Rasal, Paragouda A. Patil[1], Rajesh K. Joshi[2]

Abstract:

Objective: *Angelica glauca* Edgew (*Apiaceae*) is used in traditional medicine for treatment of several diseases including bronchial asthma. The present investigation was aimed to evaluate broncho-relaxant activity of *A. glauca* essential oil in histamine and ovalbumin (OVA)-induced broncho constriction in experimental animals.

Materials and Methods: Airway was induced using histamine aerosol in guinea pigs ($n = 24$) and OVA aerosol in albino mice ($n = 24$). The number of inflammatory cells, namely, absolute eosinophils count in blood, total immunoglobulin E (IgE) in serum, eosinophils, and neutrophils in bronchoalveolar lavage fluid (BALF) and histopathological examination of lung tissues were investigated in *A. glauca* oil and dexamethasone-treated groups. *A. glauca* oil 200 µL/kg was given orally, and dexamethasone 2 mg/kg was given intraperitoneal. Both the treatments were repeated daily for 7 days. Results were analyzed by one-way ANOVA, and $P \leq 0.05$ was considered statistically significant.

Results: Treatment with *A. glauca* essential oil significantly ($P < 0.001$) increased the time of preconvulsive dyspnea in histamine-induced guinea pigs. Oral treatment of *A. glauca* oil significantly ($P < 0.001$) decreased absolute blood eosinophil count (from 325 ± 3.69 to 200 ± 3.05 cells/mm^3), serum level of IgE (from 6.10 ± 0.05 to 0.70 ± 0.08 IU/L), and the number of eosinophils (from 11.0% ±1.41% to 3.0% ±0.51%), neutrophils (from 13.0% ±1.12% to 5.0% ±1.39%) in BALF. Histopathological changes observed in lungs of untreated group were marked suppressed by treatment with *A. glauca* oil.

Conclusion: The essential oil of *A. glauca* has bronchorelaxation in both histamine and OVA-induced bronchoconstriction in animals. The traditional use of *A. glauca* against asthma could be attributed to its bronchodilator property as observed in the present study.

Key words:

Angelica glauca, bronchorelaxation, histamine, ovalbumin

Department of Pharmacology, KLE University, College of Pharmacy, [1]Department of Pharmacology, USM KLE International Medical Programme, [2]Department of Phytochemistry, Regional Medical Research Centre, Indian Council of Medical Research, Belgaum, Karnataka, India

Address for correspondence:
Dr. Rajesh K. Joshi,
E-mail: joshirk_natprod@yahoo.com

Submission: 15-07-2016
Accepted: 10-12-2016

Bronchial asthma, a chronic disease involving the airways affects people of all ages, but it often starts during childhood. Bronchial asthma is a substantial health burden, often affecting quality of life, not only due to its physical effects but also its psychological and social effects.[1] Asthma can be classified as allergic asthma, caused by exposure to an allergen and nonallergic asthma caused by stress, exercise, illnesses like a cold or flu or exposure to extreme cold weather, irritants in the air, or some medications.[2] Bronchial asthma is characterized by inflammation of the bronchial tubes with increased production of sticky secretions inside the tubes. Currently, available treatments for asthma are managed using beta-2 agonists, anticholinergics, methylxanthines, mast cell stabilizers, leukotriene antagonist, glucocorticoids, anti-immunoglobulin E (IgE) antibody such as omalizumab are also used. Moreover, these drugs are associated with several adverse effects, such as muscle tremors, restlessness, hypotension, hyperglycemia, tachycardia, flushing, convulsions, mood changes, and adrenal crisis.[3] To minimize or possibly prevent these side effects, alternative, and complementary medicine is being sought. Some essential oils obtained from plants have been traditionally used in respiratory tract infections. Inhalation of vapors of essential oils augmented anti-inflammatory effect on trachea and reduces asthma.[4]

How to cite this article: Sharma S, Rasal VP, Patil PA, Joshi RK. Effect of *Angelica glauca* essential oil on allergic airway changes induced by histamine and ovalbumin in experimental animals. Indian J Pharmacol 2017;49:55-9.

The genus *Angelica* is an important medicinal and aromatic plant used in traditional medicine to treat various diseases. It is locally known as chora or gandrayan. The plant is found in Western Himalaya and ranks third on the list of 52 medicinal plants prioritized for consultation and conservation. *Angelica glauca* Edgew (*Apiaceae*) is considered to be crucially endangered and need to be conserved.[5] Traditionally, *A. glauca* is used in medicines, aromatic spices, and condiments. *A. glauca* has high global and domestic demands for the preparation of various drug formulations. In traditional medicine, the plant *A. glauca* is used in typhoid, bronchitis, flatulence, colic, and stomach pain.[6]

The essential oil of *A. glauca* has been reported to contain β-phellandrene, (Z)-ligustilide,[7] methyl octane, limonene, β-phellandrene, β-pinene, (Z)-ligustilide,[8] α-phellandrene, β-pinene, thujene, β-caryophyllene, β-bisabolene, germacrene D with oxygenated terpenes such as trans-carveol, β-caryophyllene oxide,[9] α-phellandrene, β-pinene, β-caryophyllene[10] as the major constituents. Various biological activities, namely, antioxidant, antimicrobial,[11] antifungal,[10] and insecticidal[12] have been reported. The essential oil of *A. glauca* has not been explored for its effect in asthma. Therefore, the aim of the present study was to evaluate effects of *A. glauca* in bronchoconstriction induced by histamine and ovalbumin (OVA) in experimental animals.

Materials and Methods

Reagents

The following reagents were commercially purchased and used: OVA (Sigma-Aldrich, USA), aluminium hydroxide (HiMedia Pvt., Ltd., Mumbai, Maharashtra, India), histamine dihydrochloride, tween 80 (HiMedia Pvt., Ltd., Mumbai, Maharashtra, India), dexamethasone (Centaur pharmaceuticals, Goa, India), Turk solution (NICE Chemical Pvt., Ltd., Cochin, Kerala, India), disodium ethylenediamine tetraacetic acid (EDTA-2Na), and *A. glauca* oil (Kshipra Biotech Private Limited, Indore, India).

Animals

The female Guinea pigs (400–600 g) purchased from J. N. Medical College, Belagavi, Karnataka, India and were housed in laboratory conditions (temperature 22°C ± 2°C and humidity 55% ±5%) for a week in 12:12 h light: Dark cycle before starting experimentation. The female albino mice (18–25 g) were purchased from Sri Venkateshwara Enterprises, Bengaluru, Karnataka, India and were treated similarly as Guinea pigs, before experimental study. The study was initiated after protocol approved by the Institutional Animal Ethics Committee. All the experiments were conducted in strict compliance with the ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (KLECOP/IEAC/Res. 22-10/10/2015).

Toxicity Study

Acute toxicity for this edible plant has been reported to be – 2.2 g/kg for mice and 11.2 g/kg for rat.[13] As per the IAEC advise to avoid duplication of studies, acute toxicity studies were not carried out, and two doses 200 and 400 µL/kg were used in the present study.

Chromatographic Analysis of the Essential Oil of *Angelica glauca*

The chemical composition of the essential oil was analyzed using a gas chromatograph (GC) (Varian 450 fitted with a fused silica capillary column TG-5 (30 m × 0.25 mm id, 0.25 µm film thickness) under the experimental conditions reported earlier.[14,15] The oven temperature was programmed from 60°C to 220°C at 3°C/min, using nitrogen as carrier gas. The injector and the flame ionization detector temperature were set at 230 and 240°C, respectively. GC-mass spectrometer (GC-MS) analysis was employed a Thermo Scientific Trace Ultra GC interfaced with a Thermo Scientific ITQ 1100 MS fitted with a BP-1 fused silica capillary column (30 m × 0.25 mm; 0,25 mm id 0.25 µm film thickness). The oven and injector temperature were same as used for GC, and helium was used as carrier gas at 1.0 mL/min for analysis. The injection volume was 0.1 µL in n-hexane, with a split ratio of 1: 50. MS was taken at 70 eV with a mass range of m/z 40–450, and other parameters used were those reported earlier.[16,17] The major constituent of the essential oil of *A. glauca* was identified and confirmed (co-injection of commercial sample from Sigma-Aldrich, India (≥98% purity).

Histamine-induced Bronchoconstriction in Guinea Pigs

Bronchospasm was induced in each guinea pig by exposing to 1% histamine aerosol under constant pressure (1 kg/cm^2) in an aerosol chamber (24 cm × 14 cm × 24 cm) made of perplex glass, and the time for onset of dyspnea which indicates preconvulsive time (PCT) was noted on day 0 as basal value. As soon as, the preconvulsion dyspnea (PCD) was recorded, the animals were removed from the chamber and positioned in fresh air to recover. After determination of PCD, the animals were randomly divided into four groups ($n = 6$ in each): group I negative control, received normal saline orally; Group II received intraperitoneal (i.p.) injection of dexamethasone 2 mg/kg (standard drug) once daily for 7 days, Group III and IV received orally *A. glauca* oil at the dose of 200 µL/kg and 400 µL/kg once daily on 7th day 2 h after the last dose, all the groups were subjected to histamine aerosol and the time for the onset of PCT was recorded.[18] The percent increase in the time of PCT was calculated using following formula.

Percentage increase in time of PCT = $(1 - T_1/T_2) \times 100$

Where; T_1 = time for PCT on day 0, T_2 = time for PCT on day 7.

Ovalbumin-sensitization in Mice

Female mice were randomly divided into four groups ($n = 6$ in each), normal, negative control, dexamethasone, and *A. glauca* oil treatment groups. Except normal mice, other groups were sensitized by i.p. injection of 50 µg OVA and 1 mg aluminum hydroxide in 200 µL of phosphate-buffered saline (PBS) on day 1 and day 7. Group I was given normal saline for 14 days. After sensitization, from day 8 to day 14 group II was given only OVA aerosols (1% w/v) in PBS for 30 min every day. Group III was given injection dexamethasone (2 mg/kg) i.p. injection daily for 7 days along with OVA aerosols. *A. glauca* oil was given orally to group IV at a dose of 200 µL/kg for 7 days along with OVA aerosols. On day 15, 24 h after the final allergen aerosol animals were sacrificed by over anesthesia to collect cardiac blood on EDTA bulbs for differential cell counts by staining with a modified Giemsa stain. Lung lavaging

was performed for collection of bronchoalveolar lavage fluid (BALF). The trachea was aspirated (3 times) with PBS until 2 mL of BALF was collected. BALF from each animal was pooled in a plastic tube, cooled in ice, and centrifuged (2 g) at 4°C for 10 min. Cell pellets were resuspended in the same buffer (1 mL). A portion of the cell suspension was mixed with Turk solution, and cells were counted in a hemocytometer. The lungs were dissected out along with bronchia for histopathological studies.

Measurement of Immunoglobulin E Levels in Serum

The serum level of OVA-specific IgE was measured by ELISA kit using commercially available reagents, according to the manufacturer's instructions. The detection limit was 0.1 ng/mL for IgE.

Histopathological Examination of Lung

Dissected out lungs were fixed in 10% buffered formalin for 24 h, dehydrated, embedded in paraffin, and the cut sections stained with hematoxylin-eosin were observed under light microscope to assess the degree of peribronchial and perivascular inflammation.

Statistical Analysis

Results were expressed as mean ± standard error of the mean and were analyzed by one-way ANOVA followed by Dunnett's multiple comparison tests (GraphPad Prism, Version 5, GraphPad Software, Inc., USA software for Windows) and $P < 0.05$ was considered statistically significant.

Results

Analysis of the Essential Oil

The GC-MS analysis of essential oil of *A. glauca* revealed that the main compound was identified as α-pinene [Figure 1].

Effect of the Essential Oil of *Angelica glauca* on Histamine-induced Bronchoconstriction in Guinea Pigs

The essential oil of *A. glauca* significantly ($P < 0.001$) and dose-dependently increased the latent period for PCD. In histamine-induced bronchoconstriction at the dose of 200 and 400 µL/kg, the percentage increase in PCT was found to be 53.74 ± 6.36 and 67.17 ± 6.48, respectively. Hence, the increase of PCT at a dose of 400 µL/kg of *A. glauca* oil was almost comparable to that of dexamethasone (standard drug)-treated group [Table 1].

Effect of the Essential Oil of *Angelica glauca* on Absolute Eosinophil Count in Blood

Control group showed maximum increase in eosinophil count 24 h after the final exposure of OVA. *A. glauca* oil-treated group at the dose of 200 µL/kg significantly ($P < 0.001$) decreased inflammatory cell in blood [Figure 2a].

Effect of the Essential Oil of *Angelica glauca* on Serum Immunoglobulin E Level in Ovalbumin-sensitized Mice

Antigens are known to induce antigen-specific IgE antibody production. Repeated OVA inhalation significantly ($P < 0.001$) increased on the day 15 (immediately after the final OVA challenge) the total serum level of IgE. The treatment with essential oil of *A. glauca* significantly ($P < 0.001$) decreased the serum IgE at a dose of 200 µL/kg [Figure 2b].

Effect of *Angelica glauca* Essential Oil on Inflammatory Cells in Bronchoalveolar Lavage Fluid

After challenging with OVA, the levels of inflammatory cells including eosinophils and neutrophils were significantly ($P < 0.001$) increased as compared with normal group. However, with *A. glauca* essential oil treatment, the inflammatory cells were significantly ($P < 0.001$) decreased as compared to that of untreated and was almost comparable to that of dexamethasone group [Figure 2c and d].

Table 1: Effect of *Angelica glauca* oil on histamine-induced bronchoconstriction in guinea pigs (*n*=24)

Grouping	Before treatment (s)	After treatment (s)	Percentage of increase in preconvulsive dyspnea time (s)
Negative control	108	120	6.17±2.49
Dexamethasone	90	180	50.53±3.77*
Angelica glauca (µL/kg)			
200	115.65	250	53.74±6.36*
400	108	328.9	67.17±6.48*

Values are expressed as mean±SEM; where *n*=6 in each group: *P<0.001 as compared with negative control by one-way analysis of variance, followed by Dunnett's test. SEM=Standard error of the mean

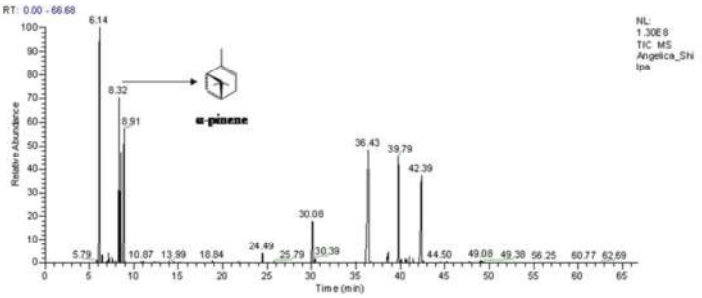


Figure 1: Gas chromatograph-total ion chromatogram of *Angelica glauca* essential oil and its major compound α-pinene

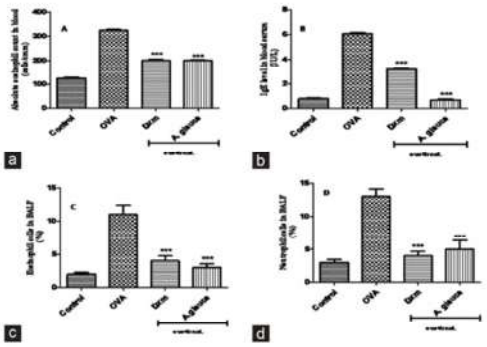


Figure 2: Effect of *Angelica glauca* essential oil on: (a) Blood eosinophils (cells/mm^3); (b) serum immunoglobulin E (IU/L); (c) eosinophils in bronchoalveolar lavage fluid (percentage); (d) neutrophils in bronchoalveolar lavage fluid (percentage) values were expressed as mean ± standard error of the mean, *$P < 0.001$ compared with ovalbumin-treated group

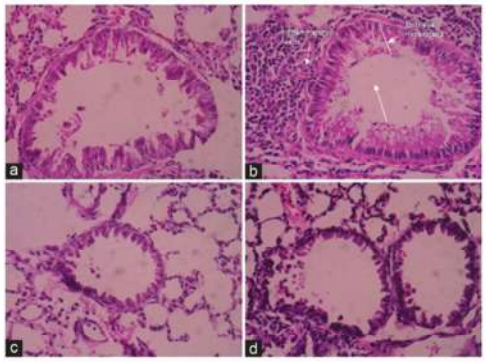


Figure 3: Effect of *Angelica glauca* oil on lung inflammatory cell infiltration in ovalbumin-induced allergic asthmatic mice (H and E, ×40,) in (a) normal group; (b) ovalbumin group; (c) ovalbumin + Dexa (2 mg/kg) group; (d) ovalbumin + *Angelica glauca* oil (200 µL/kg) treated

Effect of *Angelica glauca* on Ovalbumin-induced Histopathological Changes in Lung Tissues

The histopathological examination of lung sections of mice exposed to OVA stained with hematoxylin and eosin showed significant inflammatory changes in peribronchial and perivascular areas and also increased bronchial muscles thickening, epithelial hyperplasia [Figure 3b]. In contrast, the histological changes induced by OVA were markedly suppressed by treatment with *A. glauca* oil at a dose of 200 µL/kg [Figure 3d] as compared to untreated group [Figure 3a] and was almost similar to the dexamethasone [Figure 3c]-treated groups.

Discussion

Asthma is an allergic respiratory disease triggered by different spasmogens such as acetylcholine, leukotrienes, prostaglandins, or specific exposure of allergens, which reflect the signals of acute bronchoconstriction.[19] Histamine and other inflammatory mediators cause a host of changes in bronchial tissue by increasing the mucous secretion and simultaneous rapid constriction of bronchial smooth muscle. Bronchodilating effect of *A. glauca* oil was determined by its effect to increase the latency for PCT in guinea pigs. The study revealed that the latency for PCT was significantly ($P < 0.001$) increased suggesting bronchodilating activity of *A. glauca* oil against histamine.

OVA-induced model of allergic airway inflammation demonstrated that IgE levels in blood and eosinophilic infiltration in the lungs were markedly increased, as in asthmatic condition. Eosinophil count was elevated in response to the OVA exposure in untreated animals. OVA-induced asthma has been recognized as pathological condition that resembles chronic airway inflammation characteristically associated with the infiltration of lymphocytes, eosinophils, macrophages, and neutrophils into the bronchial lumen.[20]

Eosinophils become active when one has certain allergic diseases, infections, and other medical conditions. Treatment with the essential oil of *A. glauca* significantly reduced the total eosinophils in the blood. Asthma is almost always associated with some type of IgE-related reaction and therefore has an allergic basis.[21] Numerous epidemiologic studies have shown a highly significant relationship between asthma and sensitization to various allergens as demonstrated by skin tests or the presence of specific IgE in the serum. IgE initiates the allergic response by causing mast cells to release inflammatory mediators and by recruiting eosinophils.[22] Thus, blocking the effects of IgE is a promising strategy for preventing or ameliorating allergic symptoms.[23] This study showed significant reduction in serum IgE level by *A. glauca* oil-treated group. Eosinophil-mediated secretions in asthma have been reported in BALF in the form of granule-derived basic proteins.[24] Once established, the repetitive cycle of tissue damage and inflammatory cell recruitment becomes chronic, even in the absence of sustained allergen, the chronic inflammation persists. Eosinophil level in sputum is associated with the degree of chronic airway obstruction.[25] In the present studies, eosinophils in BALF were significantly ($P < 0.001$) decreased in *A. glauca* oil-treated groups and were comparable to that of dexamethasone-treated group. The effect of *A. glauca* essential oil was not compared with other bronchodilators. Besides eosinophils, neutrophils also have an important role in the late-phase asthmatic reaction. The findings of the present study need to be confirmed before their extrapolation to human situations.

Conclusion

The present study provides evidence that essential oil of *A. glauca* has bronchodilating and immunosuppressant actions in guinea pigs and mice. Efficacy of *A. glauca* oil in the treatment of bronchial asthma is worth exploring clinically.

Acknowledgments

The authors are grateful to Director-in-Charge Regional Medical Research Centre (ICMR), Belagavi for encouraging the study. The authors are indebted to Kshipra Biotech Private Limited, Indore, India for providing essential oil of *A. glauca*.

Financial Support and Sponsorship
Nil.

Conflicts of Interest
There are no conflicts of interest.

References

1. GAN. The Global Asthma Report 2014. Auckland, New Zealand: Global Asthma Network; 2014.
2. AAAAI. American Academy of Allergy Asthma and Immunology; Asthma Overview. 2016. Available from: http://www.aaaai.org/conditions-andtreatments/asthma. [Last cited on 2016 Jun 02].
3. Boushey HA. Basic and clinical pharmacology. In: Akporiaye ET, Aminoff MJ, Benowitz NL, Berkowitz BA, Biaggioni I, Bikle DD, editors. Drugs Used in Asthma. 12th ed. China: McGraw-Hill Companies; 2009. p. 339.
4. Inouye S, Yamaguchi H, Takizawa T. Screening of the antibacterial effects of a variety of essential oils on respiratory tract pathogens, using a modified dilution assay method. J Infect Chemother 2001;7:251-4.
5. Semwal DP, Saradhi PP, Kala CP, Sajwan BS. Medicinal plants used by local Vaidya in Ukimath block, Uttarakhand. Indian J Tradit Knowl 2010;9:480-5.
6. Prajapati ND, Purohit SS, Sharma AK, Kumar T. A Handbook of Medicinal Plants a Complete Source Book. Section II: Medicinal Plants: A to Z. Jodhpur: Agrobios (India); 2006. p. 49.
7. Kaul PN, Mallavarapu GR, Chamoli RP. The essential oil composition of *Angelica glauca* roots. Planta Med 1996;62:80-1.
8. Thappa RK, Kaul P, Chisti AM, Kapahi BK, Suri OP, Agarwal SG. Variability in the essential oil of *Angelica glauca* Edgew of different geographical regions. J Essent Oil Res 2005;17:361-3.
9. Agnihotri VK, Thappa RK, Meena B, Kapahi BK, Saxena RK, Qazi GN, *et al.* Essential oil composition of aerial parts of *Angelica glauca* growing wild in North-West Himalaya (India). Phytochemistry 2004;65:2411-3.
10. Irshad M, Aziz S, Rehman H, Hussain H. GC-MS analysis and antifungal activity of essential oil of *Angelica glauca*, *Plectranthus rugosus* and *Valeriana wallichii*. J Essent Oil Bear Plants 2012;15:15-21.
11. Irshad M, Rehman HU, Shahid M, Aziz S, Ghous T. Antioxidant, antimicrobial and phytotoxic activities of essential oil of *Angelica glauca*. Asian J Chem 2011;23:1947-51.
12. Sharma RN, Deshpande SG, Joseph M. Evaluation of natural essential oils as antifeedants against *Spodoptera litura*. Indian Perfum 1990;34:196-8.
13. Lis-Bachin M, Gardner Z, Mugufin M. American Herbal Products Association Botanical Safety Handbook. 2nd ed. U.S: CRC Press; 2013. p. 110.
14. Joshi RK. Chemical composition of the essential oil of *Baccharoides lilacina* from India. Nat Prod Commun 2013;8:401-2.
15. Joshi RK. Essential oil of flowers of *Anaphalis contorta*, an aromatic and medicinal plant from India. Nat Prod Commun 2013;8:225-6.
16. Joshi RK. GC/MS analysis of the essential oil of *Leucas indica* from India. Nat Prod Commun 2014;9:1607-8.
17. Joshi RK. GC/MS analysis of the essential oil of *Vernonia cinerea*. Nat Prod Commun 2015;10:1319-20.
18. Sagar R, Sahoo HB, Kar B, Mishra NK, Mohapatra R, Sarangi SP. Pharmacological evaluation of *Calendula officinalis* L. on bronchial asthma in various experimental animals. Int J Nutr Pharmacol Neurol Dis 2014;4:95-103.
19. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma from bronchoconstriction to airways inflammation and remodeling. Am J Respir Crit Care Med 2000;161:1720-45.
20. Wu J, Zhang Y, Liu Q, Zhong W, Xia Z. All-trans retinoic acid attenuates airway inflammation by inhibiting Th2 and Th17 response in experimental allergic asthma. BMC Immunol 2013;14:28.
21. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med 1989;320:271-7.
22. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der P I) and the development of asthma in childhood. A prospective study. N Engl J Med 1990;323:502-7.
23. Platts-Mills E, Thomas A. The role of immunoglobulin E in allergy and asthma. Am J Respir Crit Care Med 2001;164:S1-5.
24. Bloemen K, Verstraelen S, Van Den Heuvel R, Witters H, Nelissen I, Schoeters G. The allergic cascade: Review of the most important molecules in the asthmatic lung. Immunol Lett 2007;113:6-18.
25. Gibson PG, Simpson JL, Saltos N. Heterogeneity of airway inflammation in persistent asthma: Evidence of neutrophilic inflammation and increased sputum interleukin-8. Chest 2001;119:1329-36.

CERTIFICATES

ICMR Sponsored Training Program on
Good Laboratory Practices
Organised by
KLE University's J. N. Medical College, Belagavi

Certificate of Participation

Dr./Mr./Mrs./Miss SHILPA SHARMA has

Participated as Delegate in a ICMR DHR Sponsored Training Program on "GOOD LABORATORY PRACTICES"

conducted by KLE University's J. N. Medical College, Belagavi, Karnataka. vide letter No. V-25011/25/2013-HR

held from 15/12/2014 to 02/01/2015

Dr. Ganga S. Pilli
Training Program Co-ordinator
J N Medical College, Belagavi

Dr. N.S. Mahantshetti
Principal
J N Medical College, Belagavi

Dr. V. S. Patil
Registrar
KLE University, Belagavi

KLE UNIVERSITY
Accredited 'A' Grade by NAAC

JAWAHARLAL NEHRU MEDICAL COLLEGE & KLES DR. PRABHAKAR KORE HOSPITAL

CLINICAL SKILLS LAB

DR. A. S. GODHI, Chairman | DR. S. C. METGUD, Member | DR. ANIL S. HARUGOP, Member | DR. M. G. DHORIGOL, Member-Secretary

CERTIFICATE

This is to certify that SHILPA SHARMA was a

~~Resource Person~~ / Participant for BLS & ACLS WORKSHOP.

conducted on 20th - 06 - 15.

DR. (Mrs) N. S. MAHANTSHETTI
Principal, J. N. Medical College
Professor Dept. of Paediatrics
KLE University, Belgaum

DR. M. G. DHORIGOL
Member - Secretary, Clinical Skills Lab
Professor Dept. of Anaesthesiology
J. N. Medical College, KLE University, Belgaum

KLE University's

Re-Accredited "A" Grade by NAAC | Placed in Category 'A' by MHRD (GoI)

J. N. Medical College, Belagavi

Department of Pharmacology &

Indian Pharmacological Society, Belagavi Branch

This is to certify that

Ms. Shilpa Sharma

from KLE College of Pharmacy - Belagavi has participated as Delegate

in the Pharmacology CME 2016 on "Current Trends in the Management of Hypertension"

held on 22nd April 2016 at J. N. Medical College, Belagavi

Dr. (Mrs.) N. S. Mahantashetti, Principal, KLE University's J N Medical College, Belagavi

Dr. Banappa S. Unger, President, IPS Local Branch Belagavi

Dr. A. P. Hogade, Organising Chairman

Dr. Anupama M. G., Organising Secretary

Sponsored by

AD INSTRUMENTS

K.L.E. UNIVERSITY's

COLLEGE OF PHARMACY, BELAGAVI

20th NATIONAL CONVENTION OF SOCIETY OF PHARMACOGNOSY

Certificate

This is to certify that Mr./Ms./Dr./Prof. SHILPA SHARMA.

has participated as Delegate / in Poster Presentation at 20th National Convention of Society of Pharmacognosy

on "NEWER APPROACHES FOR DEVELOPMENT AND PROMOTION OF NATURAL PRODUCTS"

held on 26th and 27th March 2016 organized by KLE University's College of Pharmacy, Belagavi, Karnataka.

Dr. V. P. RASAL
Chairman LOC

Dr. S. S. JALALPURE
Organizing Secretary

K.L.E. UNIVERSITY'S

(Accredited 'A' Grade by NAAC & Placed in Category 'A' by MHRD, GoI)

SHRI B. M. KANKANAWADI AYURVED MAHAVIDYALAYA

Post Graduate Studies and Research Centre

Shahapur, Belagavi - 590003 - Karnataka, India.

Ph. 0831-2486286, Fax : 0831-2424157, E-mail : kleayurworld.edu.in, bmkayurveda@rediffmail.com

Workshop on Analytical & Experimental Techniques

Organized by

Central Research Facility

CERTIFICATE

It gives us immense pleasure to express our sincere gratitude to Dr. Shilpa Sharma for his / her participation as a Delegate in Workshop on Analytical & Experimental Techniques held on 22nd & 23rd May 2015.

Dr. M. B. Gundakalle
Organizing Secretary

Dr. Rudramma R. Hiremath
Organizing Secretary

Dr. B. Sreenivasa Prasad
Chairman, Organizing Committee