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Synergistic protection: Integrating in silico and in vivo evidence for *Moringa oleifera* flavonoids as potent Mitigators of Deltamethrin toxicity

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ABSTRACT

Deltamethrin (DM), a highly effective synthetic pyrethroid, has been associated with neurotoxicity, hepatotoxicity, and pronounced oxidative stress, primarily through the generation of reactive oxygen species that damage cellular components. The present study integrates in silico molecular modelling with in vivo experimentation using a Japanese quail model to evaluate the toxic effects of DM and the protective potential of *Moringa oleifera* (MO) flavonoids, namely quercetin and kaempferol. In silico analysis revealed that DM possesses high predicted toxicity, whereas MO flavonoids exhibited favorable pharmacokinetic properties, lower toxicity, and strong binding affinities (up to −9.7 kcal/mol) toward key targets including acetylcholinesterase (AChE), caspase-3, and aspartate aminotransferase (AST), suggesting their potential to modulate oxidative stress and apoptosis. Functional enrichment and protein–protein interaction analyses further highlighted oxidative stress, reactive oxygen species metabolism, and apoptotic signaling as central mechanisms underlying DM toxicity. For in vivo evaluation, Japanese quails were fed a basal diet supplemented with 30 mg/kg DM as a positive control (PC) group, along with diets containing MO (0.3–0.7 g/kg) for 42 days. The PC group exhibited significant impairment in growth performance and hematological parameters, severe hepato-renal injury (elevated AST, ALT, creatinine, and urea), and marked oxidative stress (increased MDA with decreased SOD and CAT activities). Dietary supplementation with MO, particularly at 0.7 g/kg, resulted in a significant ($P < 0.05$) and dose-dependent improvement in growth performance, normalization of hematobiochemical parameters, and restoration of antioxidant defense systems. These findings corroborate computational predictions and demonstrate that MO flavonoids effectively mitigate DM-induced toxicity by reducing oxidative stress, enhancing antioxidant defenses, and inhibiting apoptosis. In conclusion, dietary supplementation of MO at 0.7 g/kg acts as a potent natural phytogenic intervention against DM-induced oxidative damage, organ dysfunction, and performance decline, supporting its application in environmental and clinical toxicology.

1. Introduction

The global demand for high-quality animal protein continues to rise, with the poultry industry playing a pivotal role in meeting this need. In this domain, the Japanese quail (*Coturnix coturnix japonica*) is the one that is taking the spotlight as a sustainable and efficient protein production model made possible due to its rapid growth rate, high feed efficiency, and resistance to diseases, (Lobato et al., 2025). Yet, the economic aspect of intensive poultry farming including quail production, keeps being under threat from toxins in feeds and feed additives which are the main reason for impaired health, and lower performance (Li et al., 2025). One of the most widely used environmental contaminants is synthetic pyrethroid pesticides, and among them, DM is one of the most widely used not only for crop protection but also for ectoparasite control in livestock (Hamidipoor et al., 2015). Being lipophilic, DM gets stored in fatty tissues and is very persistent in grains; hence, it enters poultry feeds through various means (Rano and Singh, 2020). Chronic exposure to DM even at sub-lethal levels has been shown to create a chain of toxicological impacts.

The primary mechanism underlying DM-induced toxicity involves the excessive generation of reactive oxygen species (ROS), resulting in oxidative stress and disruption of cellular redox homeostasis (Jiang

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et al., 2024). DM primarily impairs mitochondrial function, leading to increased ROS production and subsequent depletion of endogenous antioxidant defenses, including superoxide dismutase (SOD) and catalase (CAT) (Kumar et al., 2015). This redox imbalance promotes extensive lipid peroxidation, damaging cellular membranes and generating toxic by-products such as malondialdehyde (MDA). In this context, assessment of enzymatic antioxidant defense systems is essential to elucidate the mechanistic basis of DM-induced toxicity. Superoxide dismutase (SOD) serves as the first line of defense by catalyzing the dismutation of superoxide radicals into hydrogen peroxide, which is subsequently detoxified by catalase (CAT) and glutathione peroxidase (GPx). GPx further reduces hydrogen peroxide and lipid hydroperoxides using glutathione as a substrate, thereby protecting membrane integrity from oxidative injury. Lipid peroxidation (LPO), a hallmark of oxidative membrane damage, is commonly evaluated through quantification of malondialdehyde (MDA), a stable end-product of polyunsaturated fatty acid oxidation. Therefore, measurement of SOD, CAT, GPx, and MDA provides a comprehensive and mechanistically relevant evaluation of oxidative imbalance and cellular damage induced by DM exposure. The inter-organ oxidative damage then develops into clinical signs of the liver- and kidney dysfunction, evidenced by the increased levels of the liver enzymes i.e., alanine transaminase (ALT), aspartate transaminase (AST), creatinine and urea (Jiang et al., 2024). This physiological harm is directly associated with the performance of the growth reflected in the poor feed intake, the lower feed conversion ratio, and the producers' economic losses being substantial (Hamed et al., 2022).

To accomplish these challenges scientists are searching for safe, natural, and effective (phytobiotics) feed additives, and this was further aggravated by a global cutback on antibiotic growth promoters (Biswas et al., 2024). MO is considered a multi-functional tropical plant and considered a leading candidate due to its great nutritional composition and more importantly its strong antioxidant efficacy (Ahmad et al., 2018). MO leaves are rich sources of active compounds like flavonoids, phenolic acids, tannins, and vitamins C and E (Ibrahim et al., 2022). These compounds are very effective in scavenging free radicals and induce the expression of internal antioxidant enzymes, thus giving cells protection against oxidative stress (Ahmad et al., 2018).

While the benefits of MO against various stressors like heat stress and heavy metals have been documented, its specific protective role against DM-induced toxicity in Japanese quails is not well-elucidated. Therefore, this study was designed to investigate the ameliorative potential of dietary MO leaf powder supplementation on growth performance, feed utilization, haemato-biochemical parameters, and the antioxidant status of Japanese quails challenged with DM.

2. Materials and methods

2.1. Ethical approval

All experimental procedures involving animals were conducted in strict accordance with the guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the ethical review board of Cholistan university of veterinary and animal sciences Bahawalpur, Pakistan under approval number ORIC-318.

2.2. Functional enrichment analysis

Functional enrichment analysis was performed using the Metascape platform (https://metascape.org/) to identify biological processes and pathways associated with oxidative stress. Gene Ontology (GO) and pathway enrichment analyses were conducted using default parameters, with a significance threshold set at $p < 0.05$ (hypergeometric test with Benjamini–Hochberg correction). Significantly enriched terms were visualized using bar plots and enrichment network diagrams. Only oxidative stress–related and apoptosis-associated pathways were considered for downstream interpretation to ensure biological relevance.

2.3. Ligand selection and preparation

In this study, Deltamethrin (CID: 40585) was used as the reference toxicant, while Quercetin (CID: 5280343) and Kaempferol (CID: 5280863), two major flavonoids from MO, were selected as potential ameliorative agents. The 3D conformers of all compounds were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). (Fig. 1).

2.4. Pharmacokinetic evaluation

The pharmacokinetic and drug-likeness properties of Quercetin and Kaempferol were assessed using the SwissADME (https://www.swissadme.ch/) web tool (Daina et al., 2017). The Bioavailability Radar was used to evaluate six key parameters lipophilicity, size, polarity, solubility, flexibility, and saturation with optimal drug-like behavior indicated by positioning within the pink zone. Additionally, the BOILED-Egg model was employed to predict GIT absorption and BBB permeability based on WLOGP and TPSA values. This provided insights into the systemic availability and potential CNS activity of the phytoconstituents.

2.5. In silico toxicity prediction

The ProTox-II server (https://tox.charite.de/) was employed for performing toxicological assessments, which are based on the prediction of toxicity endpoints from the chemical structure (Banerjee et al., 2018). The chemical substances received assessment regarding their LD_{50} values along with GHS classification and the probability of resulting in the four toxic effects, namely, liver toxicity, cancer, mutation, and suppression of immune response. The outcomes of these evaluations were utilized to assess the safety levels of Quercetin and Kaempferol in comparison to DM.

2.6. Protein retrieval and preparation

The 3D crystal structures of the mentioned proteins were taken from the RCSB Protein Data Bank (https://www.rcsb.org/). The three proteins that were chosen; AChE (Cheung et al., 2012; PDB ID: 4EY7), Caspase-3 (Lee et al., 2000; PDB ID: 1GFW), and AST (Nam et al., 2010; PDB ID: 3II1)—were selected because they do possess a significant role in neurotoxicity, apoptosis, and hepatotoxicity, respectively (Fig. 2). Using AutoDock Tools (v1.5.6), protein preparation was accomplished by eliminating water molecules and heteroatoms, polar hydrogens, and assigning Kollman charges (Trott and Olson, 2010). The docking process involved saving the prepared structures in PDBQT format.

2.7. Molecular docking analysis

Molecular docking was carried out with AutoDock Vina for evaluating the binding affinities and interactions of each ligand with the target protein (Trott and Olson, 2010). The grid boxes were set around the active sites of the proteins according to the known functional residues. The docking runs were conducted using default exhaustiveness parameters, and the binding affinities (kcal/mol) were noted. The most stable docking conformations were chosen for visualization and interaction analysis with (BIOVIA, Dassault Systèmes, 2021), where special attention was given to hydrogen bonds, hydrophobic contacts, and π–π interactions.

2.8. Comparative binding and ameliorative assessment

To determine the potential competitive or inhibitory interaction of the MO compounds with DM at the target sites, a comparative docking

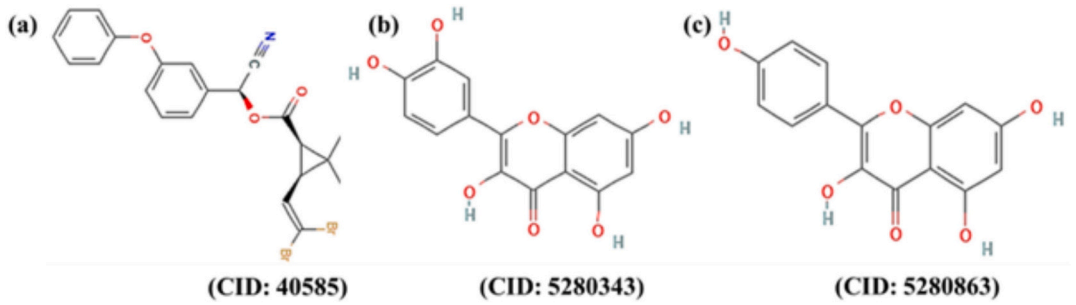

Fig. 1. Ligand Structures, (a) Deltamethrin; (b) Quercetin; (c) Kaempferol.

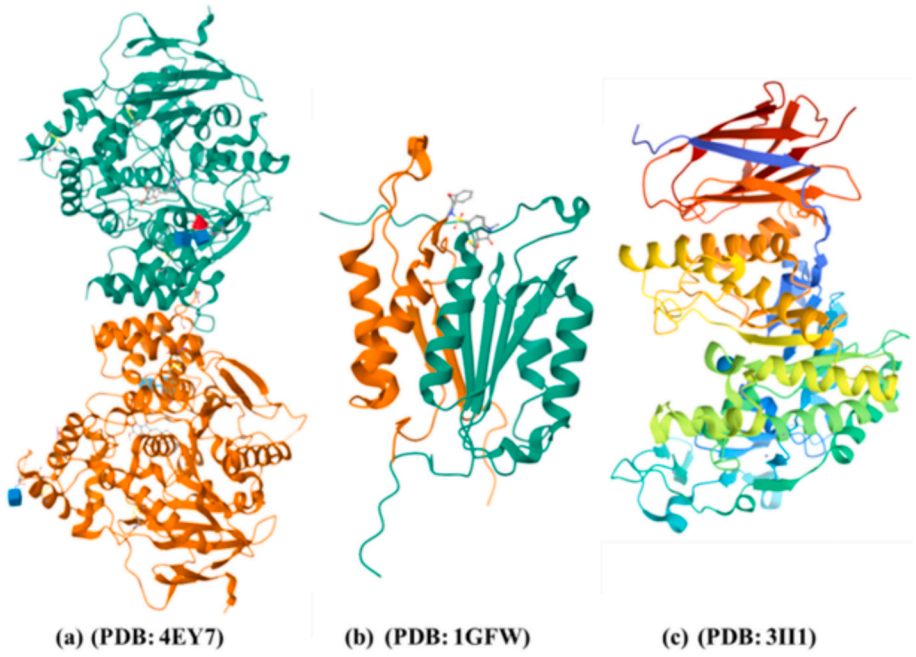

Fig. 2. PDB retrieved protein structures, (a) Acetylcholinesterase; (b) Caspase-3; (c) Aspartate aminotransferase.

approach was applied. Binding affinities of Quercetin and Kaempferol were compared directly with those of DM across the three protein targets. A higher binding affinity of the phytoconstituents was interpreted as indicative of their potential to interfere with DM binding, supporting their role as ameliorative agents in oxidative stress and toxicity modulation.

2.9. Preparation of experimental additives

Fresh, healthy MO leaves were collected from botanically garden of Cholistan university of veterinary and animal sciences Bahawalpur, Pakistan. The leaves were thoroughly washed with clean water to remove dirt and debris, then spread on mesh racks and shade-dried at room temperature (25–30 °C) for several days until they became brittle and crispy (Khan et al., 2021). Shade-drying is employed to preserve heat-labile nutrients and bioactive compounds, such as vitamins and antioxidants, which can be degraded by direct sunlight (Potisate et al., 2014).

Once completely dry, the leaves were ground into a fine powder using a mechanical hammer mill, passing through a 2 mm sieve to ensure uniform particle size. The resulting MO leaf meal was stored in airtight, labelled bags in a cool, dark, and dry place until its incorporation into the experimental diets. DM (99.5%) was procured from Sigma-Aldrich (St. Louis, MO, USA) and was dissolved in corn oil to prepare a stock solution for dietary mixing.

2.10. Experimental animals, housing, and diets

A total of 250 day-old, apparently healthy Japanese quails (*Coturnix coturnix japonica*) of mixed sex were obtained from Avian Research & Training Centre of University of veterinary and animal sciences Lahore, Pakistan. Upon arrival, the birds were weighed and randomly allocated into five (5) experimental groups in a completely randomized design (CRD). Each group consisted of 5 replicate pens, with 10 birds per replicate.

The birds were housed under controlled environmental conditions, with a 23 L:1D photoperiod, and temperature maintained at 35 ± 1 °C for the first week, then gradually reduced by 3 °C weekly until it reached 25 ± 2 °C. Feed (in mash form) and fresh water were provided ad libitum throughout the 42-day (6-week) experimental period. A basal diet was formulated to meet the nutritional requirements of Japanese quails as per NRC (1994) guidelines (Table 1).

2.11. Growth performance and feed utilization

Birds were weighed individually at the start of the trial (Day 1) and weekly thereafter. Feed intake (FI) per replicate was recorded weekly. Body Weight Gain (BWG) and Feed Conversion Ratio (FCR) were calculated for each period and overall using the following formulae:

BWG (g) = Final Body Weight–Initial Body Weight

Table 1
Ingredient composition and calculated nutrient content of the basal diet for Japanese quails.

Ingredient	Composition (g/kg)
Ingredients	
Yellow Corn	548.0
Soybean Meal (48% CP)	340.0
Fish Meal (60% CP)	50.0
Soybean Oil	20.0
Limestone (Calcium Carbonate)	18.0
Dicalcium Phosphate (DCP)	14.0
Vitamin-Mineral Premix[1]	5.0
DL-Methionine	2.5
Salt (NaCl)	2.5
Total	**1000.0**
Calculated Nutrient Content[2]	
Metabolizable Energy (kcal/kg)	2920
Crude Protein (%)	23.70
Crude Fat (%)	5.20
Crude Fiber (%)	3.10
Calcium (%)	0.99
Available Phosphorus (%)	0.45
Lysine (%)	1.38
Methionine + Cystine (%)	1.07

[1] Vitamin-Mineral Premix provided (per kg of diet): Vitamin A, 12,000 IU; Vitamin D3, 2500 IU; Vitamin E, 50 IU; Vitamin K3, 3 mg; Vitamin B1, 2 mg; Vitamin B2, 6 mg; Vitamin B6, 5 mg; Vitamin B12, 0.02 mg; Niacin, 35 mg; Pantothenic Acid, 12 mg; Folic Acid, 1 mg; Biotin, 0.1 mg; Choline Chloride, 500 mg; Mn, 100 mg; Zn, 85 mg; Fe, 60 mg; Cu, 10 mg; I, 1.2 mg; Se, 0.2 mg; Antioxidant.
[2] Calculated based on NRC (1994) values for Japanese quails.

$$\text{FCR} = \text{Total Feed Intake (g)} / \text{Total Body Weight Gain (g)}$$

2.12. Sample collection and processing

At the end of the trial (Day 42), (10 birds per group, 2 per replicate) were randomly selected and fasted for (4 h). Blood samples were collected from the brachial vein. Approximately 2 mL of blood was transferred into vacuum tubes containing K2-EDTA for hematological analysis. Another 3 mL of blood was collected in sterile tubes without anticoagulant. These tubes were left to clot at room temperature, and serum was subsequently harvested by centrifugation at 3000 $\times g$ for 15 min. The separated serum was stored at −20 °C in labelled Eppendorf tubes until biochemical analysis.

2.13. Hematological analysis

Red blood cell (RBC) count, hemoglobin (Hb) level, hematocrit (Hct), and total white blood cell (WBC) count were performed with the help of an automated hematology analyzer Sysmex XT-2000iV. For differential leukocyte counting (lymphocytes, heterophils), blood smears were prepared, stained with Giemsa stain, and then 100 cells were counted per slide under oil immersion microscopy.

2.14. Serum biochemistry and antioxidant status

Serum samples were analyzed for key biochemical indices (AST, ALT, Creatinine and Urea, Total Protein, Albumin, and Glucose using a clinical auto-analyzer Microlab 300 and commercially available diagnostic kits, following the manufacturer's protocols.

Globulin : Total Protein–Albumin

The serum antioxidant status was evaluated by measuring the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as the concentration of malondialdehyde (MDA). These analyses were performed using commercial colorimetric assay kits Cayman Chemical. All absorbance readings were taken using a microplate spectrophotometer.

2.15. Statistical analysis

The data collected were subjected to statistical analysis using SPSS (Version 25.0). All data were expressed as Mean ± Standard Error (SE). The replicate pen was considered the experimental unit. Data were analyzed using a one-way Analysis of Variance (ANOVA). When the ANOVA revealed a significant effect ($P < 0.05$), means were separated using Tukey Test.

3. Results

3.1. In silico drug prediction

3.1.1. Functional enrichment analysis

Functional enrichment analysis identified oxidative stress as the dominant biological process associated with the selected gene set. The most significantly enriched pathway was oxidative stress response (LogP = −23.87), indicating a strong involvement of redox imbalance mechanisms. (Table 2).

Multiple closely related pathways, including reactive oxygen species metabolic process, response to hydrogen peroxide, and cellular response to chemical stress, were also significantly enriched, collectively highlighting the central role of ROS-mediated cellular damage.

Furthermore, enrichment of intrinsic apoptotic signaling pathways suggests that oxidative stress may trigger apoptosis as a downstream consequence of cellular injury.

Key genes contributing to these pathways included major antioxidant enzymes such as SOD1, SOD2, CAT, GPX1, and GPX3, along with regulatory factors NFE2L2 (NRF2) and HMOX1. Additionally, apoptosis-related genes CASP3 and BCL2, and ROS-generating enzymes NOX1 and NOX4, were identified, indicating coordinated regulation of oxidative stress and cell death pathways. (Figs. 3 and 4).

These findings are consistent with in vivo observations, where deltamethrin exposure resulted in decreased antioxidant enzyme activities (SOD, CAT, GPx) and increased lipid peroxidation (MDA), confirming oxidative stress as a primary mechanism of toxicity.

3.2. Protein–protein interaction network analysis

Protein–protein interaction network analysis revealed a tightly interconnected cluster of antioxidant and apoptosis-related proteins. Core nodes included CAT, SOD1, SOD2, and GPX3, representing the primary antioxidant defense system, along with CASP3 and BCL2, which regulate apoptosis. The close interaction among these proteins indicates

Table 2
Top enriched oxidative stress-related pathways identified using Metascape analysis.

Category	Pathway / Biological Process	LogP Value	Key Genes
WikiPathways	Oxidative stress response	−23.87	CAT, GPX1, GPX3, HMOX1, NFE2L2, SOD1, SOD2, NOX1, NOX4
Reactome	Cellular response to chemical stress	−18.96	BCL2, CAT, GPX1, GPX3, HMOX1, NFE2L2, SOD1, SOD2, KEAP1, NOX4
GO BP	Reactive oxygen species metabolic process	−18.87	BCL2, CAT, GPX1, GPX3, NFE2L2, SOD1, SOD2, NOX1, NOX4
GO BP	Response to oxidative stress	−15.81	BCL2, CASP3, CAT, GPX1, GPX3, HMOX1, NFE2L2, SOD1, SOD2, KEAP1
GO BP	Response to hydrogen peroxide	−16.72	BCL2, CASP3, CAT, GPX1, HMOX1, NFE2L2, SOD1, SOD2

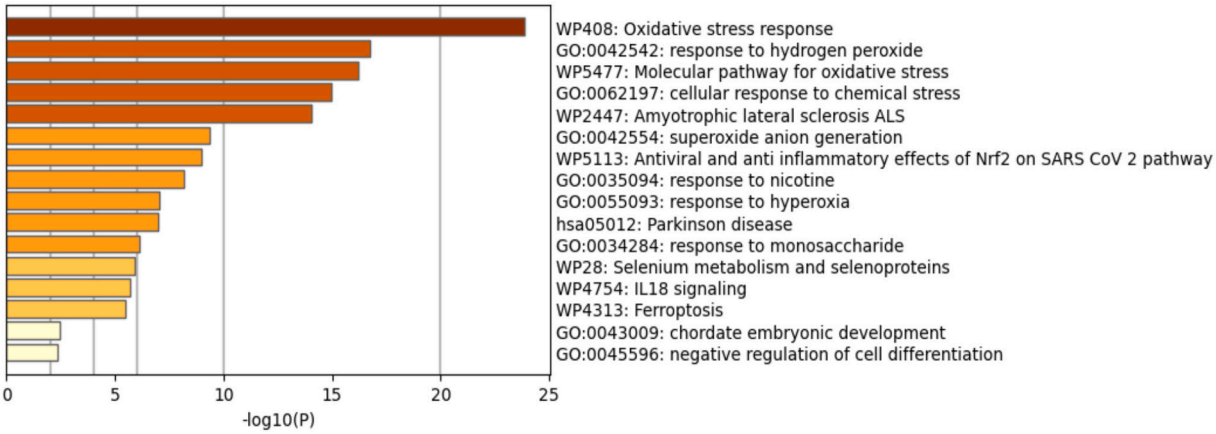

Fig. 3. Functional enrichment analysis of oxidative stress-related pathways.

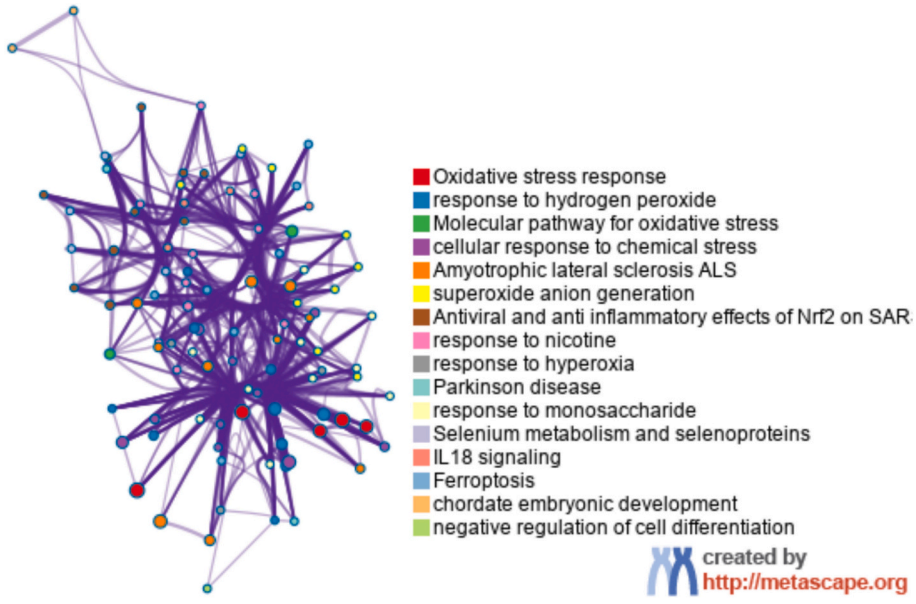

Fig. 4. Network visualization of enriched oxidative stress-related pathways.

coordinated regulation of oxidative stress and apoptotic pathways, highlighting the central role of redox imbalance in mediating cellular damage. (Fig. 5).

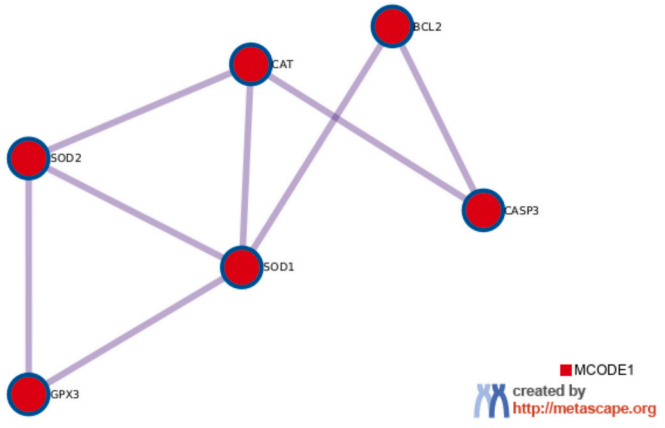

Fig. 5. Protein–protein interaction network of oxidative stress and apoptosis-related genes.

3.3. Mechanistic insight into oxidative stress

The integrated in silico analysis suggests that DM induces toxicity primarily through excessive generation of reactive oxygen species, leading to oxidative stress, depletion of antioxidant defenses, and activation of apoptotic pathways. Conversely, MO flavonoids, particularly quercetin and kaempferol, may exert protective effects by scavenging ROS and activating antioxidant defense systems, including NRF2-mediated pathways, thereby restoring redox homeostasis and inhibiting apoptosis. (Fig. 6).

3.4. Molecular docking analysis

Based on the identified oxidative stress-related pathways and key regulatory proteins, molecular docking analysis was performed to evaluate the interaction of selected compounds with target proteins involved in these mechanisms. Molecular docking analysis demonstrated differential binding affinities of the compounds toward selected protein targets associated with oxidative stress and apoptosis. Quercetin exhibited the strongest binding affinities across all targets, with docking energies reaching −9.7 kcal/mol for acetylcholinesterase (AChE) and −9.5 kcal/mol for aspartate aminotransferase (AST) and caspase-3.

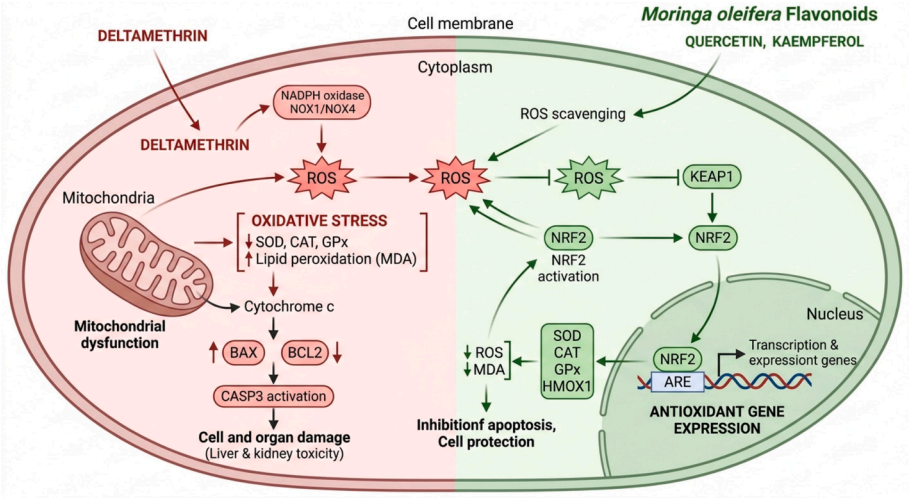

Fig. 6. Cellular mechanism of deltamethrin-induced oxidative stress and protective role of *Moringa oleifera* flavonoids.

Kaempferol also showed strong binding interactions, with values comparable to quercetin in several cases. In contrast, DM displayed moderate binding affinities, with the highest interaction observed for AST (−8.8 kcal/mol). The strong binding of quercetin and kaempferol is attributed to their ability to form multiple hydrogen bonds and π–π interactions, enhancing complex stability. Importantly, their interaction with caspase-3 suggests potential modulation of apoptosis pathways. AST and AChE are well-established markers of hepatic and neurotoxic damage associated with oxidative stress. Therefore, targeting these proteins provides mechanistic insight into organ-specific toxicity induced by DM. (Fig. 7–10).

3.5. Physicochemical properties and ADME analysis

The physicochemical properties of DM, quercetin, and kaempferol were evaluated using SwissADME to assess their drug-likeness and pharmacokinetic profiles. The bioavailability radar plots demonstrated distinct physicochemical characteristics among the compounds. DM exhibited higher lipophilicity and molecular size with lower polarity and flexibility, whereas quercetin and kaempferol showed higher polarity and flexibility with comparatively lower lipophilicity.

The BOILED-Egg model indicated that all three compounds were located outside the predicted region for BBB permeation, while demonstrating favorable GI absorption. Molecular weight analysis revealed that quercetin (302.24 g/mol) and kaempferol (286.24 g/mol) fall within the acceptable drug-like range (<500 g/mol), whereas DM (519.9 g/mol) slightly exceeds this threshold. Dose distribution analysis further indicated that DM requires a higher dose compared to the flavonoids, with kaempferol showing the lowest dose requirement. These findings suggest that quercetin and kaempferol possess more favorable pharmacokinetic profiles compared to DM. (Fig. 11).

3.6. Toxicity prediction analysis

Toxicity assessment using ProTox-II revealed significant differences among the compounds. DM exhibited a predicted LD_{50} of 5 mg/kg, corresponding to toxicity class 2 (fatal if swallowed), whereas quercetin (LD_{50} = 159 mg/kg) and kaempferol (LD_{50} = 3919 mg/kg) were classified as toxicity classes 3 and 5, respectively. Additionally, DM showed activation of toxicity pathways including neurotoxicity, respiratory toxicity, and mitochondrial dysfunction. In contrast, quercetin and kaempferol demonstrated comparatively lower toxicity profiles, although some activation in metabolic and receptor-related pathways was observed. (Fig. 12).

Collectively, these findings demonstrate that DM induces toxicity primarily through oxidative stress and apoptosis, while MO flavonoids exert protective effects by modulating antioxidant defense systems and inhibiting apoptotic pathways.

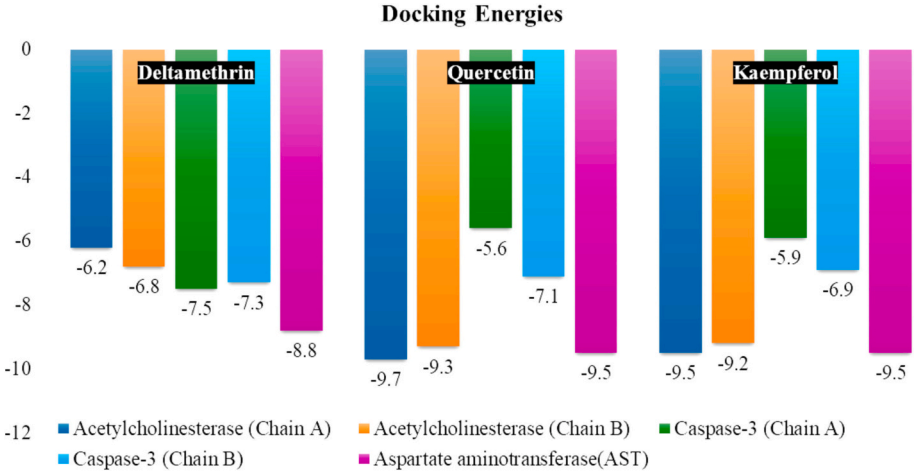

Fig. 7. Comparison of docking energies.

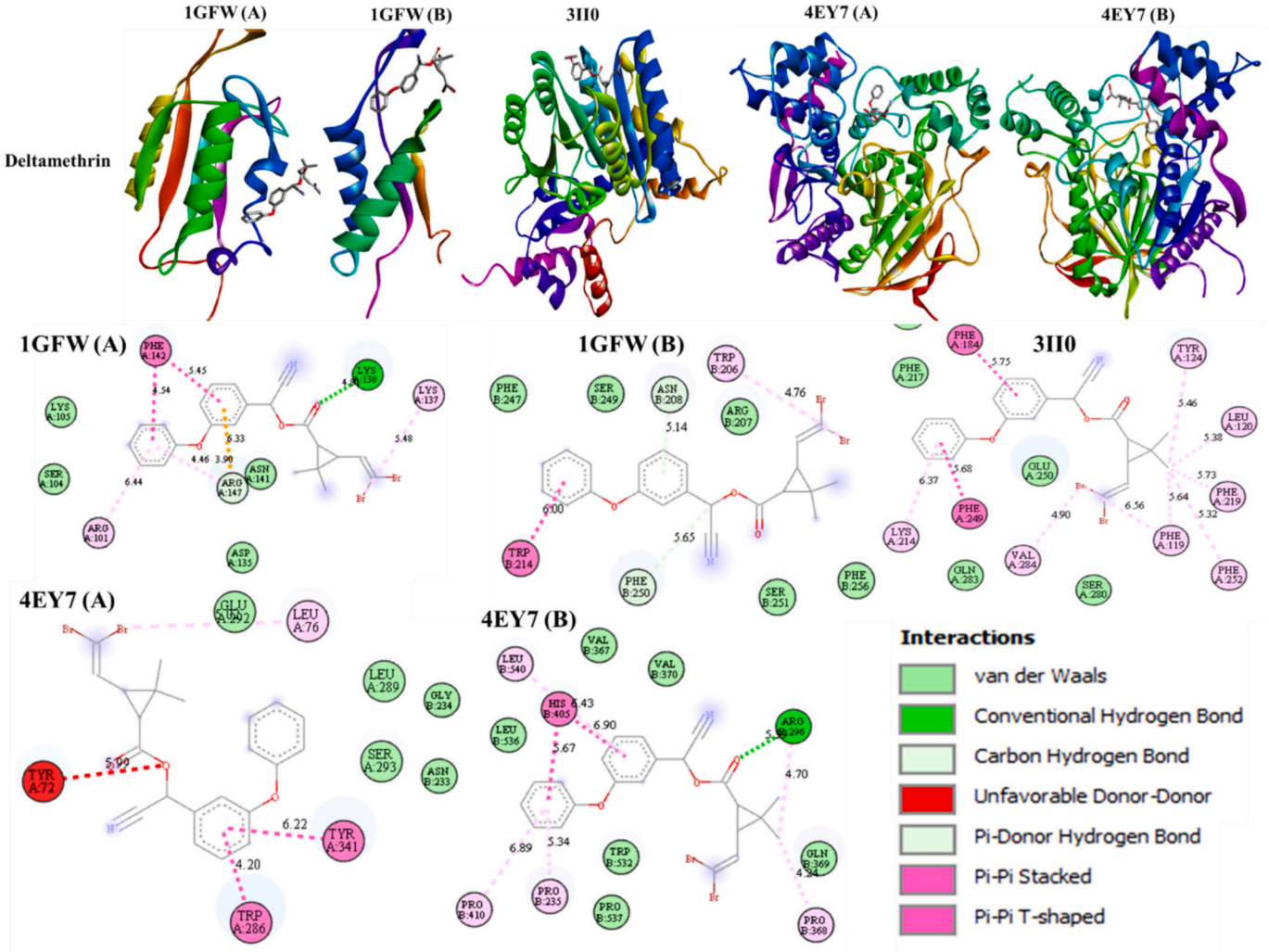

Fig. 8. Molecular docking analysis results visualization of Deltamethrin.

3.7. Growth performance and feed utilization

Initial body weight (IBW) and early growth (Day 7 and Day 14) did not differ significantly among treatment groups ($p > 0.05$), with values ranging from 8.57 ± 0.29 to 9.00 ± 0.37 g (Day 1), 21.85 ± 0.55 to 22.71 ± 0.60 g (Day 7), and 68.71 ± 2.79 to 74.89 ± 1.78 g (Day 14).

Significant differences emerged from Day 21 onward. Body weight increased from 92.00 ± 0.57 g in the positive control to 107.28 ± 0.77 g in the 0.7MO group F (4, 20)=17.229, $p < 0.001$. This trend persisted at Day 28 (127.28 ± 1.84 to 144.00 ± 0.87 g; F (4, 20)=31.499, p < 0.001, Day 35 (151.57 ± 3.96 to 202.14 ± 4.82 g; F (4, 20)=16.745, p < 0.001, and Day 42 (222.71 ± 4.29 to 273.85 ± 4.17 g; F (4, 20)=17.194, p < 0.001.

Body weight gain showed no significant differences during early stages ($p > 0.05$), but differed significantly at Day 21 (26.85 ± 5.16 to 112.57 ± 0.61 g; $p = 0.02$), Day 35 (24.28 ± 2.53 to 58.14 ± 5.00 g; $p = 0.03$), and overall (213.85 ± 4.50 to 264.85 ± 4.30 g; $p = 0.01$).

Feed intake was similar among groups at Day 7 ($p = 0.127$), but differed at Day 14 (100.28 ± 0.94 to 105.00 ± 0.43 g; F (4, 20)=4.925, $p = 0.004$. Marked differences were observed at Day 21 (115.85 ± 1.01 to 154.57 ± 0.36 g; F (4, 20)=848.460,p < 0.001, Day 28 (160.57 ± 0.68 to 187.14 ± 1.48 g; F (4, 20)=28.257,p < 0.001, and Day 35 (228.00 ± 5.57 to 248.42 ± 0.68 g; F (4, 20)=10.932,p < 0.001, while no differences were observed at Day 42 ($p = 0.949$). Overall feed intake ranged from 936.42 ± 6.39 to 1017.85 ± 3.50 g F (4, 20)=53.418,p < 0.001.

Feed conversion ratio (FCR) did not differ significantly at Day 7, Day 14, Day 28, and Day 42 (p > 0.05), but showed significant differences at Day 21 (1.24 ± 0.09 to 6.20 ± 1.13; p = 0.02) and Day 35 (4.43 ± 0.48 to 10.32 ± 1.53; p = 0.02). Overall FCR ranged from 3.84 ± 0.06 to 4.39 ± 0.11 (p = 0.03). (Table 3).

3.8. Hematology and serum biochemistry parameters

RBC count (2.8 ± 0.01 to 3.4 ± 0.09 × 10^6/μL), hemoglobin (8.1 ± 0.05 to 9.8 ± 0.05 g/dL), and hematocrit (36.66 ± 0.30 to 44.66 ± 0.33%) differed significantly among groups F (4, 20)=20.269,142.500, and 48.167,respectively;p < 0.001, respectively; $p < 0.001$ F (4, 20)= 20.269,142.500, and 48.167 respectively;$p < 0.001$. WBC count ranged from 11.9 ± 0.05 to 14.3 ± 0.15 × 10^3/μL F (4, 20)=71.882, p < 0.001, while lymphocytes (57.33 ± 0.33 to 62.00 ± 0.57%; F (4, 20)=7.559, $p = 0.005$ and heterophils (28.33 ± 0.33 to 32.00 ± 0.57%; F (4, 20)= 13.222, $p = 0.001$ were also significantly affected.

Total protein (3.5 ± 0.01 to 4.53 ± 0.03 g/dL), albumin (1.83 ± 0.01 to 2.26 ± 0.03 g/dL), and globulin (1.70 ± 0.05 to 2.26 ± 0.03 g/dL) differed significantly among groups F (4, 20)=55.115,16.214, and 16.100, respectively; $p < 0.001$. ALT (26.66 ± 0.30 to 36.00 ± 0.80 U/L) and AST (248.00 ± 0.57 to 277.66 ± 1.40 U/L) were significantly altered F (4, 20)=49.115 and 137.898, respectively; p < 0.001. Creatinine (0.30 ± 0.05 to 0.50 ± 0.05 mg/dL), urea (12.33 ± 0.33 to 18.33 ± 0.33 mg/dL), and glucose (170.00 ± 0.61 to 188.00 ± 0.43 mg/dL) also differed significantly among groups F (4, 20)=23.500,45.500, and

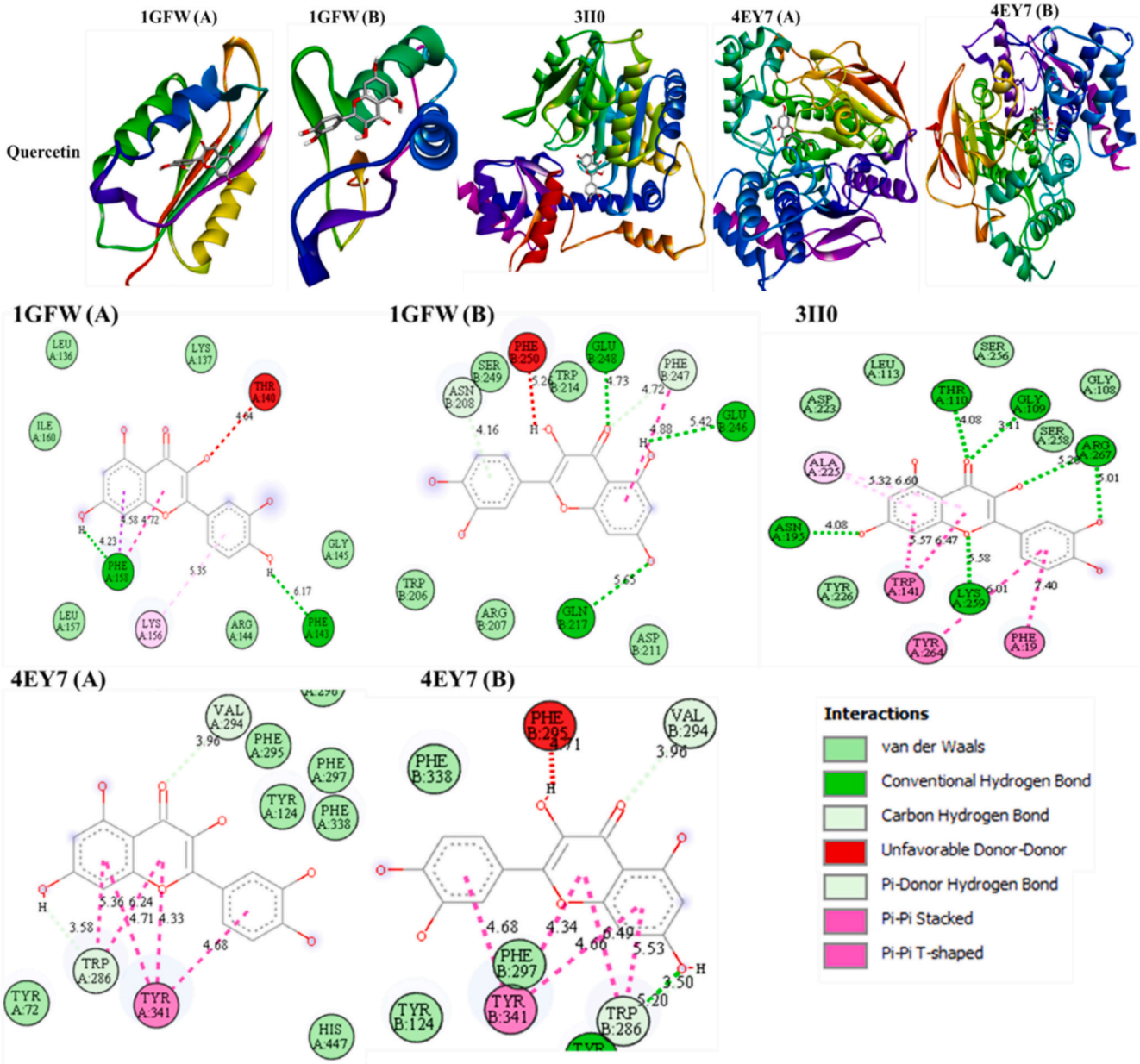

Fig. 9. Molecular docking analysis results visualization of Quercetin.

141.600, respectively; $p < 0.001$. (Table 4).

3.9. Serum antioxidant status

SOD (255.00 ± 2.88 to 392.66 ± 1.45 U/mL), CAT (31.00 ± 0.21 to 49.66 ± 0.33 U/mL), and GPx (15.33 ± 0.43 to 27.66 ± 0.54 U/mL) activities were significantly affected F (Biswas et al., 2024; Kim et al., 2015)=416.823,146.088,and149.286, respectively; $p < 0.001$. MDA levels ranged from 1.00 ± 0.01 to 2.40 ± 0.02 nmol/mL and differed significantly among groups F (Biswas et al., 2024; Kim et al., 2015)= 117.375, $p < 0.001$. (Table 5).

4. Discussion

The present study adopted an integrated approach, utilizing both in silico molecular modelling and an in vivo animal model, to comprehensively evaluate the toxicological profile of deltamethrin (DM) and the ameliorative potential of *Moringa oleifera* (MO) phytoconstituents, specifically quercetin and kaempferol. This integrative approach provides complementary mechanistic and physiological evidence supporting the protective role of MO phytoconstituents against DM-induced toxicity.

The in-silico study evaluated the pharmacokinetic and drug-likeness properties of the selected compounds. SwissADME bioavailability radar plots showed that the three compounds (DM, quercetin, and kaempferol) deviated from the ideal oral bioavailability profile in at least one physicochemical parameter. DM exhibited high lipophilicity and large molecular size, with lower polarity and flexibility compared to quercetin and kaempferol. This suggests poor aqueous solubility and limited bioavailability, which is consistent with its hydrophobic nature as a pesticide (David, 2017). In contrast, quercetin and kaempferol demonstrated higher polarity and flexibility, indicating better solubility; however, their increased polarity may reduce passive membrane permeability (Kaur and Kaur, 2014). Overall, deviations from the optimal range suggest that all three compounds may require formulation strategies to improve oral absorption.

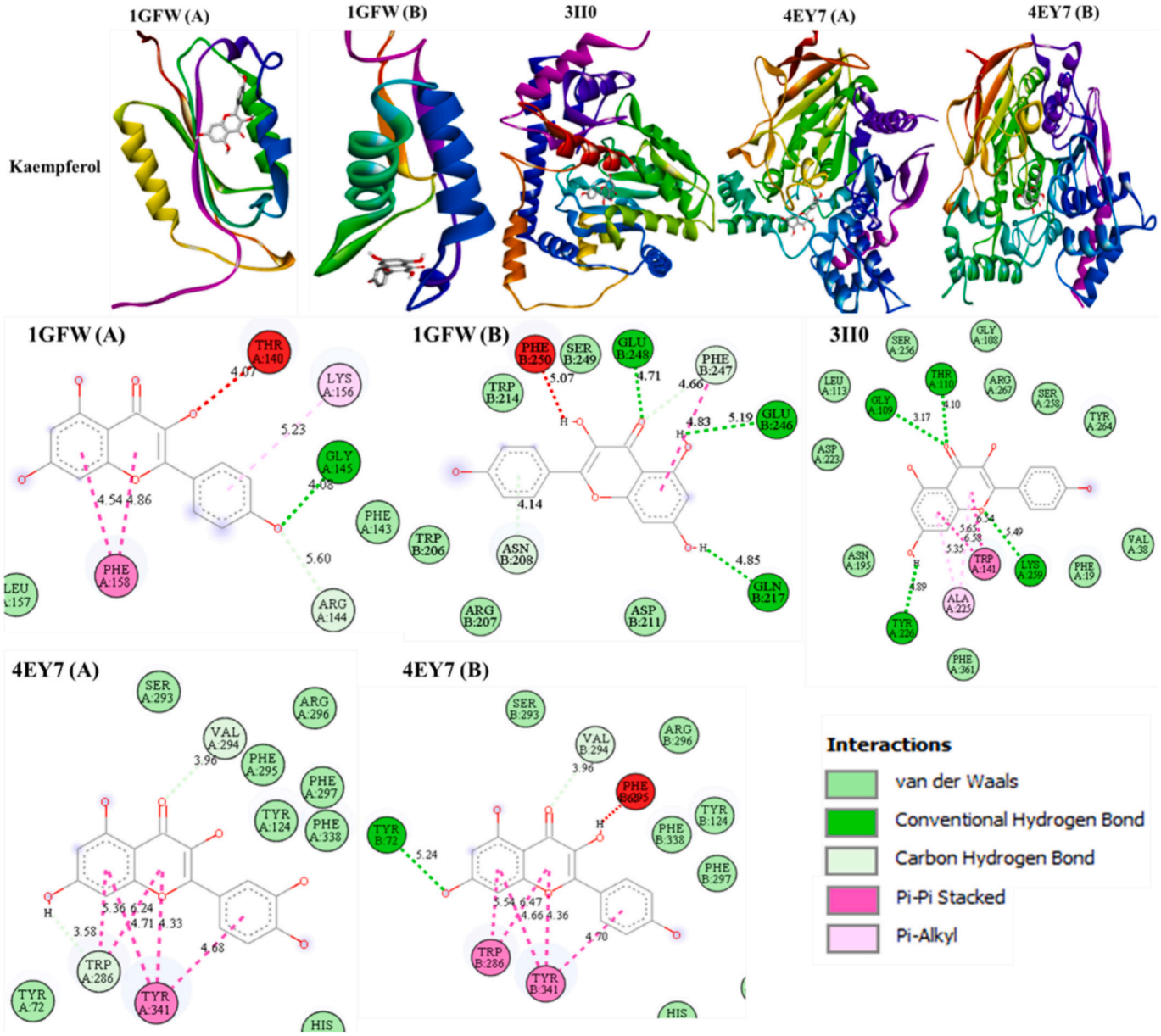

Fig. 10. Molecular docking analysis results visualization of Kaempferol.

The BOILED-Egg model further supported these findings by indicating that all compounds fall outside the regions of passive gastrointestinal absorption and blood–brain barrier permeation, suggesting limited passive diffusion and low central nervous system penetration. Molecular weight analysis revealed that DM (519.9 g/mol) slightly exceeds Lipinski's threshold, which may contribute to its reduced permeability (Lipinski, 2004). In contrast, quercetin (302.24 g/mol) and kaempferol (286.24 g/mol) fall within the acceptable range, supporting their drug-like characteristics.

ProTox-II toxicity predictions revealed marked differences in toxicological profiles. DM exhibited high acute toxicity (LD_{50}: 5 mg/kg, Class 2), consistent with its established neurotoxic effects (Rehman et al., 2014). The predicted activation of neurotoxicity, respiratory toxicity, and mitochondrial dysfunction pathways suggests that DM toxicity is closely associated with oxidative stress and mitochondrial impairment (Ramachandran et al., 2018). In contrast, quercetin (LD_{50}: 159 mg/kg) and kaempferol (LD_{50}: 3919 mg/kg) demonstrated significantly lower toxicity, supporting their relatively safer profiles.

Although both flavonoids showed predicted activation of certain pathways, including mutagenic and endocrine-related effects, these findings likely reflect concentration-dependent dual behavior, where flavonoids may act as antioxidants under physiological conditions but exhibit pro-oxidant effects at higher concentrations (Tumilaar et al., 2024). Importantly, the relatively lower toxicity of these compounds supports their potential protective role against oxidative stress-induced damage.

In addition to compound-level analysis, functional enrichment analysis provided strong biological evidence supporting oxidative stress as the central mechanism of deltamethrin toxicity. The enrichment of pathways such as oxidative stress response, reactive oxygen species metabolism, and response to hydrogen peroxide highlights the critical role of redox imbalance in mediating cellular damage. The identification of key antioxidant genes, including SOD1, SOD2, CAT, GPX1, and GPX3, along with regulatory genes such as NFE2L2 (NRF2) and HMOX1, indicates activation of endogenous antioxidant defense systems. Furthermore, enrichment of intrinsic apoptotic signaling pathways suggests that oxidative stress may trigger apoptosis as a downstream consequence of cellular injury.

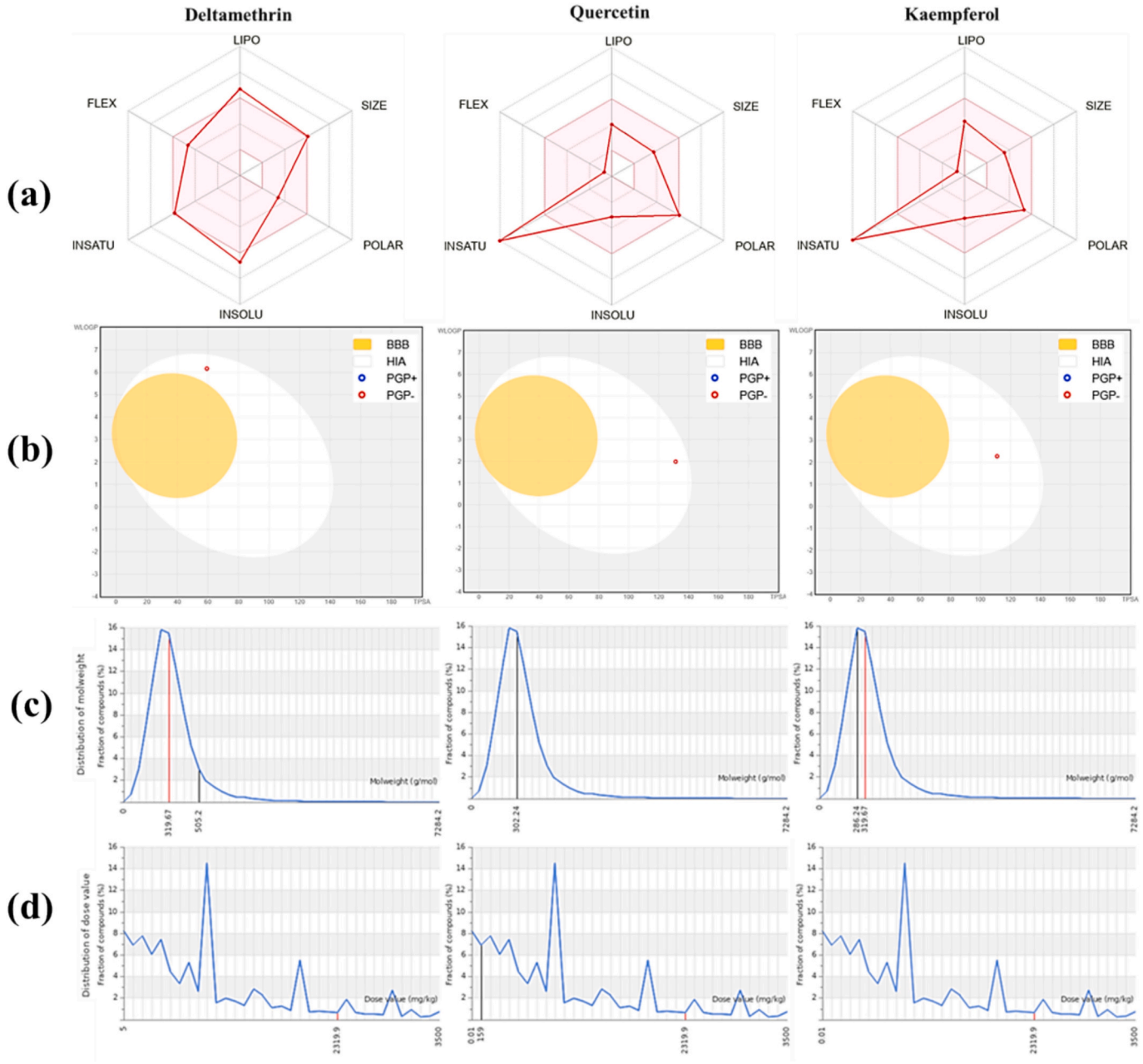

Fig. 11. Physicochemical Properties of drugs; (a) SwissADME (b) Boiled Egg (c) Distribution of molecular weight (d) distribution of dosage value.

Protein–protein interaction (PPI) analysis further revealed a tightly interconnected network of antioxidant and apoptosis-related proteins. Central nodes included CAT, SOD1, SOD2, and GPX3, representing the core antioxidant defense system, along with CASP3 and BCL2, which regulate apoptosis. The close interaction among these proteins indicates coordinated regulation of oxidative stress and apoptotic pathways, reinforcing the role of redox imbalance in toxicity.

These enrichment and network findings are consistent with in vivo observations, where deltamethrin exposure resulted in decreased antioxidant enzyme activities (SOD, CAT, GPx) and increased lipid peroxidation (MDA), confirming oxidative stress as a primary mechanism of toxicity. The identification of NFE2L2 (NRF2) further suggests that activation of antioxidant signaling pathways may underlie the protective effects of *Moringa oleifera* flavonoids.

Molecular docking analysis provided further mechanistic insight into the interaction of compounds with key proteins involved in oxidative stress and apoptosis. The selected targets, including acetylcholinesterase (AChE), caspase-3, and aspartate aminotransferase (AST), are directly associated with neurotoxicity, apoptosis, and hepatic damage, respectively, all of which are closely linked to oxidative stress.

The docking analyses revealed that DM exhibited moderate binding affinities, with the strongest interaction observed with AST (−8.8 kcal/mol), followed by caspase-3. Its interactions were primarily dominated by π–π stacking and hydrophobic interactions, with limited hydrogen bonding due to the absence of polar functional groups. This may result in less stable binding compared to flavonoids. Its moderate interaction with AChE supports its potential role in disrupting neurotransmission and contributing to neurotoxicity.

In contrast, quercetin demonstrated the strongest and most consistent binding affinities across all targets. Its multiple hydroxyl groups enabled extensive hydrogen bonding, while its planar aromatic structure facilitated π–π interactions, resulting in highly stable complexes. These interactions suggest that quercetin may effectively modulate apoptosis and oxidative stress-related pathways, particularly through inhibition of

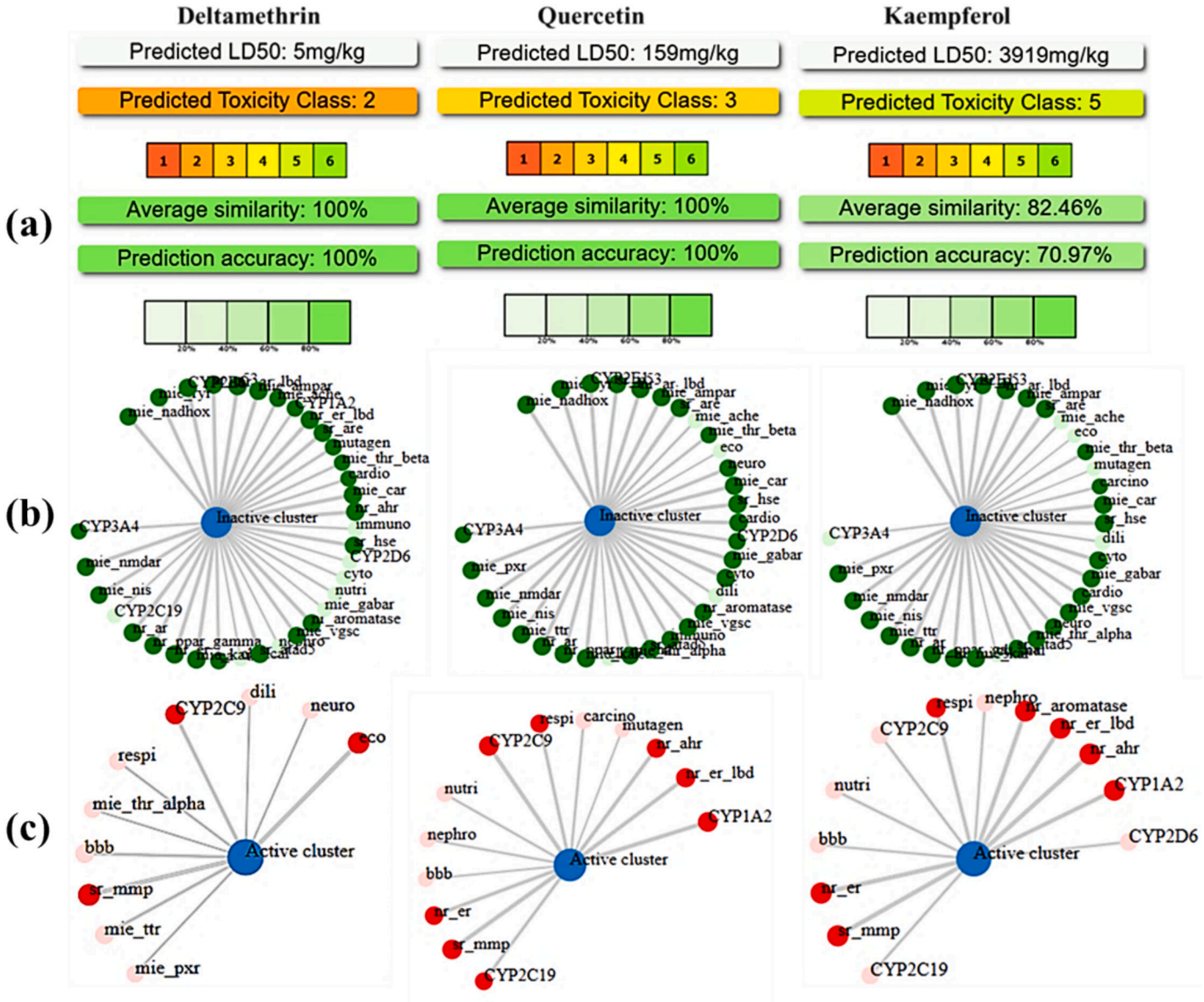

Fig. 12. Toxicity prediction by Protox II.

caspase-3 activity. These findings support experimental observations where Moringa supplementation reduced oxidative stress and improved biochemical parameters (Famurewa et al., 2020).

Kaempferol exhibited binding patterns like quercetin, although slightly weaker due to fewer hydroxyl groups. It formed stable interactions through hydrogen bonding and aromatic stacking, supporting its potential role in antioxidant and anti-apoptotic mechanisms. Minor unfavorable interactions observed in some complexes may slightly reduce binding stability, but overall, its docking profile supports its biological activity.

Overall, the integrated in silico findings demonstrate that deltamethrin induces toxicity primarily through oxidative stress and apoptosis, whereas *Moringa oleifera* flavonoids exert protective effects by enhancing antioxidant defense systems, activating NRF2-mediated pathways, and inhibiting apoptosis.

The DM-treated group showed a significant decrease in major growth performance indicators, including final body weight, total weight gain, and feed intake. The poor feed conversion ratio is consistent with previous studies reporting that pyrethroid toxicity induces anorexia, reduced activity, and metabolic disturbances, ultimately impairing growth performance (Khan et al., 2021). This decline reflects systemic physiological stress, which is further supported by biochemical alterations observed in serum parameters.

The central mechanism underlying DM-induced toxicity in the present study was oxidative stress. Compared with the negative control, the DM-exposed group exhibited significant depletion of endogenous antioxidant enzymes (SOD, CAT, and GPx), accompanied by a marked elevation in MDA levels, indicating excessive lipid peroxidation. This pattern reflects classical redox imbalance, where excessive ROS generation overwhelms antioxidant defenses (Jiang et al., 2024). Reduced SOD activity suggests impaired neutralization of superoxide radicals, while decreased CAT and GPx activities indicate compromised detoxification of hydrogen peroxide and lipid hydroperoxides. The substantial increase in MDA further confirms membrane lipid damage, reinforcing oxidative stress as the primary driver of organ dysfunction in DM-treated birds (Kumar et al., 2015).

This oxidative damage provides a direct explanation for the observed organ dysfunction. The significantly elevated serum levels of ALT and AST indicate hepatocellular damage, while increased creatinine and urea levels reflect impaired renal function (Humanes et al., 2012). The liver and kidney, being primary detoxification organs, are particularly susceptible to ROS-mediated injury during xenobiotic metabolism (Vicidomini et al., 2024). Additionally, decreased serum total protein and albumin levels suggest impaired hepatic synthetic function.

Table 3
Effect of *Moringa oleifera* and Deltamethrin on blood parameters of Japanese Qualis.

	NC	PC	0.3MO	0.5MO	0.7MO	P value
Body weight (g)						
Day 1	8.71 ± 0.28	8.85 ± 0.34	9 ± 0.37	8.57 ± 0.29	9 ± 0.30	0.85
Day 7	22.57 ± 0.61	22.42 ± 0.48	22.71 ± 0.60	21.85 ± 0.55	22 ± 0.65	0.80
Day 14	68.71 ± 2.79	70 ± 2.69	71.85 ± 1.85	74.89 ± 1.78	71.53 ± 3.52	0.53
Day 21	95.57 ± 2.85^{d}	92 ± 0.57^{cd}	98.14 ± 0.70^{bc}	102.28 ± 0.80^{ab}	107.28 ± 0.77^{a}	0.01
Day 28	131.14 ± 0.67^{c}	127.28 ± 1.84^{c}	139.14 ± 1.24^{ab}	138.42 ± 0.97^{b}	144 ± 0.87^{a}	0.03
Day 35	167.28 ± 6.02^{bc}	151.57 ± 3.96^{c}	174.14 ± 3.09^{b}	182.28 ± 4.41^{b}	202.14 ± 4.82^{a}	0.01
Day 42	238.85 ± 5.37^{bc}	222.71 ± 4.29^{c}	246.14 ± 3.29^{b}	255.28 ± 5.43^{ab}	273.85 ± 4.17^{a}	0.02
Body weight gain (g)						
Day 7	13.85 ± 0.67	13.57 ± 0.52	13.71 ± 0.71	13.28 ± 0.74	13 ± 0.65	0.89
Day 14	46.14 ± 2.83	47.57 ± 2.34	49.14 ± 2.16	53.04 ± 1.95	49.53 ± 3.4	0.43
Day 21	26.85 ± 5.16^{c}	92.71 ± 0.47^{b}	105.71 ± 0.99^{a}	108.71 ± 0.52^{a}	112.57 ± 0.61^{a}	0.02
Day 28	35.57 ± 3.28	35.28 ± 1.82	41 ± 1.70	36.14 ± 1.14	36.7 ± 1.45	0.28
Day 35	36.14 ± 5.98^{bc}	24.28 ± 2.53^{c}	35 ± 3.76^{bc}	43.85 ± 3.67^{ab}	58.14 ± 5.00^{a}	0.03
Day 42	71.57 ± 1.52	71.14 ± 1.66	72 ± 1.78	73 ± 1.41	71.71 ± 1.47	0.94
Overall	230.14 ± 5.17^{bc}	213.85 ± 4.50^{c}	237.14 ± 3.21^{b}	246.71 ± 5.41^{ab}	264.85 ± 4.30^{a}	0.01
Feed Intake (g)						
Day 7	40.57 ± 0.71	42 ± 0.95	42.42 ± 0.52	43.42 ± 0.48	41.71 ± 0.91	0.12
Day 14	105 ± 0.43^{a}	100.71 ± 1.01^{b}	102.57 ± 0.97^{ab}	100.28 ± 0.94^{b}	101.57 ± 0.71^{ab}	0.04
Day 21	135.14 ± 0.34^{d}	115.85 ± 1.01^{e}	144 ± 0.12^{c}	149.42 ± 0.29^{b}	154.57 ± 0.36^{a}	0.04
Day 28	160.57 ± 0.68^{c}	161.85 ± 0.96^{c}	171.42 ± 4.36^{b}	179.57 ± 0.61^{ab}	187.14 ± 1.48^{a}	0.01
Day 35	248.42 ± 0.68^{a}	228 ± 5.572^{b}	230.28 ± 0.64^{b}	237.85 ± 0.79^{ab}	243.85 ± 1.40^{a}	0.02
Day 42	290.71 ± 2.07	288 ± 3.32	289.57 ± 1.83	289.71 ± 2.38	289 ± 1.95	0.94
Overall	980.42 ± 3.02^{c}	936.42 ± 6.39^{d}	980.28 ± 4.54^{c}	1000.28 ± 1.88^{b}	1017.85 ± 3.50^{a}	0.05
Feed conversion ratio (g:g)						
Day 7	2.96 ± 0.13	3.12 ± 0.16	3.14 ± 0.15	3.33 ± 0.21	3.27 ± 0.22	0.64
Day 14	2.33 ± 0.17	2.15 ± 0.14	2.11 ± 0.10	1.90 ± 0.07	2.11 ± 0.14	0.27
Day 21	6.20 ± 1.13^{a}	1.24 ± 0.09^{b}	1.36 ± 0.01^{b}	1.37 ± 0.06^{b}	1.37 ± 0.04^{b}	0.02
Day 28	4.79 ± 0.51	4.67 ± 0.28	4.22 ± 0.20	4.99 ± 0.15	5.14 ± 0.19	0.27
Day 35	8.19 ± 1.43^{ab}	10.32 ± 1.53^{a}	7.11 ± 0.86^{ab}	5.66 ± 0.48^{b}	4.43 ± 0.48^{b}	0.02
Day 42	4.07 ± 0.06	4.06 ± 0.10	4.03 ± 0.11	3.97 ± 0.12	4.21 ± 0.07	0.96
Overall	4.27 ± 0.10^{a}	4.39 ± 0.11^{a}	4.13 ± 0.06^{ab}	4.06 ± 0.09^{ab}	3.84 ± 0.06^{c}	0.03

NC: Negative Control supplemented with basal diet; PC: Positive Control supplemented with Deltamethrin; 0.3MO: Supplemented with 0.3 g of *Moringa oleifera*; 0.5MO: Supplemented with 0.5 g of *Moringa oleifera*; 0.7MO: Supplemented with 0.7 g of *Moringa oleifera*. Means with different superscripts are statistically significant.

Table 4
Effect of *Moringa oleifera* and Deltamethrin on blood parameters of Japanese Qualis.

	NC	PC	0.3MO	0.5MO	0.7MO	P value
Hematology						
RBC Count (10^6/μL)	3.1 ± 0.09^{b}	2.8 ± 0.01^{a}	3.2 ± 0.07^{ab}	3.36 ± 0.03^{a}	3.4 ± 0.09^{a}	0.01
Hemoglobin (g/dL)	9.5 ± 0.05^{bc}	8.1 ± 0.05^{d}	9.4 ± 0.05^{c}	9.7 ± 0.07^{ab}	9.8 ± 0.05^{a}	0.05
Hematocrit (%)	41 ± 0.5^{c}	36.66 ± 0.3^{d}	42 ± 0.57^{bc}	43.66 ± 0.33^{ab}	44.66 ± 0.33^{a}	0.03
WBC Count (10^3/μL)	13 ± 0.15^{b}	14.3 ± 0.15^{a}	12.56 ± 0.08^{bc}	12.2 ± 0.05^{cd}	11.9 ± 0.05^{d}	0.04
Lymphocytes (%)	59.66 ± 0.88^{ab}	62 ± 0.57^{a}	60 ± 0.57^{ab}	59 ± 0.57^{b}	57.33 ± 0.33^{b}	0.05
Heterophils (%)	31 ± 0.57^{ab}	32 ± 0.57^{a}	29.66 ± 0.33^{bc}	28.33 ± 0.33^{c}	28.33 ± 0.33^{c}	0.02
Serum Biochemistry						
Total Protein (g/dL)	4.2 ± 0.05^{bc}	3.5 ± 0.01^{d}	4.1 ± 0.03^{c}	4.4 ± 0.01^{ab}	4.53 ± 0.03^{a}	0.02
Albumin (g/dL)	2.1 ± 0.04^{ab}	1.83 ± 0.01^{c}	2.03 ± 0.03^{b}	2.13 ± 0.02^{ab}	2.26 ± 0.03^{a}	0.01
Globulin (g/dL)	2.1 ± 0.1^{a}	1.7 ± 0.05^{b}	2.06 ± 0.03^{a}	2.26 ± 0.01^{a}	2.26 ± 0.03^{a}	0.05
ALT (U/L)	29 ± 0.5^{bc}	36 ± 0.8^{a}	30 ± 0.7^{b}	27 ± 0.1^{c}	26.66 ± 0.3^{c}	0.004
AST (U/L)	260 ± 1.15^{b}	277.66 ± 1.4^{a}	256.66 ± 0.8^{b}	251 ± 0.57^{c}	248 ± 0.57^{c}	0.05
Creatinine (mg/dL)	0.4 ± 0.02^{b}	0.5 ± 0.05^{a}	0.4 ± 0.01^{b}	0.3667 ± 0.03^{bc}	0.3 ± 0.05^{c}	0.02
Urea (mg/dL)	15.33 ± 0.33^{b}	18.33 ± 0.33^{a}	15.33 ± 0.3^{b}	13.66 ± 0.33^{c}	12.33 ± 0.33^{c}	0.04
Glucose (mg/dL)	181 ± 0.76^{b}	188 ± 0.43^{a}	178 ± 0.52^{c}	174 ± 0.57^{d}	170 ± 0.61^{e}	0.001

NC: Negative Control supplemented with basal diet; PC: Positive Control supplemented with Deltamethrin; 0.3MO: Supplemented with 0.3 g of *Moringa oleifera*; 0.5MO: Supplemented with 0.5 g of *Moringa oleifera*; 0.7MO: Supplemented with 0.7 g of *Moringa oleifera*. Means with different superscripts are statistically significant.

Table 5
Effect of *Moringa Oleifera* and Deltamethrin on Antioxidant activity of Japanese Qualis.

	NC	PC	0.3MO	0.5MO	0.7MO	P value
SOD (U/mL)	348.33 ± 4.40^{c}	255 ± 2.88^{d}	367.66 ± 1.45^{b}	381 ± 2.08^{a}	392.66 ± 1.45^{a}	0.001
CAT (U/mL)	43.33 ± 0.88^{c}	31 ± 0.21^{d}	46 ± 0.32^{bc}	48 ± 0.57^{ab}	49.66 ± 0.33^{a}	0.02
GPx (U/mL)	23 ± 0.57^{b}	15.33 ± 0.43^{c}	24.33 ± 0.21^{b}	26.33 ± 0.31^{a}	27.66 ± 0.54^{a}	0.01
MDA (nmol/mL)	1.5 ± 0.05^{b}	2.4 ± 0.02^{a}	1.3 ± 0.01^{bc}	1.1 ± 0.04^{cd}	1 ± 0.01^{d}	0.001

NC: Negative Control supplemented with basal diet; PC: Positive Control supplemented with Deltamethrin; 0.3MO: Supplemented with 0.3 g of *Moringa oleifera*; 0.5MO: Supplemented with 0.5 g of *Moringa oleifera*; 0.7MO: Supplemented with 0.7 g of *Moringa oleifera*. Means with different superscripts are statistically significant.

Hematological alterations, including reduced RBC count, hemoglobin, and hematocrit, indicate anemia, likely resulting from oxidative damage to erythrocyte membranes and possible suppression of hematopoiesis (Orrico et al., 2023).

One of the most significant findings of this study was the dose-dependent protective effect of MO against DM-induced toxicity. MO supplementation effectively restored antioxidant enzyme activities (SOD, CAT, GPx) and normalized MDA levels, indicating improved redox homeostasis (Hamed et al., 2022). This protective effect is attributed to the antioxidant properties of MO phytoconstituents, particularly quercetin and kaempferol, which likely act through both

direct ROS scavenging and activation of endogenous antioxidant defense systems, including NRF2-mediated pathways.

By alleviating oxidative stress, MO protected vital organs from damage, as evidenced by the normalization of liver and kidney function markers (ALT, AST, creatinine, and urea). The improvement in serum protein levels further indicates restoration of hepatic function, while normalization of hematological parameters suggests reduced systemic stress and inflammation (Aprioku et al., 2022). Consequently, improved physiological status led to enhanced feed intake, growth performance, and feed conversion efficiency.

Notably, the 0.7 MO group not only restored normal physiological parameters but also exceeded the control group in growth performance, suggesting that MO may function as a natural growth promoter. Its antioxidant capacity likely reduces basal metabolic stress, allowing more efficient energy utilization for growth. This dual role of MO in mitigating toxicity and enhancing performance highlights its potential as a functional feed additive in poultry production (Biswas et al., 2024).

5. Conclusion

This integrative study combining in silico modelling with an in vivo Japanese quail model provides mechanistic and physiological evidence of DM-induced systemic toxicity and the protective efficacy of MO supplementation. Computational analyses revealed the high predicted toxicity of DM (LD_{50}: 5 mg/kg) and its moderate binding interactions with AChE, Caspase-3, and AST, supporting its neurotoxic and organ-damaging potential. In contrast, the flavonoids Quercetin and Kaempferol exhibited stronger binding affinities (−9.3 to −9.7 kcal/mol) and lower predicted acute toxicity, indicating their molecular capacity to counteract oxidative and apoptotic pathways. In vivo findings corroborated these predictions. DM exposure significantly reduced antioxidant enzyme activities (SOD, CAT, GPx) and increased lipid peroxidation (MDA), alongside marked deterioration in growth performance and hepato-renal biomarkers. Notably, dietary MO supplementation—particularly at 0.7 g/kg—restored antioxidant enzyme activities beyond control levels, reduced MDA to near-baseline values, normalized ALT, AST, creatinine, and urea levels, and significantly improved body weight gain and feed conversion ratio. These findings confirm that oxidative stress is a central mechanism of DM toxicity, and that MO exerts dose-dependent protective effects through restoration of redox homeostasis and preservation of organ integrity. The novelty of this study lies in the combined mechanistic validation of MO flavonoids at both molecular docking and animal physiological levels, providing a multi-layered understanding of phytogenic mitigation against pesticide toxicity in poultry.

Future studies should explore molecular signaling pathways involved in antioxidant gene regulation (e.g., Nrf2-mediated responses), long-term safety profiling, bioavailability optimization of flavonoids, and large-scale field trials to validate MO as a sustainable phytobiotic feed additive. Collectively, these findings support MO as a promising natural strategy for mitigating pesticide-induced oxidative damage and enhancing poultry resilience under environmental toxicant exposure.

CRediT authorship contribution statement

Muhammad Sarfraz: Methodology, Data curation. **Irfan Baboo:** Writing – original draft, Supervision. **Zahid Farooq:** Software, Methodology. **Haseeb Khaliq:** Writing – original draft, Visualization, Validation, Methodology. **Valiollah Palangi:** Writing – review & editing, Supervision, Software.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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