



In Vivo Assessment of Anti-Asthmatic Potential of *Moringa Oleifera* and *Momordica Charantia* in Guinea Pig Model

Sandhya Suresh^{*1}, Dr. Siddharaj Singh Sisodia^{*2}

^{*1} M. Pharm (Pharmacology), Research Scholar, B. N. College of Pharmacy, Udaipur

^{*2} PhD (Pharmacology), Professor, B. N. College of Pharmacy, Udaipur

(Corresponding Author: Sandhya Suresh)

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KEYWORDS	ABSTRACT:
Asthma	<p>Introduction: Asthma is a chronic inflammatory condition of the airways marked by increased sensitivity, blockage, and repeated episodes of wheezing, dyspnoea, and coughing. Although conventional therapies such as corticosteroids and bronchodilators are beneficial, they possess limitations including adverse effects and diminished efficiency in extreme instances. This has prompted the search for plant-based alternatives for asthma management.</p> <p>Objectives: This study aimed to investigate and compare the anti-asthmatic properties of <i>Moringa oleifera</i> leaf extract (MOFL) and <i>Momordica charantia</i> leaf extract (MCFL) in an ovalbumin (OVA)-induced asthma model in guinea pigs.</p> <p>Methods: Guinea pigs were sensitized with ovalbumin and divided into nine groups: a normal control, a disease control, a positive control treated with dexamethasone (2.5 mg/kg), and six test groups receiving varying doses of MOFL (100, 200, 400 mg/kg) or MCFL (50, 100, 150 mg/kg). The evaluation included monitoring body weight, lung function (tidal volume, respiratory rate), total leukocyte count in bronchoalveolar lavage fluid (BALF), serum tumour necrosis factor-alpha (TNF-α), histamine levels in lung tissue, and histopathological analysis of lung tissue.</p> <p>Results: No significant differences in body weight were observed across the groups. The higher doses of MOFL (400 mg/kg) and MCFL (150 mg/kg) significantly reduced tidal volume, respiratory rate, total leukocyte count in BALF, serum TNF-α, and lung histamine levels when compared to the disease control group. Histopathological examination revealed that these extracts restored normal lung architecture and decreased eosinophilic infiltration, with effects comparable to the dexamethasone-treated group. <i>Moringa oleifera</i> had more efficacy than <i>Momordica charantia</i> in mitigating asthmatic changes.</p> <p>Conclusions: Both <i>Moringa oleifera</i> and <i>Momordica charantia</i> leaf extracts possess significant anti-asthmatic properties, likely due to their bioactive flavonoids and phytochemicals. <i>Moringa oleifera</i> showed superior potential, suggesting it could serve as a promising natural adjunct therapy for managing asthma.</p>
Moringa Oleifera	
Momordica Charantia	
TNF- α	
Histamine	
Inflammation	
Guinea Pigs	
Ovalbumin Model α	

1. Introduction

Asthma is a chronic inflammatory respiratory disorder characterized by airway hyper responsiveness, obstruction, and underlying inflammation, leading to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing. Globally, asthma affects an estimated 262 million people, causing substantial morbidity and healthcare burden, especially in low- and middle-income countries.^[1] The pathophysiology of asthma involves a complex interplay of inflammatory cells, mediators, and structural airway changes. Among

the key players are eosinophils, mast cells, T-helper type 2 (Th2) lymphocytes, and pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6), which orchestrate airway inflammation and remodelling.^{[2][3]} Current pharmacological management primarily relies on corticosteroids and bronchodilators, which, while effective, are often associated with side effects and limited efficacy in severe cases. This has propelled the exploration of alternative therapies, particularly those derived from natural sources with anti-inflammatory and



immunomodulatory properties. ^{[4][5]} *Moringa oleifera*, commonly referred to as the “miracle tree,” has garnered significant attention for its broad spectrum of pharmacological activities, including antioxidant, anti-inflammatory, and immunomodulatory effects. ^{[6][7]} The bioactive components of *Moringa*, such as quercetin, kaempferol, and isothiocyanates, have been demonstrated to suppress inflammatory mediators and modulate immune responses. Similarly, *Momordica charantia* (bitter melon) is recognized for its therapeutic potential against metabolic and inflammatory disorders, attributed to its rich phytochemical composition, including charantin, polypeptide-p, and flavonoids. ^{[8][9]} Both plants have a traditional history of use in respiratory ailments, but systematic scientific validation in asthma models remains limited. Animal models of asthma, particularly those employing ovalbumin (OVA)-induced sensitization and challenge, are widely established for mimicking the human disease pathology, including eosinophilic airway inflammation, elevated cytokines, and histamine release. ^{[10][11]} Guinea pigs serve as a suitable model due to their airway physiology and immunological responses being closely aligned with humans. ^[12] The present study was designed to evaluate and compare the therapeutic efficacy of *Moringa oleifera* and *Momordica charantia* leaf extracts on OVA-induced asthma in guinea pigs. The study investigates key pathological markers, including total leukocyte count in bronchoalveolar lavage fluid (BALF), serum levels of TNF- α , histamine concentrations in lung tissue, and histopathological alterations. This approach not only offers insight into the anti-asthmatic potential of these botanicals but also contributes to the growing scientific interest in plant-based interventions for chronic inflammatory diseases. By assessing dose-dependent responses and histopathological outcomes, the study aims to establish a basis for developing safer, effective, and affordable therapeutic alternatives for asthma management.

2. Material and Method

2.1 In-vivo study

2.1.1 In-vivo Study and Ethics Approval:

The Experiment was carried out on Guinea pigs aged 2-4 months and weighing 500-600g that were housed and placed under a 12 h light/dark cycle in a temperature-controlled room (22 ± 2 °C). They were provided with an

unlimited supply of water and a standard rodent pellet diet. All the animals assessments were approved by IAEC (Institutional Animal Ethics Committee) Pinnacle Biomedical Research Institute, Bhopal (Reg. No. 1824/PO/RcBi/S/15/CPCSEA). Protocol Approval Reference No. PBRI/IAEC/30-09-24/023.

2.1.2 Experimental induction of asthma and treatment

In this protocol, Guinea pigs (500-600 g) with $n = 6$ was used and divided into IX groups. Group-1 normal (no inducing of any protocol), group-2 vehicle treated; group-3 treated with standard, while groups-4 to 9 test sample treatment groups. All groups received ovalbumin (20 μ g, i.p.) and 2 mg aluminum hydroxide (Al (OH)3) prepared in 200 μ L sterile normal saline on days 1, 7, and 14. From days 15-21, these groups were exposed to 5% ovalbumin in the form of aerosol for 30 min in a chamber. Group 2 was given 200 μ L i.p normal saline 30 min before 5% ovalbumin challenge for 7 days. Group 3 received dexamethasone (2.5 mg/kg, i.p.) 15 minutes before 5% ovalbumin challenge for 7 days. Groups 4 to 9 received extract of *Moringa oleifera* and *Momordica charantia*, respectively before 5% ovalbumin inhalation for 7 days. The serum was separated and TNF- α , IL6 levels were estimated by ELISA kit. ^[13]

2.1.3 Treatment Protocol and Animal Groups

The following groups of six animals each were randomly assigned to the animals.

Table.1: In-vivo research treatment protocol

Group No.	Group	Drug and dose	Number of animals
1.	Normal control	Saline	6
2.	Disease control	Ovalbumin sensitized	6
3.	Positive control	Dexamethasone (2.5mg/kg)	6
4.	Test control 1 (MOFL)	Ovalbumin sensitized +100mg/kg	6
5.	Test control 2 (MOFL)	Ovalbumin sensitized +200mg/kg	6



6.	Test control 3 (MOFL)	Ovalbumin sensitized +400mg/kg	6
7.	Test control 4 (MCFL)	Ovalbumin sensitized +50mg/kg	6
8.	Test control 5 (MCFL)	Ovalbumin sensitized +100mg/kg	6
9.	Test control 6 (MCFL)	Ovalbumin sensitized +150mg/kg	6

2.1.3 Body weight

The body weight of guinea pigs from all groups was measured on days 0,7,14 and 21 using digital balance. Each animal was handled carefully to minimize stress during weighing. measurements were taken at the same time day to maintain consistency. ^[15]

2.1.4 Lung function test

Every guinea pig was measured for lung function parameters on day 21. Using a nebulizer, the animals' tidal volume and respiratory rate were assessed both before and after the guinea pigs were exposed to aerosolized ovalbumin (CX4-Omron Healthcare Company Ltd., Kyoto, Japan) and note the data in biopac system (model MP-35, Biopac System, Inc., Santa Barbara, CA) and in group I (normal control) animals were exposed to aerosolized saline. ^[15]

2.1.5 Bronchoalveolar Lavage Fluid (BALF)

The pigs were rendered unconscious by thiopental sodium (100 mg/kg, i.p.) 24 hours after the final ovalbumin challenge. The BALF was obtained by aspirating ice-cold phosphate buffered saline (PBS) into the trachea three times with a tracheal catheter. The fluid was then collected with a syringe and stored in an Eppendorf tube (1.5 mL) each time. The BALF thus obtained underwent centrifugation at $1000 \times g$ at $4^{\circ}C$ for 10 min which results in concentration of cells at the bottom of the tube and stored at $-80^{\circ}C$. ^[15]

2.1.6 Total leukocyte count from BALF

To determine the total leukocyte count, BALF will be mixed with 200 μL of PBS in a 1.5 mL EP tube, and the

cell suspension will be gently vortexed. The resulting fluid will be centrifuged at a condition specified before. The concentrated leukocytes will be re-suspended in 100-200 μL RBC lysis buffer solution and kept on ice for 10 min, which lysed RBCs, and then PBS (1 mL) will be mixed to stop cell-lysis. The leukocyte cell suspension will be again centrifuged at the same rate and time as before. The BALF leukocyte concentrate thus obtained will be suspended in PBS (400 μL) from which 20 μL is taken using micropipette, and leukocytes will be counted using haemocytometer under a microscope. ^[15]

2.1.7 Measurement of inflammatory mediator TNF- α

The levels of TNF- α in Serum samples were measured using ELISA (enzyme-linked immuno-sorbant assay) kits. After following the manufacturer's instructions, all plates were analyzed on an automated plate reader (Lab System Multiscan Model-51118220, Thermo Bioanalysis Co., Helsinki, Finland). ^[14]

2.1.8 Histamine assay in Lung tissue

To determine histamine levels in lung tissue using the Histamine Quantification Assay Kit (MAK432), begin by preparing the necessary reagents and ensuring all kit components are stored correctly at $-20^{\circ}C$. Throughout the process, plastic containers must be used because histamine can stick to glass surfaces. For tissue samples, first, prepare a Histamine Extraction Buffer by diluting the provided Assay Buffer in a 1:1 ratio with methanol. Next, homogenize approximately 200–400 mg of lung tissue in 500 μL of the prepared extraction buffer. The homogenate should be heated to $90^{\circ}C$ for 20 minutes and then cooled on ice to efficiently extract histamine. Centrifuge the sample at $10,000 \times g$ for 5 minutes and collect the supernatant for analysis. For the assay, ensure that all reagents and samples reach room temperature before use. In a 96-well plate, prepare a standard curve by diluting the histamine standard from 50 $\mu g/mL$ down to 0.8 $\mu g/mL$ in a serial manner. To each well designated for standards, samples, and blanks, add 50 μL of the respective solution. Prepare the Reaction Mix by combining 46 μL of Assay Buffer, 2 μL of Reaction Enzyme, and 2 μL of Detection Solution per well. Add 50 μL of this Reaction Mix to each well and incubate the plate at room temperature for 30 minutes, protecting it from light. A plate reader can be used to measure absorbance at 450 nm. To determine histamine



concentration, subtract blank absorbance values from all samples and standards, then plot a standard curve and calculate the sample histamine content using the equation derived from the curve. If necessary, adjust for sample dilution. ^[16]

2.1.9 Histopathology

After the collection of BALF, the lungs were immersed in 10% formalin solution for histopathological examination. After processing, these tissues were cleaned in toluene, dehydrated in various alcohol grades, and impregnated in molten paraffin wax for predetermined lengths of time. Freshly melted paraffin wax was used to insert processed tissues, which were then left to solidify. To show general tissue structure, sections were dried on a hot plate for 15 minutes at 3 μ and stained with hematoxylin and 1% aqueous eosin. Stained slides were dehydrated in various ascending grades of alcohol, cleared in xylene, and mounted in Canada balsam. Sections were viewed microscopically using ×10 objective lenses. ^[17]

2.1.10 Statistical Analysis

Results are provided as Mean±SD (n=6). Results were analysed statistically using one-way analysis of variance (ANOVA) followed by Bonferroni t-test. P < 0.05 was considered as level of significance while comparison between groups

3. Results of In-vivo study

3.1 BODY WEIGHT

The recorded values across all groups from Day 0 to Day 21 showed only minimal fluctuations. so there is no significantly differences were observed between normal, disease, positive and test groups over the 21 days of observation periods.

Table 2. Average body weight in kilogrammes

S.NO	GROUPS	0 DAY	7 DAY	14 DAY	21 DAY
1.	Normal control	523±1.78	525.33±3.01	536.3±1.5	545.3±2.9

2.	Disease control	523.3 3±1.9 6	527. 33± 1.96	532.6 6±1.5 0	550.6 6±2.1 6
3.	Positive control	526.5 ±2.07	527. 5±2 .07	534± 2.60	546.5 ±4.03
4.	TC-1(MOFL 100mg/kg)	527.1 ±1.04	528. 33± 1.50	533.1 6±2.9 9	544.5 ±1.64
5.	TC-2(MOFL 200mg/kg)	528.3 3±1.3 6	533 ±1. 26	534.6 6±4.3 2	546.5 ±2.42
6.	TC-3(MOFL 400mg/kg)	528.5 ±1.51	533 ±1. 26	536.1 6±2.7 1	547.8 3±2.4 8
7.	TC-4(MCFL 50mg/kg)	527.3 3±1.0 3	529. 33± 1.21	534.1 6±1.8 3	545.3 3±1.9 6
8.	TC-5(MCFL 100mg/kg)	529.5 ±2.07	532. 66± 1.21	536.6 ±1.94	548.4 ±4.09
9.	TC-6(MCFL 150mg/kg)	530.3 3±2.4 2	536. 58± 1.62	539.7 5±1.7 0	552.5 ±2.51

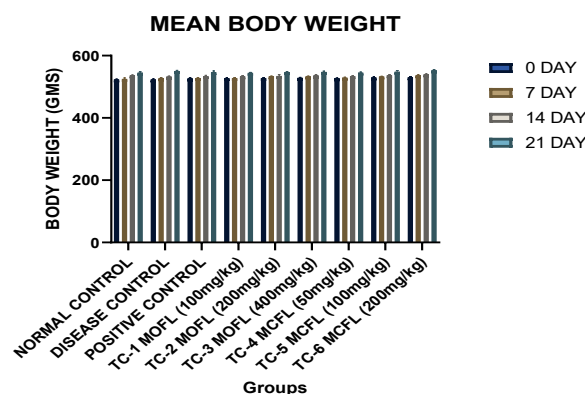


Figure 1: Histogram representing the effect of positive drug (OVA +DEX) and test drugs on Body weight. Data represented as mean ±SEM (n=6). No significance differences shown on body weight



3.2 Lung Function test (Tidal volume)

The results (mean \pm SEM, n=6) show that MOFL 400MG/kg (TC-3) and 150mg/kg (TC-6) produced the most significant reduction in Tidal volume initial and exposed stages. These effects were highly significant (*p<0.001) compared to NC, DC, PC, TC-4 and TC-6. It findings indicate strong and sustained respiratory-modulating potential at these doses.

Table.3: Mean Lung function test (Tidal volume)

S.N O	GROUPS	TIDAL VOLUME (ml/sec)	
		INITIAL	EXPOSE D
1.	Normal control (NC)	3.373 \pm 0.45 1*	2.833 \pm 0.53 1*
2.	Disease control (DC)	1.281 \pm 0.20 4*	1.133 \pm 0.07 4*
3.	Positive control (PC)	2.883 \pm 0.33 8	2.416 \pm 0.10 6
4.	Test control 1(MOFL100mg/ kg)	1.483 \pm 0.06 8	1.166 \pm 0.11 0
5.	Testcontrol 2(MOFL 200mg/kg)	1.55 \pm 0.111	1.216 \pm 0.06 8
6.	Testcontrol 3(MOFL 400mg/kg)	2.35 \pm 0.170 #	1.983 \pm 0.06 8#
7.	Test control 4(MCFL 50mg/kg)	1.516 \pm 0.06 8	1.216 \pm 0.13 4
8.	Testcontrol 5(MCFL 100mg/kg)	1.633 \pm 0.24 2	1.3 \pm 0.182
9.	Testcontrol 6(MCFL 150mg/kg)	2.066 \pm 0.18 8#	1.683 \pm 0.06 8#

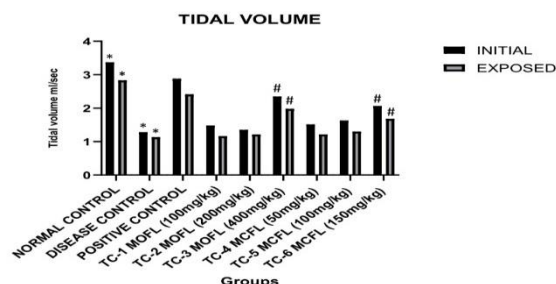


Figure 2: Data represented as mean \pm SEM (n=6). *p<0.001 vs NC, *p<0.001 vs DC, *p<0.001 vs PC, TC-4, TC-6. MOFL 400 mg/kg (TC-3) and 150mg/kg (TC-6) showed the most significant reduction in both initial and exposed stage of Tidal volume

3.3 Lung function test (Respiratory rate)

The graph illustrates the impact of the positive drug (OVA + DEX) and various test drug doses on respiratory rate in both the initial and exposed stages. Data are expressed as mean \pm SEM (n=6), with significant differences indicated at *p < 0.001 compared to NC, DC, PC, and all test groups. Among the treatments, MOFL 400 mg/kg (TC-3) and 150 mg/kg (TC-6) produced the greatest reduction in respiratory rate across both stages. This suggests a strong dose-dependent efficacy of MOFL in modulating respiratory function.

Table.4: Mean Lung function test (Respiratory rate)

S. N.	GROUPS	Respiratory rate (bpm)	
		INITIAL	EXPOSED
1	Normal control (NC)	112.33 \pm 1.5 9*	130.33 \pm 2.9 8*
2	Disease control (DC)	162.66 \pm 1.8 8*	184.66 \pm 1.4 9*
3	Positive control (PC)	126.16 \pm 2.1 1 ^s	141.66 \pm 1.2 4 ^s
4	Test control 1(MOFL100mg /kg)	144.33 \pm 1.1 05 ^s	161.83 \pm 0.8 9 ^s



5	Testcontrol 2(MOFL 200mg/kg)	142.16±1.0 6 ^s	155.33±2.2 1 ^s
6	Testcontrol 3(MOFL 400mg/kg)	140.50±3.0 19 ^s	148.33±4.7 1 ^s
7	Test control 4(MCFL 50mg/kg)	150.16±2.5 44 ^s	153.16±1.3 43 ^s
8	Testcontrol 5(MCFL 100mg/kg)	149.4±2.93 ^s	152.4±1.49 ^s
9	Testcontrol 6(MCFL 150mg/kg)	142.25±1.7 8 ^s	149.5±3.20 1 ^s

experimental groups. The disease control group (DC) showed a marked elevation in WBC count, indicating airway inflammation. Treatment with positive control and MOFL extracts significantly reduced WBC levels compared to DC. Among the test groups, MOFL 400 mg/kg (TC-3) exhibited the most pronounced effect, showing a highly significant reduction in WBC count (#p < 0.01).

Table-5: Total Leukocyte counting BALF

S.NO	GROUPS	LEUCOCYTE (%)
1.	Normal control (NC)	9.72±1.1303*
2.	Disease control (DC)	23.33±1.5680*
3.	Positive control (PC)	9.93±0.7581 [#]
4.	Test control 1 (MOFL 100mg/kg)	18.80±1.1331 [#]
5.	Test control 2 (MOFL 200mg/kg)	13.05±1.1879 [#]
6.	Test control 3 (MOFL 400mg/kg)	10.80±1.4805 [#]
7.	Test control 4 (MCFL 50mg/kg)	21.58±1.0907 [#]
8.	Test control 5 (MCFL 100mg/kg)	15.53±0.8802 [#]
9.	Test control 6 (MCFL 150mg/kg)	12.30±0.9980 [#]

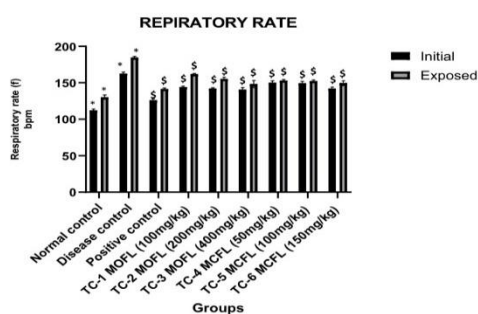


Figure 3: Histogram representing the effect of positive drug (OVA +DEX) and test drugs on Respiratory rate. Data represented as mean ±SEM (n=6). *p<0.001 vs NC, *p<0.001 vs DC, *p<0.001 vs PC, TC-1, TC-2,TC-3,TC-4, TC-5, TC-6. MOFL 400 mg/kg (TC-3) and 150mg/kg (TC-6) showed the most significant reduction in both initial and exposed stage of respiratory rate (\$p < 0.001).

3.4 Total Leukocyte Count in BALF

The graph impact of positive control (OVA+DEX) and various test drugs on WBC count in BALF of

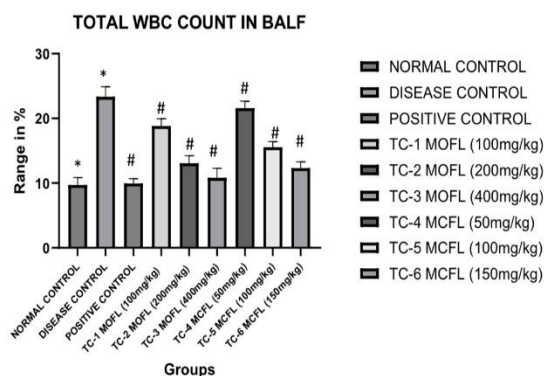


Figure 4: Histogram representing the effect of positive drug (OVA +DEX) and test drugs on WBC count on BALF. Data represented as mean ±SEM



(n=6). *p<0.001 vs NC, *p<0.001 vs DC, *p<0.002 vs PC, TC-1, TC-2,TC-3,TC-4, TC-5, TC-6. MOFL 400 mg/kg (TC-3) showed the most significant reduction in WBC in BALF (#p < 0.01).

3.5 TNF-α Estimation.

Graph demonstrate the effect of OVA+DEX and test drugs on TNF-α levels in BALF. The disease control group exhibited a sharp increase in TNF-α, confirming inflammatory response activation. Both the positive and test drugs significantly reduced TNF-α levels compared to DC (*p<0.001). Notably, MOFL 400mg/kg (TC-3) and MCFL 150mg/kg (TC-6) produced the strongest suppression of TNF-α (#p<0.001).

Table-6: Serum TNF-alpha in treatment groups

S.N O	GROUPS	TNF-α LEVEL	
		ABS	Conc. (ng/ml)
1.	Normal control	0.398±0.0025	0.917±0.1667*
2.	Disease control	0.445±0.0031	4.033±0.2073*
3.	Positive control	0.405±0.0018	1.333±0.1217#
4.	Test control 1 (MOFL100mg/kg)	0.429±0.0024	2.95±0.1575#
5.	Test control 2 (MOFL 200mg/kg)	0.421±0.0022	2.4±0.1440#
6.	Test control 3 (MOFL 400mg/kg)	0.41±0.0025	1.667±0.1721#
7.	Test control 4 (MCFL 50mg/kg)	0.436±0.0029	3.433±0.1924#
8.	Test control 5 (MCFL 100mg/kg)	0.425±0.0037	2.7±0.2464#

9.	Test control 6 (MCFL 150mg/kg)	0.415±0.0029	2.017±0.199#
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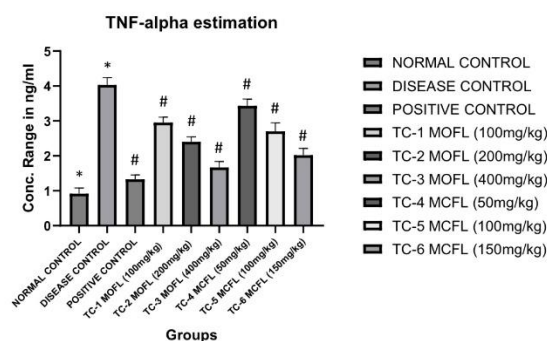


Figure 5: Histogram representing the effect of positive drug (OVA +DEX) and test drugs on TNF-alpha estimation. Data represented as mean ±SEM (n=6). *p<0.001 vs NC, *p<0.001 vs DC, #p<0.001 vs PC, TC-1, TC-2, TC-3, TC-4, TC-5, TC-6. MOFL 400 mg/kg (TC-3) and MCFL 150 mg/kg (TC-6) showed the most significant reduction (#p < 0.001).

3.3 Histamine assay.

To determine histamine levels in lung tissue Histamine Quantification Assay Kit (MAK432) was used.

Table 7.25: Values of Standard curve of Histamine

Histamine Conc. (ug/ml)	Average OD (450 nm)	Blank subtracted
50.0	3.735	3.399
25.0	2.364	2.028
12.5	1.687	1.351
6.3	1.354	1.018
3.1	1.179	0.843
1.6	1.047	0.711
0.8	0.986	0.650
0.0	0.336	0.000

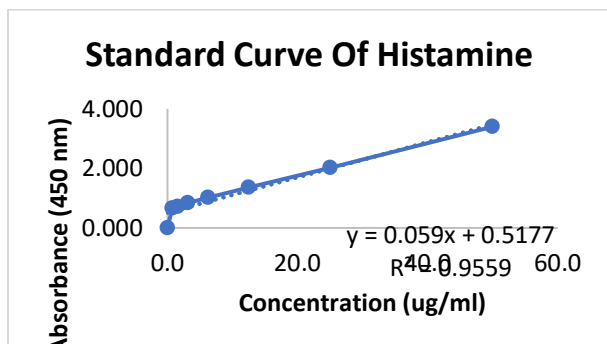


Figure 6: Standard curve of Histamine

The graph shows how several test medications and the positive control drug (OVA+DEX) affect histamine levels, with mean±SEM (n=6). A significant elevation in histamine count was observed in the disease control compared to the normal control (#p<0.001). Treatment with the positive control, as well as test compound, markedly reduced histamine levels compared to the disease control group. The pronounced reductions were recorded for MOFL 400mg/kg (TC-3) AND MCFL 150 mg/kg (TC-6). These both are showing *p<0.001 versus all other groups and mitigating airway inflammation.

Table 7.26: Histamine Assay in treatment groups

S.N o.	Group	Histamine Levels	
		ABS	Conc.(ug/ml)
1.	Normal control	0.688±0.0088	2.886±00 [#]
2.	Disease control	1.081±0.0073	9.539±0.1242 [#]
3.	Positive control	0.739±0.0060	3.747±0.1020 [*]
4.	Test control 1 (MOFL100mg/kg)	0.998±0.0075	8.145±00 [*]
5.	Test control 2 (MOFL 200mg/kg)	0.930±0.0055	6.988±0.0928 [*]
6.	Test control 3 (MOFL 400mg/kg)	0.803±0.0065	4.831±0.1102 [*]

7.	Test control 4 (MCFL 50mg/kg)	1.020±0.0056	8.518±00 [*]
8.	Test control 5 (MCFL 100mg/kg)	0.977±0.0056	7.789±0.0942 [*]
9.	Test control 6 (MCFL 150mg/kg)	0.850±0.0076	5.628±0.1294 [*]

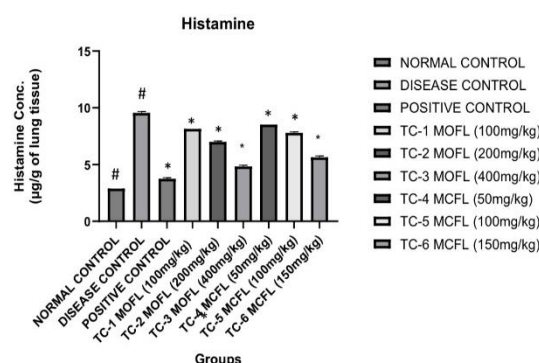
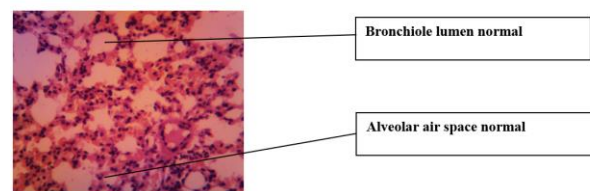


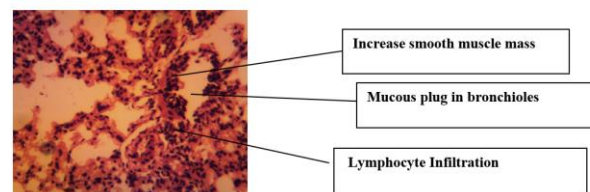
Figure 7: Histogram representing the effect of positive drug (OVA +DEX) and test drugs on Histamine count. Data represented as mean ±SEM (n=6). #p<0.001 vs NC, #p<0.001 vs DC, *p<0.001 vs PC, TC-1, TC-2, TC-3, TC-4, TC-5, TC-6. MOFL 400 mg/kg (TC-3) and MCFL 150 mg/kg (TC-6) showed the most significant reduction in WBC in BALF (*p < 0.001).

3.3 Histopathological analysis

Group I (Normal control)



Group II (Disease control)





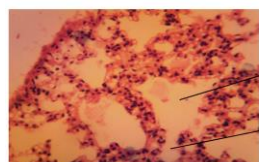
Group III (Positive control-)



Thin airway smooth muscles

Mucous plug is less

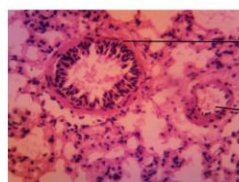
Group IV (Test control-1)



Respiratory duct slightly filled with mucus

Alveolar is slightly impairing for normal gas exchange

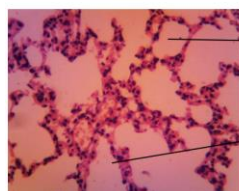
Group V (Test control-2)



Bronchial lumen is narrowing

Artery congestion

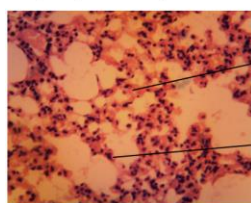
Group VI (Test control-3)



Bronchioles is thin and reduced mucous plug

Alveolar space restored and indicating reduction of obstructions

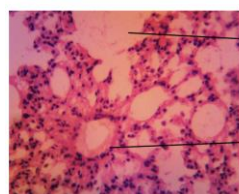
Group VII (Test control-4)



Capillaries in alveolar wall exhibit changes

Bronchiolar wall thickening

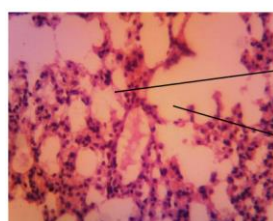
Group VIII (Test control-5)



Respiratory duct slightly filled with mucus

Pseudo stratified respiratory epithelium

Group IX (Test control-6)



Thin airway smooth muscles

Mucous plug is less to improve airway patency

4. Discussions

In the present study, methanolic, ethyl acetate, and petroleum ether extracts of *Momordica charantia* (MC) and *Moringa oleifera* (MO) leaves were prepared using maceration. Methanolic extracts showed the highest yield (MC: 2.58%; MO: 1.63%), consistent with reports highlighting methanol's polarity and extraction efficiency (Kumar *et al.*, 2022). Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, glycosides, tannins, and phenolics, with methanolic extracts showing strong positive responses, corroborating previous findings (Sharma *et al.*, 2023; Patel *et al.*, 2022). Quantitative analysis indicated high total phenolic content (TPC) and total flavonoid content (TFC), with MO consistently higher than MC (TPC: 109.18 vs. 70.96 mg GAE/g; TFC: 93.10 vs. 60.43 mg RE/g), confirming its superior antioxidant potential (Chahar *et al.*, 2017; Adegbesan *et al.*, 2024). TLC and column chromatography, followed by UV-Vis, FT-IR, ¹H NMR, and mass spectrometry, identified flavonoid-type compounds, including catechin derivatives (MC: C₁₅H₁₄O₆; MO: C₁₅H₁₀O₈), known for antioxidant and anti-inflammatory properties (Yadav *et al.*, 2024; Singh *et al.*, 2023). In vivo, ovalbumin-induced asthma in guinea pigs was used to evaluate anti-asthmatic effects. No significant changes in body weight, appetite, or water intake were observed. MOFL 400 mg/kg and MCFL 150 mg/kg significantly reduced tidal volume and respiratory rate (*p<0.001), indicating strong respiratory modulation (Mahajan *et al.*, 2009). Total leukocyte counts and eosinophil infiltration in bronchoalveolar lavage fluid were elevated in disease controls but significantly decreased in MOFL and MCFL groups, comparable to dexamethasone (Rahman *et al.*, 2020; Bhattacharya *et al.*, 2022). TNF-α levels, elevated in disease controls, were dose-dependently reduced by both extracts (MOFL 1.66 ng/ml; MCFL 2.01 ng/ml), highlighting anti-inflammatory activity (Shukla *et al.*, 2021). Histamine levels were also significantly lowered (MOFL 4.83 μg/ml; MCFL 5.62 μg/ml), suggesting mast cell stabilization (Verma *et al.*, 2023). Histopathology confirmed dose-dependent protection of lung architecture, with the highest MOFL dose showing comparable effects to dexamethasone (Singh *et al.*, 2021). Overall, *Moringa oleifera* and *Momordica charantia* leaves are rich in flavonoids and phenolics,



with MO showing higher yield and compound diversity. Both extracts demonstrated significant protective and anti-asthmatic effects, validating their traditional medicinal use and potential as natural therapeutic agents.

5. Conclusion

Finally, we concluded that phytochemical, pharmacological, and anti-asthmatic potential of *Moringa oleifera* (MO) and *Momordica charantia* (MC) leaves. Extraction using solvents of increasing polarity revealed methanolic extracts as the richest in phytochemicals, with *Moringa oleifera* exhibiting higher yields of phenolic and flavonoid contents. Phytochemical screening, TLC, chromatographic separation, and spectroscopic characterization confirmed the presence of catechin-type flavonoids, known for potent antioxidant and anti-inflammatory activities. In vivo studies using ovalbumin-induced guinea pig models demonstrated that both MO and MC extracts significantly improved respiratory parameters, reduced leukocyte infiltration in BALF, suppressed TNF- α and histamine levels, and restored lung histoarchitecture. The effects were dose-dependent, with MO at 400 mg/kg showing the greatest efficacy, closely comparable to the standard drug dexamethasone. Importantly, no adverse impact on body weight, appetite, or general health was observed, indicating good tolerability. Overall, these findings scientifically validate the traditional use of MO and MC leaves in respiratory disorders and highlight their potential as flavonoid-rich natural therapies for allergic asthma. *Moringa oleifera* demonstrated superior efficacy, positioning it as a promising clinical investigation aimed at developing safe and effective adjunct treatments for asthma.

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