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Development and Evaluation of Polyherbal Formulation for Treatment of Asthma

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Abstract: *Asthma is a chronic disorder associated with airways in lungs. Phyto Pharmaceuticals are the leading alternative over the conventional pharmaceutical treatment to reduce the side effects of allopathic drugs used for long term treatment of asthma. Ajwain oil, Clove oil and Garlic oil are known to possess a number of medicinal properties like, expectorant, bronchodilatory, anti tussive, antimicrobial, antispasmodic, antioxidant and traditionally used in common cold, cough and asthma since decades. The purpose of the present investigation was to develop a dry powder inhaler containing essential oil loaded biodegradable nanoparticles for pulmonary target delivery for the treatment of asthma. 27 L8 array Taguchi screening design method was applied for screening of most effective variables using design expert software (13.0.1). Screening was carried out to choose the suitable biodegradable polymer for targeted pulmonary delivery. The NPs were formulated using ionic gelation method and estimated for particle size and entrapment efficiency. Based on the results of trial batches prepared from each polymer, nanoparticles prepared using Chitosan was having very good particle size, entrapment efficiency, poly dispersive index, drug loading and favorable zeta potential for pulmonary delivery of essential oils. Fourier transform infrared spectroscopy (FTIR) had employed to study essential oils-excipients compatibility. 32 factorial design was applied for formulation optimization. Stirring speed and Sonication time were the significant factors. Essential oil loaded chitosan nano particles have been formulated and optimized individually for Ajwain oil (ANP), Clove oil (CNP) and Garlic oil (GNP). Following freeze drying of NPs, the powder obtained was mixed with lactose pre-blend to obtain a respirable powder. Optimized batch of dry powder inhaler having a particle size of 109.7±9.3 nm with a zeta potential value of +14.4 mV, thus revealing the positively charged behavior of NPs due to the presence of cationic polymer, which may facilitate the high uptake by the negatively charged sialic acid on the alveolar macrophages. The design batches' polydispersity index values were found to be in range of 0.0790 to 0.4630 and the drug loading was found to be between 2.54 ± 0.1 to 6.89 ± 2.1. In vitro deposition studies were performed using non-viable cascade impactor. The freeze-dried powder showed FPF of 29.23±0.9% with MMAD of 3.28±0.02 µm represents good lung deposition and better local targeting into the lungs. Less than 5 µm MMAD suggested that the powder may retain in the lower region of the lung for a long duration. Franz diffusion cell was used to evaluate the in-vitro drug release study of pure essential oil mixture and the optimized batch of dry powder biodegradable nanoparticles using a dialysis bag. Pure essential oil mixture showed 91 % drug release within 3 hours where drug was released within 7 hours from the NPs shows sustain release. Stability study shows dry powder inhaler was more stable at room temperature condition. Animal Study shows that formulation- treated rats showed a significant reduction in inflammatory cell infiltration and mucus production compared to asthmatic rats. The present study demonstrated that herbal dry powder inhaler is suitable for pulmonary drug delivery and had great potential for targeting Asthma.*

Keywords: Dry powder inhaler, Biodegradable nanoparticles, Herbal formulation, Asthma, Chitosan nanoparticles

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I. INTRODUCTION

INTRODUCTION TO ASTHMA

Asthma is a chronic disorder associated with airways in the lungs. The basic symptoms are chronic inflammation and hyperresponsiveness. Asthma is not curable, but it can be managed. Exposure to indoor allergens, especially as a child, and a family history of asthma or allergy are the two most powerful risk factors for developing asthma.

Allopathic medicines are used for the long term, producing many side effects. Like, 1) Beta 2 agonists causes Fine tremor particularly in the hands Nervous tension, Headache, Muscle cramps, Palpitation, 2) Antimuscarinics causes Dry mouth, Gastro-intestinal motility disorder, Cough, Headache, 3) Corticosteroids causes Fragile bones, High blood pressure, Diabetes, Weight gain, Cataracts, and glaucoma, Thinning of the skin, Easy bruising, Muscle weakness, 4) Leukotriene inhibitors causes Abdominal pain, Thirst, Headache, Hyperkinesia (in young children), 5) Xanthines causes Nausea, Vomiting, Gastric irritation, Diarrhoea, Palpitation, Tachycardia, Arrhythmias, Headache, Insomnia.

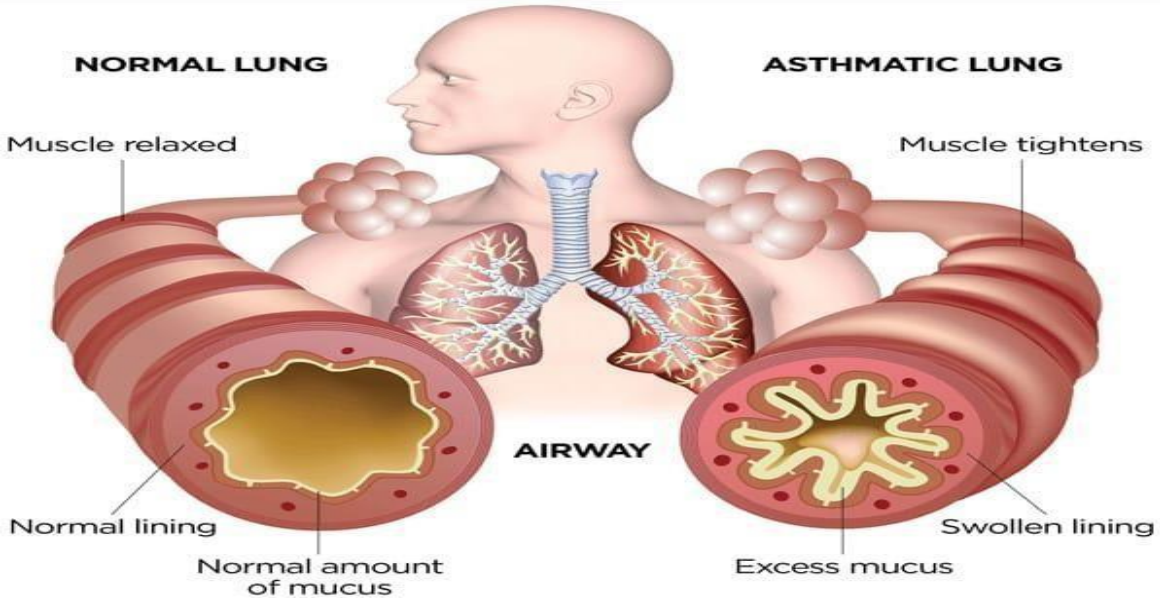

Figure 1: Anatomy of asthma attack. Asthma is characterized by a complex interaction of airflow obstruction, airway hyperresponsiveness and inflammation. The symptoms, which occur during an asthma attack, are spasmodic contraction of the airway smooth muscles, increased mucus secretion and infiltration by immune cells. These structural and cellular changes impede breathing to a greater or lesser extent.

TYPES OF ASTHMA

• There are Asthma is broken down into types based on the cause and theseverity of symptoms

1. Allergic asthma
2. Non-allergic Asthma
3. Seasonal Asthma
4. Occupational asthma
5. Exercise Induced Asthma

• Allergic asthma

Allergic (or atopic) asthma is asthma that's triggered by allergens like pollen, petsand dust mites. About 80% of people with allergic asthma have a related condition like hay fever, eczema or food allergies.

If you have allergic asthma your doctor is likely to prescribe a preventerinhaler to take every day anda reliever inhaler to use when you have asthmasymptoms. It's also important to avoid your asthma triggers as much as possible.

• An allergy is when the immune system mistakes a harmless substance, such as pollen, as dangerous. The bodyreleases chemicals to attack the substance.

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• Another Type of asthma caused by the allergens is called allergic asthma.this type of asthma is triggered by allergens such as dust ,dust mites,mold,pollen,cockroaches,or pet dangens.Asit turns out,allergic asthma is the most common subcategory of the condition.

• Non-allergic asthma

Non-allergic asthma, or non-atopic asthma, is a type of asthma that isn't related to an allergy trigger like pollen or dust, and is less common than allergic asthma.

The causes are not well understood, but it often develops later in life, and can bemore severe.If you think you have non-allergic asthma, see your GP or asthma nurse, who canhelp you find the best way to manage your asthma Intrinsic asthma, also known asnonallergic asthma or nonatopicasthma, has a range of triggers, including weather conditions, exercise, infections, and stress.

Non-allergic asthma is caused by the different irritants we encounter in the air like perfume, fresh paint, room deodorizers, wood smoke, and tobacco smoke.

• Seasonal asthma

Some people have asthma that only flares up at certain times of the year, such as during hay fever season, or when it's cold.

While asthma is always a long-term condition, it's possible to be symptom-free when your triggers aren't around.If you've been diagnosed with seasonal asthma or think you have it, speak to your GP or asthma nurse about the best ways to manage it.

Seasonal asthma is exactly that-when asthma symptomsoccur during different times of the year. This asthma is often due to the change in weather and can be triggered bydifferent allergens such as trees, weeds, and grasses.

You might, for example, only need to take asthma medicines during the season when your asthma bothers you most, and for a short time afterwards.

Occupational asthma Occupational asthma is asthma that's caused directly bythe work you do. You might have occupational asthma if: your asthma symptoms started as an adult and your asthma symptoms improve on the days you're not at work. Occupational asthma is usually a type of allergic asthma .

• Occupation Asthma

Occupational asthma is a type of asthma. It occurs when you breathe in chemical fumes, gases, dust or other substances on the job. When that happens, it causes an allergic or immunological response.

Many substances in the workplace can trigger asthma symptoms, leading to occupational asthma. The most common triggers are wood dust, grain dust, animal dander, fungi, or chemicals.

Occupational asthma is often a reversible condition, which means the symptoms may disappear when the irritants that caused the asthma are avoided. However, permanent damage can result if the person experiences prolonged exposure Factors that increase the risk for developing occupational asthma include existing allergies or asthma, a family history of allergies or asthma, and cigarette smoking. According to the National Institutes of Health, the following workers are at increased risk of developing occupational asthma: Bakers

• Exercise Induced Asthma

Exercise-induced asthma occurs because of the narrowing of the airways in the lungs during strenuous exercise.Suffering from exercise-induced asthma doesn't mean you can't remain active; just be sure you take the necessary treatment when you exercise.

Exercise-induced asthma is when the airways narrow or squeeze during hard physical activity. It causes shortness of breath, wheezing, coughing, and other symptoms during or after exercise.

The medical term for this condition is exercise-induced bronchoconstriction. Many people with asthma have exercise-induced bronchoconstriction. But people without asthma also can have it.

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Most people with exercise-induced bronchoconstriction can continue to exercise and remain active if they treat symptoms. Treatment includes asthma medicines and taking steps to prevent symptoms before physical activity starts.

CAUSES

➢ Causes of Asthma

• Airborne allergens, such as pollen, dust mites, mold spores, pet dander or particles of cockroach waste
• Respiratoryinfections, such as the common cold
• Physical activity
• Cold air
• Tobacco Smoke.
• Dust Mites.
• Outdoor Air Pollution.
• Pests
• Mold.
• Cleaning and Disinfection
• Air pollutants and irritants, such as smoke
• Certain medications, including beta blockers, aspirin, and nonsteroidal anti-inflammatory drugs, such as ibuprofen (Advil, Motrin IB, others) and naproxen sodium (Aleve)
• Strong emotions and stress • Sulfites and preservatives added to some types of foods and beverages, including shrimp, dried fruit, processed potatoes, beer and wine
• Gastroesophageal reflux disease (GERD), a condition in which stomach acids back up into your throat.

SYMPTOMS

• Asthma symptoms vary from person to person. You may have infrequentasthma attacks, have symptoms only at certain times such as when exercising or have symptoms all the time.

➢ Asthma signs and symptoms include:-

• Shortness of breath
• Chest tightness or pain Wheezing
• when sleeping exhaling ,caused which by shortness of breath is a common sign of asthma in children Trouble
• coughing

worsened by a respiratory virus, such as a cold or the flu or wheezing Coughing or wheezing attacks that are

➢ Signs that your asthma is probably worsening include:

• Asthma signs and symptoms that are more frequent and bothersome
• Increasing difficulty breathing, as measured with a device used to check how well your lungs are working (peak flow meter)
• The need to use a quick-relief inhaler more often

1. PULMONARY ROUTE

In recent years, pulmonary drugs have attracted a lot of scientific and biomedical attention, and they've advanced in the sense of local care for lung disorders, appreciations to advances in local targeting and decreased systemic side effects, as well as the administration of minute (shorter) drug dosages.

The pulmonary drug delivery of therapeutic agents has been recognised, but less understood, for many years in the twenty-first century transition in therapy with the use of systemic, with the high surface area and permeability of the lung. The most common route for active substances to be attracted is to treat local pulmonary (e.g., asthma, chronic obstructive pulmonary disease (COPD), and microbial infections) as well as systemic diseases (e.g., diabetes).

For the treatment of various disease states, a wide variety of agents have been administered to the lungs through oral inhalation. Inhalation therapy is most often used to treat obstructive airway conditions with medications like short and

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long-acting sympathomimetics, corticosteroids, and anticholinergic agents. Since the early 1990s, the respiratory route has received more attention as a viable alternative to parenteral drug delivery, especially for inhaled insulin and peptide and protein therapeutics.

1.2.1 Advantages of Pulmonary Drug Delivery[8]

It is a non-invasive method. Useful for drug delivery as it is having a large surface area (80m2- 140m2). Without permeation enhancers, rapid absorption of the drug is possible.

After absorption of the drug no hepatic (first pass) metabolism effect will be seen.

1.2.2 Structure and Function of Lungs [8,9]

Airways: The human respiratory system is split into two functional areas:

1) Conducting airways and 2) Respiratory zone

The pharynx, larynx, trachea, bronchi, and bronchioles filter and condition the inspired air, as do the conducting airways, which regulate the nasal cavity and associated sinuses, the pharynx, larynx, trachea, bronchi, and bronchioles. The airways repeatedly branch shortening into two daughter branches of smaller diameters and shorter lengths than the parent branch from the trachea to the periphery of the airway tree. The number

of divisions doubles with each new generation of airways, and the cross-sectional areagrows exponentially. Generations 0 (trachea) to 16 (terminal bronchi) make up the airway control area.

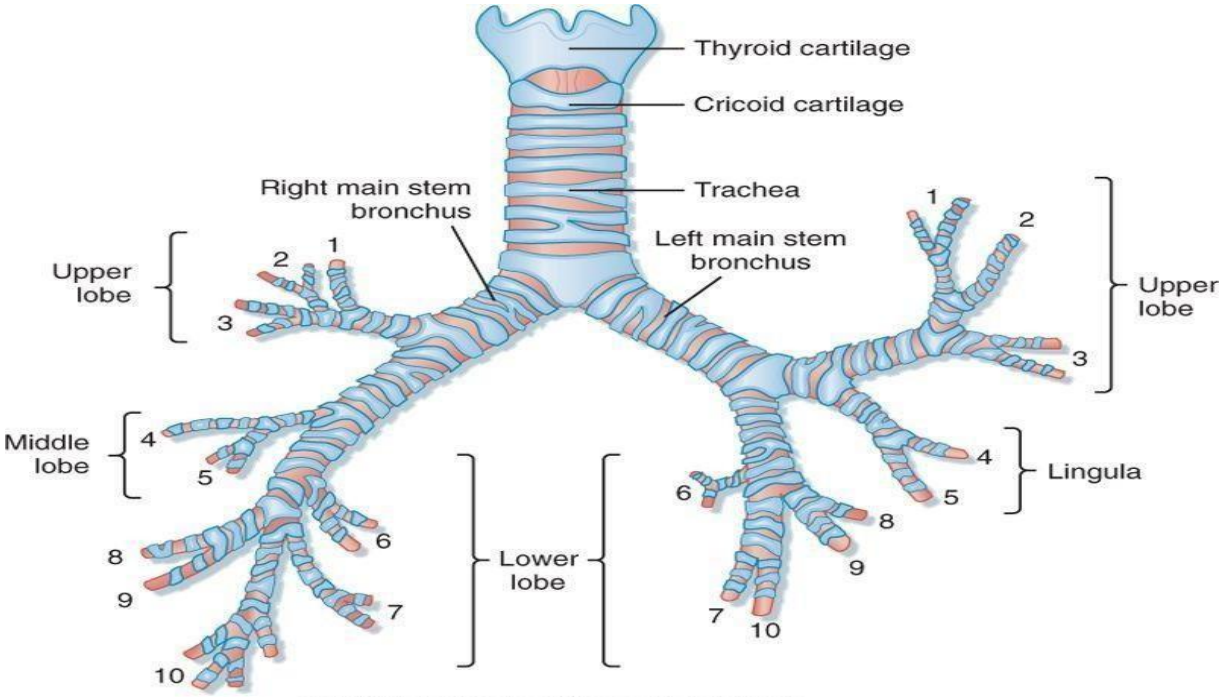

Figure 1.2 Structure of the airways

1.2.3 Mechanisms of Drug Deposition

Drugs are delivered as aerosols or dried powders for inhalation therapy. A suspension of liquid or solid in the form of fine particles scattered in gas is referred to as an aerosol.

For effectiveness, the ability of the aerosolized drug to enter the peripheral airways is critical. The pathology of lung disease may have a significant impact on aerosol deposition. Impaction (inaction deposition), sedimentation (gravitational attraction deposition), Brownian diffusion, interception, and electrostatic precipitation are some of the mechanisms by which particles settle in the respiratory tract. The relative value of both is determined by the properties of the inhaled particles, as well as breathing habits and anatomy of the respiratory tract.

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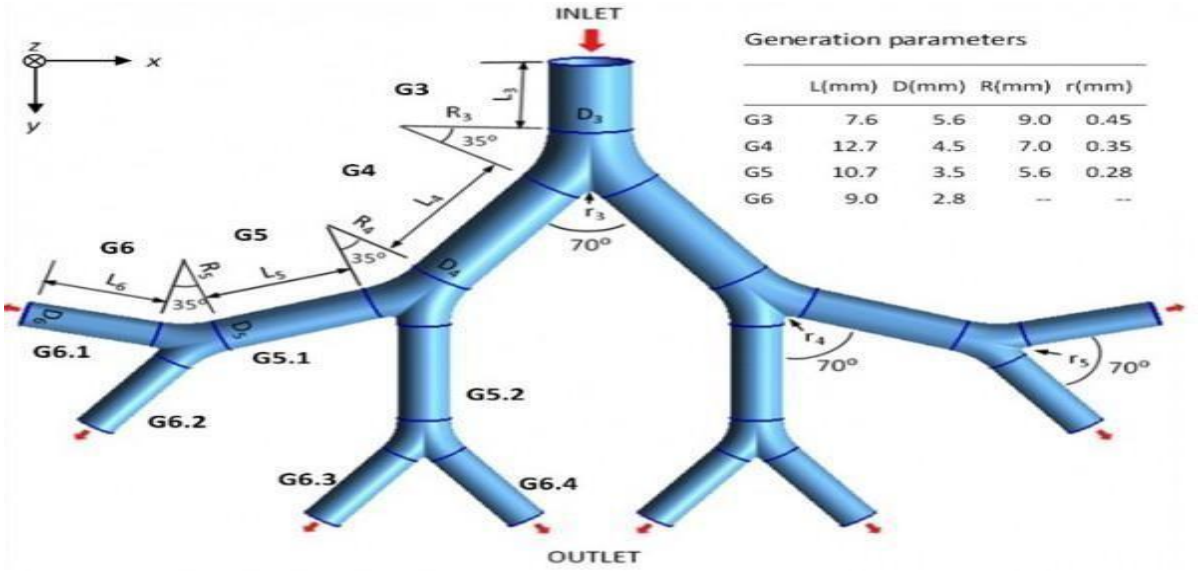

Figure 1.3: Three major deposition mechanisms in lung

Both processes are active at the same time, but the first two are the most critical for large-particle removal in the airways (1 m MMAD 10 mm). Dispersing is also the most important factor in the accumulation of tiny particles in the lung's periphery

1.2.4 Mechanism of Drug Absorption

Accordingly, some in-depth research has been conducted to clarify the absorption processes of pharmaceutical inhaled formulations. Physiological studies of the lungs indicate that the airway and alveolar epithelia, rather than the interstitium and endothelium, are the major barriers that control the passage of drugs and solutes from the airway lumen into the blood circulation. Passive and active transport pathways are needed for drug absorption through the epithelium, including paracellular and transcellular transport, orifice development, vesicular transport, and effluent into the lymphatics, depending on the drug and absorption site.

2. Polyherbal Formulation

Formulations containing more than 2 herbs are called polyherbal formulation. In the Ayurvedic system of medicine mainly polyherbal compounds are used for treatment of various infections. The Unani system of medicine is also gaining global acceptance due to the amazing clinical efficiency of the formulations. Although Unani medicines have long been used, there isnegligible documented evidence regarding their safety and effectiveness. The lack of evaluation has, in turn, slowed down thedevelopment of regulations and legislations.[8] Drug combination often produces a promising effect in treatment of diseases over a single drug. The concept of drug combination has been well established in Western medicine and remarkable success has been achieved over the decades. In recent years, drug combination therapies in cancer and infectious diseases have offered new hope to patients [8].

Advantages of polyherbal formulation

Ayurvedic and herbal medicinal products contain a combination of botanicals; each of these contains a number of chemical compounds that may give the anticipated activity in combination. Herbal medicines are in widespread use and although many believe herbal medicines are safe, they are often used in combination and are drawn from plant sources with their own variability in species, growing conditions, and biologically active constituents. A major hypothetical advantage of botanicals over conventional single-component drugs is the presence of multiple active compounds that together can provide a potentiating effect that may not be achievable by any single compound. Polyherbal formulations have plant-based pharmacological agents which may exert synergistic, potentiative, agonistic antagonistic actions by virtue of its associated diverse active principles themselves.

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These pharmacological principles work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects Based on the nature of the interaction, there are two mechanisms on how synergism acts (i.e., pharmacodynamics and pharmacokinetic) [9]. In terms of pharmacokinetic synergism, the ability of herb to facilitate the absorption, distribution, metabolism and elimination of the other herbs is focused. Pharmacodynamics synergism on the other hand, studies the synergistic effect when active constituents with similar therapeutic activity are targeted to a similar receptor or physiological system [10].

Combination of herbals may act on multiple targets at the same time to provide a thorough relief. Due to synergism, polyherbalism offers some great benefits which lacks in single herbal formulation. It is evident that better therapeutic effect can be reached with a single multi-constituent formulation. For this, a lower dose of the herbal preparation would be needed to achieve desirable pharmacological action, thus reducing the risk of deleterious side-effects. Besides, PHFs bring to improved convenience

for patients by eliminating the need of taking more than one different single herbal formulation at a time, which indirectly leads to better compliance and therapeutic effect. All these benefits have resulted in the popularity of PHF in the market when compared to single herbal formulation.

Polyherbal formulation also having multiple types of molecules against a disease complication so different molecules cure a disease by different mechanism so provide a complete therapy against a disease condition [11].

Limitations of polyherbal formulation

When combinations of plants with these constituents are combined together it may show better activity when compared to the individual extract. But at the same time presence of many constituents may lead to chemical incompatibility which may result in instability [12]

3. Herbal Drugs

Herbal medicine is the leading alternative to conventional pharmaceutical treatment to reduce the side effects. The herbal treatment aims to relieve infections and strengthen immunity using immuno-stimulants and anti-infectives. It reduces swelling and obstruction-using anti- inflammatories. Also relaxes respiratory muscles, increase the elimination of mucus and improve circulation using circulatory tonics. Ajwain oil is known to possess many medicinal properties like expectorant, bronchodilatory, antitussive, antimicrobial, antispasmodic, and antioxidant traditionally used in common cold, cough and asthma. Garlic is a popular medicinal herb used to treat asthma. It is having anti-inflammatory properties which help clear congestion in the lungs. Its antibacterial and antiviral capabilities also assist to build immunity and combat infections that might cause asthma. It promotes histamine breakdown and reduces

histamine release in the body. Histamine, a natural substance produced by the body, is involved in numerous allergic reactions and has been linked to asthmatic inflammation.

It also boosts the capacity of the body to create prostacyclin which is a lipid molecule that helps keep the air passages of the lungs open and thus help ease breathing in asthmatic individuals. There are advantages of herbs over conventional allopathic drugs which prevent the side effects on long term usage.

There are advantages of pulmonary delivery of drugs to treat a respiratory disease like it delivers high drug concentrations directly to the disease site, minimizes the risk of systemic side effects, rapid clinical response, bypass the barriers to therapeutic efficacy, such as poor gastrointestinal absorption and first-pass metabolism in the liver and achieve a similar or superior therapeutic effect at a fraction of the systemic dose.

1.3.1 Traditional herbs / phytomedicine used for treatment of Asthma[10,11]

Phyto Pharmaceuticals are the leading alternative over the conventional pharmaceutical treatment to reduce the side effects of allopathic drugs used for long term treatment.

The herbal treatment relieves infections and strengthens immunity using immunostimulants and anti-infectives. It reduces swelling and obstruction- using antiinflammatories. Also, it loosens up respiratory muscles and increases the elimination of mucus and improving circulation using circulatory tonics.

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In phytomedicine and aromatherapy as well as in pharmaceutical industries, essential oils (EOs), volatile and odour products derived from medicinal plants and aromatic herbs have widespread use and application. Fragrance and pharmacological activities of medicinal plants and aromatic herbs are responsible for the main phytochemical compounds of EOs, e.g. oxygenated sesquiterpenes, monoterpenes, terpenoids like thymol and eugenol and phenylpropanoids (Allicin), including alcohols, aldehydes, carbohydrates, ethers and ketones. There are many biological properties of different EOs and their signature substances, such as antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, wound-healing and in vitro and in vivo anti-cancer effects. These EO includes Thymol (Nadjib BM et al. 2020)10. Recent research into the resistance of SARS-CoV 2 compounds in Garlic Essential Oil shows that 17 organosulfur compounds containing 99.4% of garlic essential oil have good interactions with ACE2 protein amino acids and the key protease PDB6LU7 of SARS-CoV-2. The strongest anticoronavirus activity is expressed in allyl disulfide and allyl trisulfide, which account for the highest content in the essential oil of garlic (51.3 %), (Bui Thi Phuong Thuy, et al 2020)11

1. AJWAIN OIL
2. CLOVE OIL
3. GARLIC OIL

2.3.2 Phyto profile of Potential Herbs

1.3.2.1 Phyto profile of Ajwain Oil: Ajwain, also known as ajowan, caraway, thymol seeds, bishop's weed, or carom, is an annual plant of the Apiaceae family.

Figure 1.5: Photograph of Ajwain Essential Oil12

The phytoprofile of ajwain is as follows in table 1.1 Table 1.1: Phyto profile of Ajwain13

Scientific Classification:

Ajwain Kingdom Plantae

Binomial name Trachyspermum ammi Family Apiaceae

Plant parts used for essential oil Seeds Chemical constituents

Thymol, p-cymene, γ-terpinene, β-pinene, terpinen-4-ol, oleic acid, linoleic acid, palmitic acid.

Other constituents With essential oil, Ajwain seeds also consist of moisture, protein, fat, minerals, fiber, carbohydrates, calcium, phosphorus, iron, carotene, thiamin, riboflavin and niacin.

Organoleptic Properties of oil

Brownish liquid with characteristic odor and sharp hot taste Solubility of oil Insoluble in water and soluble in organic solvent

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The six major constituents present in ajwain are having medicinal properties like Antiseptic, Fungicidal, antispasmodic, antioxidant, Antimicrobial, Anti-inflammatory, antiproliferative, anti- spasmodic, Antibacterial, Fungicidal, anti-Infective Bronchitis Virus

Properties Major Constituents of Ajwain

(1) Thymol14-16 (2) p-Cymene17 (3) γ-terpinene18 Chemical Name 5-Methyl-2-(propan-2-yl)phenol 4-isopropyltoluene or 1-isopropyl-4-methylbenzene 4-Methyl-1-(1-methylethyl)-1,4-cyclohexadiene Mol.Formula C10H14O C10H14 C10H16

Mol.Weight 150.22 g·mol−1 134.21 g/mol 136.24 g·mol−1

Boiling point 232 °C (450 °F; 505 K) 177 °C (351 °F; 450 K) γ: 183 °C

Solubility in water

0.9 g/L (20 °C) Insoluble in water, miscible with ethanol 8.68 mg/L Medicinal properties

Induce Asthma, Antiseptic, Fungicidal, antispasmodic, antioxidant13-16Effective in asthma, Antimicrobial, Antioxidant16Effective in Asthma, Antioxidant, Antiinflammatory, Antiproliferativeproperties

Antiinflammatory, anti-spasmodic, Antibacterial, Fungicidal, anti-Infective Bronchitis Virus properties18 Antibacterial, antifungal18Antioxidant, antiinflammatory

1.3.2.2 Phyto profile of Clove Oil: Cloves are the aromatic flower buds of a tree in the family Myrtaceae, Syzygium aromaticum.

Figure 1.6: Photograph ofClove Essential Oil22

The Phyto profile of Clove is as follows in table 1.2. Table 1.2: Phyto profile of Clove23 Scientific Classification: Clove Kingdom Plantae

Binomial name Eugenia caryophyllus Family Myrtaceae

Plant parts used Flower buds Chemical constituents

Eugenol, Eugenol acetate, Tyranton , Caryophyllene , 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-,Z,Z,Z.

Other constituents

The trace amounts (<1%) of other constituents are vanillin, crategolic acid, tannins such as bicornin, methyl salicylate (painkiller), gallotannic acid, the flavonoidseugenin, rhamnetin, kaempferol, and eugenitin, triterpenoids such as oleanolic acid, campesterol and stigmasterol, and several sesquiterpenes

Organoleptic Properties of oil

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Light yellow liquid with characteristic odor
Solubility of oil Slightly soluble in water, Miscible with alcohol, chloroform, ether, soluble in glacial acetic acid
Six major constituents present in Clove are having medicinal properties like anti H. pylori agents and anti-bacterial agents. It can also act like a fungicidal agent, which can completely inhibit the growth of Saccharomyces cerevisiae, a species of yeast, .antibacterial and anti-virulence agent against drug- resistant Acinetobacterbaumanniias
Table 1.2.1: Major constituents of Clove-124-26 Properties Major Constituents of Clove
(1) Eugenol24 (2) Eugenol acetate25 (3) Tyranton26 Chemical Name
2-Methoxy-4-(prop-2-en-1-yl)phenol 4-Allyl-2-methoxyphenyl acetate 4-Hydroxy-4-methyl-2- pentanone
Mol.Formula $C_{10}H_{12}O_2$ $C_{12}H_{14}O_3$ $C_6H_{12}O_2$
Mol.Weight 164.2 g/mol 206.24 g/mol 116.158 g/mol Boiling point 254 °C 281-286 °C 168.1°C
Solubility Soluble in Organic solvents Soluble in Organic solvents Soluble in Organic solvents Medicinal properties
Anti H. pylori agents and anti-bacterial agents.It can also act like a fungicidal agent, which can completely inhibit the growth of Saccharomyces cerevisiae, a species of yeast.
Antifungal25, antibacterial and antivirulence agent against drugresistant Acinetobacterbaumannii. antibacterial
Table 1.2.2: Major Constituents of Clove 227-29 Properties Major Constituents of Clove
(4) Caryophyllene27 (5) 1,4,7-Cycloundecatriene28(6) 1,5,9,9-tetramethyl-,Z,Z,Z29 Structure
Chemical Name
(1R,4E,9S)-4,11,11- Trimethyl-8-methylidenebicycloundec-4-ene (1E,4E,7E)- cycloundeca-1,4,7-triene 1,5,9,9-tetramethyl-,Z,Z,Z
Mol.Formula $C_{15}H_{24}$ $C_{11}H_{16}$ $C_{15}H_{24}$
Mol.Weight 204.36 g /mol 148.249 g/mol 204.351g/mol Boiling point 126–129 °C 121–125 °C 125–130 °C
Solubility Soluble in organic solvents Soluble in organic solvents Soluble in organic solvents
Medicinal properties Anti-inflammatory,analgesic, antipyretic, and platelet-inhibitory actions
Anti-inflammatory Antiinflammatory
1.3.2.3 Phyto profile of Garlic Oil: Garlic (Allium sativum) is a species of bulbous flowering plant in the genus Allium.

Figure 1.7: Photograph of Garlic Essential Oil30

The Phyto profile of Garlic is as follows in table 1.3:
Table 1.3: Phyto profile of Garlic31 Scientific Classification: Garlic Kingdom Plantae
Binomial name Allium sativum Family Alliaceae
Plant parts used Cloves
Mode of extraction Steam distillation
Chemical constituents Sulphur-containing compounds such as allicin, alliin, ajoene, diallylsulphide, dithin, S-allyl cysteine

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Other constituents It also contains enzymes, vitamin B, proteins, minerals, saponins and flavonoids. Organoleptic Properties of oil

Brownish liquid with characteristic odor

Solubility of oil Insoluble in water and soluble in organic solvent

The major constituents present in Garlic are having medicinal properties as presented below in table 1.3.1 and 1.3.2.

Table 1.3.1: Major Constituents of Garlic-132

Properties Major Constituents of Garlic Allicin32 Alliin32 Ajoene32 Chemical Name

S-Prop-2-en-1-yl prop-2-ene-1-sulfinothioate (2R)-2-amino-3-[(S)prop-2-enylsulfinyl]propanoic acid 2-propen-1-yl (1E)-3-(2-propen-1-ylsulfinyl)-1-propen1-yl Mol.Formula C6H10OS2 C6H11NO3S C9H14OS3

Mol.Weight 162.26 g·mol−1 177.22 g/mol 234.4 g/mol Boiling point decomposes decomposes decomposes Solubility soluble in ethanol and oils soluble in water soluble in ethanol

Medicinal properties

Antiviral, Antifungal effectiveness to prevent the common cold Antioxidant, anti- atherosclerotic , antiinflammatory Antioxidant, antithrombotic (anticlotting), virucidal , anti-leukemia

Table 1.3.2: Major Constituents of Garlic-232

Properties Major Constituents of Garlic Diallyl sulphide32 Dithiane32 S-allyl cysteine32 Structure

Chemical Name

3-[(Prop-2-en-1-yl)disulfanyl]prop-1-ene 1,3-Dithiacyclohexane (R)-2-Amino-3-prop-2-enylsulfanylpropanoic acid

Mol.Formula C6H10S2 C4H8S2 C6H11NO2S

Mol.Weight 146.28 g/mol 120.228 g/mol 161.22 g/mol Boiling point 180 °C 207-208°C 300°C Solubility soluble in ethanol and

oils soluble in ethanol soluble in ethanol Medicinal properties

Detoxication, Antimicrobial, Antioxidant, Antimicrobial, Antioxidant, anticancer, 4. MECHANISM OF ACTION

Thymol, Eugenol and Alliin are considered as phytomarkers for the evaluation activities as they are having medicinal properties against asthma and also they are available in high concentrations in ajwain oil, clove oil and garlic oil respectively. Thymol has been shown to abrogate hyperresponsiveness (AHR) and allergic airway inflammation by attenuating infiltration of inflammatory cells, Th2 cytokines and ovalbumin (OVA)-specific IgE and suppressing the pathological changes due to its NF-

κB activation blocking property. In OVA-induced mice, thymol suppressed the antigenspecific immune response by inducing reductions in TH cells [TH1, TH2 and T-helper cell 17 (TH17)]-related

cytokines and key transcription factors, revealed their ability to influence T-cell activation and the resulting damaging immunological responses3

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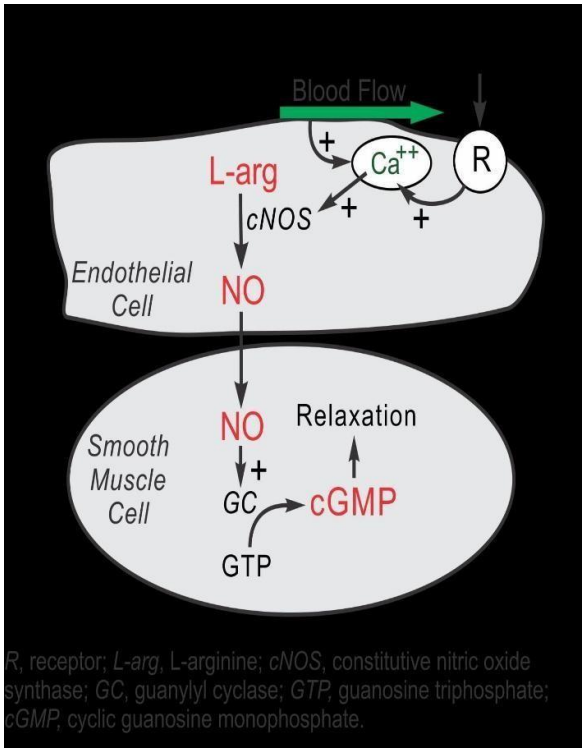

Figure 1.9: Nitric oxide cycle35

Allicin and Diallyldisulphide decreased nitric oxide production with a reduction in the levels of interleukins (IL)-1β and IL-6 in RAW264.7 cells stimulated with LPS. Proinflammatory proteins such as inducible nitric oxide synthase (iNOS), nuclear factor (NF)-kB, and matrix metalloproteinase (MMP)-9 were reduced by DADS, whereas antioxidant proteins such as Nrf-2 and heme oxygenase (HO)-1 were increased36.Eugenol having an inhibitory effect on leukotriene. Eugenol was found to significantly inhibit the formation of LTC(4) in calcium ionophore A23187 and arachidonic acid (AA) stimulated PMNL cells37

5. ANALYTICAL METHODS

1.5.1 Standardization of Herbal essential oils38For characterising samples, quantification of biomarkers and/or chemical markers, and fingerprint profiles, the World Health Organization (WHO) emphasises the importance of qualitative and quantitative approaches. It is most rational to quantify a principal active component if one is identified. Botanical formulations that contain proven active ingredients that contribute to therapeutic effectiveness should be standardised to these compounds. When the active ingredients of a botanical are unknown, a marker substance that is unique to that botanical may be chosen for analytical purposes.

"Standardization refers to the body of knowledge and control required to produce content of fair consistency," according to the American Herbal Product Association.

This is accomplished by using quality assurance practices in agricultural and industrial processes to reduce the inherent difference in natural product composition.

All aspects that contribute to the quality of herbal drugs should be considered in standardisation methods, including sample identity, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and bifurcation testing. The phytochemical profile is particularly important because it has a direct impact on the action of herbal drugs.

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1.5.2 Identification and Quantification of essential oils

Identification and Quantification of essential oils are very important as the Essential oils are complex matrices that are primarily made up of terpenoids. Due to the close resemblance of these compounds, accurate identification is difficult. The phyto markers composition in any oil varies greatly depending on many factors, including the stage of production, the environment, and other factors. And also the essential oil's market value varies depending on the amount of the marker compound present in it which is therapeutically active. Based on the identification it proves the purity of the material and also based on the quantification of the phytomarkers it helps in finalizing the dose of the finished product. Different analytical tools and techniques are used like FTIR, RP- HPLC, GC-MS, LC-MS method etc.

1.5.3 Analytical Method development and validation of simultaneous estimation of phyto markers present in essential oils39Validation aims to show that an analytical technique is appropriate for its intended purpose. Validation is evidence that is recorded that offers a high level of certainty for a particular procedure.

1.5.3.1 Validation Parameters39

1.5.3.1.1 Specificity

It is the capacity to assess the analyte unequivocally in the presence of components that may be present. Impurities, degradants, matrices, and other substances are examples of these. This can be demonstrated in the assay by spiking pure substances (drug substance or drug product) with appropriate levels of impurities and/or excipients and demonstrating that the assay result is unaffected by the presence of these materials; practically, this can be done by spiking pure substances (drug substance or drug product) with suitable quantities of contaminants and/or excipients, indicating that their presence has no effect on the assay result (by comparison with the assay result obtained on unspiked samples).

1.5.3.1.2 Accuracy

The closeness of agreement between the value accepted as a standard true value or an accepted reference value and the value found is represented by the accuracy of an analytical method. By spiking samples in the sample, accuracy is calculated as the percentage of analyte recovered by assay. A known volume of analyte was spiked in the sample mixture at a concentration of 50 % to 150 %. A minimum of 9 determinations of at least three concentrations in the given range should be used to determine accuracy.

Criteria for acceptance: % The recovery should be between 98 % and 102 %. 1.5.3.1.3 Precision The degree of scattering (closeness of agreement) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the specified conditions is expressed by the precision of an analytical method. There are three degrees of precision: repeatability, intermediate precision, and reproducibility.

Acceptance criteria: % Relative standard deviation should be less than 21.5.3.1.4 RepeatabilityThe term "repeatability" refers to the accuracy of a measurement made under the same operating conditions over a short period. Intra-assay precision is another word for repeatability.A minimum of 9 determinations covering the procedure's defined spectrum (e.g., 3 concentrations/3 replicates each) OR a minimum of 6 determinations at 100% of the test concentration should be used to determine repeatability.

1.5.3.1.5 Intermediate precision

Variations within laboratories are expressed by intermediate precision: different days, different analysts, different equipment, and so on. 1.5.3.1.6 ReproducibilityThe term "reproducibility" refers to the consistency of results across laboratories (collaborative studies, usually applied to standardisation of methodology).

1.5.3.1.7 Detection Limit

The lowest quantity of analyte in a sample that can be detected but not generally quantified as an exact value is the detection limit of an individual analytical technique.

Depending on whether the technique is non-instrumental or instrumental, there are many approaches to deciding the detection cap.

Acceptance criteria: S/N ratio > 3 or 2:1

1.5.3.1.8 Quantitation limit

The lowest quantity of analyte in a sample that can be quantitatively measured with sufficient precision and accuracy is the quantitation limit of an individual analytical technique. The quantitation limit is a parameter of quantitative assays

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for low levels of compounds in sample matrices, and it is used to determine impurities and/or degradation products in particular.

Acceptance criteria: S/N ratio > 10:1

1.5.3.1.9 Linearity

The ability of an analytical technique to produce test results that are directly proportional to the concentration of analyte in the sample is known as linearity. The suggested technique can be used to demonstrate it directly on the drug material (by dilution of a regular stock solution) and/or separate weighing of synthetic mixtures of the drug product components. The latter element may be investigated as part of the range investigation. Acceptance criteria: The Correlation coefficient should not be less than 0.999.

1.5.3.1.10 Range

The range of an analytical technique is the interval between the analyte sample's upper and lower concentrations. The defined range is usually derived from linearity studies and is dependent on the procedure's intended implementation.

1.5.3.1.11 Robustness

The robustness of an analytical technique is a measure of its ability to remain unaffected by minor yet deliberate changes in system parameters, and it indicates its reliability in regular use.

Acceptance criteria: % Relative standard deviation should be less than 2. 1.5.3.1.9 Linearity The ability of an analytical technique to produce test results that are directly proportional to the concentration of analyte in the sample is known as linearity. The suggested technique can be used to demonstrate it directly on the drug material (by dilution of a regular stock solution) and/or separate weighing of synthetic mixtures of the drug product components. The latter element may be investigated as part of the range investigation.

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Acceptance criteria: The Correlation coefficient should not be less than 0.999. 1.5.3.1.10

Range The range of an analytical technique is the interval between the analyte sample's upper and lower concentrations. The defined range is usually derived from linearity studies and is dependent on the procedure's intended implementation.

6. FORMULATION DEVELOPMENT OF NANOPARTICLES

Development of nanoformulation for pulmonary delivery: The nanocarrier delivery offers various advantages such as faster- onset, prolonged effect, greater regular dosing and efficiency equivalent at the lower level of doses8 Emerging drug delivery innovations such as nanoparticles (NPs) will improve the therapeutic index of medications and eventually lead to improving patient compliance (De Jong and Borm, 200840; FernándezTena and CasanClarà, 201241). The small size of the NPs (10- 1000 nm) can also promote their fast absorption of alveolar macrophages, thereby demonstrating a stronger local and target selective distribution of drugs (Paranjpe and Müller-Goymann, 201442; Singh and Lillard, 200943). The polymeric NPs were selected in this context because they display higher encapsulation, strong surface engineering properties, resistance to enzymatic degradation and longer-lasting controlled release of drugs. Chitosan, polylactic acid, poly (ε-caprolactone), polylactic-co-glycolic acid, etc., are different polymers used in the processing of NPs (Grottkau et al.,201344; Dutta et al., 2012; Gelperina et al., 200545).

There are two opposing strategies for making nanosuspensions:

1. Bottom-up technologies
2. Top-down technologies

Bottom-up technology is characterized as a method of forming solid particles that begin at the molecular level and progresses through molecular association. Top-down strategies include beginning with a large-sized powder and growing its bulk.

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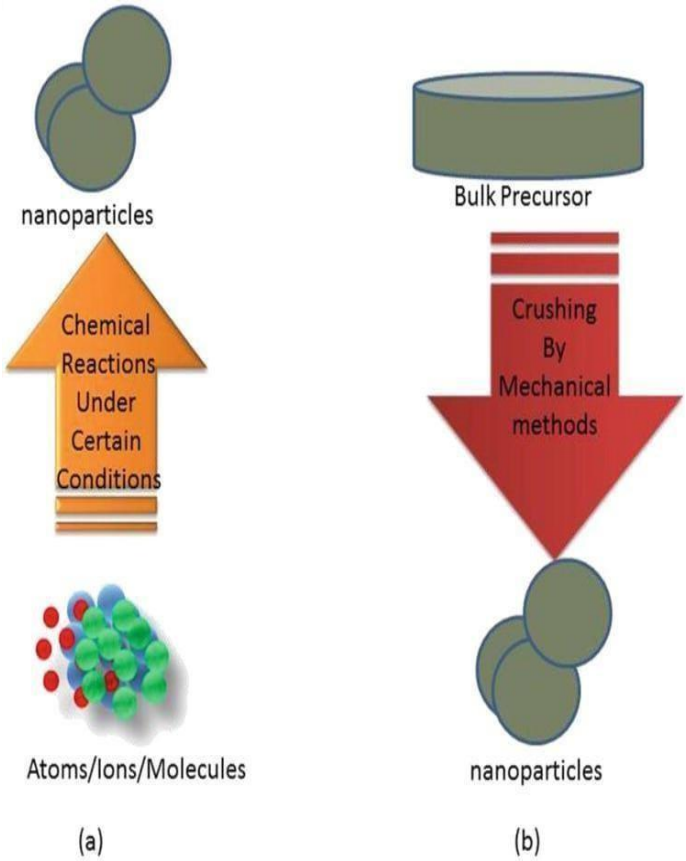

Figure 1.10- Schematic of top down and bottom up techniques

The Bottom-up techniques primarily include emulsion/microemulsion templates, nanoprecipitation, and melt emulsification process while Top-down techniques include high-pressure homogenization, milling and sonication methods. Top-Down Technologies Sonication Method: Ultrasonic waves are formed in a liquid suspension by rinsing an ultrasound probe into it or by placing the sample container with the suspension in a bath containing a liquid that raises ultrasonic waves. (Sonication
is used indirectly). The sound waves that produce into the liquid media at high force on sonication liquid effect like result in alternating high-pressure and low-pressure (rarefaction) periods, with rates that are depending on the frequency. During a high-pressure loop, the bubbles explode cruelly as they reach a volume where they can no longer absorb energy.

7. SCREENING OF POLYMERS USING TAGUCHI DESIGN

Taguchi Design was applied for the screening of polymers. Design expert 13 software was used for determining the factors of selected responses. Here, the particle size and % entrapment efficiency are the two factors considered to be independent factors. Amount of polymer influences the particle size of the final formulation. The seven others factors were selected and considered to be the dependent variables. The batches obtained were prepared according to the low value and high value given by the software and evaluated for particle size and % entrapment efficiency. The response result obtained was added in the design and further analysis obtained the Pareto chart. After that, the Validation batches were prepared and the significant and non-significant factors were obtained from the Pareto chart. The significant factors obtained will be considered while applying the factorial design.

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8. INTRODUCTION TO STATISTICAL CALCULATION

The compilation, presentation, study, and interpretation of numerical data are all examples of statistics. When a new analytical method is being developed, it is common to practise to equate the new method's mean and precision to that of existing procedures. The statistical analysis entails the use of a variety of methods to compare findings in different formats. The most common tools used are as follows:

ANOVA:

Method for evaluating data from planned experiments to compare the means of two or more groups. The one-way analysis of variance is the right statistical approach to compare the means when more than two assay methods are being compared (ANOVA). It is a method of dividing total variability in a collection of data into component sections, which is expressed by a statistical model.

The application of statistical tools among the newly developed method is defined in this section, which reveals whether the method is statistically significant or not by comparison.

The statistical analysis was carried out with the aid of the QI macros programme and Excel.

9. LYOPHILIZATION

The term lyophilization refers to the process of making a solvent-loving substance. In terms of operation, freeze-drying can be described as a controllable method of vacuum desiccation for dehydrating labile materials. Freezing of the liquid sample, preceded by the conversion of water into ice and crystallisation of crystallizable solutes, as well as forming of an amorphous matrix containing non-crystallizing solutes associated with unfrozen moisture. Moisture in the amorphous matrix evaporates.

Desorption of chemisorbed water in the cake that is dry Lyophilization, commonly known as freeze- drying, is a process that involves freezing water and removing it from a sample, first by sublimation (primary drying) and subsequently by desorption (secondary drying) Freeze drying is a drying method in which water is evaporated from a commodity after it has been frozen. A drying method applicable to the manufacturing of some drugs, pharmaceuticals, and biologicals that are thermolabile or otherwise unstable in aqueous solutions that are stable in the dry state is used for long-term storage. The term lyophilization refers to the method of creating a substance that prefers to be dry. To allow ice to sublimate, lyophilization is done at temperatures and pressures below the triple point of water.

In general, lyophilization is characterised as a stabilising process in which a sample is frozen, followed by sublimation and desorption to reduce the water content to levels that prevent biological growth or chemical reactions.

The first freeze-dried agricultural items were used to store potatoes about a thousand years ago in ancient South America. It was first recorded in 1890 that biological specimens can be stored under vacuum and at temperatures below 0 degrees Celsius.

With the number of antibiotics and other susceptible pharmaceuticals, interest in freezedrying, now commonly referred to as lyophilization, grew dramatically. Lyophilization has developed into a well- established technology for the preservation of biopharmaceutical materials by the turn of the century. Fundamental principles in process design and formulation production have been developed during this period.

10. DRY POWDER INHALER

Inhaled particles move down the trachea and into the bronchi, then into finer and finer bronchioles, finally arriving at the microscopic, sac-like alveoli of the lungs, where gaseous exchange with the blood is primarily performed. Small compounds, such as essential oil ingredients, are transported into the bloodstream very efficiently by the alveoli. The rate of blood flow through the lungs, the rate and depth of breathing, and the fat-solubility of the molecules all contribute to this efficiency (Breuninger et al 1970; Römmelt et al 1987).

This inhaler system is critical to the successful production of DPI drugs. There are threemain types of inhaled drug distribution systems:

1) Pressurized metered-dose inhalers (pMDIs) such as Becorideforte and Becoride plus,

2) dry powder inhalers (DPIs) such as Asthalin and Beclate, and

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3) nebulizers Consider the following scenario: (Aracort, Duolin,)

Each class has its own set of advantages and disadvantages. In contrast to both pMDIs and DPIs, nebulizers remove or suspend the substance in a polar solvent, usually water.

Since they are bigger and less suitable, and the aerosol is distributed continually for an increasing period, nebulizers are often used in hospital and ambulatory treatment settings and are not usually used for chronic disease treatment. pMDIs and DPIs are two types of bolus drug delivery systems that have solid drugs suspended or dissolved in a nonpolar volatile propellant or a dry powder mix (DPI) that is fluidized as the patient inhales. Dry powder inhalers (DPIs) are instruments that distribute a dry powder formulation of an active drug through the pulmonary route for local or systemic effect. Inhalable dry powders are either loose clusters of micronized drug particles of aerodynamic particle sizes smaller than 5m, or carrier-based connective mixtures of micronized drug particles adhered to the surface. Particle size of 2–5 m71,72 is required for topical respiratory drug delivery, while a particle size of less than 2 m is required for drug deposition in the narrow peripheral airways for systemic results. Due to impaction in the throat (i.e., oropharyngeal delivery) and oral absorption, particles larger than 5m can cause systemic effects. The powder composition is aerosolized using a DPI unit, which separates the drug particles from the carrier (from drug-carrier mixtures) of deagglomerates drug particles and delivers the dosage deep into the patient's lungs.

Particle size and flow properties, formulation, drug–carrier fixing, respiratory flow rate, and DPI device configuration all contribute to good efficiency in these systems. There is a wide variety of DPI products available on the market, including single and multipledose devices, breath controlled and power operated devices. DPI systems are currently divided into three groups depending on their design: first-generation DPIs, secondgeneration DPIs, and third-generation DPIs.

11. STABILITY STUDIES

Since stability is one of the most important factors in maintaining the quality and effectiveness of drug drugs, its evaluation has become increasingly important in the pharmaceutical industry.

However, in the pharmaceutical sector, stability issues and assessing the stability of drug-loaded nanoformulations remain a major challenge. Drug nanoparticle stability issues could occur during processing, storage, and shipping. While recent advances in analytical technology have provided a plethora of instruments for assessing the stability of nanopharmaceuticals, they all have performance limitations.

Pharmaceutical stability research is a large analysis that looks at how the nature of a drug substance varies over time as a result of environmental conditions like temperature, humidity, and light.

Stability monitoring is usually advised during the manufacture of new products to determine the drug's shelf life and prescribe the best storage conditions. Stability tests on all potential drug formulations, including nanomedicines, should include all conditions that are vulnerable to modification during shipment and storage and are likely to affect the final product.

12. ANIMAL STUDIES

Clinical trials of asthma cannot resolve all facets of the disease's pathophysiology. Animal models have been created to help understand these pathways and to assess the effectiveness and effectiveness of treatments before they are tested in humans. Animals such as Drosophila, rodents, guinea pigs, kittens, goats, pigs, monkeys, and equines have been used in laboratory asthma models. Rat / Mice, especially BALB/c mice, have been the most commonly studied species in the last two decades. Animal asthma models attempt to replicate the pathophysiology of the human condition.

Traditionally, they have two phases: sensitization and challenge. Standard methods of sensitization include intraperitoneal and subcutaneous injections, but intranasal instillation of allergens is becoming more common since allergen inhalation causes human asthma. Allergen challenges may be administered through aerosol, intranasal, or intratracheal instillation. Few trials, however, have compared different sensitization and challenge paths. Another significant consideration in creating a healthy animal model is the causative allergen. Ovalbumin has been substituted by aeroallergens, such as house dust mites, to use the allergens that cause human illness, while being more traditional and causing severe inflammation.

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1.12.1 Allergens and agents

The most common allergen is OVA. It's made from chicken eggs and can be massproduced in vast quantities, making it more affordable. OVA has been used in asthma research and has been shown to cause severe allergic pulmonary inflammation.

Nonetheless, OVA does not cause airway inflammation in humans, and its suitability as an asthma allergen has been debated. In animal models, HDM (House Dust Mite - Dermataphagoides pteronyssinus)) generally used to successfully cause asthma. This allergen is ideal because it has an inherent enzymatic function, immunogenicity, and direct activation of innate immune cells through the Dectin-2 receptor, which promotes allergic inflammation.

1.12.2 Routes of sensitization and challenge

Several methods in terms of sensitization and difficulty have been studied to improve both acute and chronic animal models of asthma. Since the 1980s, the IP route has most likely been the most popular method of inducing sensitization. Animal sensitization with two IP injections separated by 7– 14 days and a challenge with the culprit allergen one week later was one of the most commonly used protocols.

In terms of pulmonary inflammation, an influx of eosinophils can be seen in experimental asthma models, especially in the airways, peribronchial space, and parenchyma. In addition to a rise in lymphocytes, macrophages, and neutrophils, there is an increase in eosinophil count in blood and BALF. Dendritic cells may also move into the ganglia from the outside, interacting with sensory neurons and stimulating or protecting allergic airway inflammation.

13 . THERAPEUTIC POTENTIAL OF POLYHERBAL FORMULATIONS

Polyherbal formulation having Antiasthmatic activity:

1. A polyherbal formulation (PHF) was prepared by using ethanolic extract of Adhatoda vasica, Clerodendrum serratum, Curcuma longa, Solanum xanthocarpum and Piper longum in the proportion of 40%, 30%, 10%, 10% and 10%, respectively. The mast cell stabilizing and anti- anaphylactic property of this PHE was investigated against compound 48/80-induced mast cell degranulation as well as triple antigen-induced anaphylaxis in rats. The polyherbal formulation produced significant reduction in the mortality of rats subjected to triple antigen-induced anaphylactical shock. It also depicted marked protection of rat mesenteric mast cells from disruption by compound 48/80 in dose dependant manner. Their study suggested anti-anaphylactic and mast cell stabilizing properties of the polyherbal formulation [17].

2. HK-07 is a polyherbal formulation containing mainly the extracts of Curcuma longa, Zingiber officinale, Piper longum, Emblica officinalis, Terminalia belerica, Ocimum sanctum, Adhatoda vasica and Cyperus rotundus was prepared. The antianaphylactic activity of HK-07 was investigated in rats using the active anaphylaxis model. Treatment with HK-07 at different test concentrations showed significant reduction in signs and severity of symptoms ($P<0.05$), onset ($P<0.001$) and mortality rate ($P<0.05$) following anaphylactic shock-induced bronchospasm. HK-07 also significantly reduced the serum IgE levels ($P<0.001$) in animals compared to untreated controls [18].

3. Bharangyadi is a polyherbal compound having Clerodendrum serratum, Hedychium spicatum and Inula racemosa as an ingredient herbs. Evaluation of the anti-asthmatic activity of Bharangyadi through various in-vitro and in-vivo experimental models was carried out by Divya Kajaria et al. The results demonstrate that Bharangyadi has potent histamine antagonism property with significant mast cell stabilizing and spasmolytic activity in the experimental animals [19].

4. Thuthuvalayathy Chooranam is a polyherbal formulation contains Solanum trilobatum, Aristolochia indica, Alpinia officinarum, Nigella sativa, Madhuca lonifolia, Zingiber officinale, Piper nigrum, Piper longum, Terminalia chebula, Ferula asafetida and Piper longum. Evaluation of safety of the Thuthuvalayathy Chooranam through acute and sub-acute toxicity study was carried out. The study revealed that Thuthuvalayathy chooranam formulation at different doses of 270, 1350, 2700 mg/kg did not show toxic effects in the animal's tissues and it was safe when administered to bronchial asthma patients [20].

5. An herbal compound formulation Pentapala-04 prepared from five medicinal plants namely, Adhatoda vasica, Ocimum sanctum, Coleus aromaticus, Glycyrrhiza glabra and Alpiania galangal. The effect of "Pentapala-04" on ova albumin and aluminium hydroxide induced lung damage in albino wistar rats was investigated. The rats were divided

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into three groups of four animals each. Group I, II and III serves as control, toxic and post treatment group respectively. The results showed that there was increased level of lipid peroxidation and decreased level of antioxidants in toxic group animals.

But the levels of antioxidant enzymes were restored in post treated groups of animals, which might be due to the ability of Pentapala-04 to scavenge the reactive oxygen species. Thus they demonstrated that 'pentapala-04' prevents ova albumin and aluminum hydroxide induced oxidative stress, lung injury and inflammatory changes and can be used as an antiasthmatic drug [21].

6. A polyherbal formulation prepared form ethanolic extract of the leaves of Solanum xanthocarpum, Murraya koenigii, Aegle marmelos and Caesalpinia bonduc and evaluated for antiasthamatic activity using animal models. The result reveals mast cell stabilization, antihistaminic and anticholinergic actions of polyherbal formulation [22].

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