DEVELOPING NANOEMULSION, MOLECULAR DOCKING AND *EX-VIVO* STUDIES OF SELECTED TRADITIONAL ANTIASTHMATIC MEDICINAL PLANTS

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Abstract

Asthma is a chronic inflammatory respiratory disorder that requires long-term management due to its recurrent nature. Traditional Siddha medicine has been widely used to manage respiratory ailments, with several medicinal plants exhibiting anti-asthmatic properties. This study explores the molecular docking, ex vivo pharmacological screening, and nanoemulsion formulation of a traditional anti-asthmatic herbal extract composed of Solanum trilobatum, Piper longum, and Ocimum tenuiflorum. Molecular docking studies using AutoDock 1.5.6 revealed that quinic acid and eugenol exhibited strong binding affinity to IL-4 (2D48), a cytokine involved in asthma pathogenesis. Ex-vivo pharmacological evaluations, including mast cell stabilization and histamine-induced contraction studies, demonstrated significant anti-asthmatic activity of the extract, comparable to standard drugs. Furthermore, a nanoemulsion formulation of the herbal extract was developed and characterized, showing favorable physicochemical properties for improved bioavailability and therapeutic efficacy. These findings provide scientific validation for the traditional use of this herbal formulation in asthma treatment and highlight its potential as an alternative therapeutic strategy. Further in vivo and clinical studies are required to assess its safety and efficacy in human subjects.

Keywords: Asthma, molecular docking, mast cell stabilization, IL-4 inhibition, herbal medicine, nanoemulsion.

1. Introduction

Asthma is a chronic inflammatory disease of the airways, characterized by airway hyper responsiveness, excessive mucus secretion, and bronchial remodeling, leading to persistent respiratory distress. The standard treatment options, including corticosteroids and bronchodilators, provide symptomatic relief but may lead to adverse effects with long-term use. As a result, there is an increasing focus on natural alternatives for managing asthma, particularly herbal-based therapies known for their safety and efficacy. [1]

Traditional Siddha medicine has long incorporated medicinal plants with anti-inflammatory, bronchodilatory, and immunomodulatory properties to alleviate asthma symptoms. This study investigates a compound herbal extract formulated into a Nano emulsion to enhance bioavailability and therapeutic efficiency. Nano emulsions are advanced drug delivery systems that improve the absorption of bioactive compounds, ensuring prolonged activity in the body. [2]

This research focuses on: Molecular docking interactions of active phytoconstituents with IL-4 (2D48), a key cytokine implicated in asthma pathogenesis, Ex vivo pharmacological evaluations, including mast cell stabilization and histamine-induced contraction studies, to assess the extracts anti-asthmatic potential, ddevelopment and characterization of a Nano emulsion formulation to optimize drug delivery. [3]

These study aims to provide scientific validation for the traditional use of this compound herbal extract in asthma treatment and to explore its potential as an alternative therapeutic approach to conventional drugs.

2. Materials and methods

2.1. Preparation of ligands

The structure of 2,3-dimethyl hydroquinone, Hexahydro-1,6-pentalenedione, Cycloocta- 2,7-dienone, Quinic acid and Eugenol were imported from PubChem in sdf format and drawn in Marvin sketch. The sdf file was converted to pdb format using OpenBabel-2.3.1 and finally it was saved in pdb format. [4,5,6]

2.2. Structure of ligands

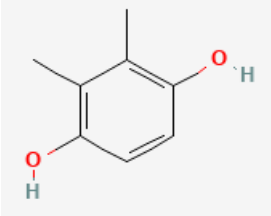

Fig.1. 2,3-dimethyl hydroquinone

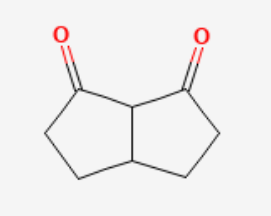

Fig.2.Hexahydro-1,6-pentalenedione

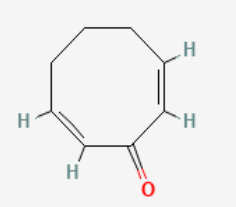

Fig.3.Cycloocta - 2,7-dienone

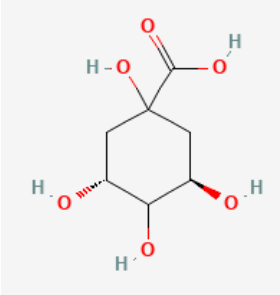

Fig.4.Quinic acid

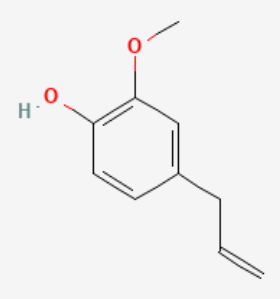

Fig.5.Eugenol

3. Selection of IL-4 as Target Protein from Asthma pathway

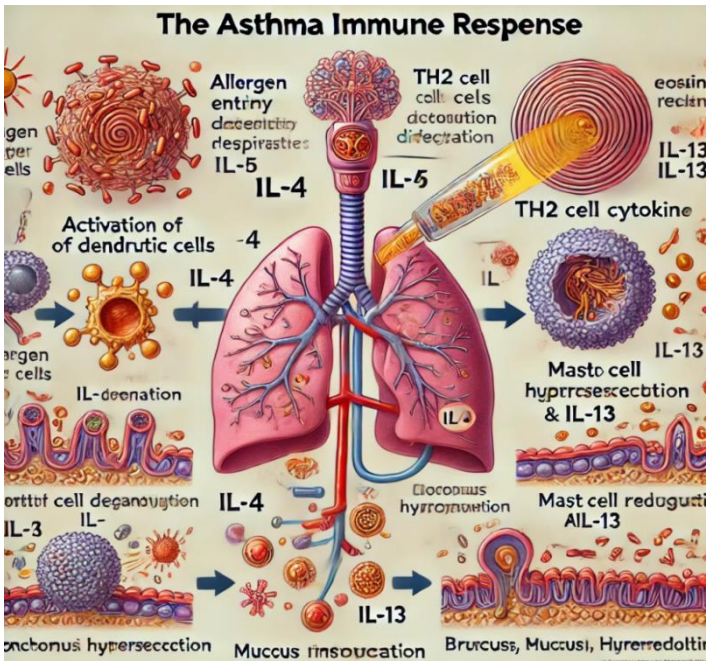

Fig.6. Targeted Pathway- IL-4

Interleukin-4 (IL-4) is a pivotal cytokine involved in the pathogenesis of asthma, playing a central role in driving the Th2 immune response. It facilitates the differentiation of naïve T-helper cells into Th2 cells, which produce other pro-inflammatory cytokines such as IL-5 and IL-13. IL-4 also induces B cells to produce IgE, which sensitizes mast cells to allergens, leading to their degranulation and the subsequent release of histamine and other mediators. These processes result in airway inflammation, bronchoconstriction, and excessive mucus secretion. [7] Additionally, IL-4 contributes to the recruitment of eosinophils and chronic airway remodeling, including smooth muscle hypertrophy and subepithelial fibrosis, which exacerbate asthma symptoms. Given its crucial role in the asthma pathway, IL-4 (PDB ID: 2D48) was selected as the target protein for this study. Molecular docking was employed to evaluate the binding affinity and inhibitory potential of bioactive compounds in the herbal extract against IL-4, aiming to identify compounds capable of modulating its activity. [8]

4. Molecular docking studies using Autodock version 1.5.6

Molecular docking studies were conducted using AutoDock 1.5.6to determine the inhibitory constant (Ki), binding energy, amino acid residues involved in interactions, and hydrogen bonds formed between the active compounds of the traditional herbal extract and the target protein. The 3D structure of the target protein was retrieved from the Protein Data Bank (PDB), followed by the removal of water molecules and non-essential heteroatoms. Polar hydrogens were added, and Kollman charges were assigned before saving the prepared protein in PDBQT format. The ligands were prepared by obtaining their 3D structures from PubChem and subjecting them to energy minimization using the Universal Force Field (UFF). Rotatable bonds were assigned, and the ligands were converted to PDBQT format. The active site of the target protein was identified based on literature data, and a grid box was created to encompass the binding pocket. Docking was performed using the Lamarckian Genetic Algorithm (LGA) with 100 docking runs per ligand. Post-docking analysis was conducted to extract binding energy values (kcal/mol), inhibitory constants, and details of interactions such as hydrogen bonding and hydrophobic contacts. Visualization of docked complexes using PyMOL and Discovery Studio confirmed the amino acid residues and specific binding interactions involved in the protein-ligand complexes. [9]

5. Plant collection and Authentication

Solanum trilobatum, *Piper longum*, and *Ocimum tenuiflorum* were collected from wild resource in Puducherry, India and taxonomically identified in the Department of Ecology, French Institute of Puducherry, under the joint supervision of the French Ministry of Foreign Affairs and the French National Centre for Scientific Research, Puducherry, India. Voucher specimens (Herbarium No. MTPGRIHS / HBVS / COP - DOPG / 2024 / 01 - *Solanum trilobatum,* MTPGRIHS / HBVS / COP - DOPG / 2024 / 02 - *Ocimum tenuiflorum*)

6. Preparation of Compound Herbal Extract

Decoction Method

The decoction method was employed for the preparation of a herbal extract using *Solanum trilobatum, Piper longum,* and *Ocimum tenuiflorum*. Initially, the plant materials were thoroughly washed with distilled water to eliminate dust and impurities. They were then shade-dried at room temperature to preserve their phytochemical constituents. Once dried, the plant materials were coarsely powdered and mixed in appropriate proportions. The powdered mixture was boiled in distilled water at a 1:10 (w/v) ratio and simmered for 30–45 minutes to facilitate the extraction of bioactive compounds. After boiling, the decoction was filtered through muslin cloth or Whatman filter paper to remove plant residues. The filtrate was then subjected to evaporation using a water bath at a 100 to prevent the degradation of heat-sensitive compounds. Upon complete evaporation, a solid mass of herbal extract was obtained. The extract was stored in an airtight container at 4°C for further studies. [10]

7. Ex-vivo Pharmacological evaluation for assessing Anti-Asthmatic efficacy

Discarded chick intestines were collected from local poultry shops for evaluating mast cell stabilization activity and histamine-induced contraction studies.

7.1. Mast Cell Stabilization Activity Using Chick Ileum Method

The mesentery from the chick ileum was carefully excised, ensuring the removal of fat and blood vessels. The isolated mesentery was then immersed in Ringer-Locke physiological solution to maintain tissue viability. For experimental evaluation, five mesentery samples were placed in separate petri dishes, each assigned to a specific treatment group:

a) Petri Dish 1: Normal control (distilled water)
b) Petri Dish 2: Allergic control (0.1 ml of 1% egg albumin)
c) Petri Dish 3: Standard drug (Ketotifen fumarate, 100 µg/ml) + 0.1 ml of 1% egg albumin
d) Petri Dish 4: Test drug (Compound herbal extract, 200 µg/ml) + 0.1 ml of 1% egg albumin
e) Petri Dish 5: Test drug (Compound herbal extract, 400 µg/ml) + 0.1 ml of 1% egg albumin

The mesentery samples were incubated for 30 minutes with their respective treatments. Following incubation, the samples were stained by immersing them in 0.1% toluidine blue containing 4% formaldehyde solution for 30 minutes to visualize mast cells. The stained tissues were then subjected to sequential treatment with acetone and xylene, followed by mounting on slides for microscopic observation.To assess mast cell stabilization, the number of intact and degranulated mast cells was counted, and the percentage protection of mast cells was determined using the formula:

Percentage protection of mast cell= (Total mast cells – Total degranulated cells)/Total mass cells ×100

A total of five samples were analyzed for each drug concentration. The results were statistically evaluated using one-way ANOVA, followed by Dunnett's multiple comparison test to determine the significance of the mast cell stabilizing effects of the test compounds. [11]

7.2. Histamine-Induced Contraction Studies

Tissue contraction studies were performed using a kymograph connected to a Sherrington rotating drum machine. The ileum tissue was mounted under a resting tension of 1g and allowed to equilibrate for 60 minutes before initiating the experiment. During this period, the Tyrode solution was replaced every 15 minutes.After equilibration, a concentration-response curve was generated for histamine by assessing the contractile effects of Histamine (100 µg/ml), Standard drug (histamine antagonist Chlorpheniramine maleate) andTest drug (Compound herbal extract-400µg/ml).The contractile responses were recorded, plotted on graphs, and subjected to regression analysis to determine statistical significance. [12]

8. Formulation of Compound Herbal Extract Nanoemulsion:

O/W Nano emulsion Preparation

An O/W nanoemulsion was prepared using the high-speed homogenization method with Tween 80 as the surfactant and Span 20 as the co-surfactant. A 12.5 ml aqueous phase, containing 25 mg of extract dissolved in distilled water, was placed in a 50 ml beaker. Tween 80 (0.25 ml, 20% v/v) was mixed with the aqueous phase and homogenized using a high-

speed homogenizer. Meanwhile, Span 20 (0.25 ml) was blended with 12.5 ml of macerated oil to form the oil phase. The homogenized aqueous phase with Tween 80 was then gradually added to the homogenized oil phase with Span 20 under high-speed homogenization (T18 Ultra Turrax, IKA) at 10,000 rpm for 1 hour. [13]

9. Characterization of nano emulsion

9.1. Determination of Density

The density of sunflower oil was determined using a pycnometer. First, the pycnometer or density bottle was thoroughly cleaned with nitric acid and rinsed two to three times with purified water. If needed, an organic solvent such as methanol or acetone was used for rinsing and drying. The dry, empty density bottle with its stopper was then weighed. Next, the bottle was filled with purified water, and the stopper was inserted, allowing excess water to escape through the capillary. The exterior was wiped with a tissue to remove any excess liquid, and the bottle with water was weighed using a balance. After emptying the water, the bottle was rinsed with the test liquid and refilled in the same manner. The density bottle with the test liquid was then weighed. The experiment was repeated at least three times, and the average value was recorded. [14]

Density of Liquid = (Weight of Water/ Weight of Liquid) × Density of Water

9.2. Determination of Viscosity

Viscosity in liquids is influenced by intermolecular forces that determine their resistance to flow. The viscosity of a liquid can be measured based on its flow time, as it is directly proportional to this parameter. This principle is applied in Oswald's viscometer, which consists of two bulbs connected by a U-shaped tube containing a capillary section.To measure viscosity, the viscometer must first be thoroughly cleaned with chromic acid solution, rinsed with distilled water, followed by water and ether, and then dried. The instrument should be securely positioned in a perfectly vertical orientation using a burette stand. Distilled water is then added to the lower bulb until it is completely filled. Using a rubber tube, the liquid is drawn above the calibration mark A, after which it is allowed to flow freely. A stopwatch is started as soon as the liquid level crosses mark A and stopped once it reaches mark B. The flow time is recorded, and the process is repeated three times for accuracy. The same procedure is followed for the experimental liquid to determine its viscosity. [15]

$$\eta_L = \eta_w \times \rho_L \times t_L / \rho_w \times t_w$$

Where,

η_L = Absolute viscosity of liquid

η_w = Absolute viscosity of water

ρ_L = Density of liquid

t_L = Density of water

ρ_w = Time flow of liquid

t_w = Time flow of water

9.3. Determination of pH

A pH meter is an electronic instrument used to determine the hydrogen ion activity (acidity or alkalinity) of a solution. It primarily consists of a voltmeter connected to a pH-sensitive electrode and a reference electrode. The pH-sensitive electrode is typically made of glass, while the reference electrode is commonly a mercury-mercurous chloride electrode.When both electrodes are immersed in a solution, they function as a battery. The glass electrode generates an electric potential that corresponds to the hydrogen ion activity in the solution, and the voltmeter measures the potential difference between the glass electrode and the reference electrode. This measurement is then used to determine the pH of the solution. [16]

pH Measurement

To measure the pH of a solution using a pH meter, the electrode is first connected to the device. pH 7.0 and pH 4.0 buffer solutions are prepared for calibration. The electrode is then rinsed with double-distilled water and gently dried using tissue paper. For calibration, the electrode is immersed in the pH 7.0 buffer solution, and the pH mode is selected. After allowing it to stabilize for at least 60 seconds, the pH is adjusted using the CAL control. The electrode is then removed, washed, and dried before being calibrated again using the pH 4.0 buffer solution in the same manner. To measure the pH of an unknown solution, the electrode is immersed in the sample and left undisturbed for 60 seconds until the display stabilizes. The final pH value is then recorded. [17]

9.4. Particle size Analysis

The particle size and polydispersity index (PDI) of the nanoemulsion were analyzed using the Malvern Analyzer, which utilizes dynamic light scattering (DLS) for precise measurement. The nanoemulsion sample was appropriately diluted to ensure optimal scattering conditions before being introduced into the instrument’s measurement chamber. The analyzer detected fluctuations in light scattering intensity to determine the size distribution of the droplets. The average particle size was recorded to assess the overall droplet dimensions, while the PDI, which indicates the uniformity of the particle size distribution, was measured to evaluate the stability and consistency of the formulation. A lower PDI value signifies a more uniform and stable nanoemulsion, whereas a higher PDI indicates broader size distribution and potential instability. [18]

10. Results and Discussion

10.1. Table 1.Docking score of IL-4(2d48) with various ligands using Autodock tools 4.2.(Version 1.5.6)

S. NO	Ligands	Protein with PDB ID	Binding energy (kcal/ mol)	Inhibition constant (µM)	No. of H-bonds	Amino acid residues
1	2,3-dimethyl hydroquinone	IL-4 (2D48)	-5.83	52.84um	3	2d48:A:GLN54:OE1 2d48:A:THR30:HN 2d48:A:THR28:O
2	Hexahydro pentalin 1,6-dione		-6.09	34.4um	1	2d48:A:THR30:HN
3	Cycloocta- 2,7-dienone		-5.5	93.64um	1	2d48:A:THR30:HN
4	Quinic acid		-8.16	1.05	3	2d48:A:THR28:O 2d48:A:THR30:HN 2d48:A:THR30:HG1
5	Eugenol		-6.8	10.39	3	2d48:A:GLN54:OE1 2d48:A:THR30:HN 2d48:A:THR30:HG1

10.2. Docked images of Ligands with Protein-IL-4 (2D48)

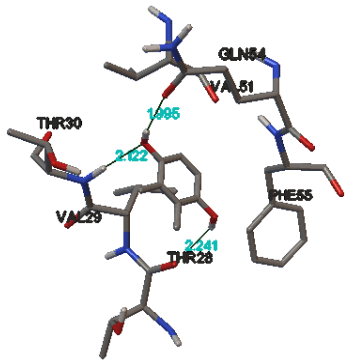

Fig.7. 2,3-dimethyl hydroquinone

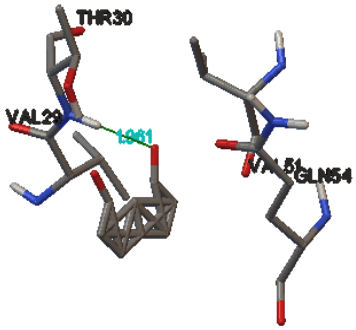

Fig.8. Hexahydro pentalin 1,6-dione

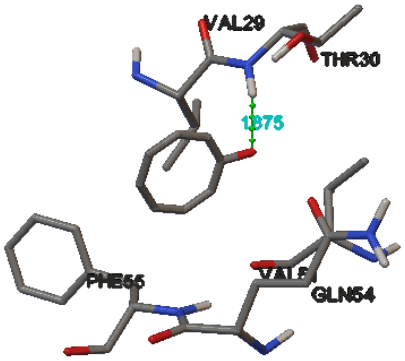

Fig.9. Cycloocta- 2,7-dienone

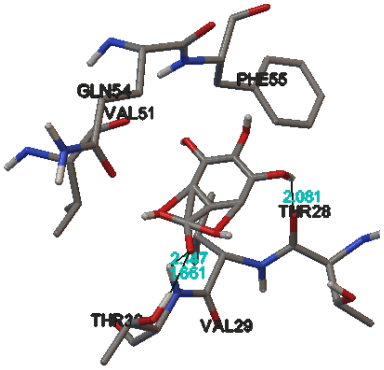

Fig.10.Quinic acid

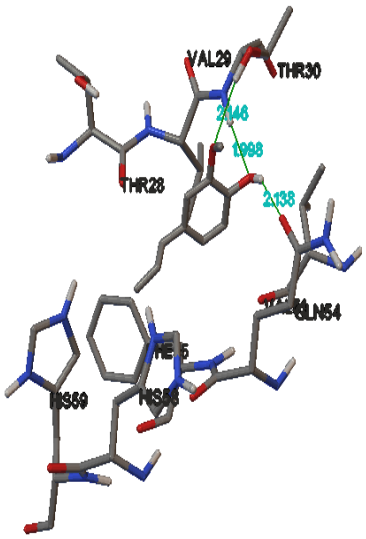

Fig.11. Eugenol

The molecular docking analysis of bioactive compounds from the compound herbal extract against IL-4 (PDB ID: 2D48) revealed significant interactions, providing insights into their potential anti-asthmatic effects. Among the tested ligands, Quinic acid (Fig.10**)** exhibited the highest binding affinity (-8.16 kcal/mol) and the lowest inhibition constant (1.05 µM), indicating its strong interaction with IL-4. The multiple hydrogen bond formations with THR28 and THR30 suggest that quinic acid may play a key role in modulating IL-4 activity, thereby reducing airway inflammation associated with asthma. Eugenol (Fig.11**)** also showed notable binding affinity (-6.8 kcal/mol) and interacted with GLN54 and THR30, indicating its potential role in stabilizing IL-4 and disrupting its pro-inflammatory function. Other ligands (Fig.7,8,9) including 2,3-dimethyl hydroquinone (-5.83 kcal/mol), Hexahydro-1,6-pentalenedione (-6.09 kcal/mol), and Cycloocta-2,7-dienone (-5.5 kcal/mol), exhibited moderate interactions, suggesting varying degrees of inhibitory potential.The docking results suggest that the bioactive compounds present in the extract may effectively modulate IL-4, a cytokine known for driving asthma pathophysiology. Quinic acid and eugenol emerged as the most promising candidates, exhibiting strong binding interactions and multiple hydrogen bonds, which could contribute to mast cell stabilization and reduced airway inflammation. [19]

10.3. Mast Cell Stabilization activity

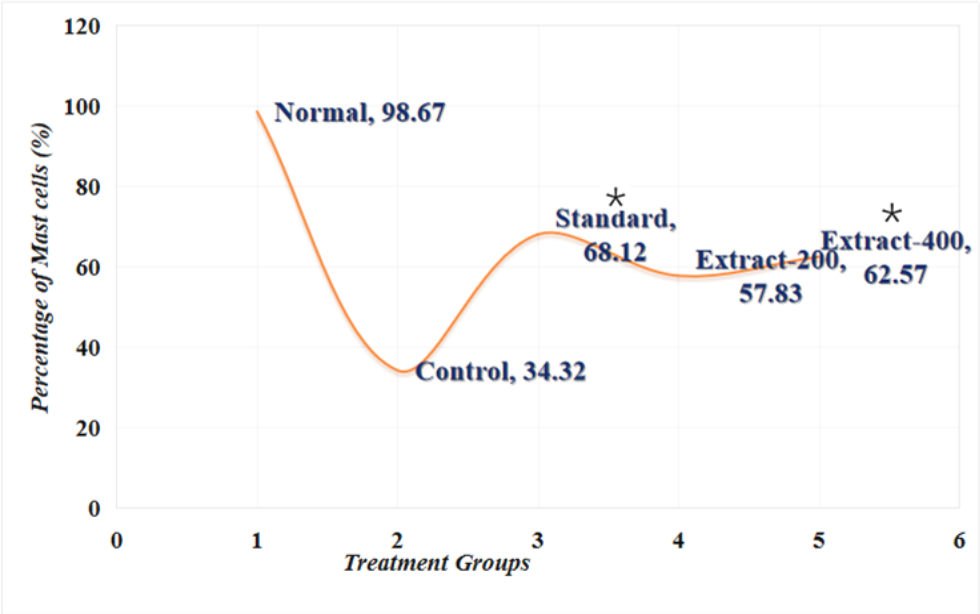

Fig.12. Percentage of Mast Cell stabilization (*p < 0.05)

Mast cell degranulation plays a crucial role in allergic asthma, leading to the release of histamine and other inflammatory mediators that cause airway inflammation and bronchoconstriction. The present study evaluated the mast cell stabilizing potential of the compound herbal extract using an ex vivo model. The results demonstrated that the normal group had the highest percentage of stable mast cells (98.67%), indicating the absence of any allergen-induced degranulation. In contrast, the control group exhibited a significant reduction in mast cell stability (34.32%), confirming the successful induction of an allergic response. [20]

Treatment with the standard drug significantly stabilized mast cells (68.12%, $p < 0.05$), serving as a positive reference for comparison. The compound herbal extract at 200 mg/kg showed moderate mast cell stabilization (57.83%), while the higher dose (400 mg/kg) exhibited an increased effect at 62.57%, suggesting a dose-dependent response. The observed stabilization indicates that bioactive compounds in the extract may act by inhibiting calcium influx in mast cells, thereby preventing histamine release and reducing hypersensitivity reactions. The dose-dependent enhancement further suggests that higher concentrations of the extract may exert stronger mast cell-stabilizing effects. [21]

10.4. Histamine induced contraction in chick ileum

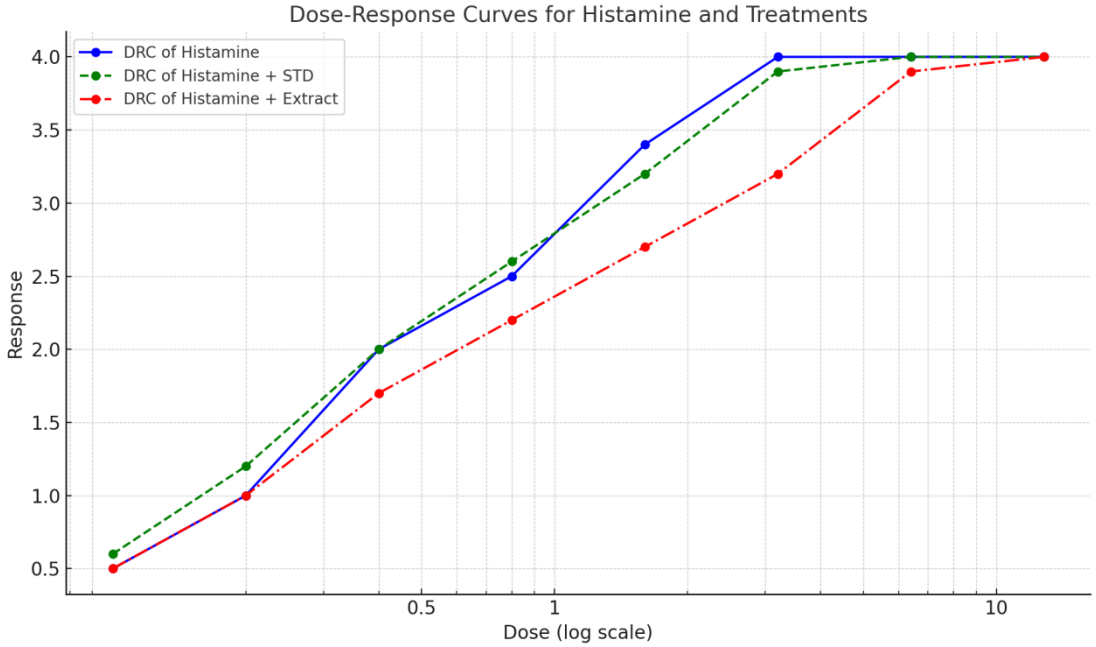

Fig.13. Dose Response curve of various treatments - Histamine induced contraction studies

Histamine plays a critical role in the pathophysiology of asthma by inducing bronchoconstriction and airway hyper responsiveness. The histamine-induced contraction study evaluates the ability of the compound herbal extract to modulate airway smooth muscle contraction, which is a key determinant of asthma severity. The dose-response curve (Fig. 8) illustrates the contractile response of airway tissues following histamine exposure across different treatment groups.In the control group, a sharp increase in contractile response was observed, indicating significant airway constriction due to histamine exposure. This confirms the model's validity in mimicking asthmatic conditions. [22] The standard drug-treated group demonstrated a marked reduction in histamine-induced contraction, validating its bronchodilatory and anti-spasmodic effects.Treatment with the compound herbal extract at 200 mg/kg showed a moderate reduction in airway contraction compared to the control, suggesting partial relaxation of bronchial smooth muscle. The higher dose (400 mg/kg) exhibited a more pronounced inhibitory effect, indicating a dose-dependent bronchodilatory response. This suggests that bioactive compounds within the extract may interfere with histamine receptor activation or modulate intracellular calcium levels, leading to smooth muscle relaxation. [23]

10.5. Compound Herbal extract Nanoemulsion

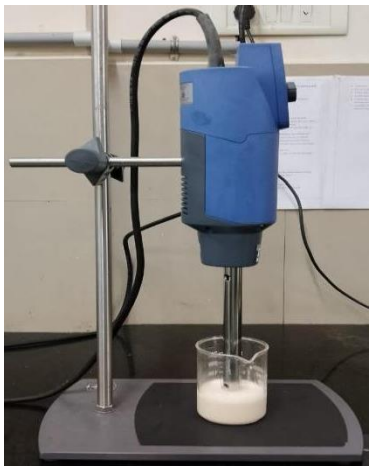

Fig.14. High speed Homogenization of Nanoemulsion

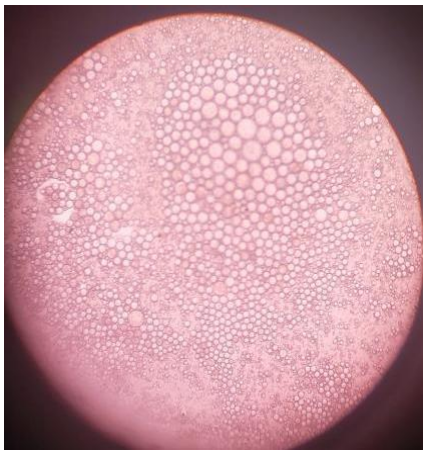

Fig.15. Microscopic view of Herbal formulation (10x)

Nanoemulsion technology has gained significant attention in drug delivery due to its ability to enhance the bioavailability and therapeutic efficacy of bioactive compounds. In this study, a compound herbal extract nanoemulsion was developed using high-speed homogenization (Fig. 14), a widely used technique to achieve uniform droplet size and improved dispersion of active ingredients. This method ensures better solubility and absorption of the extract, which is crucial for its pharmacological effectiveness.The microscopic analysis (Fig. 15) of the nanoemulsion at 10x magnification revealed a well-distributed, stable formulation with uniform particle size. The nanoscale size of the formulation is expected to facilitate better cellular uptake, enhance permeability through biological membranes, and prolong the systemic circulation of the bioactive compounds. The reduced particle size also contributes to improved solubility, ensuring efficient delivery of active constituents to target sites, such as bronchial tissues in asthma treatment.The nanoemulsion formulation of the compound herbal extract holds significant potential for enhancing its therapeutic efficacy in asthma management. [24]

10.6. Characterization of Nanoemulsion

Table 2. Characterization of O/W Compound herbal extract Nanoemulsion

SI.NO	Parameters	Physical Characters
1	Colour	White
2	Odour	Characteristic

3	Taste	Acrid
4	Nature	Viscous
5	Density	0.86g/ml
6	pH	6.06
7	Particle size (Z Aveage in d.nm)	363.6
8	Poly DispersityIndex (Pdi)	0.553

The characterization of the Oil-in-Water (O/W) nanoemulsion of the compound herbal extract was performed to assess its physicochemical properties, ensuring its stability and suitability for therapeutic applications. The results, as presented in Table 2, provide insights into the formulation's key attributes.The nanoemulsion appeared white in color, indicating uniform dispersion of the extract within the emulsion system. It exhibited a characteristic odor and an acrid taste, which are inherent to the bioactive compounds present in the extract. The viscous nature of the formulation suggests good emulsification, which is essential for maintaining the stability of nano-sized droplets.Physicochemical measurements revealed that the density of the nanoemulsion was 0.86 g/mL, suggesting an appropriate balance between the aqueous and oil phases, which is crucial for maintaining phase stability. The pH of the formulation was measured at 6.06, which falls within an acceptable range for biological applications, ensuring compatibility with physiological conditions. [25]

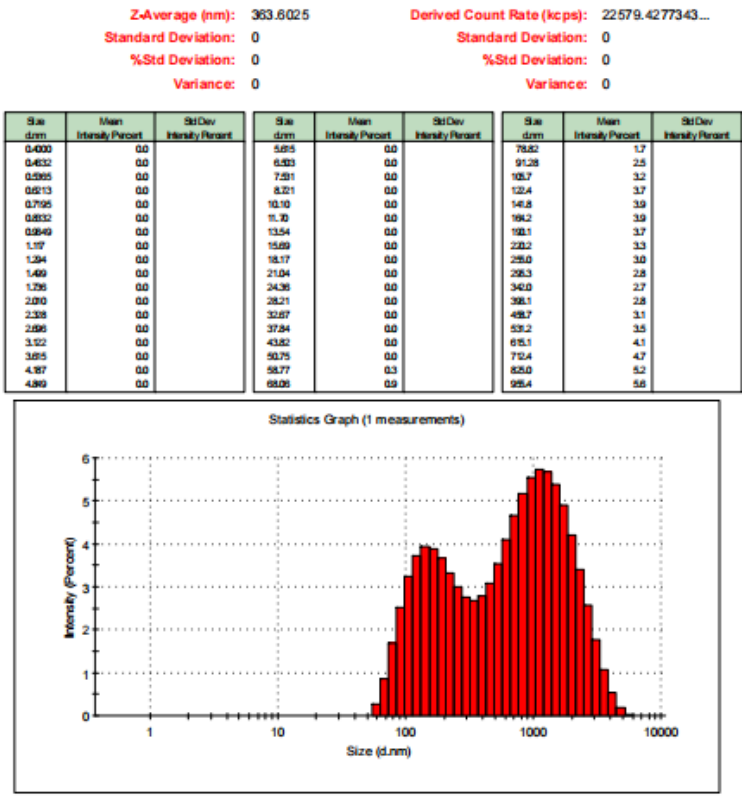

Fig.16. Dynamic Light Scattering for Particle size Analysis

The mean particle size (Z-average) was found to be 363.6 nm, indicating a nano-sized dispersion that facilitates better bioavailability and penetration through biological membranes. The polydispersity index (PDI) was 0.553, suggesting a moderately uniform particle size distribution, which is an important factor for maintaining the reproducibility of the nanoemulsion. [26]

Conclusion

This study successfully demonstrated the potential of a compound herbal extract nanoemulsion in asthma management. Molecular docking analysis revealed strong binding interactions of key bioactive compounds with IL-4 (2D48), indicating their potential role in inhibiting inflammatory pathways associated with asthma. Ex vivo studies further confirmed the mast cell stabilization and bronchodilatory effects of the extract, supporting its pharmacological efficacy.The nanoemulsion formulation improved the physical and chemical properties of the extract, enhancing its bioavailability and therapeutic potential. Characterization parameters, including particle size, pH, and polydispersity index, confirmed the suitability of the nanoemulsion for pharmaceutical applications. These findings highlight the promise of herbal nanoformulations in respiratory disease management, offering a natural and effective alternative to conventional asthma treatments. Further in vivo and clinical studies are warranted to validate these results and facilitate the development of herbal-based therapeutic solutions for asthma control.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript. All research and findings presented in this study are based solely on scientific data, and no financial or personal relationship have influenced the results or conclusions drawn.

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