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Protective effects of *Moringa oleifera* L. Leaves extract against ethylene glycol–induced lung injury: An integrated *In Vivo* and In Silico study

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ABSTRACT

Ethylene glycol (EG) intoxication induces multi-organ toxicity characterized by oxidative stress, inflammation, and pulmonary injury secondary to metabolic acidosis and renal damage. This study aimed to evaluate the protective effect of *Moringa oleifera* L. leaves extract against EG-induced lung damage using integrated *In Vivo* and in silico approaches. Twenty-five male Wistar rats were randomly divided into five groups: negative control, EG control (265 mg/kg BW/day), and three treatment groups receiving EG followed by *M. oleifera* extract at doses of 200, 316, and 500 mg/kg BW for 21 days. Lung tissues were examined histopathologically using hematoxylin and eosin staining to assess alveolar congestion and interalveolar septal thickening. Molecular docking analysis was performed to evaluate interactions between major phytoconstituents and apoptosis- and inflammation-related targets, namely Caspase-3 and Tumor Necrosis Factor Receptor 1 (TNFR1). EG exposure significantly increased congestion and septal thickening compared to the negative control ($p < 0.05$). Administration of *M. oleifera* extract produced dose-dependent improvement. The 500 mg/kg BW dose showed the greatest protective effect, reducing alveolar congestion by 48% and septal thickening by 78.3%, with no significant difference compared to the negative control ($p > 0.05$). Phytochemical screening identified quercetin, kaempferol, β-carotene, tocopherol, and ascorbic acid as potential bioactive compounds. Docking results demonstrated strong binding affinities of β-carotene (−8.1 kcal/mol) toward Caspase-3 and flavonoids toward TNFR1 (−6.4 to −6.5 kcal/mol), suggesting inhibition of apoptotic and inflammatory pathways. In conclusion, *M. oleifera* L. leaves extract, particularly at 500 mg/kg BW, exhibits significant protective effects against EG-induced pulmonary injury. The integrated *In Vivo* and in silico findings support its potential as a natural therapeutic candidate targeting oxidative stress–mediated lung damage.

Introduction

Ethylene glycol (EG) is the simplest diol compound, chemically characterized as a clear, colorless, odorless liquid with a sweet taste (Fowles *et al*., 2017). Due to its palatable sweetness and non-irritative properties upon ingestion, EG is often referred to as the "sweet killer" (Al-Kasabera *et al*., 2024). Despite its widespread industrial use in antifreeze, solvents, and pharmaceutical preparations, accidental and intentional poisoning remain significant public health concerns (Sharma and Sebastian, 2025). In 2022, the Indonesian Ministry of Health reported 199 pediatric deaths associated with contaminated liquid syrup medications containing EG and diethylene glycol (Ministry of Health Republic of Indonesia, 2022). In addition to human cases, EG poisoning has also been documented in animals (Meles *et al*., 2024). Recent reports described EG intoxication in sport horses exposed through contaminated drinking water and fatal cases in calves, in which necropsy findings revealed pulmonary edema, petechial hemorrhage, and dark-reddish swollen lungs on macroscopic and microscopic examination (Daradics *et al*., 2025).

Following ingestion, EG is rapidly metabolized in the liver by alcohol dehydrogenase into glycolaldehyde, glycolic acid, glyoxylic acid, and ultimately oxalic acid (Ghannoum *et al*., 2023). Accumulation of glycolic acid contributes to high anion gap metabolic acidosis, while oxalic acid binds calcium ions to form calcium oxalate crystals (Ukita *et al*., 2022). Deposition of these crystals, particularly in renal tubules, leads to acute kidney injury (AKI) (Xu *et al*., 2026). Beyond renal toxicity, EG metabolism triggers oxidative stress through activation of NADPH oxidase and excessive generation of reactive oxygen species (ROS) (Caliri *et al*., 2021). ROS disrupt membrane lipids, impair ion transport, alter membrane fluidity, and initiate apoptotic signaling cascades, ultimately resulting in cellular injury and death (Chen *et al*., 2025).

Kidney injury secondary to EG poisoning can exacerbate pulmonary damage through systemic inflammatory mechanisms (Taira *et al*., 2025). The release of pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8) contributes to endothelial dysfunction in distant organs, including the lungs (Somade *et al*., 2020b). Activation of TNF receptor 1 (TNFR1) stimulates the nuclear factor kappa B (NF-κB) pathway and promotes caspase-3–mediated apoptosis in pulmonary endothelial cells (Ruiz *et al*., 2021). Increased capillary permeability, fluid retention, and metabolic acidosis further predispose to pulmonary edema, congestion, and interalveolar septal thickening (Zanza *et al*., 2023). The cardiopulmonary stage of EG toxicity typically occurs within 12–24 hours post-exposure, characterized by vascular congestion and inflammatory lung injury (Tanasescu *et al*., 2014).

Given the role of oxidative stress and inflammation in EG-induced lung damage, natural antioxidants have attracted attention as potential protective agents (Somade *et al*., 2020a). Herbal medicines are increasingly explored due to their multi-target activity and relatively favorable safety profiles (Wang *et al*., 2023). *Moringa oleifera* L., commonly known

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as drumstick tree or miracle tree, is rich in bioactive phytochemicals including flavonoids (quercetin and kaempferol), phenolic acids, vitamins (ascorbic acid and tocopherol), and carotenoids (Pareek *et al*., 2023). These compounds exhibit antioxidant, anti-inflammatory, and anti-apoptotic properties (Kashyap *et al*., 2022). Previous studies have demonstrated that *M. oleifera* leaf extract modulates inflammatory mediators and reduces oxidative stress in experimental models (Tilaoui *et al*., 2026). However, its specific protective effect against EG-induced pulmonary injury has not been comprehensively investigated.

Therefore, the present study aimed to evaluate the effect of *M. oleifera* . leaves extract on ethylene glycol–induced lung damage using an integrated *In Vivo* and in silico approach. The *In Vivo* analysis focuses on histopathological parameters, namely alveolar congestion and interalveolar septal thickening in Wistar rats. Complementary in silico molecular docking analysis was conducted to elucidate potential interactions between major phytoconstituents and key molecular targets involved in inflammation and apoptosis, particularly TNFR1 and caspase-3. This dual approach is expected to provide mechanistic insight into the therapeutic potential of *M. oleifera* leaves extract in mitigating EG-induced pulmonary injury.

Materials and methods

Ethical approval

All *In Vivo* experimental procedures in this study were conducted in accordance with ethical standards for the care and use of laboratory animals. Ethical clearance was obtained from the Health Research Ethical Clearance Commission, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia, under protocol number 158/HRECC.FODM/XI/2025.

The in silico component of this study did not involve human participants or live animals and therefore did not require additional ethical approval.

Study design

This study employed a completely randomized experimental design integrating both *In Vivo* and in silico approaches to evaluate the protective effect of *M. oleifera* leaves extract against ethylene glycol–induced lung damage. The *In Vivo* experimental procedures were conducted at the Experimental Animal Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. The study was carried out from August to October 2023.

The in silico molecular docking analysis was performed using computational platforms to investigate the interaction between major phytoconstituents of *M. oleifera* and target proteins associated with inflammation and apoptosis involved in ethylene glycol–induced pulmonary injury.

In Vivo Study

Preparation of *Moringa oleifera* L. leaves extract

M. oleifera leaves were taxonomically authenticated by the UPT Laboratorium Herbal Materia Medica Batu, East Java Provincial Health Office, Indonesia.

A total of 500 g of dried leaves were extracted using the maceration method with 2 L of 96% ethanol as solvent. The maceration process was carried out at room temperature for 72 hours with periodic stirring. The filtrate was collected and concentrated using a rotary evaporator at 40°C and 50 rpm to obtain a thick ethanolic extract (Sarumpaet *et al*., 2025).

Experimental animals

Twenty-five healthy male Wistar rats (*Rattus norvegicus*), aged 2–3 months and weighing 150–200 g, were used in this study. The animals were acclimatized for 7 days before treatment. Rats were housed under standard laboratory conditions, provided with commercial pellets and water ad libitum, and maintained at the Animal Experiment Unit, Faculty of Veterinary Medicine, Universitas Airlangga.

Experimental design and treatment

The rats were randomly divided into five groups (n = 5 per group):
K− (Negative control): Received 1% sodium carboxymethyl cellulose (CMC-Na) and distilled water orally for 21 days.
K+ (Positive control): Received ethylene glycol at a non-lethal toxic dose of 265 mg/kg BW/day orally for 21 days (Meles *et al*., 2024).
P1: Received ethylene glycol (265 mg/kg BW/day) followed by *M. oleifera* extract at 200 mg/kg BW/day.
P2: Received ethylene glycol (265 mg/kg BW/day) followed by *M. oleifera* extract at 316 mg/kg BW/day.
P3: Received ethylene glycol (265 mg/kg BW/day) followed by *M. oleifera* extract at 500 mg/kg BW/day.

Ethylene glycol was dissolved in distilled water and administered orally once daily. The *M. oleifera* extract was administered orally 4 hours after ethylene glycol induction each day for 21 consecutive days (Meles *et al*., 2024).

Termination and organ collection

On day 29, all rats were anesthetized with ketamine (100 mg/kg BW) and xylazine HCl (5 mg/kg BW) administered intraperitoneally. Intracardiac perfusion was subsequently performed. The lungs were collected through necropsy for histopathological examination.

Histopathological examination

Lung tissues were fixed in 10% neutral buffered formalin. After fixation, tissues were dehydrated in graded ethanol series (70%, 80%, 90%, 96%, and 100%), cleared in xylene, and embedded in paraffin. Sections were cut using a microtome and stained with hematoxylin and eosin (H&E) (Webster *et al*., 2009).

Histopathological changes were observed using a light microscope equipped with Optilab at 400× magnification. Lung damage was evaluated using a semi-quantitative scoring system adapted from previously reported criteria (Meles *et al*., 2024). The scoring guidelines are presented in Tables 1 and 2.

Table 1. Congestion scoring criteria.

Score	Histopathological condition
0	No congestion observed
1	<10% of field affected
2	11–25% of field affected
3	26–50% of field affected
4	51–75% of field affected
5	>75% of field affected

Table 2. Interalveolar septal thickening scoring criteria

Score	Histopathological condition
0	No septal thickening observed
1	<10% of field affected
2	11–25% of field affected
3	26–50% of field affected
4	51–75% of field affected
5	>75% of field affected

All histological procedures were conducted at the Laboratory of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Airlangga.

In Silico study

Ligand and receptor preparation

Bioactive compounds of *M. oleifera* were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

The target receptors used in this study were Caspase-3 (UniProt ID: P55213) and Tumor Necrosis Factor Receptor 1 (TNFR1; UniProt ID: P22934) of *Rattus norvegicus*, retrieved from the UniProt database (https://www.uniprot.org/).

Compound screening

Screening of bioactive compounds was conducted using the SCF-Bio web server based on Lipinski's Rule of Five to evaluate drug-likeness properties. The parameters assessed included molecular weight, hydrogen bond donors, hydrogen bond acceptors, logP value, and molar refractivity. Compounds fulfilling at least two of the five criteria were considered potential drug candidates (Mendie and Hemalatha, 2022).

Molecular docking and visualization

Molecular docking simulations were performed using PyRx 0.8 software. The docking process evaluated the binding affinity between selected ligands and target receptors. Binding affinity values were expressed in kcal/mol, where more negative values indicated stronger ligand–receptor interactions (Rana *et al*., 2021).

Visualization of ligand–receptor interactions was carried out using PyMOL v1.7.4 and BIOVIA Discovery Studio 2019.

Statistical analysis

Histopathological scoring data were analyzed using the Kruskal–Wallis test to determine differences among groups. When significant differences were detected, pairwise comparisons were performed using the Mann–Whitney test. Statistical analysis was conducted using SPSS software, with significance set at $p < 0.05$.

Results

In Vivo evaluation of lung histopathology

This study evaluated the protective effect of *M. oleifera* leaves extract against ethylene glycol (EG)–induced lung damage in Wistar rat (*Rattus norvegicus*) using both *In Vivo* and in silico approaches. The primary histopathological parameters assessed were alveolar congestion and interalveolar septal thickening.

Histological examination using hematoxylin and eosin (H&E) staining (Figures 1 and 2) demonstrated that the negative control group (K−) exhibited normal pulmonary architecture, characterized by thin interalveolar septa, intact alveolar spaces, and minimal vascular congestion. In contrast, the positive control group (K+) exposed to EG showed marked pathological alterations, including severe vascular congestion and prominent septal thickening.

Quantitative scoring (Table 3; Figure 3) showed that the K+ group had the highest mean congestion score (3.08 ± 0.66) and septal thickening score (4.24 ± 0.96), significantly higher ($p < 0.05$) than all other groups. Administration of *M. oleifera* leaves extract resulted in a dose-dependent improvement in lung histoarchitecture.

The P1 group (200 mg/kg BW) showed partial reduction in congestion (2.52 ± 0.94) and septal thickening (3.84 ± 0.62). The P2 group (316 mg/kg BW) demonstrated moderate improvement (2.36 ± 0.65 and 2.64 ± 0.52, respectively). Notably, the P3 group (500 mg/kg BW) exhibited the greatest protective effect, with congestion (1.60 ± 0.25) and septal thickening (0.92 ± 0.30) scores approaching those of the negative control group. Statistical analysis using Kruskal–Wallis followed by Mann–Whitney tests indicated no significant difference ($p > 0.05$) between K− and P3.

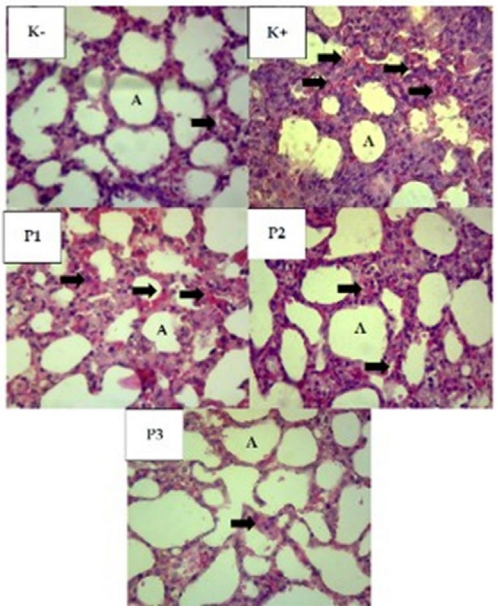

Figure 1. Haematoxylin and eosin (H&E) staining results in the lung section showing alveoli (A), vessel (V) and arrow (congestion). [K-: Control group, K+: Ethylene glycol group, P1: EG+Moringa 200 mg/kg BW, P2: EG+Moringa 316 mg/kg, P3: EG+Moringa 500 mg/kg BW]. Scale bar: 100 µm.

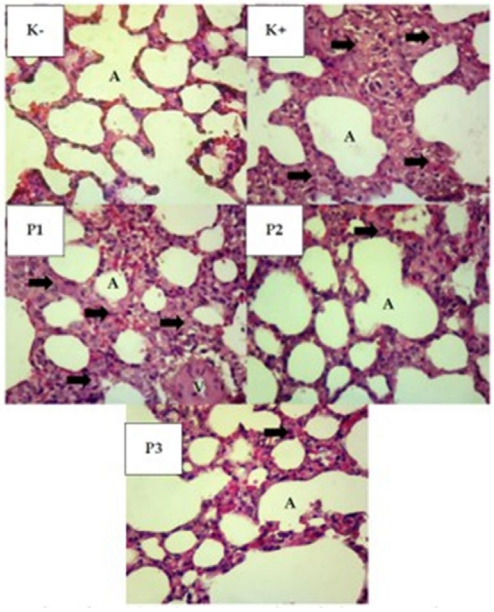

Figure 2. Haematoxylin and eosin (H&E) staining results in the lung section showing alveoli (A), vessel (V) and arrow (septa thickening). [K-: Control group, K+: Ethylene glycol group, P1: EG+Moringa 200 mg/kg BW, P2: EG+Moringa 316 mg/kg, P3: EG+Moringa 500 mg/kg BW]. Scale bar: 100 µm.

Table 3. Lung damage scores in ethylene glycol–induced rats

Group	Congestion (Mean ± SD)	Septal Thickening (Mean ± SD)
K−	$1.32^{a} \pm 0.36$	$0.64^{a} \pm 0.26$
K+	$3.08^{c} \pm 0.66$	$4.24^{c} \pm 0.96$
P1 (200 mg/kg)	$2.52^{b} \pm 0.94$	$3.84^{c} \pm 0.62$
P2 (316 mg/kg)	$2.36^{b} \pm 0.65$	$2.64^{b} \pm 0.52$
P3 (500 mg/kg)	$1.60^{a} \pm 0.25$	$0.92^{a} \pm 0.30$

Note: Different superscripts within the same column indicate significant differences ($p < 0.05$).

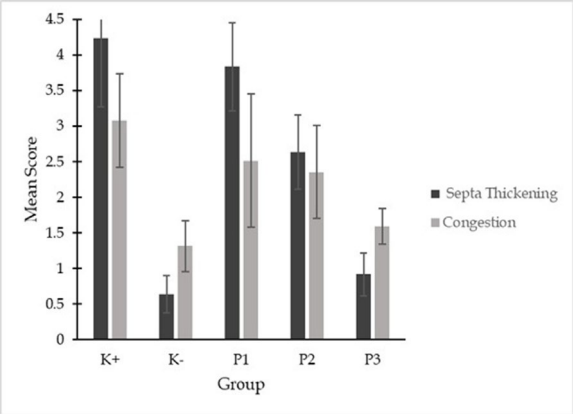

Figure 3. The effects of Moringa leaves extract on lungs tissue. [K-: Control group, K+: Ethylene glycol group, P1: EG+Moringa 200 mg/kg BW, P2: EG+Moringa 316 mg/kg, P3: EG+Moringa 500 mg/kg BW].

Phytochemical profile and drug-likeness evaluation

Phytochemical screening (Table 4) revealed that *M. oleifera* leaves contain several bioactive compounds, including quercetin, kaempferol, caffeic acid, ascorbic acid, tocopherol, β-carotene, and oxalic acid. All identified compounds met Lipinski's Rule of Five criteria, indicating favorable oral bioavailability and acceptable pharmacokinetic properties.

Table 4. Phytochemical compounds and lipinski's rule of five evaluation.

Compound	MW (g/mol)	HBA	HBD	LogP	MR	Drug-Like
Ascorbic acid	176.12	6	4	−1.41	35.12	Yes
β-carotene	536.87	0	0	12.61	184.43	Yes
Caffeic acid	180.16	4	3	1.09	47.16	Yes
Kaempferol	266.24	6	4	2.28	76.01	Yes
Oxalic acid	90.03	4	2	−0.84	15.27	Yes
Quercetin	302.24	7	5	1.99	78.03	Yes
Tocopherol	416.68	2	1	8.53	134.31	Yes

In Silico molecular docking analysis

To elucidate the molecular mechanism underlying the protective effects of *M. oleifera*, molecular docking was performed against Caspase-3 and TNFR1 receptors (Table 5; Figures 4 and 5).

Table 5. Molecular docking results

Compound	Receptor	Binding Affinity (kcal/mol)
β-carotene	Caspase-3	−8.1
Quercetin	Caspase-3	−7.0
Kaempferol	Caspase-3	−7.0
Quercetin	TNFR1	−6.4
Kaempferol	TNFR1	−6.5
Caffeic acid	Caspase-3	−5.4
Tocopherol	Caspase-3	−6.6

β-carotene exhibited the strongest binding affinity toward Caspase-3 (−8.1 kcal/mol), followed by quercetin and kaempferol (−7.0 kcal/mol). For TNFR1, quercetin (−6.4 kcal/mol) and kaempferol (−6.5 kcal/mol) demonstrated notable binding affinities.

Interaction analysis (Figure 4) showed that quercetin and kaempferol bind to key catalytic residues of Caspase-3, including Cys163, Arg207, Gln217, and Trp214. Similarly, interactions between flavonoids and TNFR1 residues (Lys128, Cys125, Glu160) were identified (Figure 5).

Discussion

The present study demonstrated that exposure to Ethylene glycol (EG) induced marked pulmonary structural alterations, as evidenced by severe vascular congestion and interalveolar septal thickening in the positive control (K+) group. These findings confirm that EG intoxication is not limited to renal injury but also exerts significant secondary effects on lung

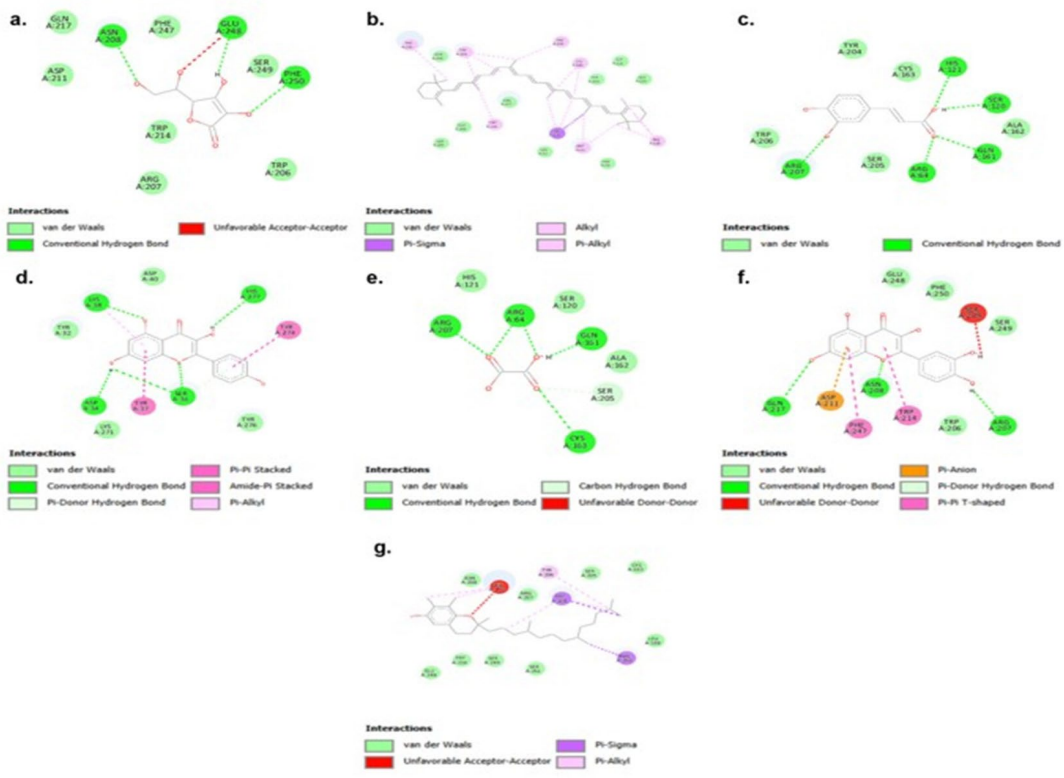

Figure 4. The binding visualization of *M. oleifera* organic compounds and Caspase-3 [a: ascorbic acid, b: betacarotene, c: caffeic, d: caempferol, e: oxalic acid, f: quercetin, g: tocopherol].

tissue, consistent with previous reports describing pulmonary edema, endothelial injury, and inflammatory infiltration following EG exposure (Cao *et al*., 2020).

Administration of *M. oleifera* leaves extract resulted in progressive histopathological improvement across increasing doses, indicating a clear dose–response relationship. At 200 mg/kg BW (P1), partial attenuation of congestion and septal thickening was observed, suggesting early protective activity. Greater improvement at 316 mg/kg BW (P2) and near-complete restoration of lung architecture at 500 mg/kg BW (P3) demonstrate that higher doses confer more substantial protection. Importantly, the absence of significant differences between the negative control (K−) and P3 group indicates that the highest dose effectively restored pulmonary morphology to near-normal conditions.

These results are in agreement with prior experimental studies reporting the protective effects of *M. oleifera* against oxidative and inflammatory lung injury (Pareek *et al*., 2023; Sidharta *et al*., 2025). The observed restoration of alveolar structure suggests that the extract not only limits ongoing injury but may also support tissue recovery processes, potentially through modulation of redox balance and inflammatory signaling (Ceci *et al*., 2024).

EG intoxication produces systemic toxicity primarily through its toxic metabolites, particularly glycolic acid and oxalic acid. Following ingestion, EG is metabolized within 4–12 hours, leading to glycolic acid accumulation and high anion gap metabolic acidosis. Subsequent formation of calcium oxalate crystals contributes to multi-organ injury (Stepanova, 2023).

Renal injury caused by oxalate deposition may initiate systemic inflammatory response syndrome (SIRS) (Ermer *et al*., 2016). This systemic inflammatory cascade promotes the release of pro-inflammatory cytokines such as TNF-α, which binds to Tumor Necrosis Factor Receptor 1 (TNFR1) expressed on endothelial cells (Wilson *et al*., 2018). TNFR1 activation triggers downstream signaling pathways, including NF-κB activation and subsequent induction of Caspase-3–mediated apoptosis (Shundo *et al*., 2023).

Endothelial apoptosis compromises vascular integrity, increasing capillary permeability and promoting plasma leakage into alveolar spaces (Wu *et al*., 2024). This process results in pulmonary edema, vascular congestion, and thickening of interalveolar septa, as observed in the present study. Additionally, persistent systemic inflammation may induce pulmonary vasoconstriction and elevate capillary hydrostatic pressure, further aggravating congestion and structural distortion (Mocan *et al*., 2025). Thus, EG-induced lung injury appears to involve a complex interplay between metabolic acidosis, oxidative stress, inflammatory cytokine release, and apoptosis-mediated endothelial damage.

Phytochemical screening confirmed that *M. oleifera* leaves contain multiple bioactive compounds with favorable drug-likeness properties (Vergara-Jimenez *et al*., 2017). Flavonoids such as quercetin and kaempferol are well recognized for their potent antioxidant and anti-inflammatory activities (Al-Khayri *et al*., 2022). These compounds neutralize reactive oxygen species (ROS), reduce lipid peroxidation, and inhibit activation of NF-κB, thereby decreasing the production of pro-inflammatory cytokines including TNF-α, IL-6, and IL-8 (Jomova *et al*., 2025).

Furthermore, flavonoids modulate intrinsic and extrinsic apoptotic pathways by regulating caspase activation, thereby limiting endothelial and epithelial cell death (Rana and Mumtaz, 2025). Ascorbic acid (vitamin C) and tocopherol (vitamin E) complement these effects by stabilizing cellular membranes and preventing oxidative degradation of phospholipids (Huang *et al*., 2002). β-carotene also contributes as a singlet oxygen quencher and free radical scavenger, enhancing overall antioxidant defense capacity (Miazek *et al*., 2022).

The dose-dependent histopathological improvements observed in this study strongly align with these mechanisms. Reduced congestion and septal thickening in treated groups likely reflect decreased oxidative injury, attenuation of inflammatory signaling, and preservation of endothelial integrity (Hsia *et al*., 2016).

The in silico docking analysis provided mechanistic insight into potential molecular interactions underlying the observed protective effects. Strong binding affinities of β-carotene, quercetin, and kaempferol toward Caspase-3 and TNFR1 suggest that these phytoconstituents may directly modulate apoptotic and inflammatory pathways (Ali *et al*., 2024).

Binding of flavonoids to catalytic residues of Caspase-3 may inter-

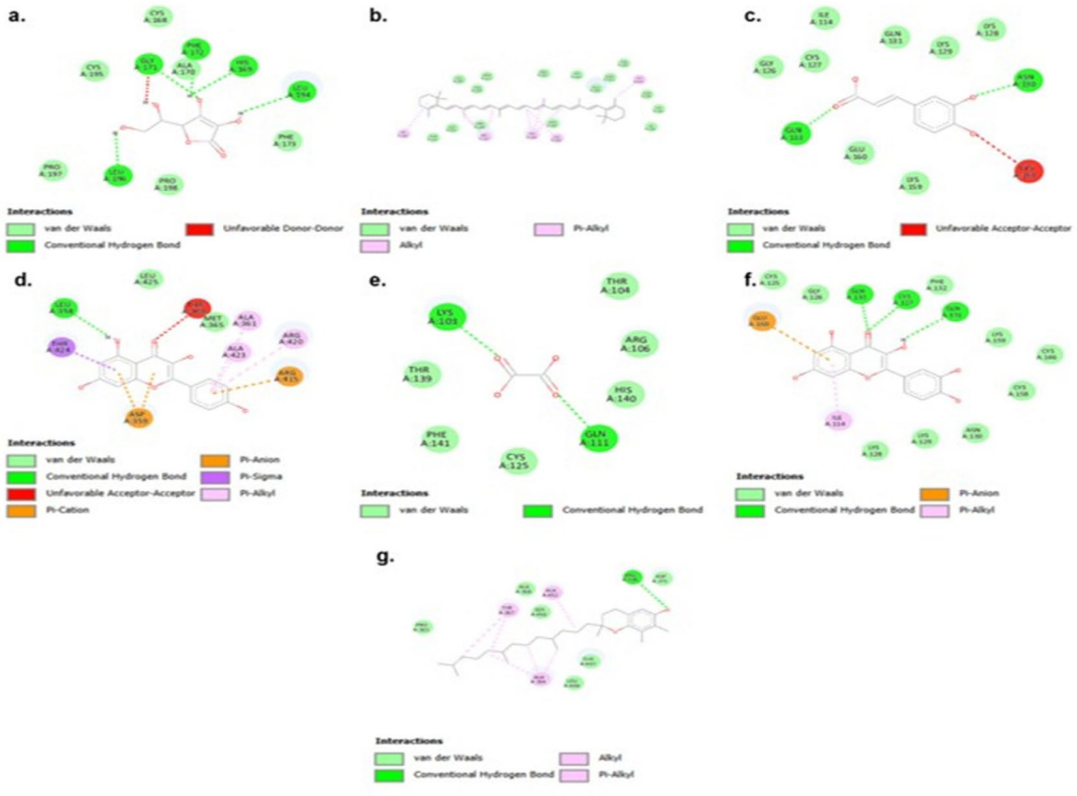

Figure 5. The binding visualization of *M. oleifera* organic compounds and receptor TNR1A [a: ascorbic acid, b: betacarotene, c: caffeic, d: caempferol, e: oxalic acid, f: quercetin, g: tocopherol].

fere with its proteolytic activation, thereby reducing apoptosis-mediated tissue damage (Shrestha *et al*., 2021). Similarly, interaction with TNFR1 residues indicates possible inhibition of TNF-α receptor signaling and downstream NF-κB activation (di Gesso *et al*., 2015). Through simultaneous suppression of apoptosis and inflammatory signaling, *M. oleifera* extract may exert a dual protective mechanism.

Importantly, the docking results complement the *In Vivo* findings. The reduction in histopathological injury observed in EG-exposed rats treated with *M. oleifera* is consistent with inhibition of TNFR1-mediated inflammation and Caspase-3–dependent apoptosis (Afolabi *et al*., 2022). Although molecular expression levels were not directly measured in this study, the combined computational and morphological evidence supports a plausible mechanistic pathway.

Overall, integration of *In Vivo* histopathological evaluation and in silico molecular docking suggests that *M. oleifera* leaves extract mitigates EG-induced pulmonary injury through interconnected antioxidant, anti-inflammatory, and anti-apoptotic mechanisms (Zahra *et al*., 2024). The near-complete restoration of lung architecture at 500 mg/kg BW further highlights its therapeutic potential.

While the present findings are promising, additional studies evaluating molecular expression, oxidative stress biomarkers, and inflammatory mediators are warranted to confirm the proposed mechanisms. Nevertheless, the current results provide strong experimental and computational evidence supporting the potential of *M. oleifera* as a natural therapeutic candidate for managing ethylene glycol–induced lung injury (Ebhohon and Miller, 2022).

Conclusion

The present study demonstrates that *M. oleifera* leaves extract, obtained through maceration, exerts significant protective effects against ethylene glycol–induced lung damage in Wistar rats, as evidenced by both *In Vivo* and in silico findings. *In Vivo* results showed that administration of 500 mg/kg BW markedly reduced alveolar congestion and interalveolar septal thickening by 48% and 78.3%, respectively, compared to the ethylene glycol control group, restoring lung histoarchitecture to near-normal conditions.

Phytochemical screening revealed the presence of bioactive compounds such as quercetin, kaempferol, β-carotene, ascorbic acid, and tocopherol, which met drug-likeness criteria. Molecular docking analysis further supported these findings by demonstrating strong binding affinities of key phytoconstituents toward caspase-3 and tnfr1, suggesting inhibition of apoptosis and inflammatory signaling pathways as underlying protective mechanisms.

Taken together, the integrated *In Vivo* and in silico approaches indicate that *M. oleifera* leaves extract, particularly at a dose of 500 mg/kg bw, has promising therapeutic potential in mitigating ethylene glycol–induced pulmonary injury. However, further studies are required to evaluate long-term safety, determine the toxic dose threshold, and clarify the molecular mechanisms through advanced pharmacokinetic and molecular dynamic analyses before clinical application can be considered.

Acknowledgments

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Conflict of interest

The authors have no conflict of interest to declare.

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