Original Article

Bronchodilatory Activity of Moringa Oleifera: An in-Vitro and in-Silico Analysis

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Abstract

Background: Bronchodilation is a critical therapeutic approach in managing asthma. Traditional medicine documents bronchodilatory effects of Moringa oleifera, suggesting that it can help alleviate airway constriction and improve respiratory function.

Objective: To explore the bronchodilatory effect of Moringa oleifera leaf extract, through in-vitro and in-silico analysis.

Methods: An in-vitro experimental study and in-silico analysis was performed in the Pharmacology Department of CMH Lahore Medical College – From October 2022 to July 2023.

Swiss albino mice were used for the study. Trachea was dissected and mounted in organ baths connected to the PowerLab data acquisition system. Contractions were induced using acetylcholine (ACh) and high potassium chloride (Kcl), and incremental doses of the Moringa oleifera leaf extract were cumulatively added to observe bronchodilator activity. This was followed by in-silico analysis of catechin, gallic acid, quercetin and isoquercetin. Pharmacokinetic profiling and molecular docking were carried out.

Results: Moringa oleifera leaf extract reduced contractions induced by Ach and KCl, leading to tracheal relaxation, with an IC50 of 1.223 mg and 4.574 mg, respectively. A significant difference between the IC50 values of ACh and KCl induced contractions was observed (p=0.0008).

Pharmacokinetic profiling documented drug likeness for catechin, gallic acid, and quercetin. Molecular docking analysis revealed that quercetin exhibited the highest binding affinity to the voltage gated calcium channel.

Conclusion: In-vitro investigation demonstrated the significant bronchodilatory effect of Moringa oleifera. The in-silico analysis provided insights into potential active compounds, with quercetin showing promising interactions with the target protein.

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Introduction

Asthma is a chronic respiratory condition manifested as bronchiolar constriction and inflammation of the airways. It affects millions globally, leading to significant morbidity and imposing a considerable burden

on healthcare systems. In this context, the potential of Moringa oleifera in addressing asthma-related concerns, particularly its bronchodilatory properties, has attracted attention.

Moringa oleifera is a highly resilient perennial plant renowned for its rapid growth and ability to withstand drought conditions. It is commonly known as the drumstick tree or horseradish tree and cultivated extensively in Asia, including Pakistan, India, Sri Lanka, Malaysia, and the Philippine Islands. The roots, bark and leaves of Moringa oleifera harbor remarkable medicinal as well as nutritional properties, earning it the popular title of the "Miracle tree".[1]

Among its diverse uses in traditional folk medicine, Moringa oleifera has been historically employed to address various health issues, ranging from anemia, malnutrition, diabetes, and depression to arthritic disorders.[2–5]. Modern scientific investigations have revealed additional beneficial attributes, such as its anti-inflammatory, anticancer, and antioxidant properties. Notably, studies have reported no adverse effects associated with the consumption of Moringa oleifera leaves, even at doses achievable through oral ingestion. Recent research has further highlighted its antioxidant and anti-inflammatory activities. It has also been shown to be effective against depression, diabetes mellitus and hyperlipidemias.[6–8]

A treasure trove of bioactive compounds such as alkaloids, saponins, terpenoids, flavonoids, tannins, glycosides, phenolic acids, and steroids enrich the Moringa oleifera plant, contributing to its remarkable medicinal potential. Notable compounds like tannic acid, isoquercetin, and catechin exert antioxidant and anti-inflammatory effects, safeguarding against oxidative stress and inflammation. The plant's antioxidant activity shields against cellular damage inflicted by free radicals, while its anti-inflammatory effects counteract inflammatory processes within the body.[9,10] In-silico studies are increasingly being carried out to observe the receptor-ligand interactions at molecular level, in order to identify the active compounds in plant extracts that could serve as a potential drug precursor.[11,12] Multiple studies have documented the phytochemicals present in M. oleifera leaves, and rigorous research is being carried out to elucidate the pharmacological potential of different compounds through bioinformatic studies. Encouragingly, this miraculous plant exhibits a favorable safety profile, with no reported adverse effects in humans or animals, making it a widely embraced component of traditional folk medicine without significant toxicity or adverse reactions.[13]

Bronchodilation, the widening of the airways in the lungs, is a critical therapeutic approach in managing asthma. Traditional medicine documents bronchodilatory effects of Moringa oleifera, suggesting that it can help alleviate airway constriction and improve respiratory function. This property is of particular interest for asthmatic patients who experience difficulty breathing due to narrowed air passages. As research unfolds and sheds light on its specific mechanisms of action, Moringa oleifera may emerge as a valuable adjunctive therapy in the management of asthma, offering hope for improved respiratory function and enhanced quality of life for those living with this chronic respiratory condition.[5,6]

The purpose of this study was to investigate the effect of leaf extract of M. oleifera on isolated trachea, in vitro, shedding light on its bronchodilator potential as a natural remedy for asthma.[14] Subsequently, in-silico studies were carried out to analyze molecular interactions between the phytochemicals of M. oleifera and target receptor. Clinical trials and human studies are essential to validate these findings and develop safe and effective treatments for asthma patients.

Methods

An in-vitro experimental study was conducted in the Pharmacology laboratory of CMH Lahore Medical College, from October 2022 to July 2023. The study was conducted following ethical guidelines and after obtaining the ethical approval from the Ethics Committee of CMH Lahore Medical College (Certificate no. 609/ERC/CMH/LMC). Healthy Swiss albino mice, of male gender, within the weight range of 25-35 grams were obtained from the Animal Laboratory at CMH Lahore Medical College. All animal care and handling procedures strictly complied with the protocol detailed in the 'Guide for the care and use of laboratory animals.'[15]

Approximately 800 grams leaves of Moringa oleifera, in dried form, were acquired from a local market. The plant was verified by Botany Department at Government College University, Lahore. A sample of the plant was stored in the Herbarium, with voucher number GC-Herb-BOT-3785. Aqueous extract of the leaves was prepared using standard extraction protocol.

The mice were administered ketamine for anesthesia. Tracheal tissue was extracted, and adjacent fatty tissues were removed. The tissue was then sliced into 2-3 mm rings, each containing two cartilages. These isolated tracheal segments were mounted in the organ bath filled with Krebs solution. The temperature of the solution was kept at 37°C. One gram of pre-tension was applied

to the trachea, and it was left to stabilize for an hour. To induce contractions, acetylcholine (1 μM) and high KCl (80 mM) were introduced. After a 30-minute period, incremental doses of the extract were cumulatively added to observe its bronchodilator activity. PowerLab data system, developed by AD Instru-ments Australia, was used for recording isometric responses.[16]

The data was analyzed, statistically, with the latest available version of GraphPad Prism (8.0.1). Quantitative variables were presented as mean ± standard error of mean (SEM). All the data was normalized and expressed in the form of percentage inhibition, in order to draw multiple comparisons. The number of tissues per group was denoted as n. Dose-response curves were plotted and analyzed with non-linear regression. Half-maximal inhibitory dose (IC50) was calculated, keeping the confidence interval at 95 percent. To determine whether there was a statistically significant difference between the groups, the IC50 of the various groups were compared, and $p < 0.05$ was considered significant.

After gathering data from multiple phytochemical studies[4,5,17,18], four most promising compounds were retrieved from the PubChem database. These included catechin, gallic acid, quercetin and isoquercetin. SMILE Canonicals of these compounds were submitted SwissADME, for assessment of pharmacokinetic profiles and drug likeness.[17] The target protein was calcium voltage-gated channel subunit alpha 1C. Its 3-dimensional configuration was taken from RCSB protein data bank (PDB ID: 8FD7). It was visualized with Biovia Discovery Studios software. All unnecessary molecules were removed and only protein chains were selected to be visualized. This test protein structure was stored in PDB form. The three dimensional configurations of test compounds were also visualized with Biovia Discovery Studios software and stored as a PDB file.

Docking analysis was carried out with Autodock Vina/ PyRx software. Test protein was loaded as macromolecule. Test compounds were added as test ligands, one by one. Autogrid was maximized and Vina Wizard was run. Maximum binding affinities (kcal/mol) were recorded. The docked complex was then visualized with Biovia Discovery Studios software and receptor-ligand interactions were analyzed.[12]

Results

The leaf extract consistently reduced the contractions induced by acetylcholine in the trachea, leading to tracheal relaxation. The IC50 was 1.223 mg and the dose range within 95% confidence interval was 0.6016-2.320 mg. The leaf extract also reduced the contractions induced by KCl in the trachea, leading to tracheal relaxation. The IC50 value was calculated to be 4.574 mg and the dose range within 95% confidence interval was 2.934-7.003.

Figure 1a and 1b visually represent the response of tracheal tissue, to increasing doses of the extract, recorded on PowerLab software LabChart.

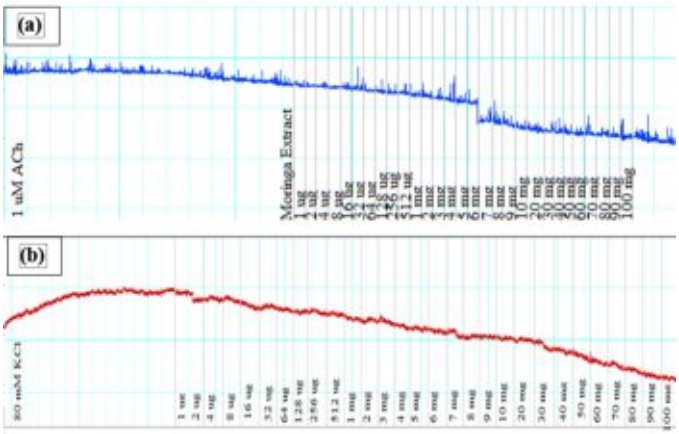


Figure. 1: ***(a)*** *Effect of Moringa oleifera leaf extract on trachea pre-treated with 1 uM Acetylcholine* ***(b)****Effect of Moringa oleifera leaf extract on trachea pre-treated with 80 mM KCl (n=6)*

Non-linear regression was applied to compare the IC50 values for the tracheal tissue response to M. oleifera leaf extract between Acetylcholine and potassium-induced contractions. A statistically significant difference was observed between IC50 values of the two groups (p=0.0008).

Table 1 and Fig. 2 present the comparative results of the relaxant activity of Moringa oleifera against Acetylcholine and potassium-induced tracheal contraction.

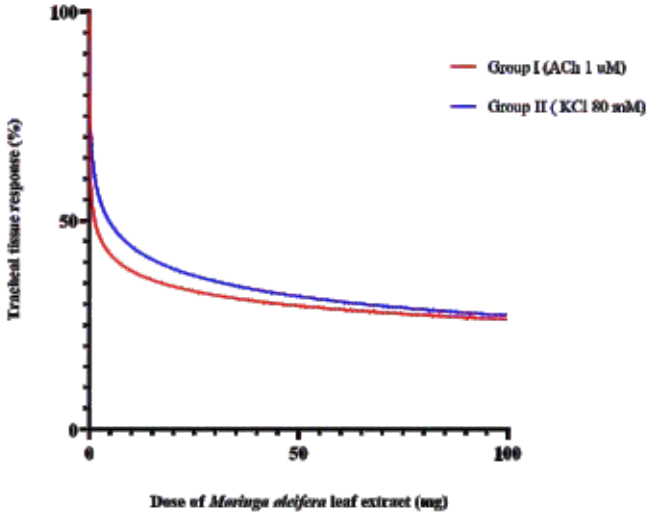


Figure. 2: *Comparison of relaxant effect of Moringa*

oleifera leaf extract against Acetylcholine and KCl-induced tracheal contraction (n=6)

Table 1: *Effect of Moringa oleifera extract on in-vitro tracheal contraction (n=6)*

Non-linear regression analysis			
Para-meters	**Group I (1 uM Acetylcholine)**	**Group II (80 mM KCl)**	**p-value**
IC50	1.223	4.574	0.0008
95 % CI (IC50)	0.602 to 2.320	2.934 to 7.003	
HillSlope	-0.234	-0.318	
R squared	0.428	0.566	

Table 2 reports the test ligands' information obtained from PubChem, including the compound name, PubChem CID, SMILES Canonicals, 2D and 3D structures.

Pharmacokinetic profiling documented drug likeness for Catechin, Gallic acid, and Quercetin. Isoquercetin did not fulfil the Lipinski's criteria. Table 3 provides detailed description of the pharmacokinetic profiles of test compounds.

Based upon the pharmacokinetic data and drug likeness, molecular docking was carried out with Catechin, Gallic

Table 2: *PubChem data of test compounds selected from Moringa oleifera*

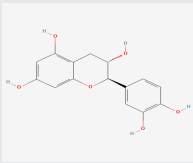
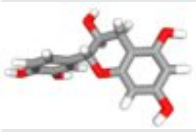
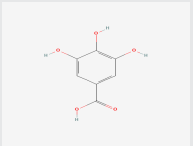
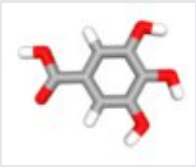
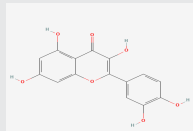
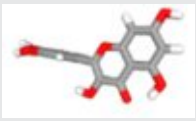
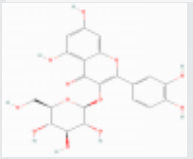
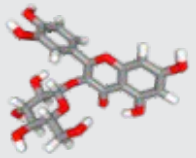
Compound	PubChem CID	2D Structure	3D Structure
Catechin	9064		
Gallic acid	370		
Quercetin	5280343		
Isoquercetin	5280804		

Table 3: *Pharmacokinetic profile of test compounds selected from Moringa oleifera*

Parameters	Catechin	Gallic acid	Quercetin	Isoquercetin
Absorption and bioavailability				
Blood brain barrier (BBB)	No	No	No	No
Human intestinal absorption	High	High	High	Low
P-glycoprotein substrate	Yes	No	No	No
Bioavailability score	0.55	0.56	0.55	0.17
Lipophilicity (LogP)	0.85	0.21	1.23	-0.25
Topological polar surface area (TPSA)	110.38	97.99	131.36	210.51
Metabolism				
CYP450 1A2 Inhibitor	No	No	Yes	No
CYP450 2C19 Inhibitor	No	No	No	No
CYP450 2C9 Inhibitor	No	No	No	No
CYP450 2D6 Inhibitor	No	No	Yes	No
CYP450 3A4 Inhibitor	No	Yes	Yes	No
Lipinski's rule				
Molecular weight (g/mol)	290.27	170.12	302.34	464.38
Molar refractivity	74.33	39.47	78.03	110.16
Lipophilicity (LogP)	0.85	0.21	1.23	-0.25
No. of H-bond acceptors	6	5	7	12
No. of H-bond donors	5	4	5	8
Drug likeness	Yes	Yes	Yes	No
Lead likeness	Yes	No	Yes	No

acid and Quercetin. Docking analysis revealed that Quercetin had the strongest binding affinity (-8.2 kcal/mol), followed by Catechin (-7.8 kcal/mol), while Gallic acid showed the weakest binding affinity (-6.4 kcal/mol).

Since the highest binding affinity was recorded with Quercetin, the test protein-quercetin docked complex was visualized with Biovia Discovery Studio software for receptor-ligand interaction. Quercetin formed hydrogen bonds with Thr361, Met362, Glu363, Asn741, Glu1135, Thr1462, and Gly1463. Figure 3a and 3b visualize the 3D and 2D interaction models of Quercetin with the calcium channel subunit alpha 1C.

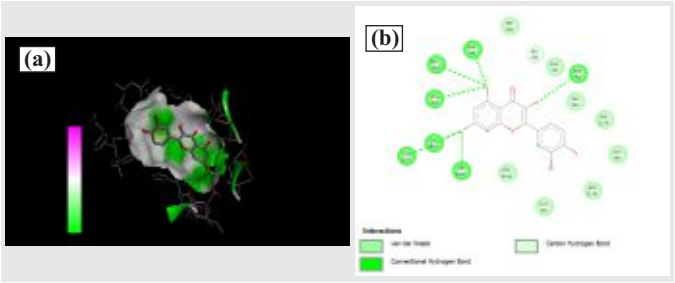


Figure 3: *3D (a) and 2D (b) interaction model of Quercetin with the calcium channel subunit alpha 1C*

Discussion

Asthma, a prevalent respiratory disorder, poses significant challenges in its management. Traditional medicine has frequently utilized Moringa oleifera for alleviation of bronchospasm associated with asthma. While its bronchodilator effects have been observed in in-vivo animal studies, further investigation is required to elucidate the underlying mechanism of action.[19,20]

The leaf extract of Moringa oleifera has demonstrated a noteworthy bronchodilatory effect, effectively counteracting tracheal smooth muscle contractions induced by both acetylcholine and KCl. The meticulous comparison of IC50 values between these two groups revealed a compelling statistical significance (p=0.0008), further accentuating the extract's remarkable potential as a bronchodilator. These findings build upon the foundation laid by prior research endeavors, which have already explored and documented the beneficial effects of Moringa oleifera in animal studies focused on bronchoconstriction induced by histamine and acetylcholine, as well as allergic asthma induced by ovalbumin. While previous in-vivo studies have reported the bronchodilatory activity of Moringa oleifera, these primarily involved oral or intraperitoneal administration of methanolic or ethanolic extracts, and measured outcomes such as changes in respiratory rate or allergic airway inflammation in ovalbumin-sensitized animal models. In contrast, our study utilized aqueous extract directly applied to isolated tracheal smooth muscle, allowing direct observation of contractility in response to acetylcholine and KCl. The extract concentrations in our study (IC_{50} of 1.223 mg for ACh-induced and 4.574 mg for KCl-induced contractions) provide dose-specific pharmacological insights that are not available from systemic in-vivo models, which are confounded by metabolism, distribution, and immune modulation.[5,6,19]

The IC_{50} values observed in our study can be contextualized by comparing them to similar in-vitro investigations on plant-based bronchodilators. For instance, Eugenia jambolana bark extract has shown bronchodilatory IC_{50} values ranging between 1–3 mg/mL in in-vitro tracheal models,[19] while Astragalus sarcocolla demonstrated effects at even lower concentrations, attributed to potassium channel activation.[22] Our IC_{50} for M. oleifera against ACh (1.223 mg/mL) aligns with moderate potency, suggesting promising efficacy, though slightly less potent than some spasmolytics.

Despite the growing body of knowledge on the pharmacological prowess of Moringa oleifera, it is indeed surprising that, until now, no in-vitro studies have been dedicated to investigating its direct impact on tracheal smooth muscle contractility.[19,21–23] This study has emerged as a significant breakthrough, addressing a critical gap in our understanding of Moringa oleifera's effects on bronchoconstriction. The relaxation induced by the Moringa oleifera leaf extract could possibly result from its profound influence on the intricate mechanisms governing tracheal smooth muscle contraction. These mechanisms primarily involve Gq-coupled pathways and potassium-induced depolarization. In the current study, the cholinergic and KCl-induced bronchoconstriction were both mitigated by the Moringa extract, suggesting that a common pathway such as calcium channel blockade must be involved. This would decrease the calcium influx and impede the calcium-calmodulin dependent smooth muscle contraction, leading to a bronchodilatory effect. Another explanation would be the hyperpolarization of the smooth muscle cell membrane brought on by an opening of potassium channels, which would then cause the voltage-operated calcium channels to close.[6,14,24] However, this could not be elucidated in the present study due to limitation of resources, and requires further research.

Results of the in-vitro study advocate calcium channel as one of the target proteins. The alpha-1 subunit of voltage operated calcium channels is responsible for calcium influx into the cell. Hence, the pharmacological

properties of the calcium channel are primarily dependent on this subunit.[25] Therefore, calcium channel subunit alpha 1C was taken as the "test protein". Exploring the bioactive constituents of Moringa oleifera, the phytochemical analysis has spotlighted the abundance of catechin, gallic acid, quercetin and isoquercetin, in the botanical. An in-silico analysis was conducted to forecast how these substances might interact with the calcium channel subunit alpha 1C. Virtual pharmacokinetic screening of test compounds was performed according to Lipinski's rule. According to Lipinski's rule of five, a chemical exhibits drug-like effect if at least three of the following conditions are met: a molecular mass of less than 500 Da, five hydrogen donors at most, ten hydrogen bond acceptors at most, and lipophilicity (LogP) of less than five.[20] Pharmacokinetic profiling documented drug likeness for catechin, gallic acid and quercetin. Isoquercetin did not fulfil the Lipinski's criteria and was henceforth, eliminated from docking studies. Molecular docking was carried out with AutoDock Vina, using grid-based approach. The docking results revealed strong interaction of catechin, gallic acid and quercetin, with the test protein. The highest binding affinity was recorded with quercetin (-8.2 kcal/mol). Therefore, it was carried forward for analysis of receptor-ligand interactions and the remaining two compounds were excluded. The docked complex of quercetin and the calcium channel subunit alpha 1C was visualized with Biovia Discovery Studio software. RCSB data reports that the binding site of calcium channel subunit alpha 1C is located on Glu363, Thr706, and Glu1135 (https://www.rcsb.org/sequence/8FD7). Quercetin formed hydrogen bonds with Thr361, Met 362, Glu363, Asn741, Glu1135, Thr1462, and Gly1463. This indicates that quercetin could form a stable complex with the active site of calcium channel subunit alpha 1C, resulting in calcium blocking activity.

The study had a few limitations. Firstly, the extrapolation of in-vitro and in-silico results to in-vivo outcomes is inherently limited, as systemic factors such as bioavailability, metabolism, and immune interactions are not accounted for. Additionally, we could not investigate the extract's effects on low K^+-induced contractions, β-adrenergic receptor activity, or specific ion channel subtypes, which could further clarify the extract's precise mechanism of action.

Conclusion

Moringa oleifera leaf extract exhibits promising bronchodilatory activity in vitro, likely mediated by calcium channel blockade, with quercetin identified as a key active compound through in-silico analysis. However, translating these findings to in-vivo contexts remains a challenge due to the inherent limitations of in-vitro and computational models. Further research involving comparisons with standard β-agonists, and ion-channel-specific electrophysiological studies is recommended.

Ethical Approval: The Institutional Review Board, CMH Lahore Medical College & Institute of Dentistry approved this study vide letter case#. 609/ERC/CMH/LMC.

Conflict of Interest: The authors declare no conflict of interest.

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Author's Contribution:

SZ: Conception & design, acquisition of data, analysis & interpretation of data, drafting of article

MQ: Conception & design, Final approval of the version to be published

MA: Analysis & interpretation of data, drafting of article

MRZ: Acquisition of data, analysis & interpretation of data

WAS: Drafting of article, analysis & interpretation of data

RB: Conception & design, critical revisions for important intellectual content

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