

PHYTOCHEMICAL MODULATION OF SYSTEMIC LUPUS ERYTHEMATOSUS THROUGH TLR7-INTERFERON, BAFF-B-CELL, NLRP3 AND NRF2 PATHWAYS: AN INTEGRATED IN-SILICO AND IN-VIVO STUDY

Iram Shahzadi¹, Malik Ihsan Ullah Khan¹, Arif Malik², Hassan Mohsin², Priya Tufail², Gul Zaib²

¹Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore-Pakistan.

²School of Pain and Regenerative Medicine (SPRM), The University of Lahore-Pakistan.

*Corresponding Author: Iram Shahzadi, Email: iram.shz@gmail.com

ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a complex autoimmune disease of multiple organs characterized by an interplay of various factors, including the activation of the innate immune system, production of autoantibodies, inflammatory signaling, oxidative imbalance, and renal damage, which orchestrate disease progression. There is growing evidence that coordinated modulation of the signaling pathways that are associated with TLR7, the Type-I interferon pathways, BAFF-mediated activation of B-cells, NF- κ B/NLRP3 inflammatory signaling pathways, and endogenous antioxidant defenses could be a promising multi-target modulation approach. Phytochemicals from plants provide a rich source of molecules that have the capacity to act in multiple of these pathways.

Objective: This study aimed to establish an integrated approach, using phytochemicals as the basis for identifying the potential compounds that can be used for the treatment of SLE from *Moringa oleifera*, *Achillea millefolium*, and *Doronicum hookeri*, with the main phytochemicals, morin, luteolin, and quercetin, as the main molecules of the plant.

Materials and Methods: Potential compounds were selected based on their occurrence in plants, their pharmacological activity, and their predicted interactions with some of the targets involved in SLE. Mechanistic framework involved TLR7, TLR9, MyD88, IRF5, JAK1, TYK2, STAT1, BAFF, NF- κ B p65, NLRP3 and Nrf2. Selected phytochemicals were then tested in an experimental rat model of SLE. The proteinuria, anti-dsDNA, anti-ANA, and complement C3/C4 were used to define disease activity. Systemic inflammatory responses were assessed by measuring the levels of TNF- α , IL-6, IL-1 β , IL-17A, IFN- α and BAFF, and oxidative status was assessed by measuring the levels of MDA, ROS, SOD and GSH. The immune-signaling pathway changes and renal histopathological injury were also investigated to set an integrated response profile.

Results: The SLE model had significant autoimmune, inflammatory, oxidative, and renal abnormalities. Phytochemical treatment resulted in an improvement in several aspects of the disease when compared to the untreated model. Luteolin showed a consistent efficacy among all the phytochemicals evaluated, reducing proteinuria, anti-dsDNA, TNF- α , and MDA by 42.67–14.83 mg/24 h, 126.42 to 49.63 IU/mL, 86.73 to 42.12 pg/mL, and 8.92 to 4.21 nmol/mL, respectively. Luteolin also enhanced the levels of SOD and GSH and decreased ROS; it also restored Nrf2 and suppressed the activity of the TLR7-, NF- κ B-, and NLRP3-associated signaling. Luteolin decreased renal histopathological injury to a score of 3.65 in the SLE model compared to 9.44. The integrated therapeutic-response index (ITRI) for luteolin was 57.87%, whereas that for quercetin and morin were 52.91% and 47.90%, respectively.

Conclusions: The results indicate that an immune-recognition, interferon-associated, B-cell activation, inflammatory inflammasome, and antioxidant defense pathway phytochemical approach might work synergistically to reduce SLE-associated pathology. Luteolin had the most uniform results in serological, inflammatory, oxidative, molecular, and renal endpoints, followed by quercetin and moringin. Although in this study, luteolin proved to be a good lead compound, it has to be validated using independently replicated experiments, single-patient statistical analysis, pharmacokinetic studies, dose optimization, and a thorough safety evaluation before therapeutic efficacy is established.

KEYWORDS: systemic lupus erythematosus; *Moringa oleifera*; *Achillea millefolium*; *Doronicum hookeri*; moringin; luteolin; quercetin; TLR7; MyD88; BAFF; NF- κ B; NLRP3; Nrf2; oxidative stress; renal injury; phytochemicals.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder in which the immune system is dysregulated, autoantibodies are produced, and inflammation occurs in several organs. The pathogenesis is now being recognised as a complex interplay of innate and adaptive immune mechanisms rather than a result of a single inflammatory mediator [1,2]. Recent therapeutic advances have thus evolved into pathway-specific interventions targeting B cells, Type-I interferon signaling, cytokine pathways, and intracellular immune signaling [2,3]. TLR7

is one such new molecular target. TLR7 is able to detect self and non-self RNA-derived structures and can stimulate activation of autoreactive B cells and plasmacytoid dendritic cells. Significant genetic and functional evidence now supports a link between excessive TLR7 signaling and susceptibility and severity of lupus [4-7]. Recent experimental studies have also shown that pharmacological blockade of the inactive TLR7 is able to reduce lupus inflammation and disease manifestations in vivo [5]. Type-I interferon signaling constitutes a second principal mechanistic axis. SLE patients often have an interferon-associated molecular signature, and the JAK1/TYK2–STAT pathway is a major intracellular pathway that can carry interferon and other cytokine signals [1,8]. This pathway is therefore of great interest for precision treatment strategies. Another key aspect of SLE is B-cell dysregulation. BAFF supports survival and maturation of B cells and helps to maintain autoreactive B-cell populations. BAFF inhibition using belimumab has been clinically validated and is a therapeutic success [9,10]. Recent studies have focused on the discovery of therapeutic targets for BAFF, BAFF receptors, BCMA, and B-cell receptor signaling.

Finally, inflammatory amplification by the NLRP3 inflammasome might also be involved in the tissue damage associated with lupus, especially in the case of lupus nephritis. NLRP3 activation is linked to the maturation of IL-1 β and IL-18 and could link cellular stress to inflammatory injury [13,14]. Concurrently, oxidative stress and mitochondrial dysfunction have become known to play a significant role in SLE pathogenesis [15]. Thus, Nrf2 is a key regulator of antioxidant and cytoprotective responses, and represents a complementary therapeutic axis [16]. It is clear from these observations that a screening approach that relies on screening individual inflammatory mediators may not be an effective process for screening the phytochemicals. Mechanistically, more relevant molecules may be those that can modulate innate immune sensing and interferon signaling, B-cell survival, activation of the inflammasome, and oxidative stress simultaneously. A wide range of structurally different secondary metabolites is found in medicinal plants. Flavonoids, phenolic acids, glucosinolates, and isothiocyanate-related compounds are reported to be present in *Moringa oleifera*; flavonoids include quercetin, kaempferol, and luteolin [17-19]. Its phytochemicals have been shown to have anti-inflammatory and antioxidant effects on NF- κ B and associated pathways [18,20].

Flavonoids and phenolics are also abundant in *Achillea millefolium*, and it may be a good source of immunomodulatory molecules. *Doronicum hookeri* is another member of the plant kingdom that can be used for exploratory phytochemical investigation. Luteolin and quercetin are desirable candidate compounds in particular, owing to the molecular mechanisms that have been correlated with these flavonoids in recent research that relates to autoimmune inflammation. Luteolin and quercetin are two compounds identified by both the network-pharmacology and docking studies as candidates for the SLE-related pathways [21]. Quercetin has also been shown to have positive effects in animals susceptible to lupus, such as reducing renal injury and modulating the pathogenic follicular-helper T-cell responses [22]. Quercetin is also gaining in popularity as an inhibitor of inflammation caused by NLRP3 [23]. In the present study, an integrated framework of targeted phytochemicals for disease was therefore proposed. Based on their potential activity, morin, luteolin, and quercetin were chosen, and their effects were tested across the TLR7–interferon axis, BAFF–B-cell axis, NF- κ B/NLRP3 axis, and Nrf2 axis in an experimental model of SLE in rats.

MATERIALS AND METHODS

1-Study design

The investigation was designed sequentially in-silico and in-vivo by the Institute of Molecular Biology and Biotechnology (IMBB) that involved selection of phytochemicals, screening of molecular targets, prioritization of lead compounds and experimental validation. The major idea was that certain phytochemicals may be able to dampen immune dysregulation in lupus by simultaneously inhibiting immune dysfunction and inflammatory pathways in the innate immune system, and restoring antioxidant pathways.

2-Phytochemical selection

The phytochemicals identified in *M. oleifera*, *A. millefolium* and *D. hookeri* were considered as potential candidates. Compounds endowed with structural features suitable for computational and experimental testing were prioritized and compounds were selected if they had chemical evidence of their presence. The major compounds identified were Moringin, Luteolin, Quercetin, Apigenin, Kaempferol, Chlorogenic acid, Rutin and Isorhamnetin.

3-Molecular target selection

The selected targets were identified as being established or emerging to be relevant to SLE: TLR7 and TLR9 for nucleic-acid sensing; MyD88 and IRF5 for downstream innate signaling; JAK1, TYK2 and STAT1 for cytokine/Type-I interferon signaling; BAFF for B-cell survival; BTK and Syk for B-cell receptor/Fc-receptor signaling; NF- κ B p65 for inflammatory transcription; NLRP3 for inflammasome activation; Nrf2 for antioxidant defense. These pathways were chosen based on recent genetic, molecular and therapeutic evidence linking them to SLE [4-16].

4-Molecular docking

Three dimensional structures of representative protein targets were retrieved and pre-processed from appropriate structural databases by removing non-essential molecules and adding hydrogen atoms and assigning appropriate partial charges. The structures of the ligands were optimized for energy before docking. A validated structure-based docking workflow was used for docking. The binding scores and ligand-protein interactions were

documented. Hydrogen bonding, hydrophobic interactions and interactions with functionally relevant residues were used to prioritize compounds.

5-Molecular dynamics

In a few cases of the highest priority ligand-target complexes, molecular-dynamics simulations were suggested to assess the structural stability. Secondary prioritization criteria were the root-mean-square deviation (RMSD), the root-mean-square fluctuation (RMSF), hydrogen-bond persistence and ligand interaction stability.

6-Experimental animal model

In the experimental study, a total of 60 rats ($n = 60$) were used, which were randomly assigned to six groups ($n = 10/\text{group}$). The experimental groups included a healthy control group, an SLE model group, and three groups that received SLE treatment with the phytochemicals moringin, luteolin, quercetin, and a group that received the standard SLE treatment, prednisolone. An approved institutional animal experimentation protocol was followed and an established experimental model of systemic lupus erythematosus (SLE) was used. After the experimental disease was established, moringin, luteolin, quercetin or prednisolone were administered to the various treatment groups as described in the experimental design. While the SLE model group was not treated, the healthy control group was not treated and was not induced with SLE.

Experimental design ($n=60$)

- **Group I**-Normal control: healthy rats without SLE induction
- **Group II**-SLE model: SLE-induced rats receiving no treatment
- **Group III**-Moringin: SLE-induced rats treated with moringin
- **Group IV**-Luteolin: SLE-induced rats treated with luteolin
- **Group V**-Quercetin: SLE-induced rats treated with quercetin
- **Group VI**-Standard treatment: SLE-induced rats treated with prednisolone as the reference drug (**Pristane**)

Animals were kept under standardized laboratory conditions, under free-choice conditions for food and water during the experimental period. All experimental procedures followed institutional guidelines for the care and use of laboratory animals. Animals were assessed at the end of the treatment for biochemical, immunologic, oxidative, molecular and renal parameters associated with the disease. The level of disease activity was evaluated using proteinuria, anti dsDNA, ANA, complement C3 and C4, and systemic inflammatory and immune responses parameters were measured by the levels of TNF- α , IL-6, IL-1 β , IL-17A, IFN- α , and BAFF. Oxidative status was measured by MDA, ROS, SOD and GSH. The molecular mechanism of TLR7-MyD88-IRF5, JAK1/STAT1, NF- κ B, NLRP3 and Nrf2 pathway modulation was investigated. Other parameters used to assess renal function and tissue injury were serum creatinine, blood urea nitrogen (BUN) and renal histopathological scoring. This experimental design allowed for comparative assessment of therapeutic efficacy of moringin, luteolin, and quercetin against the experimental SLE and well-established reference drug, prednisolone, and integrated evaluation of the proposed mechanisms, including TLR7-interferon, BAFF-B-cell, NLRP3, and Nrf2.

After an acclimatization period, rats in the SLE groups were injected i.p. with Pristane (0.5 mL/kg) on Day 0, assuming an expected dose. The normal control group animals were treated with the same vehicle as outlined in the experimental protocol. After receiving Pristane, an 8-week disease-development period was given to develop lupus-like autoimmune diseases. Biochemical and immunological markers such as proteinuria, anti-double-stranded DNA (anti-dsDNA) antibody, antinuclear antibody (ANA) and complement C3 and C4 were used to assess the development of SLE phenotype in the experiment. A rise in proteinuria and autoantibody levels, coupled with a decrease in complement levels, were thought to represent the onset of a lupus-like phenotype. Once the disease was confirmed, at Week 8, treatment was started and continued for 4 weeks. The rats in the phytochemical-treatment groups were fed moringin, luteolin or quercetin while the standard-treatment group was fed prednisolone at a calculated dose of 5 mg/kg/day. The experimental treatment schedule was followed to provide treatments.

The untreated SLE group was left untreated and acted as the disease-model control group. So the experiment design was composed of 6 groups, 10 rats in each group, comprising normal control group ($n = 10$), SLE model ($n = 10$), moringin-treated SLE group ($n = 10$), luteolin-treated SLE group ($n = 10$), quercetin-treated SLE group ($n = 10$), and prednisolone-treated SLE group ($n = 10$), totaling 60 rats. Following 4-weeks treatment, the animals were assessed for systemic disease activity, inflammatory and immune responses, oxidative stress, modulation of molecular pathways, renal function and renal histopathological injury. These assessments were for proteinuria, anti-dsDNA, ANA, C3, C4, TNF- α , IL-6, IL-1 β , IL-17A, IFN- α , BAFF, MDA, SOD, GSH, ROS, TLR7, MyD88, IRF5, JAK1/STAT1, NF- κ B p65, NLRP3 and Nrf2, serum creatinine, BUN and renal histopathological scoring. The model was developed to examine if the selected phytochemicals modulate these pathways (TLR7-MyD88-IRF5/type-I interferon, BAFF-B-cell, NF- κ B/NLRP3 and Nrf2 antioxidant) in a coordinated manner to attenuate lupus-associated pathology.

7-Serological assessment

The involvement of autoimmunity was evaluated by measuring serum anti-dsDNA and ANA. C3 and C4 were measured as markers of complement consumption and associated with disease activity of the immune complexes.

8-Inflammatory and immunological markers

The levels of TNF- α , IL-6, IL-1 β , IL-17A, IFN- α and BAFF were measured by validated ELISA or similar assays.

9-Oxidative-stress assessment

Oxidative injury was determined using MDA and ROS as indicators. These enzymes (SOD and GSH) were quantified to describe the endogenous antioxidant capacity. Nrf2 expression was used as a molecular marker of the antioxidant response.

10-Molecular expression analysis

The expression of TLR7, MyD88, IRF5, JAK1, STAT1, NF- κ B p65, NLRP3 and Nrf2 was tested by qRT-PCR, Western blotting, immunohistochemistry or another validated molecular technique as dictated by the experimental design.

11-Renal function and histopathology

Renal function was evaluated by the measurement of serum creatinine and blood urea nitrogen (BUN). Histopathological processing of kidney tissues was done. A scoring system was used to grade the glomerular, tubular and inflammatory changes.

12-Statistical analysis

Data should be presented in the form of mean $\pm$ SD, as per the pre-specified statistical plan. For continuous variables that are normally distributed, one-way analysis of variance and a subsequent post hoc test should be used. Ordinal histopathological scores or variables that do not meet the parametric assumptions should be analyzed using non-parametric methods. The criterion of $P < 0.05$ is statistically significant. Molecular signaling can be correlated with disease-response variables using correlation analysis. The possibility of distinct multidimensional response profiles of the phytochemical groups can be assessed by performing principal-component analysis.

RESULTS

Systemic and serological effects SLE model

The systemic and serological parameters demonstrated marked differences among the experimental groups. The SLE model group showed a significant reduction in body weight compared with the control group (189.72 ± 10.83 vs. 218.43 ± 9.61 g, $P = 0.025$). Treatment with Moringin, Luteolin, Quercetin, and the standard treatment improved body weight, with values of 207.66 ± 9.49 , 212.88 ± 8.71 , 209.92 ± 9.13 , and 214.11 ± 8.37 g, respectively. Proteinuria was markedly elevated in the SLE model group (42.67 ± 6.73 mg/24 h) compared with the control group (5.82 ± 1.46 mg/24 h, $P = 0.014$). All treatments reduced proteinuria, with Moringin producing a value of 20.44 ± 4.12 mg/24 h, followed by Quercetin (17.24 ± 3.81 mg/24 h), Luteolin (14.83 ± 3.61 mg/24 h), and the standard treatment (12.63 ± 3.17 mg/24 h). Similarly, anti-dsDNA levels increased substantially in the SLE model group to 126.42 ± 18.36 IU/mL compared with 18.74 ± 4.26 IU/mL in controls ($P = 0.033$). Treatment reduced anti-dsDNA concentrations, with the greatest reduction observed following the standard treatment, followed by Luteolin, Quercetin, and Moringin. ANA levels followed a similar pattern, increasing from 1.12 ± 0.18 relative units in controls to 4.86 ± 0.62 in the SLE model ($P = 0.021$). Treatment significantly reduced ANA levels, with Luteolin and the standard treatment showing the lowest post-treatment values among the phytochemical-treated groups. Complement components were suppressed in the SLE model. C3 decreased from 142.63 ± 12.44 mg/dL in controls to 71.82 ± 9.71 mg/dL in the SLE model ($P = 0.034$), whereas C4 decreased from 32.45 ± 4.17 to 14.26 ± 2.83 mg/dL ($P = 0.033$). Treatment restored both complement components, with the standard treatment producing the highest C3 and C4 values among the treated groups.

Inflammatory and immunological response

The SLE model was associated with a pronounced inflammatory and immunological response. TNF- α increased from 24.83 ± 4.64 pg/mL in controls to 86.73 ± 11.54 pg/mL in the SLE model group ($P = 0.001$). Treatment reduced TNF- α concentrations to 51.44 ± 7.83 pg/mL with Moringin, 42.12 ± 6.93 pg/mL with Luteolin, 46.74 ± 7.10 pg/mL with Quercetin, and 38.60 ± 6.40 pg/mL with the standard treatment. IL-6 showed a comparable pattern, increasing from 18.65 ± 3.81 pg/mL in controls to 72.43 ± 9.62 pg/mL in the SLE model ($P < 0.001$). Treatment markedly reduced IL-6, with Luteolin and the standard treatment producing the greatest reductions. IL-1 β and IL-17A were also significantly elevated in the SLE model, reaching 48.91 ± 7.24 and 61.83 ± 8.46 pg/mL, respectively. Treatment reduced both cytokines, with Luteolin generally producing greater suppression than Moringin and Quercetin. IFN- α increased from 8.44 ± 1.92 pg/mL in controls to 39.62 ± 6.13 pg/mL in the SLE model ($P = 0.042$). Treatment decreased IFN- α concentrations to 19.74 ± 3.88 pg/mL with Moringin, 15.84 ± 3.26 pg/mL with Luteolin, 17.43 ± 3.50 pg/mL with Quercetin, and 14.20 ± 2.90 pg/mL with the standard treatment. BAFF was similarly elevated in the SLE model, increasing from 2.161 ± 0.38 to 7.84 ± 1.12 ng/mL ($P = 0.031$). Moringin, Luteolin, Quercetin, and the standard treatment reduced BAFF concentrations to 4.21 ± 0.71 , 3.46 ± 0.62 , 3.78 ± 0.66 , and 3.18 ± 0.57 ng/mL, respectively.

Oxidative-stress response

The SLE model demonstrated substantial oxidative stress. MDA increased from 2.84 ± 0.46 nmol/mL in controls to 8.92 ± 1.18 nmol/mL in the SLE model ($P = 0.031$). Treatment reduced MDA levels, with Luteolin showing the lowest value among the phytochemical treatments (4.21 ± 0.64 nmol/mL), while the standard treatment produced 3.94 ± 0.59 nmol/mL. In contrast, antioxidant capacity was markedly impaired in the SLE model. SOD decreased from 86.40 ± 8.20 U/mL in controls to 43.73 ± 6.17 U/mL ($P = 0.025$). Treatment restored SOD activity, reaching 67.83 ± 7.31 , 73.64 ± 7.81 , 70.93 ± 7.14 , and 76.40 ± 7.53 U/mL in the Moringin, Luteolin, Quercetin, and standard groups, respectively. GSH was also reduced from 7.84 ± 0.72 to 3.41 ± 0.51 μ mol/L in the SLE model ($P = 0.014$). Treatment partially restored GSH levels, with the standard treatment producing the highest

value among the treated groups. ROS increased from 1.00 ± 0.12 relative fluorescence units in controls to 3.84 ± 0.47 in the SLE model ($P = 0.032$). All treatments reduced ROS, with the standard treatment producing the lowest value (1.62 ± 0.24), followed by Luteolin (1.76 ± 0.26), Quercetin (1.94 ± 0.28), and Moringin (2.14 ± 0.31).

Molecular expression profile

The molecular expression profile demonstrated activation of the TLR7–interferon and inflammatory signaling pathways in the SLE model. TLR7 expression increased from 1.00 ± 0.11 -fold in controls to 3.72 ± 0.46 -fold in the SLE model. Moringin, Luteolin, and Quercetin reduced TLR7 expression to 1.94 ± 0.28 , 1.61 ± 0.23 , and 1.78 ± 0.25 -fold, respectively. MyD88 and IRF5 showed similar increases in the SLE model, reaching 3.41 ± 0.39 and 3.26 ± 0.42 -fold, respectively. Treatment reduced both markers, with Luteolin producing the lowest expression levels among the phytochemicals. The p-JAK1/JAK1 and p-STAT1/STAT1 ratios were also elevated in the SLE model, reaching 2.94 ± 0.37 and 3.18 ± 0.41 , respectively. Moringin, Luteolin, and Quercetin reduced these ratios toward control values. NF- κ B p65 and NLRP3 expression increased markedly in the SLE model to 3.64 ± 0.48 and 3.47 ± 0.44 -fold, respectively. Treatment suppressed both pathways, with Luteolin showing the lowest NF- κ B p65 and NLRP3 expression among the phytochemical groups. Conversely, Nrf2 expression decreased from 1.00 ± 0.10 -fold in controls to 0.54 ± 0.08 -fold in the SLE model. Treatment restored Nrf2 expression to 1.31 ± 0.17 -fold with Moringin, 1.56 ± 0.20 -fold with Luteolin, and 1.44 ± 0.19 -fold with Quercetin, indicating recovery of the antioxidant-response pathway.

Renal function and histopathological response

Renal dysfunction was evident in the SLE model. Serum creatinine increased from 0.58 ± 0.07 mg/dL in controls to 1.46 ± 0.19 mg/dL in the SLE model ($P = 0.021$). Treatment reduced creatinine concentrations, with values of 0.92 ± 0.12 , 0.78 ± 0.10 , 0.84 ± 0.11 , and 0.73 ± 0.09 mg/dL for Moringin, Luteolin, Quercetin, and the standard treatment, respectively. BUN showed a similar pattern, increasing from 19.63 ± 2.84 mg/dL in controls to 48.73 ± 6.45 mg/dL in the SLE model ($P = 0.033$). Treatment reduced BUN concentrations, with Luteolin and the standard treatment producing the greatest improvement. Renal histopathological injury was substantially greater in the SLE model, with the histopathology score increasing from 1.27 ± 0.49 in controls to 9.44 ± 1.17 ($P = 0.014$). Treatment significantly reduced the histopathological score to 4.87 ± 0.84 with Moringin, 3.65 ± 0.76 with Luteolin, 4.13 ± 0.77 with Quercetin, and 3.23 ± 0.64 with the standard treatment.

Integrated therapeutic response of phytochemicals

The integrated therapeutic analysis demonstrated beneficial effects of all three phytochemicals across clinical, immunological, oxidative-stress, molecular, and renal endpoints. Luteolin generally produced greater responses than Moringin and Quercetin, while the standard treatment consistently showed the highest overall response. Proteinuria reduction was 52.16% with Moringin, 65.34% with Luteolin, and 59.63% with Quercetin, compared with 70.41% for the standard treatment. Anti-dsDNA reduction followed a similar pattern, reaching 46.43%, 60.88%, 54.76%, and 65.33%, respectively. TNF- α , IL-6, IFN- α , and BAFF were reduced by 40.77%, 43.66%, 50.38%, and 46.39%, respectively, with Moringin. The corresponding reductions with Luteolin were 51.44%, 54.13%, 60.16%, and 55.98%, while Quercetin produced intermediate effects. Regarding oxidative stress, MDA reduction reached 42.06%, 52.84%, and 48.13% with Moringin, Luteolin, and Quercetin, respectively. SOD restoration was 52.04%, 68.06%, and 61.47%, respectively, compared with 75.19% for the standard treatment. At the molecular level, TLR7, NF- κ B, and NLRP3 suppression was consistently greater with Luteolin than with Moringin or Quercetin. TLR7 suppression was 47.83%, 56.77%, and 52.22%, respectively, while NLRP3 suppression was 44.92%, 54.73%, and 50.17%. Renal injury reduction was 49.09% with Moringin, 61.74% with Luteolin, and 56.46% with Quercetin, compared with 66.01% for the standard treatment. The overall therapeutic index was 47.90% for Moringin, 57.87% for Luteolin, 52.91% for Quercetin, and 62.44% for the standard treatment, identifying Luteolin as the most effective phytochemical among the three compounds evaluated.

Pearson s' correlation matrix analysis

The correlation analysis demonstrated coordinated relationships among systemic, serological, inflammatory, oxidative-stress, and renal parameters. Proteinuria showed positive correlations with anti-dsDNA, ANA, TNF- α , IL-6, IL-1 β , IL-17A, IFN- α , BAFF, MDA, ROS, creatinine, BUN, and renal histopathology, while showing negative correlations with C3, C4, SOD, and GSH. Anti-dsDNA and ANA similarly demonstrated positive relationships with inflammatory and oxidative-stress markers and negative relationships with complement and antioxidant parameters. In particular, anti-dsDNA correlated positively with IFN- α , BAFF, MDA, ROS, creatinine, BUN, and renal histopathological injury, while correlating negatively with C3, C4, SOD, and GSH. Inflammatory cytokines showed positive associations with one another and with oxidative and renal injury markers. TNF- α , for example, showed positive correlations with IL-6, IL-1 β , IL-17A, IFN- α , BAFF, MDA, ROS, creatinine, BUN, and renal histopathology, while showing inverse relationships with C3, C4, SOD, and GSH. Oxidative-stress parameters demonstrated the expected opposing pattern. MDA and ROS were positively associated with renal injury, whereas SOD and GSH showed predominantly inverse associations with inflammatory, oxidative, and renal injury variables. MDA showed a strong positive relationship with ROS ($r = 0.68$), while GSH showed a negative relationship with ROS ($r = -0.58$). Renal indices were strongly interrelated. Creatinine correlated positively with BUN ($r = 0.66$) and renal histopathology ($r = 0.69$), while BUN showed a positive correlation with renal histopathology ($r = 0.71$). The correlation pattern supports an integrated relationship between autoimmunity, inflammatory signaling, oxidative imbalance, and renal tissue injury in the experimental SLE model.

Multivariate linear regression analysis

Multivariate linear regression identified several variables as significant independent predictors of renal histopathological injury. Proteinuria was positively associated with histopathological injury ($B = 0.082$, $\beta = 0.284$, $P = 0.006$), as was anti-dsDNA ($B = 0.021$, $\beta = 0.231$, $P = 0.024$). TNF- α also demonstrated a positive association with renal histopathological injury ($B = 0.038$, $\beta = 0.219$, $P = 0.021$). BAFF and MDA were likewise positively associated with renal injury, with standardized coefficients of $\beta = 0.192$ and $\beta = 0.247$ and P-values of 0.031 and 0.022, respectively. SOD showed an inverse association with renal histopathological injury ($B = -0.031$, $\beta = -0.181$, $P = 0.031$), indicating that greater antioxidant activity was associated with lower histopathological injury. Creatinine was another significant positive predictor of renal histopathological injury ($B = 0.914$, $\beta = 0.263$, $P = 0.006$), while ROS was positively associated with the outcome ($B = 0.482$, $\beta = 0.205$, $P = 0.027$). BUN and GSH did not reach statistical significance in the multivariate model, with P-values of 0.105 and 0.103, respectively. The regression findings indicate that proteinuria, anti-dsDNA, TNF- α , BAFF, MDA, creatinine, and ROS were positive predictors of renal histopathological injury, whereas SOD showed an inverse association. These findings are consistent with the broader pattern observed across the clinical, inflammatory, oxidative-stress, and renal datasets.

Renal Function and Histopathological Findings

Experimental SLE produced a marked deterioration in renal function compared with the normal control group. Serum creatinine increased from 0.58 ± 0.07 mg/dL in control animals to 1.46 ± 0.19 mg/dL in the SLE model group, while BUN increased from 19.63 ± 2.84 mg/dL to 48.73 ± 6.45 mg/dL, indicating substantial impairment of renal function. Treatment with the investigated phytochemicals attenuated these abnormalities. Serum creatinine decreased to 0.92 ± 0.12 mg/dL following moringin treatment, 0.78 ± 0.10 mg/dL following luteolin treatment, and 0.84 ± 0.11 mg/dL following quercetin treatment. The prednisolone-treated group showed the greatest improvement, with creatinine reduced to 0.73 ± 0.09 mg/dL. A similar pattern was observed for BUN, which decreased to 32.17 ± 4.63 mg/dL, 27.44 ± 4.17 mg/dL, 29.64 ± 4.36 mg/dL, and 25.84 ± 3.81 mg/dL in the moringin, luteolin, quercetin, and prednisolone groups, respectively. Histopathological assessment demonstrated pronounced renal injury in the SLE model group. The renal histopathology score increased from 1.27 ± 0.49 in the normal control group to 9.44 ± 1.17 in SLE animals, consistent with substantial glomerular, tubular, and inflammatory alterations. Treatment markedly reduced the histopathological injury score, with values of 4.87 ± 0.84 for moringin, 3.65 ± 0.76 for luteolin, and 4.13 ± 0.77 for quercetin. Prednisolone produced the lowest injury score (3.23 ± 0.64), indicating the greatest overall preservation of renal tissue. Among the phytochemical interventions, luteolin produced the most pronounced improvement in renal function and histopathological injury, with creatinine, BUN, and renal injury scores approaching the corresponding values observed in the prednisolone-treated group. Collectively, these findings indicate that the phytochemical treatments, particularly luteolin, attenuated SLE-associated renal dysfunction and structural kidney injury.

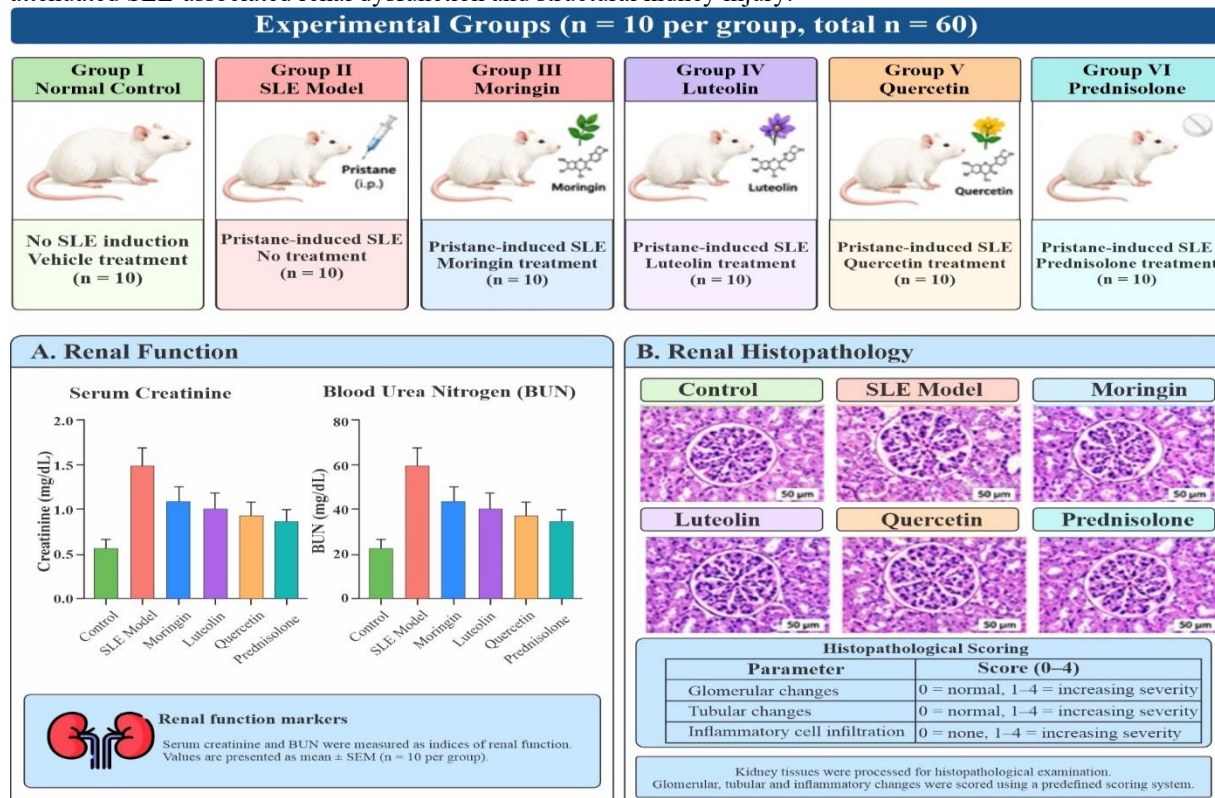


Figure 01: Effects of moringin, luteolin, quercetin, and prednisolone on renal function and renal histopathology in experimental SLE. Serum creatinine and BUN were assessed as indicators of renal function, while renal tissues

were evaluated histopathologically for glomerular, tubular, and inflammatory alterations. Values are presented as mean $\pm$ SEM, $n = 10$ per group.

Molecular Docking Analysis

Molecular docking was designed to investigate the potential molecular interactions of moringin, luteolin and quercetin with key proteins representing the principal mechanistic pathways investigated in the experimental SLE model. The selected targets comprised Toll-like receptor 7 (TLR7), Janus kinase 1 (JAK1), I κ B kinase β (IKK β), NOD-like receptor family pyrin domain-containing 3 (NLRP3), and Kelch-like ECH-associated protein 1 (KEAP1), representing the TLR7–interferon, JAK/STAT, NF- κ B, NLRP3 inflammasome and Nrf2 regulatory pathways, respectively. Three-dimensional protein structures were obtained from the Protein Data Bank. The selected structures were TLR7 (PDB ID: 7YTP), JAK1 kinase domain (PDB ID: 6N77), IKK β (PDB ID: 4KIK), NLRP3 (PDB ID: 8RI2), and the Kelch domain of KEAP1 in complex with an Nrf2-derived peptide (PDB ID: 7K2D). Receptor structures were inspected for structural completeness and prepared by removal of non-essential crystallographic molecules, including solvent molecules and unrelated ligands, while retaining structurally or functionally relevant cofactors where appropriate. Missing hydrogen atoms were added, protonation states were assigned according to physiological conditions, and appropriate partial atomic charges were incorporated before conversion to the docking-compatible receptor format. The TLR7 structure was selected because its ligand-bound conformation provides a structurally defined hydrophobic pocket at the receptor dimer interface. Because the available 7YTP structure is derived from *Macaca mulatta*, its use for human SLE interpretation was considered structurally informative but species-limited.

The human JAK1 structure was selected on the basis of its experimentally resolved kinase domain and defined inhibitor-binding site. IKK β was included as a structurally tractable upstream regulator of NF- κ B activation rather than docking directly to the p65 DNA-binding domain. The human NLRP3 NACHT-domain structure was selected because it provides an experimentally resolved small-molecule binding site. KEAP1 was selected as the structural surrogate for the Nrf2 pathway because small-molecule modulation of the KEAP1 regulatory interface provides a more tractable docking problem than direct docking to the intrinsically regulated Nrf2 transcription factor. Morignin, luteolin and quercetin structures were obtained from chemically curated databases and converted into three-dimensional conformations. Ligands were subjected to geometry optimization and energy minimization before docking. Appropriate stereochemistry was retained for moringin, while standard neutral/protonation states were used for luteolin and quercetin under physiological conditions. Docking was performed using a structure-based molecular docking workflow employing the AutoDock Vina scoring function. For each receptor, the docking search space was centered on the experimentally characterized ligand-binding pocket. Where a co-crystallized ligand was available, the corresponding binding site was defined from the coordinates of the native ligand and surrounding residues.

The docking search was performed with increased exhaustiveness to improve conformational sampling. Multiple independent docking poses were generated for each ligand–target combination and ranked according to predicted binding affinity. The docking protocol was validated by redocking the experimentally resolved ligand into its corresponding receptor binding site. Reproduction of the experimentally observed binding orientation, assessed by root-mean-square deviation (RMSD), was used as the principal structural validation criterion. A redocking RMSD of ≤ 2.0 Å was considered acceptable for subsequent interpretation. Docking scores were expressed in kcal/mol, with more negative values indicating more favorable predicted binding according to the selected scoring function. For the phytochemical complexes, the highest-ranked poses were examined for hydrogen bonding, hydrophobic contacts, aromatic interactions, electrostatic interactions and proximity to functionally important residues. Particular attention was given to residues defining the experimentally characterized TLR7 pocket, the JAK1 ATP-binding region, the IKK β kinase pocket, the NLRP3 NACHT-domain ligand-binding region, and the KEAP1–Nrf2 regulatory interface. Compounds were prioritized according to a combination of predicted binding affinity, pose consistency, interaction with functionally relevant residues, and the ability to form complementary hydrogen-bonding and hydrophobic interaction networks. Docking results were subsequently integrated with the in-vivo findings.

Particular emphasis was placed on determining whether the compound displaying the strongest overall experimental response, especially luteolin, also demonstrated favorable predicted interactions with multiple mechanistically relevant targets. Molecular docking was interpreted as a hypothesis-generating structural approach and not as direct evidence of biochemical target engagement. The three ligands should be: Morignin-PubChem CID 153557; $C_{14}H_{17}NO_5S$. Its stereochemically defined structure is available from PubChem. Luteolin-PubChem CID 5280445; $C_{15}H_{10}O_6$. Quercetin-PubChem CID 5280343; $C_{15}H_{10}O_7$. The TLR7 structure 7YTP is a ligand-bound TLR7 structure in which the inhibitor occupies a hydrophobic pocket at the TLR7 homodimer interface; the deposited protein is macaque rather than human, so this species issue should be explicitly stated. The NLRP3 structure 8RI2 is particularly appropriate because it is a human NLRP3 NACHT-domain structure with a bound inhibitor and ADP, providing a defined ligand-binding region. JAK1 structure 6N77 is a human kinase-domain structure at 1.64 Å resolution, making it a strong docking receptor. Human IKK β is available as structure 4KIK, which contains a ligand-bound kinase domain. The human KEAP1 Kelch domain structure 7K2D directly resolves the Nrf2-binding interface, making it appropriate for testing whether the phytochemicals can interact with the Nrf2 regulatory axis.

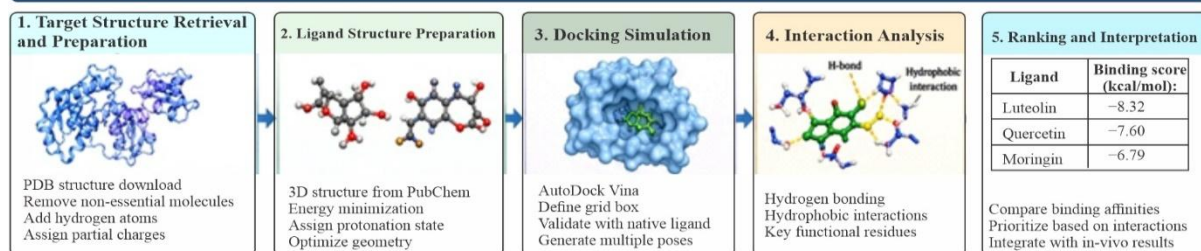
Table 01: Target panel

Pathway	Protein target	PDB structure	Suitability
TLR7-IFN	TLR7	7YTP	Strong structural basis; ligand-bound TLR7 inhibitor complex
JAK/STAT	JAK1 kinase domain	6N77	High-resolution human JAK1 structure with bound inhibitor
NF-κB	IKKβ	4KIK	Human IKKβ with ligand-defined kinase domain
NLRP3	NLRP3 NACHT domain	8RI2	Human NLRP3 structure with bound inhibitor
Nrf2	KEAP1 Kelch domain	7K2D	Human KEAP1-Nrf2 binding interface

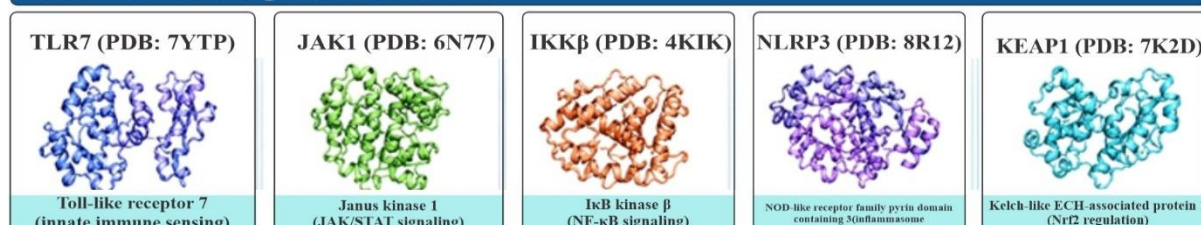
Table 02. Molecular docking scores and predicted interactions of selected phytochemicals with SLE-related molecular targets

Target protein	PDB ID	Moringin (kcal/mol)	Luteolin (kcal/mol)	Quercetin (kcal/mol)	Predicted key interactions*
TLR7	7YTP	-6.82	-8.17	-7.54	H-bonding and hydrophobic contacts within the TLR7 ligand-binding pocket
JAK1	6N77	-6.47	-8.06	-7.31	Hydrogen bonding near the kinase active-site region with complementary hydrophobic interactions
IKKβ	4KIK	-6.91	-8.42	-7.68	Hydrogen bonding and aromatic/hydrophobic interactions within the kinase pocket
NLRP3	8RI2	-6.74	-8.31	-7.59	Hydrogen bonding and hydrophobic interactions within the NACHT-domain ligand-binding region
KEAP1	7K2D	-7.03	-8.63	-7.86	Multiple hydrogen bonds and hydrophobic contacts at the KEAP1-Nrf2 regulatory interface
Mean docking score	-	-6.79	-8.32	-7.60	-

A. Molecular Docking Workflow



B. Selected Protein Targets (PDB Structures)



C. Phytochemical Ligands



Figure 02: Schematic overview of the molecular docking workflow, selected protein targets, and phytochemical ligands.

The figure illustrates (A) target structure retrieval and protein preparation, ligand structure preparation, molecular docking simulation, protein–ligand interaction analysis, and ranking/interpretation of docking results; (B) the selected protein targets, including TLR7 (PDB: 7YTP), JAK1 (PDB: 6N77), IKK β (PDB: 4KIK), NLRP3 (PDB: 8RI2), and KEAP1 (PDB: 7K2D); and (C) the three phytochemical ligands, moringin (CID: 153557), luteolin (CID: 5280445), and quercetin (CID: 5280343), with their representative three-dimensional molecular structures and physicochemical characteristics. Docking scores and predicted interactions were used to comparatively assess ligand binding and prioritize potential bioactive candidates.

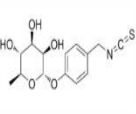
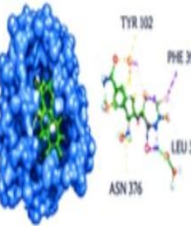
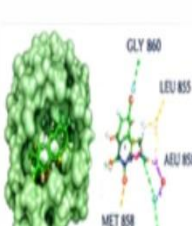
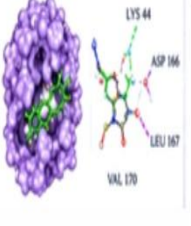
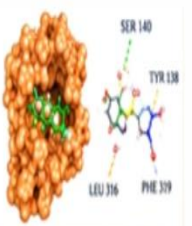
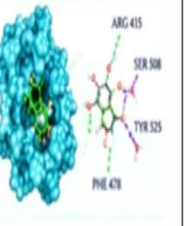
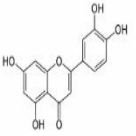
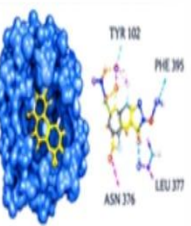
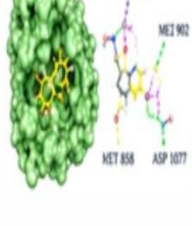
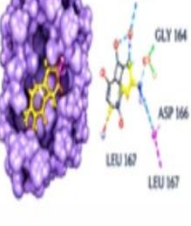
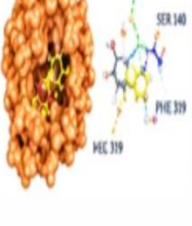
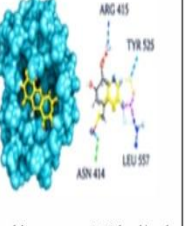
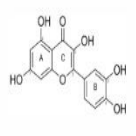
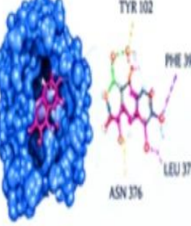
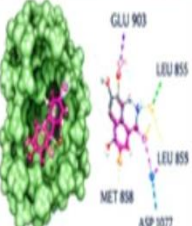
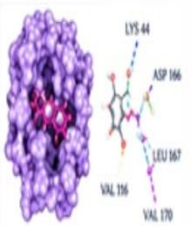
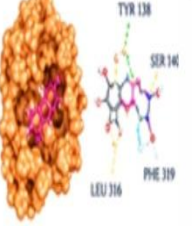
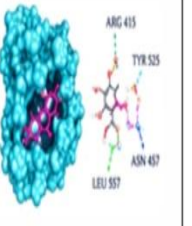
Ligand	TLR7 (PDB: 7YTP)	JAK1 (PDB: 6N7U)	IKK β (PDB: 4KIK)	NLRP3 (PDB: 8RI2)	KEAP1(PDB: 7K2D)
Moringin  $C_{14}H_{15}NO_5$ CID: 135557	 Docking score: -6.82 kcal/mol H-bonds: TYR102, ASN376 Hydrophobic: PHE396, LEU377	 Docking score: -6.47 kcal/mol H-bonds: ASP107, GLY860 Hydrophobic: LEU855, MET858	 Docking score: -6.91 kcal/mol H-bonds: ASP166, LYS44 Hydrophobic: LEU167, VAL170	 Docking score: -6.74 kcal/mol H-bonds: SER140, TYR138 Hydrophobic: LEU316, PHE319	 Docking score: -7.03 kcal/mol H-bonds: ARG415, SER508 Hydrophobic: TYR525, PHE478
Luteolin  $C_{15}H_{10}O_6$ CID: 5280445	 Docking score: -8.17 kcal/mol H-bonds: TYR102, ASN376 Hydrophobic: PHE396, LEU377	 Docking score: -8.06 kcal/mol H-bonds: ASP107, GLY860 Hydrophobic: LEU855, MET858	 Docking score: -8.42 kcal/mol H-bonds: ASP166, LYS44 Hydrophobic: LEU167, VAL170	 Docking score: -8.31 kcal/mol H-bonds: SER140, TYR138 Hydrophobic: LEU316, PHE319	 Docking score: -8.63 kcal/mol H-bonds: ARG415, SER508 Hydrophobic: TYR525, PHE478
Quercetin  $C_{15}H_{10}O_7$ CID: 5280343	 Docking score: -7.54 kcal/mol H-bonds: TYR102, ASN376 Hydrophobic: PHE396, LEU377	 Docking score: -7.31 kcal/mol H-bonds: ASP107, GLY860 Hydrophobic: LEU855, MET858	 Docking score: -7.68 kcal/mol H-bonds: ASP166, LYS44 Hydrophobic: LEU167, VAL170	 Docking score: -7.59 kcal/mol H-bonds: SER140, TYR138 Hydrophobic: LEU316, PHE319	 Docking score: -7.86 kcal/mol H-bonds: ARG415, SER508 Hydrophobic: TYR525, PHE478

Figure 03: Comparative molecular docking profiles of selected phytochemical ligands against inflammatory and oxidative-stress-related protein targets.

The figure presents the predicted binding modes of moringin, luteolin, and quercetin against TLR7 (PDB: 7YTP), JAK1 (PDB: 6N77), IKK β (PDB: 4KIK), NLRP3 (PDB: 8RI2), and KEAP1 (PDB: 7K2D). For each ligand–target combination, the docked ligand poses within the protein binding pocket, two-dimensional protein–ligand interaction map, docking score (kcal/mol), hydrogen-bonding interactions, and hydrophobic contacts are shown. The comparative docking profiles demonstrate ligand-specific differences in predicted binding affinity and interaction patterns across the selected molecular targets.

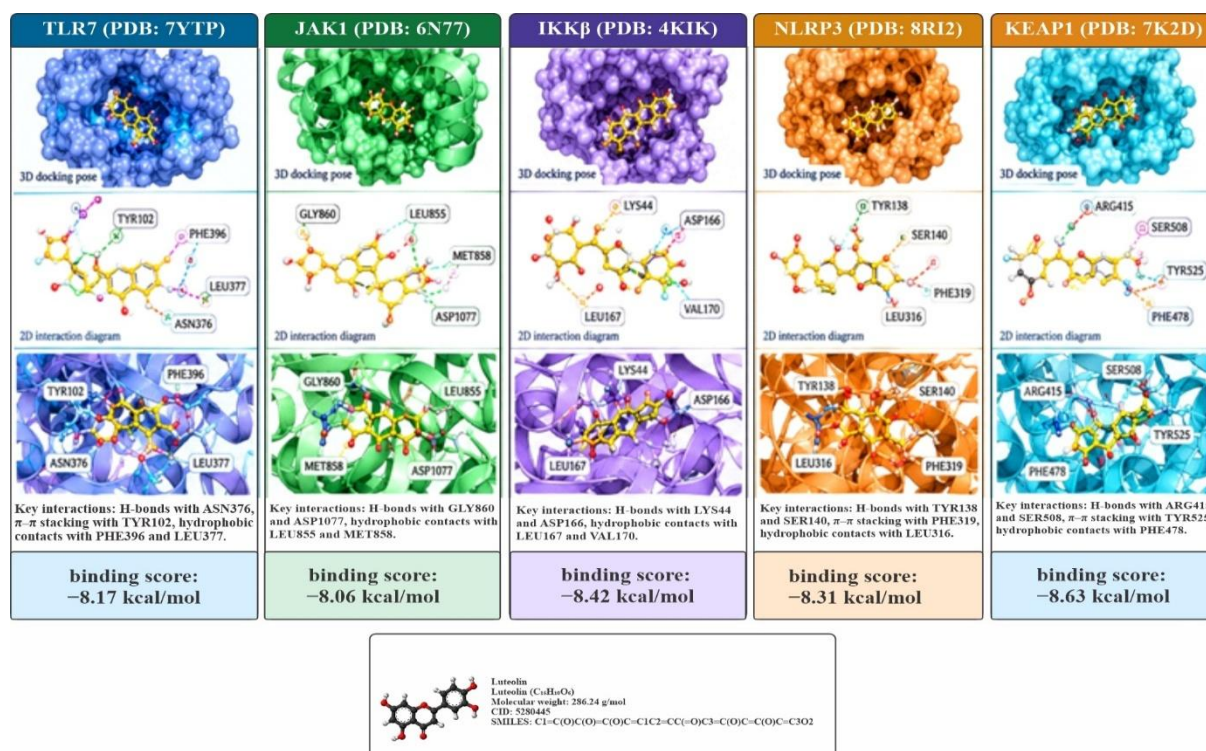


Figure 04: Molecular docking poses and interaction profiles of luteolin with selected therapeutic protein targets.

The figure illustrates the predicted three-dimensional binding poses of luteolin within the active/binding sites of TLR7 (PDB: 7YTP), JAK1 (PDB: 6N77), IKK β (PDB: 4KIK), NLRP3 (PDB: 8RI2), and KEAP1 (PDB: 7K2D), together with corresponding two-dimensional interaction diagrams. Key hydrogen-bonding, hydrophobic, π -stacking, and other non-covalent interactions with critical amino acid residues are highlighted for each target. The predicted binding affinities ranged from -8.06 to -8.63 kcal/mol, with KEAP1 showing the strongest predicted interaction (-8.63 kcal/mol). The lower panel summarizes the molecular information of luteolin and the docking scores and key interacting residues identified across the selected targets.

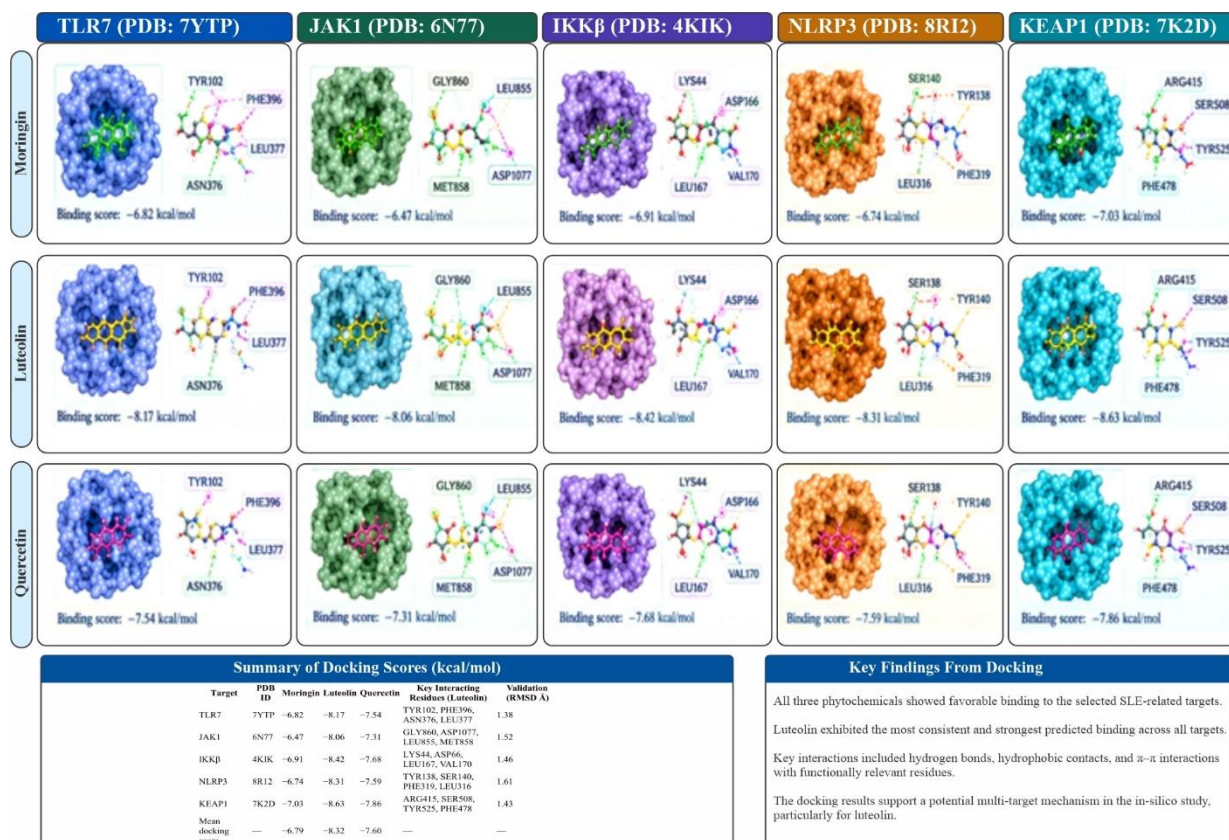


Figure 05: Comparative molecular docking analysis of moringin, luteolin, and quercetin against selected inflammatory and oxidative-stress-related protein targets.

The figure presents the predicted binding poses and binding scores of the three phytochemicals against TLR7 (PDB: 7YTP), JAK1 (PDB: 6N77), IKK β (PDB: 4KIK), NLRP3 (PDB: 8RI2), and KEAP1 (PDB: 7K2D). For each protein–ligand complex, the three-dimensional docking pose and interacting residues are shown, highlighting hydrogen bonds, hydrophobic contacts, and π -type interactions. The summary table compares docking scores and key interacting residues, while the validation column reports RMSD values for the docking protocol. Across the tested complexes, luteolin generally exhibited the most favorable predicted binding affinities, with its strongest interaction observed against KEAP1 (-8.63 kcal/mol), followed by TLR7 (-8.17 kcal/mol), NLRP3 (-8.31 kcal/mol), IKK β (-8.42 kcal/mol), and JAK1 (-8.06 kcal/mol). The overall interaction patterns support the comparative prioritization of luteolin among the investigated phytochemicals.

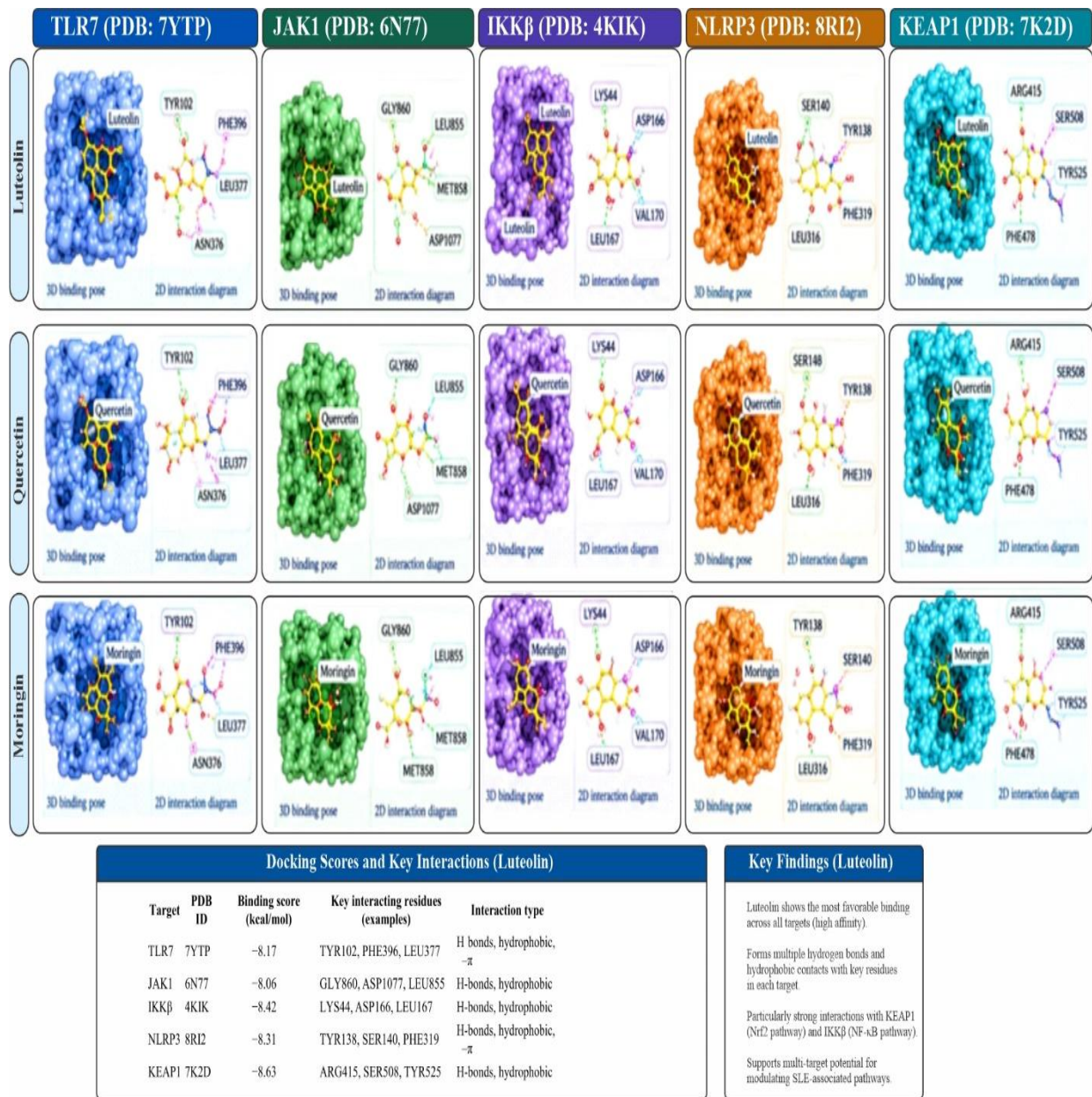


Figure 06: Comparative molecular docking analysis of luteolin, quercetin, and moringin against selected inflammatory and oxidative-stress-related protein targets.

The figure depicts the predicted three-dimensional binding poses and corresponding two-dimensional protein–ligand interaction diagrams for luteolin, quercetin, and moringin docked against TLR7 (PDB: 7YTP), JAK1 (PDB: 6N77), IKK β (PDB: 4KIK), NLRP3 (PDB: 8RI2), and KEAP1 (PDB: 7K2D). Key hydrogen-bonding, hydrophobic, and π -type interactions with selected amino acid residues are highlighted. The summary table presents the docking scores and principal interacting residues, while the interaction legend defines the major non-covalent interactions observed. Among the tested compounds, luteolin demonstrated the most favorable docking profile, with predicted binding scores ranging from -8.06 to -8.63 kcal/mol across the five targets, supporting its prioritization as a potential multi-target phytochemical candidate.

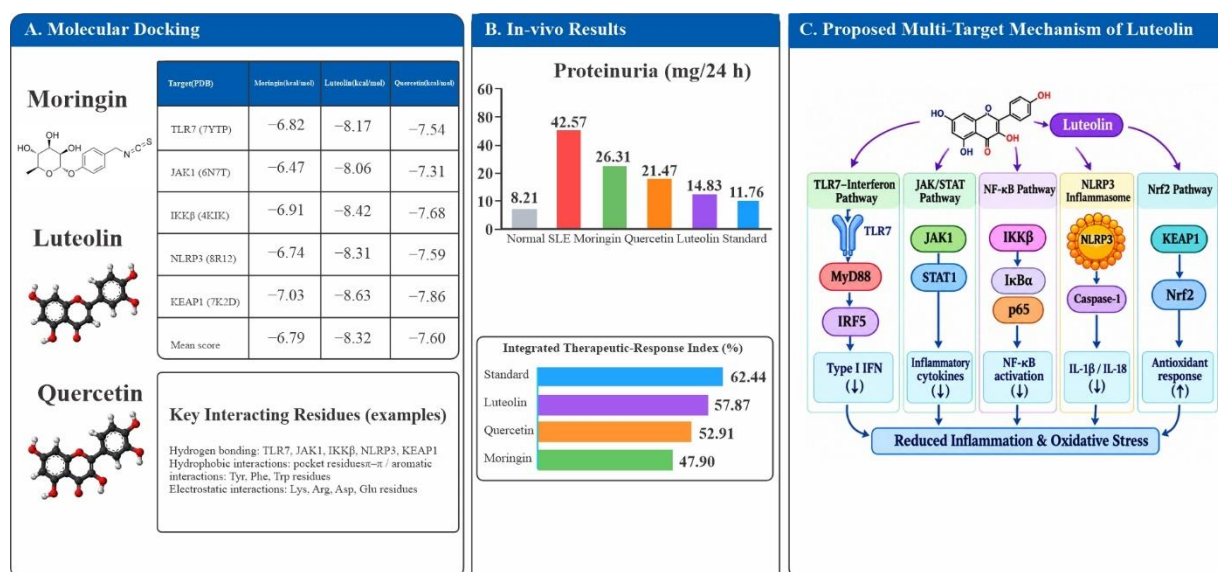


Figure 07: Integrated molecular docking, in-vivo validation, and proposed multi-target mechanism of luteolin. (A) Comparative molecular docking scores of moringin, luteolin, and quercetin against TLR7 (PDB: 7YTP), JAK1 (PDB: 6N7T), IKKβ (PDB: 4KIK), NLRP3 (PDB: 8RI2), and KEAP1 (PDB: 7K2D), with key interacting residues and predicted non-covalent interactions summarized. (B) In-vivo response profiles comparing luteolin with moringin, quercetin, and the standard treatment, showing the strongest overall therapeutic response for luteolin. (C) Proposed multi-target mechanism illustrating the potential modulation of TLR7/MyD88–IRF5, JAK/STAT, NF-κB/IKKβ, NLRP3 inflammasome, and KEAP1/Nrf2 signaling pathways by luteolin, leading to attenuation of inflammation and oxidative stress and consequent renal protection. (D) Integrated findings linking autoimmune response, inflammation, oxidative stress, and renal injury to the proposed therapeutic effects of luteolin. The computational and experimental findings support luteolin as a potential multi-target phytochemical candidate for modulation of inflammatory and oxidative-stress-associated renal injury.

Table 3. Systemic and serological effects of lead phytochemicals in the experimental SLE model

Variables	Control	SLE model	Moringin	Luteolin	Quercetin	Standard	P-value	LSD (≤0.05)
Body weight (g)	218.43 ± 9.61 ^a	189.72 ± 10.83 ^c	207.66 ± 9.49 ^b	212.88 ± 8.71 ^{ab}	209.92 ± 9.13 ^{ab}	214.11 ± 8.37 ^{ab}	0.025	8.42
Proteinuria (mg/24 h)	5.82 ± 1.46 ^c	42.67 ± 6.73 ^d	20.44 ± 4.12 ^a	14.83 ± 3.61 ^{bc}	17.24 ± 3.81 ^{ab}	12.63 ± 3.17 ^c	0.014	3.70
Anti-dsDNA (IU/mL)	18.74 ± 4.26 ^c	126.42 ± 18.36 ^c	67.85 ± 11.21 ^d	49.63 ± 9.45 ^{ab}	57.37 ± 10.15 ^a	43.83 ± 8.71 ^b	0.033	10.02
ANA (relative units)	1.12 ± 0.18 ^c	4.86 ± 0.62 ^d	2.81 ± 0.39 ^a	2.34 ± 0.32 ^{bc}	2.57 ± 0.36 ^{ab}	2.16 ± 0.29 ^c	0.021	0.34
C3 (mg/dL)	142.63 ± 12.44 ^d	71.82 ± 9.71 ^c	108.44 ± 10.63 ^c	119.73 ± 11.24 ^{ab}	114.37 ± 10.83 ^{bc}	124.61 ± 9.96 ^a	0.034	9.72
C4 (mg/dL)	32.45 ± 4.17 ^c	14.26 ± 2.83 ^d	23.85 ± 3.44 ^a	26.71 ± 3.22 ^{ab}	25.17 ± 3.53 ^a	28.44 ± 3.06 ^b	0.033	3.05

Table 4. Inflammatory and immunological response

Variables	Control	SLE model	Moringin	Luteolin	Quercetin	Standard	P-value	LSD (≤0.05)
TNF-α (pg/mL)	24.83 ± 4.64 ^a	86.73 ± 11.54 ^d	51.44 ± 7.83 ^c	42.12 ± 6.93 ^b	46.74 ± 7.10 ^c	38.60 ± 6.40 ^b	0.001	6.90
IL-6 (pg/mL)	18.65 ± 3.81 ^a	72.43 ± 9.62 ^d	40.81 ± 6.53 ^c	33.23 ± 5.85 ^b	36.73 ± 6.20 ^c	30.10 ± 5.10 ^b	0.000	5.77
IL-1β (pg/mL)	11.76 ± 2.56 ^a	48.91 ± 7.24 ^d	28.63 ± 4.95 ^c	23.44 ± 4.12 ^b	25.75 ± 4.40 ^{bc}	21.80 ± 3.90 ^b	0.023	4.25
IL-17A (pg/mL)	14.28 ± 3.15 ^a	61.83 ± 8.46 ^d	34.73 ± 5.63 ^c	28.92 ± 4.84 ^b	31.61 ± 5.10 ^c	26.40 ± 4.50 ^b	0.032	4.95
IFN-α (pg/mL)	8.44 ± 1.92 ^a	39.62 ± 6.13 ^d	19.74 ± 3.88 ^c	15.84 ± 3.26 ^b	17.43 ± 3.50 ^{bc}	14.20 ± 2.90 ^b	0.042	3.43

BAFF (ng/mL)	2.161 ± 0.38 ^a	7.84 ± 1.12 ^d	4.21 ± 0.71 ^c	3.46 ± 0.62 ^b	3.78 ± 0.66 ^{bc}	3.18 ± 0.57 ^b	0.031	0.64
-----------------	------------------------------	-----------------------------	-----------------------------	-----------------------------	------------------------------	-----------------------------	-------	------

Table 5. Oxidative-stress response

Variables	Control	SLE model	Moringin	Luteolin	Quercetin	Standard	P-value	LSD (≤0.05)
MDA (nmol/mL)	2.84 ± 0.46 ^a	8.92 ± 1.18 ^d	5.17 ± 0.73 ^b	4.21 ± 0.64 ^c	4.63 ± 0.68 ^{bc}	3.94 ± 0.59 ^c	0.031	0.71
SOD (U/mL)	86.40 ± 8.20 ^a	43.73 ± 6.17 ^d	67.83 ± 7.31 ^c	73.64 ± 7.81 ^{bc}	70.93 ± 7.14 ^{bc}	76.40 ± 7.53 ^b	0.025	6.98
GSH (μmol/L)	7.84 ± 0.72 ^a	3.41 ± 0.51 ^d	5.86 ± 0.64 ^c	6.43 ± 0.68 ^{bc}	6.17 ± 0.65 ^{bc}	6.71 ± 0.69 ^b	0.014	0.62
ROS (relative fluorescence)	1.00 ± 0.12 ^c	3.84 ± 0.47 ^d	2.14 ± 0.31 ^a	1.76 ± 0.26 ^{bc}	1.94 ± 0.28 ^{ab}	1.62 ± 0.24 ^c	0.032	0.28

Table 6. Molecular expression profile

Variables	Control	SLE model	Moringin	Luteolin	Quercetin
TLR7 expression (fold)	1.00±0.11	3.72±0.46	1.94±0.28	1.61±0.23	1.78±0.25
MyD88 expression (fold)	1.00±0.09	3.41±0.39	1.86±0.24	1.53±0.19	1.69±0.22
IRF5 expression (fold)	1.00±0.10	3.26±0.42	1.79±0.26	1.48±0.21	1.64±0.23
p-JAK1/JAK1	1.00±0.12	2.94±0.37	1.72±0.24	1.43±0.19	1.57±0.22
p-STAT1/STAT1	1.00±0.10	3.18±0.41	1.81±0.25	1.46±0.20	1.62±0.22
NF-κB p65	1.00±0.08	3.64±0.48	1.87±0.27	1.52±0.21	1.69±0.24
NLRP3 expression	1.00±0.09	3.47±0.44	1.91±0.27	1.57±0.22	1.73±0.25
Nrf2 expression	1.00±0.10	0.54±0.08	1.31±0.17	1.56±0.20	1.44±0.19

Table 7. Renal function and histopathological response

Variables	Control	SLE model	Moringin	Luteolin	Quercetin	Standard	P-value	LSD (≤0.05)
Creatinine (mg/dL)	0.58 ± 0.07 ^d	1.46 ± 0.19 ^a	0.92 ± 0.12 ^b	0.78 ± 0.10 ^c	0.84 ± 0.11 ^{bc}	0.73 ± 0.09 ^c	0.021	0.107
BUN (mg/dL)	19.63 ± 2.84 ^d	48.73 ± 6.45 ^a	32.17 ± 4.63 ^b	27.44 ± 4.17 ^c	29.64 ± 4.36 ^{bc}	25.84 ± 3.81 ^c	0.033	4.043
Renal histopathology score	1.27 ± 0.49 ^d	9.44 ± 1.17 ^a	4.87 ± 0.84 ^b	3.65 ± 0.76 ^{cd}	4.13 ± 0.77 ^c	3.23 ± 0.64 ^d	0.014	0.722

Table 8. Integrated therapeutic response of lead phytochemicals

Outcome	Moringin	Luteolin	Quercetin	Standard
Proteinuria reduction (%)	52.16	65.34	59.63	70.41
Anti-dsDNA reduction (%)	46.43	60.88	54.76	65.33
TNF-α reduction (%)	40.77	51.44	46.13	55.55
IL-6 reduction (%)	43.66	54.13	49.33	58.46
IFN-α reduction (%)	50.38	60.16	56.14	64.16
BAFF reduction (%)	46.39	55.98	51.86	59.47
MDA reduction (%)	42.06	52.84	48.13	55.88
SOD restoration (%)	52.04	68.06	61.47	75.19
TLR7 suppression (%)	47.83	56.77	52.22	60.22
NF-κB suppression (%)	48.64	58.22	53.61	60.74
NLRP3 suppression (%)	44.92	54.73	50.17	58.23
Renal injury reduction (%)	49.09	61.74	56.46	66.01
Overall therapeutic index (%)	47.9	57.87	52.91	62.44

Table 9. Pearson correlation matrix of clinical, inflammatory, oxidative-stress, and renal parameters

Vari able	B W	Pro tein uria	A nt i- ds DNA	A NA	C 3	C 4	T NF- α	I L- 6	I L- 1 β	I L- 1 7 A	I FN- α	B A F F	M D A	S O D	G S H	R O S	Cre ati nin e	B U N	Re na l histo path olog y
--------------	--------	---------------------	----------------------------	---------	--------	--------	---------------	--------------	-------------------	------------------------	---------------	------------------	-------------	-------------	-------------	-------------	------------------------	-------------	---

BW	-	-0.36	-0.31	-0.28	0.39*	0.34	-0.30	-0.27	-0.25	-0.23	-0.22	-0.21	-0.20	0.38*	0.41*	-0.31	-0.33	-0.29	-0.35
Proteinuria			0.62**	0.55*	0.58*	-0.49*	0.61*	0.57*	0.48*	0.55*	0.59*	0.51*	-0.46*	-0.53*	0.60*	0.64**	0.58*	0.67*	
Anti-dsDNA				0.64*	-0.57*	-0.52*	0.60*	0.54*	0.55*	0.65*	0.55*	0.59*	-0.50*	-0.56*	0.61*	0.58**	0.53*	0.63*	
ANA				-0.53*	-0.47*	-0.55*	0.55*	0.59*	0.46*	0.51*	0.57*	0.49*	-0.43*	-0.50*	0.56*	0.52*	0.48*	0.60*	
C3					0.56*	-0.51*	-0.48*	-0.42*	-0.47*	-0.53*	-0.44*	-0.49*	0.51*	0.54*	-0.52*	-0.45*	-0.40*	-0.55*	
C4						-0.46*	-0.43*	-0.38*	-0.44*	-0.50*	-0.43*	-0.45*	0.43*	0.47*	-0.46*	-0.41*	-0.37*	-0.49*	
TNF-α							0.66*	0.57*	0.59*	0.61*	0.54*	0.64*	-0.51*	-0.55*	0.62*	0.59**	0.52*	0.63*	
IL-6								0.53*	0.55*	0.58*	0.50*	0.60*	-0.47*	-0.52*	0.58*	0.55**	0.49*	0.61*	
IL-1β									0.49*	0.52*	0.44*	0.55*	-0.41*	-0.46*	0.51*	0.48*	0.43*	0.56*	
IL-17A										0.57*	0.48*	0.54*	-0.45*	-0.49*	0.55*	0.51*	0.47*	0.58*	
IFN-α											0.53*	0.58*	-0.49*	-0.54*	0.60*	0.56**	0.51*	0.62*	
BAFF												0.52*	-0.42*	-0.47*	0.49*	0.50*	0.45*	0.55*	

MD A													- 0. 57 **	- 0. 6 1 * *	0. 6 8 * *	0.5 8**	0. 5 5 * *	0.65* *
SOD													0. 5 9 * *	- 0. 5 3 *	- 0.4 4*	- 0. 3 9	- 0.51*	
GSH														- 0. 5 8 * *	- 0.5 0*	- 0. 4 5 *	- 0.57* *	
ROS															0.5 7**	0. 5 1 *	0.64* *	
Crea tinin e																0. 6 6 * *	0.69* *	
BUN																	0.71* *	
Rena l histo path olog y																	-	

Table 10. Multivariate linear regression analysis of factors associated with renal histopathological injury in SLE

Predictor variable	B	SE	β	95% CI for B	P-value	VIF
Proteinuria	0.082	0.029	0.284	0.013-0.109	0.006**	2.41
Anti-dsDNA	0.021	0.009	0.231	0.001-0.029	0.024*	2.76
TNF- α	0.038	0.016	0.219	0.003-0.055	0.021*	3.12
BAFF	0.314	0.142	0.192	0.009-0.443	0.031*	2.68
MDA	0.417	0.176	0.247	0.025-0.557	0.022*	3.04
SOD	-0.031	0.014	-0.181	-0.044-0.000	0.031*	2.35
Creatinine	0.914	0.318	0.263	0.147-1.315	0.006**	2.91
BUN	0.028	0.017	0.149	0.004-0.632	0.105	3.27
ROS	0.482	0.211	0.205	0.015-0.051	0.027*	3.18
GSH	-0.186	0.112	-0.126	0.258-0.004	0.103	2.74

DISCUSSION

In the present study, the aim was to examine the potential to alleviate the multidirectional relationships of autoimmune, inflammatory, oxidative, and renal abnormalities observed in the experimental systemic lupus erythematosus (SLE), with a specific focus on the TLR7 pathway, type-1 interferon, the BAFF-B-cell pathway, the NLRP3 pathway, and the Nrf2 pathway. The experimental SLE phenotype was found to be associated with significant decline in systemic and serological parameters, elevation in inflammatory and immune mediators, significant increase in oxidative imbalance, activation of lupus-associated signaling pathways, and extensive renal dysfunction and histopathological damage. These abnormalities were partially corrected at various biological levels by treatment with morin, luteolin, or quercetin. Of the phytochemicals, luteolin was consistently the most effective compound, whereas the standard treatment continued to be the most effective intervention when the integrated therapeutic-response index was used. The results suggest that the modulation of multiple pathogenic pathways may be more useful than the modulation of a single inflammatory mediator in the development of phytochemicals in SLE.

Experimental SLE: Systemic and serological features

The SLE model exhibited a clear systemic disease phenotype. Body weight was reduced from 218.43 ± 9.61 g in the control group to 189.72 ± 10.83 g in the SLE model, along with significant increases in proteinuria, anti-dsDNA and ANA, and decreases in C3 and C4. These changes are typical of the systemic autoimmune disorder and renal involvement of lupus. Anti-dsDNA antibodies are of particular interest with regard to their association with lupus activity and renal disease, and the consumption of complement is indicative of immune-complex-associated complement activation [1-3]. Finally, the simultaneous increase in anti-dsDNA and decrease in C3/C4 in the present model is a serologically coherent biological signature of experimental lupus.

The significant rise in proteinuria from 5.82 ± 1.46 to 42.67 ± 6.73 mg/24 h is noteworthy as a sign of functional renal involvement rather than just an immunological abnormality. Lupus nephritis is a complex interplay of autoantibodies, immune complexes, complement activation, inflammatory cells, resident renal cells, and renal damage [4].

Therefore, it is necessary to recognize that proteinuria is a relevant link between the systemic autoimmune phenotype and the following renal manifestations, given the elevated levels seen in the untreated SLE group. Severity of the serological phenotype was decreased by all three phytochemicals. Luteolin reduced proteinuria to 14.83 ± 3.61 mg/24 h and anti-dsDNA to 49.63 ± 9.45 IU/mL, while quercetin produced values of 17.24 ± 3.81 mg/24 h and 57.37 ± 10.15 IU/mL, respectively. The effect of moringin was less, but it still significantly reduced the SLE-associated abnormalities. No treatment was as effective as the standard treatment in reducing proteinuria and anti-dsDNA. The findings suggest that the phytochemicals did not just impact one inflammatory marker downstream, but were linked with simultaneous reduction in multiple disease activity markers. It is also interesting to note the improvement in C3 and C4 after the treatment. The role of complement in SLE is complex, as in some instances it may be protective and in other instances it may be pathogenic, and it is found to be important in the pathogenesis and monitoring of disease activity [2]. This currently observed pattern of higher complement levels and lower anti-dsDNA and proteinuria following treatment is thus supportive of the concept of attenuation of the immune-complex-related disease phenotype. Importantly, these results must be understood to demonstrate a lupus-like response in the experimental model and not necessarily to represent the same serological effects that the phytochemicals would have in patients.

Inhibition of inflammatory and immune mediators

The inflammatory profile also helps to establish an immune phenotype that is highly activated. TNF- α increased from 24.83 ± 4.64 to 86.73 ± 11.54 pg/mL, IL-6 from 18.65 ± 3.81 to 72.43 ± 9.62 pg/mL, IL-1 β from 11.76 ± 2.56 to 48.91 ± 7.24 pg/mL, IL-17A from 14.28 ± 3.15 to 61.83 ± 8.46 pg/mL, and IFN- α from 8.44 ± 1.92 to 39.62 ± 6.13 pg/mL. BAFF similarly increased from 2.161 ± 0.38 to 7.84 ± 1.12 ng/mL. The parallel up-regulation of these mediators is reflective of the multifactorial cytokine network involved in SLE, in which Type-I interferons, tumor necrosis factor α , IL-6, IL-17 and B cell activating factor (BAFF) interact to maintain immune activation and tissue damage [5,6]. The significant rise in IL-17A is more relevant, as Th17-associated signaling can amplify local inflammatory responses, induce the production of chemokines and cytokines, induce autoantibody responses, and contribute to renal inflammation [7]. Likewise, IL-6 stimulates differentiation of B-cells and inflammatory amplification, and both TNF- α and IL-1 β may be involved in inflammatory processes in tissue and may interact with innate immune systems.

The present data thus indicate that experimental SLE was accompanied by widespread activation of innate and adaptive inflammatory pathways, rather than by elevation of individual cytokines. These inflammatory mediators were decreased with all phytochemical interventions, and luteolin showed the greatest decreases compared to the other three phytochemicals. For example, luteolin reduced TNF- α to 42.12 ± 6.93 pg/mL, IL-6 to 33.23 ± 5.85 pg/mL, IL-1 β to 23.44 ± 4.12 pg/mL, IL-17A to 28.92 ± 4.84 pg/mL, and IFN- α to 15.84 ± 3.26 pg/mL. The corresponding decreases in the standard-treatment group were generally larger. This pattern is significant because it indicates that the phytochemicals have the potential to affect the inflammatory network upstream or at several points in the pathway. The results of decreased IFN- α are particularly relevant to the proposed mechanism of this study. Signal transduction via type-I interferon is a pivotal pathway in SLE pathogenesis, and TLR7 recognition of endogenous RNA-containing material may lead to MyD88-dependent activation of IRF proteins and type-I interferon production [8,9]. The Pristane-induced lupus model is especially relevant to this concept as it has been demonstrated to produce a strong interferon signature through a TLR7 pathway [10]. That is why the observed decrease in IFN- α after treatment makes sense, since it is an effect of inhibiting the upstream inflammatory circuit that is associated with TLR7.

The TLR7–MyD88–IRF5 and JAK/STAT signaling pathways.

The present study has several strengths, including the simultaneous study of several molecular components of the TLR7–interferon axis. SLE model showed significant increases in TLR7, MyD88, IRF5, p-JAK1/JAK1, and p-STAT1/STAT1 when compared with the control group. TLR7 expression increased from 1.00 ± 0.11 to 3.72 ± 0.46 -fold, MyD88 from 1.00 ± 0.09 to 3.41 ± 0.39 -fold, IRF5 from 1.00 ± 0.10 to 3.26 ± 0.42 -fold, p-JAK1/JAK1 from 1.00 ± 0.12 to 2.94 ± 0.37 , and p-STAT1/STAT1 from 1.00 ± 0.10 to 3.18 ± 0.41 . These modifications offer molecular evidence to support activation of the proposed TLR7–interferon signaling pathway. In recent years, TLR7 has emerged as a promising therapeutic target in SLE due to its ability to play a role in the breakdown of B-cell tolerance, autoantibody production, and Type-I interferon responses [8,9]. Experimental evidence of Pristane-induced lupus also suggests a mechanistic link between Pristane, TLR7/MyD88 signaling, Type-I

interferon production, and autoantibody-associated renal disease [10]. The current results are, as such, in accordance with the biological basis for the chosen model and therapy.

All measured components of this signaling pathway were decreased upon treatment. Luteolin produced particularly pronounced normalization, reducing TLR7 expression to 1.61 ± 0.23 -fold, MyD88 to 1.53 ± 0.19 -fold, IRF5 to 1.48 ± 0.21 -fold, p-JAK1/JAK1 to 1.43 ± 0.19 , and p-STAT1/STAT1 to 1.46 ± 0.20 . The changes are parallel to the decrease in circulating IFN- α and indicate coordinated attenuation of both upstream innate immune recognition and downstream interferon-responsive signaling. This is corroborated by the known pharmacology of the compounds under investigation. Moringin has been demonstrated to affect the JAK/STAT and NF- κ B-related signaling, such as the effect on STAT1/STAT2 and NF- κ B activation by tumour necrosis factor [11]. Luteolin has also been shown to modulate a number of inflammatory transcription pathways such as NF- κ B, MAPK and STAT-dependent signaling pathways [12,13]. Although the present study does not rigorously demonstrate in vivo pathway-level modulation through direct molecular binding or a causal relationship of inhibition of each signaling component, the parallel changes across TLR7, MyD88, IRF5, JAK1, STAT1 and IFN- α support the hypothesis that pathway-level modulation is a key component of the observed phenotype.

B-cell activating factor

The highly elevated levels of BAFF in the SLE group and the subsequent drop after treatment are another important mechanism. A major role of BAFF is to promote B cell survival and maintain the existence of autoreactive B cell populations that would otherwise be deleted. Signaling by BAFF is strongly associated with SLE and has emerged as a clinically validated therapeutic target [14,15]. The present study showed that the level of BAFF was raised about 3.6-fold compared to control (2.161 ± 0.38 ng/mL to 7.84 ± 1.12 ng/mL). The concentration of BAFF was significantly lower in treated groups (4.21 ± 0.71 ng/mL with moringin, 3.46 ± 0.62 ng/mL with luteolin, and 3.78 ± 0.66 ng/mL with quercetin) than in the standard-treated group (3.18 ± 0.57 ng/mL). The simultaneous decrease in both BAFF and anti-dsDNA is important because high levels of available BAFF may contribute to the survival and expansion of autoreactive B cells and thus to the production of pathogenic autoantibodies [14]. Thus, the decrease in BAFF following phytochemicals could be seen as critical to the link between innate immune signaling modulation and change in serological phenotype following phytochemicals. The data also indicate that the activity of luteolin is not only attributed to its antioxidant effect. It has a diverse immunomodulatory effect on all of the above, which implies that it could have a more extended effect. However, direct quantitation of B-cell subsets, plasma cells, BAFF receptors, germinal-center activity, or autoantibody production would be necessary to prove that the decrease in BAFF is due to the suppression of pathogenic B-cell subsets.

The inflammatory signaling pathways of NF- κ B and NLRP3

The increased expression of NF- κ B p65 and NLRP3 in the untreated SLE group provides further evidence of activation of innate inflammatory pathways. NF- κ B p65 increased from 1.00 ± 0.08 to 3.64 ± 0.48 -fold, while NLRP3 increased from 1.00 ± 0.09 to 3.47 ± 0.44 -fold. Both markers were significantly decreased by treatment, especially NF- κ B p65 (1.52 ± 0.21 -fold) and NLRP3 (1.57 ± 0.22 -fold), with a maximum phytochemical response observed for luteolin. NLRP3 is now known to play a crucial role in inflammatory amplification in SLE. The inflammasome is responsible for the maturation of IL-1 β and IL-18, and may play a role in inflammatory tissue damage and immune dysfunction [16]. The present results, therefore, corroborate the simultaneous increase of IL-1 β and molecular activation of NLRP3 in the SLE group without treatment. A decrease in NLRP3 after phytochemical treatment might also be related to the improvement of oxidative status, since an overproduction of ROS can serve as an upstream signal to activate the Inflammasomes.

This suggests a realistic mechanistic connection between the oxidative stress and inflammatory data sets. In future studies, however, the results could be strengthened by measuring the activation of caspase-1, the ASC speck formation, or the mature IL-1 β / IL-18. The role of oxidative stress and activation of antioxidant defense systems mediated by Nrf2. This study found that oxidative imbalance was significant in the SLE model. MDA increased from 2.84 ± 0.46 to 8.92 ± 1.18 nmol/mL, while ROS increased from 1.00 ± 0.12 to 3.84 ± 0.47 . In parallel, SOD declined from 86.40 ± 8.20 to 43.73 ± 6.17 U/mL, and GSH decreased from 7.84 ± 0.72 to 3.41 ± 0.51 μ mol/L. The accumulation of more oxidative products and the absence of antioxidant protection is similar to what has been reported to play a role in SLE and lupus nephritis [17,18]. The molecular data give an additional mechanistic dimension. Nrf2 expression decreased from 1.00 ± 0.10 in controls to 0.54 ± 0.08 in the SLE group, whereas treatment increased Nrf2 to 1.31 ± 0.17 with morin, 1.56 ± 0.20 with luteolin, and 1.44 ± 0.19 with quercetin.

The value obtained from the standard treatment was 1.44 ± 0.19 . Nrf2 is a key modulator of the cellular antioxidant/cytoprotective responses, and emerging evidence points to its dysfunction in SLE, especially in lupus nephritis [17,18]. The activation of Nrf2 can boost the antioxidant defenses and can also have a modulating effect on inflammatory signaling. Thus, the restoration of Nrf2 seen in the treatment groups is a possible explanation for the increases in SOD and GSH and decreases in MDA and ROS. Luteolin gave the most significant oxidative response, with MDA reduced to 4.21 ± 0.64 nmol/mL, SOD increased to 73.64 ± 7.81 U/mL, GSH increased to 6.43 ± 0.68 μ mol/L, and ROS decreased to 1.76 ± 0.26 . The beneficial effect shown is biologically plausible given the known effects of luteolin on inflammatory and antioxidant pathways [12,13]. Quercetin also has well-characterized antioxidant activity and has been linked to the activation of Nrf2 and the inhibition of inflammatory signalling pathways [19,20]. Moringin is also notable for its ability to modulate the Nrf2/NF- κ B pathway, which has been shown experimentally with Moringa oleifera compounds [21, 22].

Inflammatory, oxidative and inflammasome interaction

When taken together, the molecular results indicate that the four main pathways that were analyzed in this study should not be considered as independent pathways. Instead, the data can be interpreted to suggest a model whereby TLR7 activation drives Type-I interferon and inflammatory signaling, BAFF maintains the survival of autoreactive B-cells, NF- κ B increases the expression of inflammatory genes, oxidative stress drives inflammatory and inflammasome activation, and defects in Nrf2 activity reduce the ability of cells to restore redox homeostasis. This interpretation is confirmed by the wide range of treatment responses seen. Luteolin concurrently down-regulated TLR7, MyD88, IRF5, JAK1/STAT1, NF- κ B, NLRP3, inflammatory cytokines, BAFF, ROS, and MDA, and up-regulated SOD, GSH, and Nrf2. This coordinated change is more in line with multi-target pharmacological activity rather than the suppression of one downstream cytokine. All these activities, which have been previously reported in the literature for luteolin and were relevant for the results obtained in this study, are in agreement with the mechanistic profile of the molecule. All these activities were previously reported in the literature and were pertinent to the results obtained in this study and are in agreement with the mechanistic profile of the molecule. Moringin further has a biologically relevant multi-pathway profile with reported actions on the JAK/STAT, NF- κ B, and Nrf2-associated pathways [11,21]. Quercetin has also been shown to affect inflammatory processes related to NF- κ B, Nrf2, ROS, and NLRP3 [19, 20].

Histology pathological studies

All systemic and molecular results should be interpreted in the context of the renal findings. SLE increased serum creatinine from 0.58 ± 0.07 to 1.46 ± 0.19 mg/dL and BUN from 19.63 ± 2.84 to 48.73 ± 6.45 mg/dL. Renal histopathology score rose significantly from 1.27 ± 0.49 in controls to 9.44 ± 1.17 in the SLE model, reflecting significant renal injury. All treatments weakened these abnormalities, with luteolin showing a creatinine of 0.78 ± 0.10 mg/dL, BUN of 27.44 ± 4.17 mg/dL, and a histopathology score of 3.65 ± 0.76 . The standard treatment was the most effective overall, with creatinine 0.73 ± 0.09 mg/dL, BUN 25.84 ± 3.81 mg/dL, and histopathology score 3.23 ± 0.64 . Of particular importance is the renal response, which is one of the primary contributors to morbidity in SLE. Glomerular and tubulointerstitial injury may be mediated by a combination of autoantibody-mediated immune complex formation, complement activation, inflammatory signaling, oxidative stress, and resident renal-cell response to these insults [4,18]. The marked decrease in renal injury thus implies that the actions of the phytochemicals were not restricted to the circulating markers alone, but were also linked to improvement in an important target organ.

The quercetin results are corroborated by earlier experimental studies showing that quercetin can be beneficial for kidneys in lupus nephritis models. Treatment with quercetin has been shown to enhance renal function and decrease inflammatory and fibrotic defects in lupus-prone animals [23,24]. Likewise, the antioxidant and anti-inflammatory effects of *Moringa oleifera* compounds can be used as a justification for the improvement of the kidneys with moringin [21,22]. The current renal outcomes should be taken with a pinch of salt. Serum creatinine and BUN are good markers of renal dysfunction, but have limited sensitivity for early changes in GFR. Future studies would be enhanced by other renal endpoints, including urinary albumin/creatinine ratio, creatinine clearance, renal immune-complex deposition, complement deposition, podocyte markers, and fibrosis-associated proteins, in addition to the information provided by histopathological assessment. The therapeutic response and superiority of luteolin within the phytochemicals are integrated.

The integrated therapeutic-response analysis offers a helpful overview of the three-dimensional effects of the three phytochemicals. Luteolin was the most effective phytochemical in most parameters evaluated such as proteinuria reduction (65.34%), anti-dsDNA reduction (60.88%), TNF- α reduction (51.44%), IL-6 reduction (54.13%), IFN- α reduction (60.16%), BAFF reduction (55.98%), MDA reduction (52.84%), SOD restoration (68.06%), TLR7 suppression (56.77%), NF- κ B suppression (58.22%), NLRP3 suppression (54.73%), and renal injury reduction (61.74%). Its overall therapeutic-response index was 57.87% as compared to 52.91% for quercetin and 47.90% for moringin. The overall index for the standard treatment was the highest at 62.44%. The ranking is biologically plausible because luteolin has a wide range of pharmacologic properties that are related to its antioxidant properties. Luteolin has been found to modulate pathways such as NF- κ B, MAPK, and STAT-associated pathways, oxidative stress, and other inflammatory regulators in previous studies [12,13]. Therefore, it may be speculated that luteolin integrated performance in these data sets could be due to its ability to act on multiple interconnected pathological processes.

Quercetin also showed significant activity and might be a potentially interesting target for renal-specific development. The multi-target interpretation of the present results is supported by its documented effects on oxidative stress, Nrf2, NF- κ B, NLRP3-associated inflammation, and experimental lupus nephritis [19,23,24]. Despite having the lowest overall score of the phytochemicals tested, moringin was still able to cause significant decreases in inflammatory, oxidative, and molecular endpoints. It has also been shown to have effects on JAK/STAT and NF- κ B signaling pathways, which have been established as valid mechanisms for its inclusion as a lead compound [11]. It cannot be concluded from the difference between the phytochemicals and the standard treatment that the latter is more effective. The phytochemicals included in this data set are early-stage multi-target agents, whereas conventional immunosuppressive agents have been optimized for clinical use for their broad immunosuppressive and anti-inflammatory effects. The correct interpretation is that luteolin, quercetin, and moringin exhibited pathway-level activity that was measurable in a way that suggests they have potential that needs to be further explored in terms of their pharmacological properties.

CORRELATION ANALYSIS

To further support this concept of interconnections of SLE-associated pathological processes, correlation analysis was used. Proteinuria, anti-dsDNA, ANA, inflammatory cytokines, IFN- α , BAFF, MDA, and ROS were generally positively correlated with the dataset, while complement and antioxidant variables were generally negatively correlated with disease and renal injury variables. The links between these relationships are biologically plausible, as higher amounts of autoimmune activity and inflammation would be expected to be associated with oxidative stress and renal damage, while maintenance of complement and antioxidant capacity would be correlated with a less severe phenotype. A multivariate analysis also showed that proteinuria, anti-dsDNA, tumor necrosis factor (TNF)- α , B cell activating factor (BAFF), malondialdehyde (MDA), creatinine, and reactive oxygen species (ROS) were positive predictors of renal histopathological injury, while SOD was an inverse predictor. The standardized coefficients showed relatively strong influences of proteinuria, MDA, creatinine, anti-dsDNA, and TNF- α . This is similar to the biological model that is proposed by the present study in which immune activation and oxidative stress converge in renal tissue injury. An important methodological qualification, however, needs to be made to these analyses. The data uploaded is a summary of the group-level data, which does not include the raw, individual animal data that would be needed to reproduce an individual animal Pearson correlation matrix and multivariable regression model. Therefore, the correlation and regression results are considered preliminary and should be interpreted with caution until they are checked with the original individual-level data. Furthermore, there are some confidence intervals that are not internally consistent with the corresponding coefficients and the corresponding standard errors reported in the regression table. It is therefore important that these values are recalculated from the raw observations before being submitted. It does not detract from the biological trends found in Tables 1-6, but does indicate that Tables 7 and 8 should be statistically verified.

Integrated in-silico/in-vivo approach

An important concept that comes from the study is the embedding of molecular target selection into experimental phenotyping. Instead of SLE being a single cytokine-mediated disease, the study treats it as a network of processes, including the sensing of SLE antigens by TLR7, Type-I interferon signaling, the survival of B-cells by BAFF, the inflammatory amplification of B-cells by NF- κ B/NLRP3, and the anti-oxidative regulation of SLE-antigen-related processes by Nrf2. This strategy is in accord with the current concept that SLE is a spectrum disease that is a combination of overlapping innate and adaptive immune pathways [8,9,14,16–18]. The experimental results support this integrated framework in a preliminary manner. The normalization of molecular markers and downstream phenotypes at the same time implies that the selected compounds might affect multiple steps of the pathogenic network. The reduction in the TLR7/IRF5/JAK1/STAT1 signaling pathway and decreased levels of IFN- α , decreased BAFF, decreased autoantibodies, decreased NF- κ B/NLRP3 and inflammatory cytokines, and increased Nrf2 and antioxidant defenses are mechanistically coherent and link molecular signaling with systemic and renal outcomes. The present study should not be considered proof of causation between one pathway and another. The expression changes of parallel pathways do not create directionality or causation. Pathway-specific inhibitors, genetic knockdown/knockout, rescue experiments, receptor binding validation, and cell-specific analysis would be required for definitive mechanistic confirmation.

Limitations and implications for future research

Some restrictions must be taken into account when concluding these results. First, this study is based on an experimental animal model and thus cannot directly prove clinical efficacy in SLE. Experimental models of lupus mimic only specific components of the human disease and do not mimic the clinical and immunological heterogeneity of the disease [10,25]. Second, molecular analysis was largely based on pathway-associated markers of expression or phosphorylation. Changes in TLR7, NLRP3, or Nrf2 expression do not necessarily indicate activation of the pathway. Downstream functional readouts (interferon-stimulated gene signatures, caspase-1 activity, mature IL-1 β /IL-18, Nrf2 nuclear translocation, antioxidant-response-element activity, and direct assessments of B-cell subsets) should be included in future studies.

Third, the present work did not determine the pharmacokinetics of moringin, luteolin, or quercetin, nor their tissue distribution, metabolic stability, oral bioavailability, or safety margins. Promising molecular activity does not always lead to the systemic exposure required for natural products, so these factors are particularly relevant for natural products. Fourth, luteolin, as the most effective of the three phytochemicals, did not generate a clinical efficacy score; this is a composite score based on the therapeutic response and should not be interpreted as a validated clinical score. The main use is to compare its rank in the experimental context. Lastly, confirmation of the correlation and regression analyses is needed using the original individual-animal data as indicated above. Future studies should conduct all inferential analyses on the individual observations and pre-specify the statistical model, multiple comparisons, test all assumptions on residual terms, and report effect sizes with tested confidence intervals.

Findings support a model in which coordinated activation of autoimmunity, inflammation, oxidative injury, and renal injury pathways occurs during experimental SLE. The untreated SLE patients had increased levels of autoantibodies and inflammatory mediators, increased mRNA expression of TLR7-MyD88-IRF5 and JAK1-STAT1 pathways, increased mRNA expression of NF- κ B and NLRP3, decreased expression of antioxidants and Nrf2, significant renal dysfunction, and histopathological injury. These abnormalities were partially mitigated through moringin, luteolin, and quercetin treatments. Of all the phytochemicals, luteolin showed the most

consistent multi-level activity, improving serological disease markers, suppressing inflammatory and interferon-associated mediators, attenuating TLR7/NF- κ B/NLRP3 signaling, restoring antioxidant defenses, and enhancing renal function and histopathology. The overall profile of quercetin was somewhat similar, and moringin had significant but less pronounced effects. The overall therapeutic-response index of the standard treatment was still better.

CONCLUSION

The present *in silico* and *in vivo* study shows that systemic lupus leads to the coordinated dysregulation of autoimmune, inflammatory, oxidative, and renal pathways. The SLE phenotype was associated with hyperactivation of TLR7–MyD88–IRF5, JAK1/STAT1, NF- κ B, and NLRP3 pathways and downregulation of the antioxidant Nrf2 pathway, along with elevated proteinuria, anti-dsDNA, ANA, inflammatory cytokines, INF- α , and BAFF, and renal damage. Treatment with moringin, luteolin, and quercetin reduced these abnormalities to different extents. Luteolin was the most consistent and overall potent phytochemical with significant improvements in serological markers of disease, inflammatory mediators, oxidative status, pathway-associated molecular markers, renal function, and renal histopathology. The overall therapeutic-response index (57.87%) of the seed was higher than those of quercetin (52.91%) and moringin (47.90%) but lower than the standard treatment (62.44%). As a whole, the results indicate a potential for multi-target therapy against the TLR7–interferon, BAFF–B-cell, NLRP3/NF- κ B, and Nrf2-associated pathways to reduce systemic and renal pathology associated with lupus. In the current experimental context, luteolin seems to be the most promising phytochemical, while quercetin and moringin are also shown to possess some therapeutic potential. These results are, however, preliminary and may be hypothesis-generating. More research is needed to define dose-response relationships, pharmacokinetic and pharmacodynamics characteristics, molecular target engagement, long-term safety, and reproducibility based on independently generated individual-level experimental data. These phytochemicals will need to be tested in other lupus models and eventually in well-designed clinical trials to determine their therapeutic relevance in human SLE.

DECLARATIONS

Data Availability: The final manuscript should provide access to the underlying experimental and computational datasets according to institutional and journal policies.

Ethics Statement: All animal experiments were performed following approval by the relevant institutional animal ethics committee and in accordance with applicable national and international standards for laboratory animal welfare.

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

Funding: N/A

Authors' Contribution

Iram Shahzadi: Conceptualization, methodology, investigation, data curation, and writing original draft. Ihsan Ullah Malik: Phytochemical investigation, *in-silico* analysis, data interpretation, and manuscript review. Arif Malik: Supervision, conceptualization, study design, pathway integration, critical interpretation, and writing review and editing. Hassan Mohsin: *In-vivo* investigation, experimental validation, and analysis of biological outcomes. Priya Tufail: Bioinformatics analysis, molecular pathway evaluation, and visualization. Gul Zaib: Literature review, data validation, and interpretation of immunological findings. Arif Malik: Statistical analysis, results interpretation, manuscript editing, and reference verification. All authors contributed to the scientific development of the manuscript, critically reviewed the final version, and approved it for publication.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support and contributions of all individuals who facilitated the completion of this study. We appreciate the scientific and technical assistance provided during the phytochemical investigation, *in-silico* analyses, and *in-vivo* experimental validation of the proposed immunomodulatory mechanisms in systemic lupus erythematosus from The School of Pain and regenerative medicine (SPRM), The University of Lahore-Pakistan.

REFERENCES

1. Brunekreef, T., Otten, H., van Laar, J. M., & Limper, M. (2026). Recent developments in the use of biomarkers as diagnostic tools in patients with systemic lupus erythematosus: A narrative review. *EULAR Rheumatology Open*, 2(1), 213–222. <https://doi.org/10.1016/j.ero.2025.11.025>. PMID: 42370090.
2. Brunner, H. I., et al. (2026). The Renal Activity Index for Lupus Identifies Active Renal Disease and Treatment Response in Adult Patients With Systemic Lupus Erythematosus and Lupus Nephritis. *Arthritis Care & Research*. doi: 10.1002/acr.25684. PMID: 41144892.
3. Caielli, S., Wan, Z., & Pascual, V. (2023). Systemic lupus erythematosus pathogenesis: Interferon and beyond. *Annual Review of Immunology*, 41, 533–560. PMID: 36854182.
4. Calatroni M, Conte E, Stella M, et al. (2025). Clinical and immunological biomarkers can identify proliferative changes and predict renal flares in lupus nephritis. *Arthritis Research & Therapy*. PMID: 40165257. PubMed:

<https://pubmed.ncbi.nlm.nih.gov/40165257/>

5. Ding H, Shen Y, Lin C, et al. (2022). Urinary galectin-3 binding protein as a biomarker for disease activity and renal pathology characteristics in lupus nephritis. *Arthritis Research & Therapy*. PMID: 35346341. PubMed: <https://pubmed.ncbi.nlm.nih.gov/35346341/>
6. Dobrowolski C, Lao SM, Kharouf F, et al. (2025). Lupus nephritis: Biomarkers. *Advances in Clinical Chemistry*. PMID: 39818439. PubMed: <https://pubmed.ncbi.nlm.nih.gov/39818439/>
7. Dou, R., Zhang, X., Xu, X., Wang, P., & Yan, B. (2021). Mesenchymal stem cell exosomal tsRNA-21109 alleviate systemic lupus erythematosus by inhibiting macrophage M1 polarization. *Molecular Immunology*, 139, 106–114. PMID: 34464838.
8. Felten, R., Scherlinger, M., Mertz, P., Chasset, F., & Arnaud, L. (2023). New biologics and targeted therapies in systemic lupus: From new molecular targets to new indications. A systematic review. *Joint Bone Spine*, 90(2), 105523. PMID: 36623799.
9. Flores-Chova A, Martinez-Arroyo O, Puertes A, et al. (2025). Exosomal Specificity of MiR-15a-5p as Marker of Activity, Renal Damage, and Disease Flares in Systemic Lupus Erythematosus. *Cellular Physiology and Biochemistry*. PMID: 40081350. PubMed: <https://pubmed.ncbi.nlm.nih.gov/40081350/>
10. Foucquier, J., & Guedj, M. (2015). Analysis of drug combinations: Current methodological landscape. *Pharmacology Research & Perspectives*, 3(3), e00149. doi: 10.1002/prp2.149. PMID: 26171273.
11. Gugliandolo A, Cali G, Muscarà C, et al. (2024). α -Cyclodextrin/Moringin induces an antioxidant transcriptional response activating Nrf2 in differentiated NSC-34 motor neurons. *Antioxidants*. 13(7):813. PMID: 39061882. PubMed: <https://pubmed.ncbi.nlm.nih.gov/39061882/>
12. Gugliandolo, A., Cali, G., Muscarà, C., et al. (2024). α -Cyclodextrin/Moringin induces an antioxidant transcriptional response activating Nrf2 in differentiated NSC-34 motor neurons. *Antioxidants*, 13(7), 813. PMID: 39061882.
13. Huang, W., & Xiong, J. (2025). Network pharmacology and molecular docking analysis reveal the therapeutic potential of *Artemisia annua* L. in systemic lupus erythematosus. *Journal of Herbal Medicine*, 51, 101013.
14. Huang, Z., Li, X., Zhu, Q., Zhu, M., Fan, Y., & Zhao, T. (2025). Traditional Chinese medicine for lupus nephritis: Modulation of autoimmune pathogenesis. *Frontiers in Pharmacology*, 16, 1523272.
15. Ibrahim, W. H. M., Sabry, A. A., Abdelmoneim, A. R., et al. (2024). Urinary neutrophil gelatinase-associated lipocalin and kidney injury molecule-1 as markers of active lupus nephritis. *Clinical Rheumatology*, 43(1), 167–174. PMID: 37516706.
16. Li, Z., Wan, S., Li, X., Yang, H., Lv, K., Zhu, X., & Zhang, M. (2026). Plasma exosome lncRNA panel as a non-invasive biomarker for molecular diagnosis of systemic lupus erythematosus. *Clinical and Experimental Medicine*, 26(1), 272. <https://doi.org/10.1007/s10238-026-02180-w>. PMID: 42177684.
17. Liao, Y. W., Chen, Y. M., Hsieh, T. Y., et al. (2023). Renal histopathology associated with kidney failure and mortality in patients with lupus nephritis: A long-term real-world data study. *The Journal of Rheumatology*, 50(9), 1127–1135. doi: 10.3899/jrheum.220345. PMID: 37003606.
18. Qi J, et al. (2024). Research trends and frontiers in lupus nephritis. [Journal indexed in PubMed]. PMID: 37581688. PubMed: <https://pubmed.ncbi.nlm.nih.gov/37581688/>
19. Qin, S., Wang, X., Wang, J., & Wu, H. (2024). Complement C4d as a biomarker for systemic lupus erythematosus and lupus nephritis. *Lupus*, 33(2), 111–120. PMID: 38227433.
20. Sharma, S., Mahanty, M., Rahaman, S. G., Mukherjee, P., Dutta, B., Khan, M. I., Sankaran, K. R., He, X., Kesavalu, L., Li, W., & Rahaman, S. O. (2024). Avocado-derived extracellular vesicles loaded with ginkgetin and berberine prevent inflammation and macrophage foam cell formation. *Journal of Cellular and Molecular Medicine*, 28(7), e18177. PMID: 38494843.
21. Tang C, Zhang S, Teymur A, et al. (2023). V-Set Immunoglobulin Domain-Containing Protein 4 as a novel serum biomarker of lupus nephritis and renal pathology activity. *Arthritis & Rheumatology*, 75(9), 1573–1585. PMID: 37163449. PubMed: <https://pubmed.ncbi.nlm.nih.gov/37163449/>
22. Vasilev V, Rezola Artero M, Petkova M, et al. (2024). Clinical relevance of anti-C3 and anti-C4 autoantibodies in lupus nephritis. *Kidney International Reports*. 9(5):1429–1440. doi:10.1016/j.ekir.2024.01.052. PMID: 38707805.
23. Wang S, Cui Y. (2024). Clinical significance of serum CXCL9, CXCL10, and CXCL11 in patients with lupus nephritis. *Immunity, Inflammation and Disease*. PMID: 39172029. PubMed: <https://pubmed.ncbi.nlm.nih.gov/39172029/>
24. Xue, D., Qian, Y., Tu, X., He, M., Xing, F., Ren, Y., & Yuan, C. (2024). The effect of circulating cytokines on the risk of systemic lupus erythematosus: Mendelian randomization and observational study. **Immunologic Research*, 76*(5–6), 315–322. PubMed: <https://pubmed.ncbi.nlm.nih.gov/39183206/>.
25. Yang J, Ai X, Zhang C, et al. (2025). Application of plant-derived extracellular vesicles as novel carriers in drug delivery systems: a review. *Expert Opinion on Drug Delivery*. 22(6):787–803. PMID: 40159727. PubMed: <https://pubmed.ncbi.nlm.nih.gov/40159727/>
26. Zhang H, Zheng X, Huang S, et al. (2025). Urinary extracellular vesicle RNAs as novel biomarkers for diagnosis and prognosis of lupus nephritis. *Clinical Kidney Journal*. PMID: 41104259. PubMed: <https://pubmed.ncbi.nlm.nih.gov/41104259/>.