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Review Article

AN EXPEDIENT APPROACH TO TREAT ASTHMA THROUGH NON-STEROIDAL, NATURAL TRANSFEROSOMES AEROSOL SYSTEM

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ABSTRACT

Asthma is the most common respiratory disease, affecting an estimated 262 million people and resulting in 461,000 fatalities in 2019. The treatment is available on the market, but it is quite expensive, and it also has serious adverse effects due to the high concentration of steroids in the medicine. If given effectively, curcumin, formononetin, and matrine's anti-inflammatory properties can play a significant role in treatment. To improve the chemical stability and therapeutic potential of these active pharmaceutical ingredients (APIs) in the respiratory tract, a transferosomes system was designed, which encapsulates the APIs inside its vesicular structure and delivers them selectively to the inflamed cells. The DPPC layer will allow for efficient penetration, whereas Tween-80 will aid in deformability and lower interfacial tension, resulting in a small Z-average diameter, allowing for efficient penetration between layers of cells. The APIs' stability at alkaline pH (7.6) is ensured by the nano-vesicular structure, which significantly increases cellular antioxidant activity and ferric reducing antioxidant power values. On the RAW264.7 cell line, the formulation will be tested for anti-inflammatory activity. Nuclear factor kappa B, tumor necrosis factor, interleukin (IL)-1, IL-2, IL-6, IL-8, IL-12, nitric oxide, and cyclooxygenase-2 are all reduced by curcumin, formononetin, and matrine. They also have an inhibitory effect on the MAPK signaling pathway, preventing extracellular signal-regulated kinase, c-Jun N-terminal kinase, and p38 from causing inflammation. This formulation can effectively treat asthma without the use of steroids, has no adverse effects, and is inexpensive.

Keywords: Non-steroidal treatment of asthma, Curcumin, Formononetin, Matrine, Asthma, Nanoparticles, Transferosomes.

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INTRODUCTION

Asthma is the most common type of respiratory problem an estimation of 262 million individuals, resulting in 0.46 million fatalities [1]. Smokers are more vulnerable than non-smokers. The genetic risk of asthma is also very high. It has been seen for many years that the incidence rate in Blacks is more than in Caucasians.

There are several types of asthma: Allergic asthma, non-allergic asthma, cough-variant asthma, nocturnal asthma, exercise-induced bronchoconstriction, and occupational asthma.

The traditional techniques which include steroidal treatment used to treat asthma are highly expensive, have severe side effects, and after these exhausting stage, the reoccurring percentage of asthma is also very high. The proclivity of non-steroidal techniques is increasing due to their lesser side effects, cost efficiency, and convenience.

Curcumin is a bright yellow chemical produced by plants of the *Curcuma longa* species. It is the principal curcuminoid of turmeric (*C. longa*), a member of the ginger family, *Zingiberaceae*. It possesses one of the best anti-inflammatory potency, but shows low bioavailability and highly unstable hence treatment is strenuous. Curcumin has low oral bioavailability (0.47%) [2], it is very unstable on pH 7.4.

Matrine and formononetin also possess similar anti-inflammatory potential as of curcumin. The predominant constituent is highly soluble in water. Matrine is an alkaloid found in plants from the genus Sophora and formononetin is an O-methylated isoflavone.

Loading curcumin, formononetin, and matrine inside a transferosome nano-vesicular structure can increase the biostability of the drug by many folds. Transferosome is chosen to be the carrier because of its high permeability and high deformability. The transferosome is composed of DPPC as phosphatidylcholine component and Tween-80 as edge activator component which will be produced by thin film hydration technique/rotary evaporation-sonication method.

Further the transferosomes system will be mixed with ethanol as solvent and mixed with HFA-134a (propellant) for aerosol system.

Further, this aerosol system will be tested on different parameters such as pH effects, cellular antioxidant activity (CAA), ferric reducing antioxidant power (FRAP), shape, size, and zeta potential and assay testing on RAW264.7 cell line. The drug will be delivered through pMDI in addition to spacers for better drug consumption. Due to this novel drug delivery system, the drug directly reaches the receptor in the lungs bypassing the liver reducing t1/2 increasing clearance rate and bioavailability.

ASTHMA

Asthma is a chronic inflammatory condition of the airways. Chronic inflammation is linked to airway hyper-responsiveness (an exaggerated response by narrowing the airway in response to a specific trigger such as viruses, exercise, or allergens), which results in recurrent episodes of wheezing, dyspnea (breathlessness), chest tightness, and coughing that vary in frequency and intensity over time [3]. Because of the excessive secretion of eosinophils, mast cells, and activated T helper cells, airway hyper-responsiveness becomes more severe. These inflammatory cells produce mediators, which cause bronchoconstriction, mucus secretion, and airway remodeling. Cytokines, chemokines, growth factors, lipid mediators, immunoglobulins, and histamine are some of the inflammatory mediators that drive this process [4].

Inflammation in asthmatic airways not only involves the trachea and bronchi but also extends to the terminal bronchioles and parenchyma. The small airways are capable of producing T-helper-2 cytokines,

as well as chemokines, which have recently been recognized as a predominant site of airflow obstruction in asthma. Furthermore, in asthma, small airways show increase in IL-5 and IL-4 mRNA-positive cells [5]. The inflammation at this distal site is considered as more severe than inflammation in large airways.

PREVALENCE RATE

In 2019, asthma afflicted an estimated 262 million individuals, resulting in 0.46 million fatalities. Asthma prevalence rates vary greatly between countries, ranging from 21% in Australia to 0.2% in China [6]. The hygiene hypothesis states that repeated exposure to a variety of common infections (such as food-borne bacteria, orofecal parasites, and hookworms) and another hypothesis states that exposure to environmental microbiota during childhood is strongly linked to a healthy immune system maturation that protects against the development of asthma or allergies later in life. People who live in communities with a diversified microbiological flora have a lower risk of asthma. Although the prevalence rate in industrialized countries is higher, it has been demonstrated that asthma is more deadly in underdeveloped countries (accounting for roughly 80% of asthma deaths globally) [7].

CAUSES

Asthma can be caused by a variety of variables, including a complex mix of environmental, immunological, and host genetic factors. Asthma is caused by two types of allergens: Allergic (pollen grains, house dust mites, cockroach residues, animal hairs or fur, and molds) and non-allergic (viral infections, bacterial infections, tobacco smoke exposure, cold air, and exercise). Asthma can be induced by genetic causes, although the pattern of inheritance is unknown [8].

CURCUMIN

Curcumin (diferuloylmethane) is derived from turmeric (*C. longa* Linn). Overall curcumin comprises 3–4% of whole turmeric constituents. It is responsible for the yellow color of turmeric, and it comprises of curcumin I (90–94%), curcumin II (6–8%), and curcumin III (0.5–1%).

It has anti-inflammatory, antioxidant, anti-carcinogenic, hypotensive, anti-mutagenic, anticoagulant, antiprotozoal, antifertility, anti-aging, hepatoprotective, antidepressant, anti-venom, antidiabetic, antibacterial, anti-fibrotic, antifungal, antiviral, antiulcer, and hypocholesteremic potential [9,10]. It shows little or no side effects even at high doses. Curcumin is highly unstable at pH 7.4 and degrades quickly but quite stable at acidic pH [11] (Fig.2).

HOW CURCUMIN WORKS AGAINST INFLAMMATION

- Curcumin is a very good inhibitor of prostaglandins. It binds efficiently with cyclooxygenase-2 (COX-2) and then stops its conversion into PGG2 hence stopping the production of prostaglandins [12]
- Similarly, curcumin binds with (Lipoxygenases) 5-LOX and inhibits the productions of LTs (interleukins [ILs]) [13].
- Nitric oxide (NO) a pro-inflammatory mediator. Overproduction of NO by inducible NO synthase (iNOS) forms reactive nitrogen species, resulting in cell death in surrounding tissues and the disruption of tissue homeostasis. Curcumin inhibits iNOS which stops the production of NO
- Curcumin is also involved in PPARγ activation which suppresses airway hyper-responsiveness, which reduces inflammation and epithelial hyperplasia in the airway
- Inhibits the production of ILs: IL-1, IL-2, IL-6, IL-8, and IL-12 [14]
- It also decreases inflammatory mediator (IL-4, IL-5, and IL-13) synthesis and release which reduces mucus hypersecretion and inhibits collagen deposition
- Regulates chemokine monocyte chemoattractant protein
- Curcumin is also proven to work against the activity of histamine and β-hexosaminidase releases from IgE. Hence, it is also acts as mast cell stabilizer
- Inhibited the expression of T-helper type 2 cytokines (IL-4 and IL-13) in bronchoalveolar lavage fluid curcumin which can protect against asthmatic mucus secretion and airway hyper-responsiveness through an increment of Nrf2 molecules and HO-1 levels in lung [15]
- Curcumin inhibits the toll-like receptor 4 which stops the activation of transcription of inflammatory molecules through stimulation by MyD88 pathway hence directly regulates the release of pro-inflammatory cytokines [16]
- Curcumin also degrades the production of IκB-α, which further stops the activation of nuclear factor kappa B (NF-κB), a pro-inflammatory transcription factor
- Curcumin inhibits tumor necrosis factor alpha (TNF-α), which is mediator of inflammation [17]
- It shows inhibitory action on Janus kinase-STAT signaling which contributes to its anti-inflammatory activity.

FORMONONETIN

Formononetin is an *O*-methylated isoflavone, it is widely present in legumes, clovers (red clovers *Trifolium pratense* L.) and in Chinese herb *Astragalus membranaceus* (Fisch).

It has been known to be endowed with numerous pharmacological attributes such as anticancer, anti-inflammatory, antioxidant, anti-allergic, anti-inflammatory, antiproliferative, growth inhibitory, vasorelaxant, neuroprotective, anti-apoptotic, cardioprotective, mammary gland proliferative, and antimicrobial activities [18,19]. (Fig.3).

ANTI-INFLAMMATORY ACTION

- Formononetin inhibits the pro-inflammatory transcription factor NF-κB [20]
- With the decrease in NF-κB is a significant transcription factor for the induction of NO synthase, there is a decrease in the production of NO *in vitro*
- Formononetin demonstrated an inhibitory effect on the MAPK signaling pathway
- The MAPK pathways modulate inflammatory gene transcription through the phosphorylation of the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 proteins. With an inhibitory effect on MAPK signaling pathway, these ERK, JNK, and p38 are unable to produce any inflammatory effect [21]
- Formononetin reduces the NO production derived from iNOS which is induced by IL-1β [22]
- Formononetin reduces the prostaglandins by acting on COX-2
- Formononetin also shows inhibitory effect on TNF-α and IL6 levels [23]
- Formononetin works as a mast cell stabilizer by inhibiting the release of histamine from the mast cells. (Fig.5).

MATRINE

Matrine is extracted from the root of *Sophora flavescens*, it shows therapeutic effects on several types of cancer, Alzheimer, rheumatoid arthritis, acute lung injury, ulcerative colitis, cardiac fibrosis, cerebral infarction, psoriasis, asthma, acute respiratory distress syndrome, and ankylosing spondylitis [24,25] (Fig.4).

ANTI-INFLAMMATORY ACTION

- Matrine inhibits the pro-inflammatory transcription factor NF-κB [26]
- Matrine also has a protective effect on lung injury caused by ulcerative colitis
- It inhibits the activity of myeloperoxidase and malondialdehyde which decreases the levels of neutrophils hence works as anti-inflammatory [27]
- Matrine decreases the levels of TNF-α, IL-1β, IL-1α, IL-8, and IL-6 which lowers the inflammation caused in asthma [28]
- It reduces the expression of SOCS3 (checks the levels of eosinophils), also lowers the production of reactive oxygen species

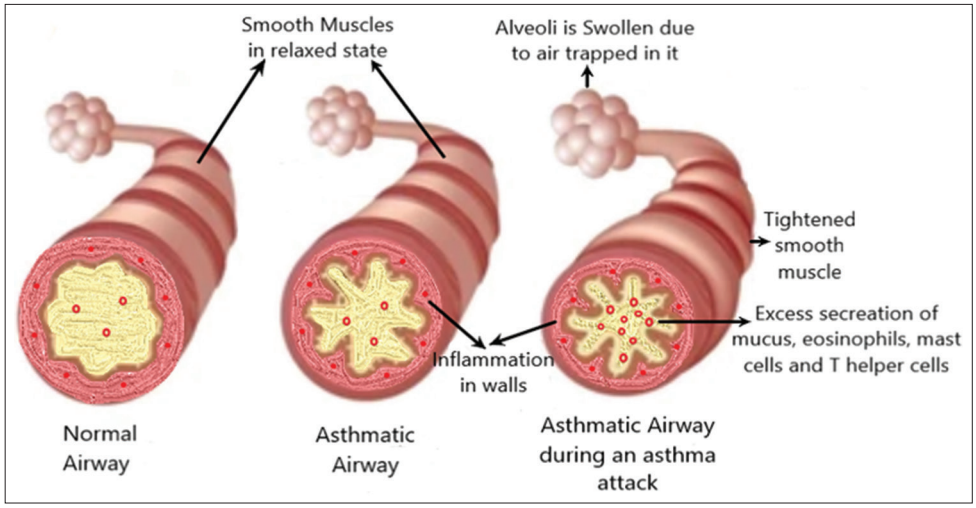

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Fig. 1: Airways during asthmatic attack

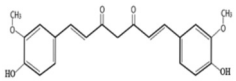

Fig. 2: Curcumin

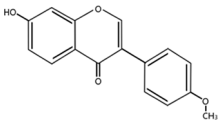

Fig. 3: Formononetin

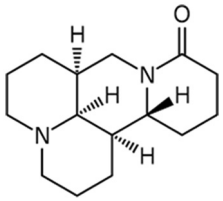

Fig. 4: Matrine

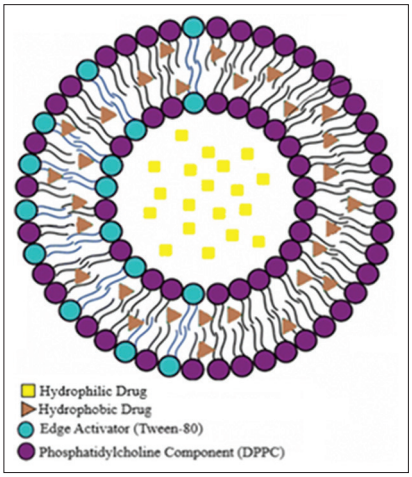

Fig. 5: Transferosomes

- It decreases the levels of pro-inflammatory cytokines COX-2 and ICAM-1.

TRANSFEROSOMES

Transferosomes are a special type of liposomes, consisting of phosphatidylcholine and an edge activator. They're soft, pliable vesicles designed to distribute active substances more effectively. They can actively transport both hydrophilic and lipophilic molecules in a single entity particle and also protects the active pharmaceutical ingredients (APIs) from distortion while transportation through the body.

Preparation of API loaded in transferosomes by thin film hydration technique/rotary evaporation-sonication method.

The phosphatidylcholine component, DPPC, and the edge activator, Tween-80, are dissolved in a round-bottom flask with an appropriate (v/v) ratio of chloroform and methanol. In this stage, the lipophilic substance curcumin is introduced. A rotary vacuum evaporator is used to evaporate the organic solvent above the lipid transition temperature under reduced pressure in order to generate a thin layer. To eliminate the last residues of the solvent, keep it under vacuum. The thin film is then hydrated using a buffer solution with a pH of 7.4 and rotated for a period of time at the appropriate temperature. Matrine and formononetin, two hydrophilic drugs, will be included at this step. To obtain tiny vesicles, the resultant vesicles are inflated at room temperature and sonicated in a bath or probe sonicator. Extrusion across a sandwich of 200 nm to 100 nm polycarbonate membranes homogenizes the sonicated vesicles [29,30].

PREPARATION OF LIQUID AEROSOL SYSTEM

The prepared transferosomes system is then immersed in ethanol solvent and then poured in the aerosol container mixed with Tween-80 and propellant used will be HFA-134a.

TESTING ON DIFFERENT PARAMETERS

- The Z-average diameter and polydispersity index of the transferosomes will be determined by dynamic light scattering as the characterization is necessary
- Change of shape and size can be seen under the TEM imaging
- Testing of the vesicular system against different pH
  The vesicular system will be tested against pH 6.2 and 7.6; these two different pH will be chosen because it represents the overall gap in the pH of the whole mouth to bronchi to alveoli
- Testing of the vesicular system against different temperatures The temperature of the body varies from 97°F (36.1°C) to 100.4°F (38°C) at normal. The vesicular system must be stable at this temperature.

FRAP

It will determine the antioxidant potential of the drug. To make FRAP reagent, acetate buffer will be mixed with TPTZ in HCl and

$FeCl_3.6H_2O$. Then, after the preparation of FRAP, the sample will be added to the FRAP and the results will be observed under the UV–VIS spectrophotometer.

CAA

For the calculation of CAA, fluorescent readings will be observed. The formula for calculation of CAA is

CAA unit = 100 – (∫S - ∫B) × 100

(∫C - ∫B)

Where, ∫ S is the integrated area under the sample fluorescence versus time curve, ∫B is the integrated area from the blank curve, and ∫ C is the integrated area under the control curve.

X-ray diffraction method

The X-ray diffraction method will determine the integrity of the vesicular system inside the respiratory system. Furthermore, the anions and cations stability will be observed at different bands.

Zeta potential and electrophoretic mobility

Both have to be calculated and if there is a relatively high surface charge of transferosomes then it will more likely to interact with negatively charged cell membranes and be taken up by the cells easily.

MTT assay can also be used to detect the cytotoxic effects of the transferosomes system.

ASSAY TESTING FOR ANTI-INFLAMMATORY RESPONSE

In this assay testing, RAW264.7 cell line is prepared and tested for the presence of different types of LPS-induced inflammatory cells. The murine macrophage RAW264.7 cell line will be maintained in Dulbecco's modified eagle medium supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin and then determination of COX-2, iNOS, TNF-α, IL-1β, and IL-6 genes by quantitative real-time PCR [31].

MECHANISM OF PENETRATION

When the drug reaches the inflamed areas of the airways, it interacts with the mucus layer of the pathway, causing deformability in the outer layer of the nano-vesicle without causing mutation or damage to the drug entrapped inside; this deformability aids in easy penetration of the mucus and epithelium layer. The medicine binds to cancer cell receptors and provides anti-inflammatory actions, relaxes the airways of the lungs, and lowers the mucus layer after reaching the mucus and epithelial layer. As a result, the inflammation in the airways is reduced. To improve the drug availability to the lungs, a spacer can be used.

RESULTS

Curcumin, formononetin, and matrine loaded in transferosomes nano-vesicular systems is prepared through thin film hydration technique/rotary evaporation-sonication method with phosphatidylcholine component, DPPC, and the edge activator, Tween-80. The transferosomes system will be tested on several bases such as for shape and size. TEM imaging will be used for surface charge and zeta potential polydispersity index is measured. Testing against RAW264.7 cells line will be done for the anti-inflammatory effect.

MTT test will be done to detect the cytotoxic effects of the nano-vesicular system. Stability will be tested on pH 7.6 and temperature at 37°C.

CAA and FRAP will also be calculated. After all these testing, it can be said that curcumin, formononetin, and matrine loaded in transferosomes will be a revolutionary cure for asthma.

CONCLUSION

Nanoparticles are used in novel drug delivery systems because of their high permeability and resistance against different body conditions. Transferosome is one of the finest nanoparticle because of its drug entrapping efficiency and cell-penetrating ability.

Curcumin, formononetin, and matrine have quite effective anti-inflammatory potential but unable to produce these effects in the body due to their instability at pH 7.4 but if loaded in transferosomes, they can utilize their potential against inflamed cells in asthma attack; even they can perform more than their potential.

Oral candidiasis, dysphonia, reflex cough or tracheal spasms, decreased bone density in adults, cataracts, and other adverse effects that occur with typical corticosteroids asthma therapy will not occur here, and the treatment will be safe, affordable, and shorter. Because the procedure is non-steroidal, patient compliance will improve. The accumulation of medications will not be visible, and patients' psychological difficulties will be lessened.

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CONFLICTS OF INTEREST

The authors confirm that the content of the article has no conflicts of interest.

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DATA AVAILABILITY

The original data that support the findings of this study are included in the article.

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Author Queries???
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