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Original Article

Psoralen Attenuates Rheumatoid Arthritis through Inhibition of the IL-1β/IL-18/NLRP3 Inflammasome Axis

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ABSTRACT

Background: Psoralen, a naturally derived compound, is known for its anti-inflammatory properties and thus it is essential to evaluate its effects on inflammatory pathways in Rheumatoid Arthritis (RA). **Aim:** To assess the anti-RA potential of psoralen by evaluating its effects on protein denaturation, cytokine and inflammasome inhibition, and cell proliferation in RA models and confirm its molecular interactions using molecular docking and simulations. **Materials and Methods:** Psoralen was tested for anti-inflammatory efficacy utilizing BSA and egg albumin protein denaturation tests and SW-982 and THP-1 cell proliferation assays. Cytokine suppression of IL-1β and IL-18 in THP-1 cells was measured by ELISA and inflammasome expression by western blotting after psoralen administration. After docking using DockThor, molecular dynamics simulations with iMODS and CABS-flex assessed the stability of psoralen interaction with IL-1β, IL-18, and NLRP3 targets. **Results:** In the BSA assay, psoralen showed significant dose-dependent inhibition, with a maximum inhibition of 88.02±1.96% at 200 mg/mL. Similarly, in the egg albumin assay, psoralen exceeded diclofenac sodium at 200 mg/mL, achieving an absorbance of 2560.80±6.02. Psoralen inhibited cell proliferation in both SW-982 and THP-1 cells, with a more significant effect in SW-982 cells. Docking results showed that psoralen exhibited the highest affinity for NLRP3 (-8.953) compared to IL-1β (-8.206) and IL-18 (-6.504). MD simulations revealed significant flexibility in the binding sites of the complexes, indicating dynamic interactions. Psoralen significantly inhibited IL-1β and IL-18 secretion in THP-1 cells in a concentration-dependent manner. Western blotting analysis demonstrated a dose-dependent inhibition of NLRP3 inflammasome activation. **Conclusion:** Psoralen effectively modulates the IL-1β/IL-18/NLRP3 inflammasome axis, reducing inflammatory responses and positioning itself as a promising candidate for therapeutic intervention in inflammatory diseases like rheumatoid arthritis. Further *in vivo* and clinical studies are essential to validate its efficacy, safety profile, and potential mechanisms as an anti-rheumatoid arthritis drug.

Keywords: Cytokines, IL-18, IL-1β, Inflammasome, NLRP3, Psoralen, Rheumatoid Arthritis.

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INTRODUCTION

RA is a long-term autoimmune disorder that mostly affects the joints of the synovial sac, which can lead to pain, swelling, and possible destruction of joint tissues.[1] It is marked by persistent inflammation and an abnormal immune response where the immune system of the body starts targeting its own tissues, primarily the synovium.[2] It is found to affect about 1% of the global population, and women are more likely to be affected than men.[3] The disease may eventually lead to severe disability because of the deformities caused in the joints and the inability to move. RA has a very complex pathogenesis and involves various factors like genetic predisposition, environmental influence, and dysregulated immune response.[4]

One of the central features of RA is occurrence when immune cells such macrophages, T- and B cells become activated, which leads to the release of pro-inflammatory cytokines like Tumor Necrosis Factor-alpha (TNF-α), Interleukin-18 (IL-18), IL-6, and IL-1β.[5] These cytokines are very important in driving the inflammatory process in RA, which leads to joint damage and systemic effects. Cytokines, such as interleukins, play significant roles in modulating adaptive and innate immunity, with a particular emphasis on regulating inflammation and cell death.[6] With the involvement of these cytokines in RA, targeted therapies have emerged, including biologics, which specifically inhibit TNF-α or interleukins, and benefit many patients with clinical response.

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In addition to cytokines, inflammasomes have garnered attention as important targets in RA. Inflammasomes control the production of pro-inflammatory cytokines including interleukins, as well as the stimulation of caspase-1. The NLRP3 inflammasome, in particular, has been implicated in RA pathogenesis.[7] Activation of the NLRP3 inflammasome under conditions of cellular stress or infection resulting in activation of these cytokines.[8] This amplification of the inflammatory response contributes to chronic inflammation and tissue destruction in RA. Targeting the NLRP3 inflammasome offers a new avenue to modulate inflammation and might be an alternative to conventional immunosuppressive therapy.

Natural products, including plant-derived compounds, now showing promise as potential RA treatments considering their anti-inflammatory and immunomodulatory properties.[9,10] Natural products offer a wealth of chemical diversity and have been used for centuries in traditional medicine. Rising interest in the possible therapeutic effects of these compounds in RA has been witnessed in past few years. Many natural products have been found to modulate different parts of the immune system, including cytokines, enzymes, and inflammasomes, which can potentially reduce inflammation and slow disease progression.[11] Psoralen, a naturally occurring compound from the plant *Psoralea corylifolia*, has been used with good results in many inflammatory diseases.[12] Psoralen has been proposed to be effective in a number of autoimmune disorders with most widely used being psoriasis.[13] Furthermore, it targets NF-κB and MAPK signalling in mouse models to induce antiinflammatory effects.[14]

Overall, the present understanding of RA emphasizes the crucial role of cytokines and inflammasomes in driving the disease process. Targeting these pathways provides new avenues for therapeutic interventions, particularly with the use of natural products like psoralen.

MATERIALS AND METHODS

Chemicals and Reagents

The study utilized the following chemicals and reagents: psoralen (99% purity by HPLC; Xi′an ZB Biotech Co., Ltd., Xi'an, Shaanxi, China; CAS: 66-97-7), Bovine Serum Albumin (BSA), fresh egg albumin, PBS, pH 6.4, 2-mercaptoethanol, Fetal Bovine Serum (FBS), Phorbol 12-Myristate 13-Acetate (PMA), Lipopolysaccharide (LPS), Adenosine Triphosphate (ATP), nigericin, Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) reagents, Polyvinylidene Fluoride (PVDF) membranes, chemiluminescence reagents, and ELISA kits.

BSA Anti-Inflammatory Assay

We tested psoralen's anti-inflammatory effects at different doses using a BSA assay. Psoralen was evaluated at various concentrations (0, 25, 50, 100, 200 μg/mL) to assess its potential to suppress protein denaturation, a critical indicator of anti-inflammatory effectiveness. The reaction mixture included 1% BSA in PBS (pH 6.4) and various psoralen concentrations. Denaturation was induced by 20 min at 37ºC and 10 min at 60ºC. A microplate reader recorded absorbance at 660 nm after cooling. The % suppression of protein denaturation for each psoralen dose revealed its anti-inflammatory effects.

Egg Albumin Anti-Inflammation Assay

Psoralen's anti-inflammatory properties were further assessed using an Egg Albumin test. In a reaction mixture with 5% fresh egg albumin in Phosphate-Buffered Saline (PBS, pH 6.4), psoralen doses of 25, 50, 100, and 200 μg/mL were compared to a control (0 μg/mL). To denaturate proteins, the samples were pre-incubated at room temperature for 15 min and heated at 70ºC for 10 min. After the reaction, a microplate reader measured sample absorbance at 660 nm. To test the anti-inflammatory effects of psoralen and compare its efficacy across doses, we calculated the protein denaturation inhibition percentage for each concentration.

Cell culture and Differentiation

THP-1 (ATCC; Manassas, VA, USA) and SW-982 (Procell Life Science & Technology Co., Ltd.; Wuhan, China) cells were cultured in RPMI 1640 media with 50 mM2-ME and 10% FBS added. For 3 hr, THP-1 cells were differentiated with 100 nm PMA.

CCK-8 assay

We used a CCK-8 assay to examine the influence of psoralen on the growth of SW982 and THP-1 cells. The cells were cultured in the presence of different concentrations of psoralen (25, 50, 100, and 200 μg/mL) or in the absence of psoralen for the control group (0 μg/mL) for 24 and 48 hr. This approach allowed us to observe psoralen's effect and thus determine its potential dose and time-dependent cytotoxic impact on these cell lines for proliferation. After incubating the plates at the recommended incubation periods, CCK-8 reagent was added to 10 μL in each of these wells, and it then again incubated at 37ºC for 2 hr with the microplate reader giving measurement at 450 nm with the help of an end reading.

Docking Assay

Molecular docking was performed using the DockThor[15] online tool ("https://dockthor.lncc.br/v2/") to analyze the interaction of psoralen with IL-1β, IL-18, and NLRP3. Protein structures were retrieved from the RCSB Protein Data Bank with IDs 8C3U (IL-1β), 7AI7 (IL-18), and 7AIV (NLRP3). Psoralen's structure was created in ChemBio3D Ultra 11.0. Protein preparation included adding polar hydrogens, removing water molecules, and energy minimization using BIOVIA Discovery Studio (Version: 24.1; Dassault Systemes, Biovia Corp., San Diego, CA, USA. The

prepared receptor and ligand files were uploaded to DockThor. Ten poses were generated, and docking scores were recorded. Visualization and 2D interaction analysis were further conducted in Discovery Studio.

Molecular Dynamics Simulations

Molecular dynamics simulations of the Psoralen-cytokine complexes were carried out using two complementary tools: iMODS and CABS-Flex.[16,17] iMODS performed Normal Mode Analysis (NMA) to analyze the flexibility and deformability of the complexes, which pointed out structural regions with high motion and potential binding site dynamics. iMODS is an online server from the Blas Cabrera Institute of Physical Chemistry, Instituto de Química Física Blas Cabrera. C/Serrano 119-28006 Madrid-Spain, providing NMA in internal coordinates for studying dynamics of nucleic acids and proteins (Availible at: "https://imods.iqf.csic.es/"). It allows visualization, animation, and simulation of conformational transitions in macromolecules. The CABS-Flex simulations produced an ensemble of structural models that provided insights into the dynamic behavior of the complexes. CABS-flex is a web server developed by the Biological and Chemical Research Center, Faculty of Chemistry, University of Warsaw, in Warsaw, Poland. It provides an efficient method for simulating the flexibility of folded globular proteins using the CABS model-a well-established coarse-grained protein modelling tool (Availible at: "https://biocomp.chem.uw.edu.pl/CABSflex2/about"). RMSF calculations were used to determine flexibility across residues, thereby defining dynamic regions critical for Psoralen binding and potential therapeutic applications.

ELISA Assay

The levels of IL-1β and IL-18 in supernatants of psoralen-treated THP-1 cells were measured using ELISA kits from Boster Biological Technology. The THP-1 cells were treated with psoralen at concentrations of 0 (control), 25, 50, 100, and 200 mg/mL. Incubated supernatants were then taken and processed as per the manufacturer's protocol. Absorbance at 450 nm was taken by microplate reader and the concentrations of cytokine were calculated using a standard curve.

Western Blotting

The effects of psoralen on inflammasome components were evaluated by Western blotting. THP-1 cells were differentiated with PMA (5×10^5 cells) and primed with LPS for 3 hr, followed by the treatment of ATP (5 mM) and nigericin (5 μM) for 1 hr. Cells were treated with psoralen at 0 (control), 25, 100, and 200 mg/mL. Total protein lysates were resolved on SDS-PAGE, transferred to PVDF membranes, and probed with primary antibodies against NLRP3 at a 1:100 dilution. The β-actin served as the loading control. Signals were detected using secondary antibodies (rabbit anti-mouse IgG (1.3 μg mL^{-1}, 1% BSA in PBST; Agilent, rabbit anti-mouse IgG (Agilent, Santa Clara, CA, United States) and chemiluminescence. The band intensity was quantifies using ImageJ Software (Version: 1.54 g, National Institutes of Health, USA).

Statistical Analysis

Data was reported as Mean±SD and analysed using Origin 2025 Software. The Tukey's *t*-test was used for multiple comparisons with One Way ANOVA. *p* values 0.05, 0.01 and 0.001 were considered significant.

RESULTS

Psoralen Induced Anti-inflammatory Effects

The BSA experiment demonstrated that psoralen had a dose-dependent anti-inflammatory activity, with notable increases in percentage inhibition correlating with higher concentrations. At lower dosages (25 and 50 mg/mL), psoralen exhibited considerable inhibition, akin to that of aspirin. At elevated dosages of 100 and 200 mg/mL, psoralen had better effectiveness compared to aspirin, achieving a % inhibition of 88.02±1.96% at 200 mg/mL, in contrast 83.36±1.00% to that of aspirin. This underscores the significant anti-inflammatory efficacy of psoralen at high dosages (Table 1).

Psoralen exhibited a significant dose-dependent reduction of protein denaturation in the egg albumin assay. At lower dosages, diclofenac sodium demonstrated marginally enhanced efficacy, with absorbance reaching 76.53 ± 0.77 at 25 mg/mL. Psoralen had equivalent effectiveness to diclofenac at concentrations of 50 and 100 mg/mL, and exceeded it at 200 mg/mL, with an absorbance of 2560.80±6.02 in contrast 528.96 ± 1.92 to that of diclofenac. These findings validate enhanced anti-inflammatory effectiveness psoralen at elevated doses, equivalent to a conventional anti-inflammatory medication (Table 1).

Table 1: The table represents the anti-inflammatory activity of psoralen in protein denaturing assays in comparison to reference control that is aspirin in BSA assay and diclofenac sodium in Egg Albumin Assay.

Concentration (mg/mL)	Psoralen (Mean±SD)	Ref. (Mean±SD)
BSA Assay		
Control	0.00	0.00
25	48.29±1.68	56.20±0.28
50	64.77±0.79	64.84±0.37
100	80.14±1.78	71.56±1.42
200	88.68±1.95	83.36±1.25
Egg Albumin Assay		
Control	0.00	0.00
25	493.00±3.56	76.53±0.77
50	1203.03±2.23	233.83±1.53
100	1795.33±4.87	339.85±1.71
200	2560.80±6.02	528.62±1.92

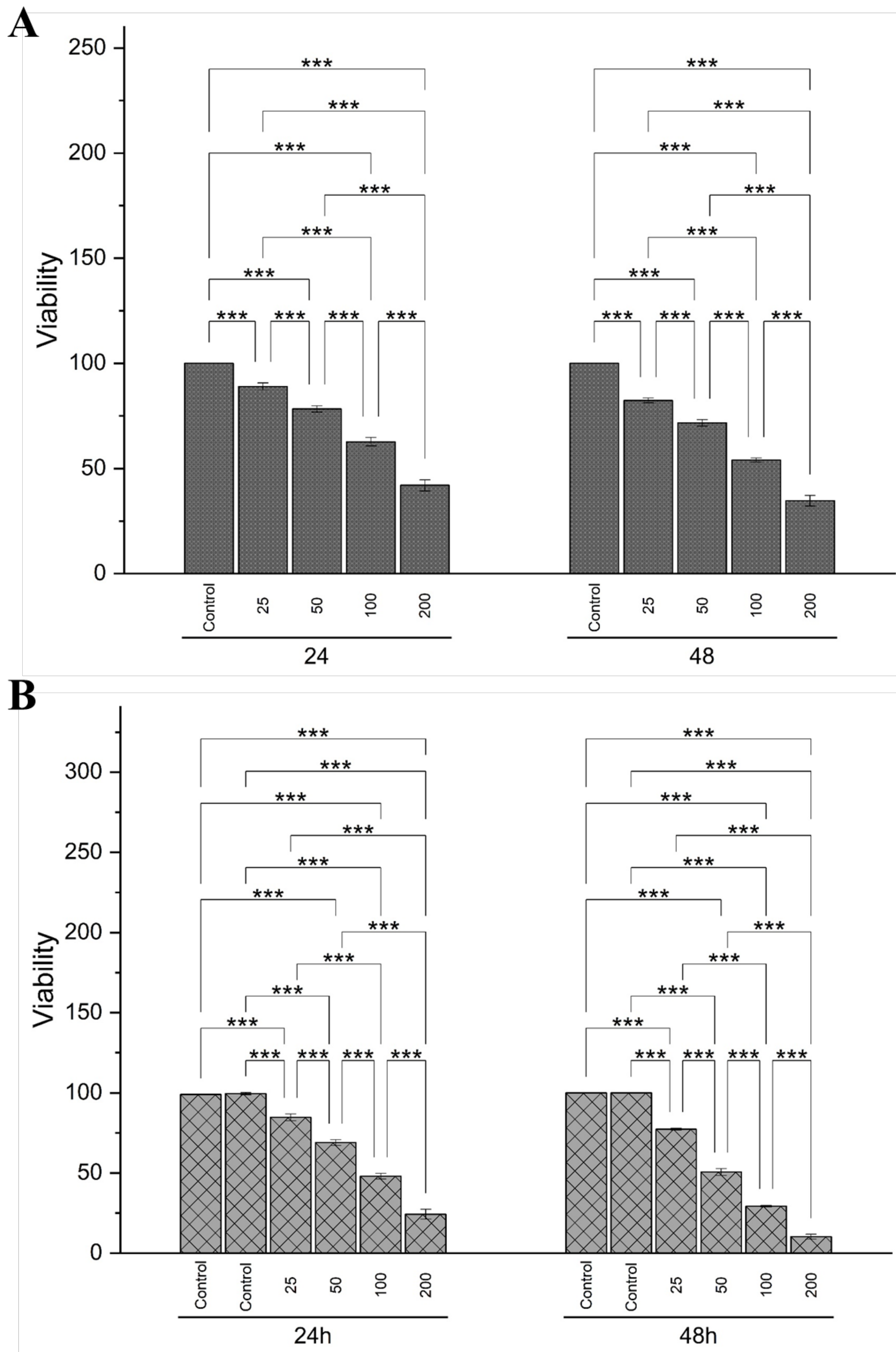

Figure 1: Psoralen's cytotoxic effects on SW-982 and THP-1 cells were assessed over time. (A) SW-982 cells exhibited significant dose- and time-dependent proliferation inhibition, with higher sensitivity to psoralen. (B) THP-1 cells also showed dose-dependent inhibition, though less pronounced compared to SW-982. Results underscore psoralen's selective cytotoxic potential against synovial and monocytic cell lines.

Psoralen Inhibited SW-982 and THP-1 Cell Proliferation

Psoralen showed concentration- and time-dependent inhibition of cell proliferation in both SW-982 and THP-1 cell lines. However, the inhibition was much more significant in SW-982 cells, which showed higher sensitivity to psoralen treatment (Figure 1A). On the other hand, although the inhibition was significant in THP-1 cells, it was relatively lower compared to SW-982 cells (Figure 1B). These findings highlight the differential cytotoxic activity of psoralen against inflammatory synovial and monocytic cell lines, which can be indicative of its application in targeting inflammatory and proliferative disorders.

Psoralen showed significant interactions with IL-1β, IL18 and NLRP3

Docking results presented psoralen with highest affinity for NLRP3 scoring of -8.953, followed by IL-1β (-8.206) and IL-18 (-6.504) (Table 2). Total energy was lowest for the psoralen-NLRP3 complex (8.316), while vdW energy was most favorable for IL-1β (-14.587). The electrostatic interaction energy of psoralen was strongest with IL-18 (-31.400). These results point to strong potential of psoralen for strong binding and modulation of NLRP3 inflammasome activity while effectively interacting with inflammatory cytokines. The 2D and 3D interactions have been shown in Figure 2.

Key interaction sites and binding energies suggest psoralen's potential to modulate inflammasome activity and inflammatory cytokines.

MD simulations

The iMODS results of the Psoralen-IL-1β (Figure 3A), Psoralen-IL-18 (Figure 3B), and Psoralen-NLRP3 (Figure 3C) complexes demonstrated distinct flexibility and deformation patterns for each of the complexes. In the case of Psoralen-IL-1β, the deformability analysis revealed that regions of IL-1β crucial to binding were relatively stable, whereas some peripheral regions were more flexible. In the case of Psoralen-IL-18, the same flexibility pattern was also observed with more motion at the regions flanking the ligand-binding site. Psoralen-NLRP3 showed significant flexibility in the regions of the NLRP3 receptor that participate in ligand binding and inflammasome activation, which can be pivotal in the modulation of inflammasome formation.

The CABS-Flex simulations for the Psoralen-IL-1β (Figure 4A), Psoralen-IL-18 (Figure 4B), and Psoralen-NLRP3 (Figure 4C) complexes presented different dynamic behaviors. Psoralen-IL-1β

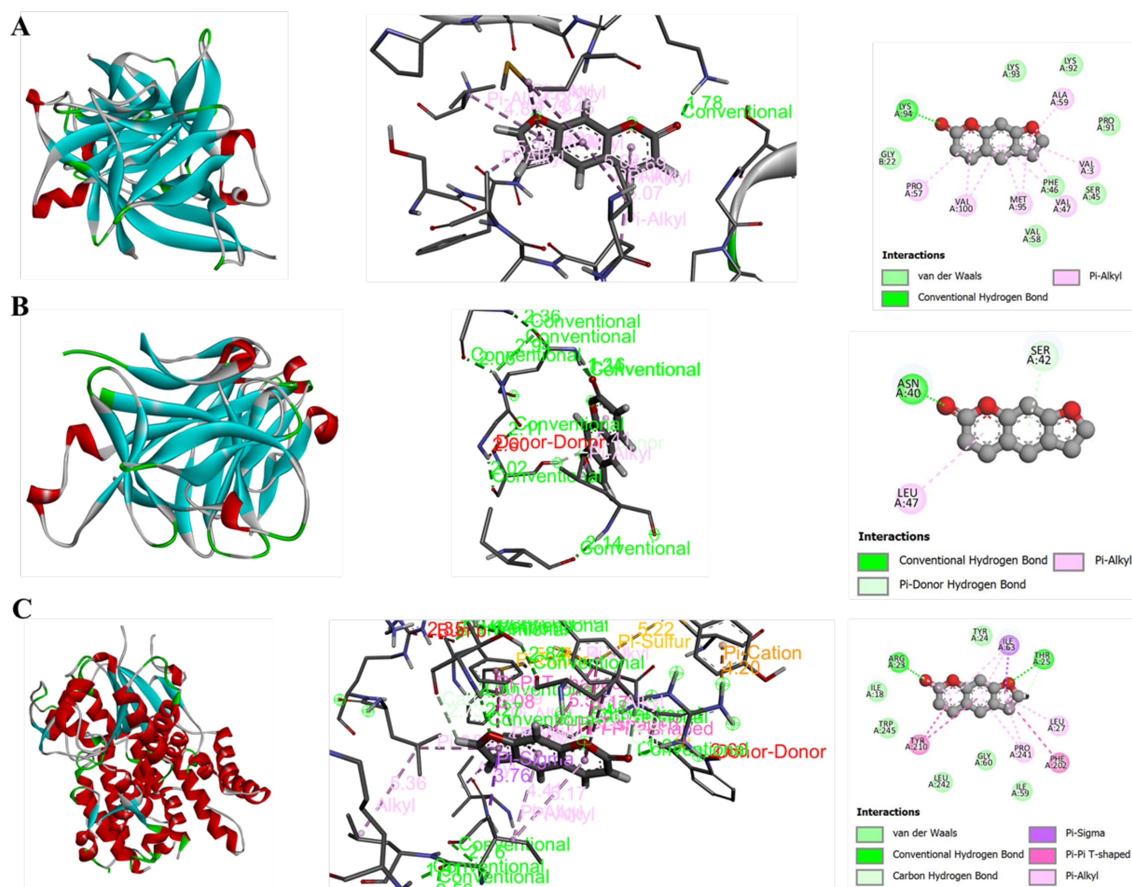

Figure 2: 2D and 3D molecular docking interactions of psoralen with (A) IL-1β, (B) IL-18, and (C) NLRP3. Psoralen demonstrated the strongest binding affinity for NLRP3 (-8.953kcal/mol), followed by IL-1β (-8.206 kcal/mol) and IL-18 (-6.504 kcal/mol).

Table 2: DockThor results for the molecular docking study for the psoralen- IL-1β, IL-18 and NLRP3 complexes.

Docked Complex	Affinity (kcal/mol)	Total Energy (kcal/mol)	vdW Energy (kcal/mol)	Elec. Energy (kcal/mol)
Psoralen-IL-1β	-8.206	14.094	-14.587	-7.645
Psoralen-IL-18	-6.504	17.008	12.082	-31.400
Psoralen-NLRP3	-8.953	8.316	-19.054	-8.956

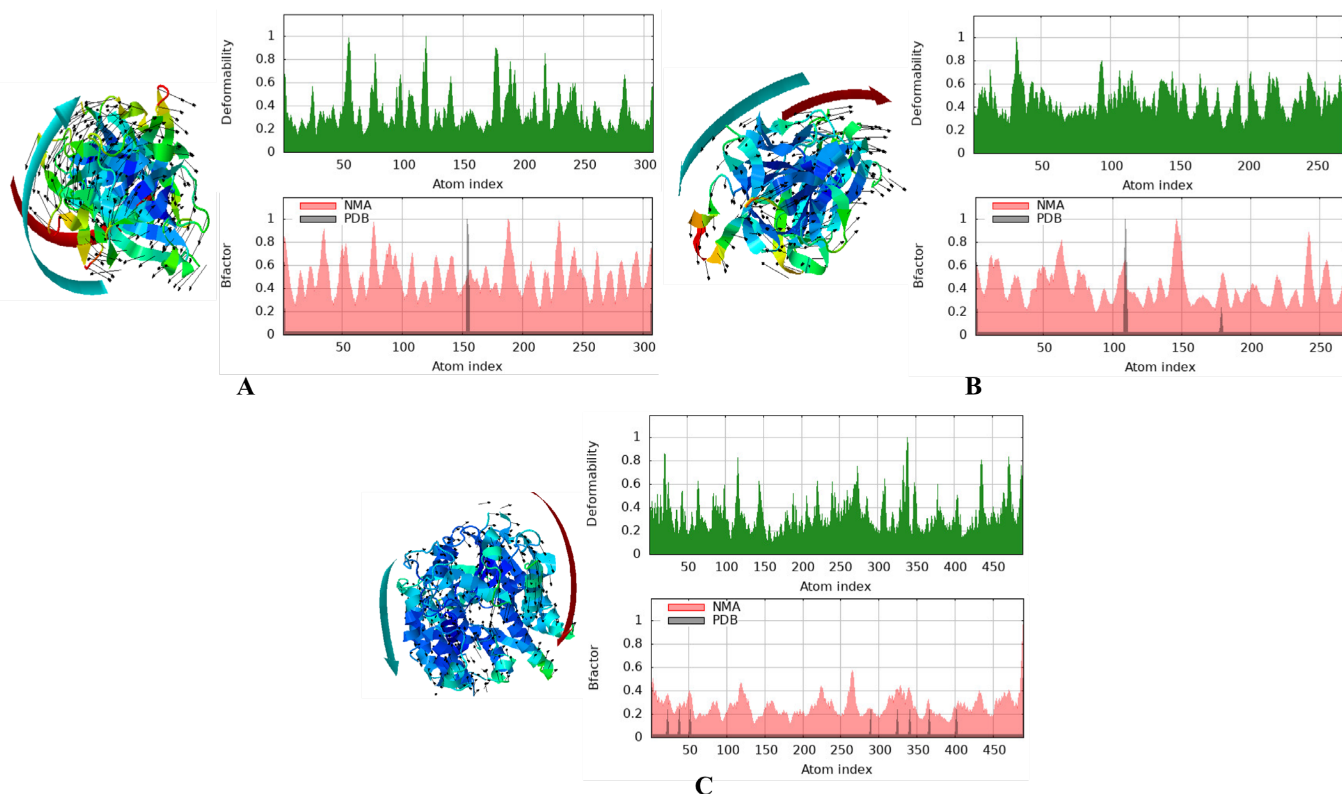

Figure 3: Molecular dynamics simulations for psoralen complexes. (A) Psoralen-IL-1β showed stability in binding regions with minimal flexibility. (B) Psoralen-IL-18 exhibited moderate deformability in binding and flanking regions. (C) Psoralen-NLRP3 displayed significant flexibility in inflammasome-related binding sites, highlighting the dynamic nature of the complex.

showed moderate RMSF, especially around the interface of the protein-ligand complex, suggesting potential sites for further optimization of binding. For Psoralen-IL-18, the RMSF values were higher, especially in loops and regions that are involved in interaction with Psoralen, which could suggest that the binding site is more adaptable. The RMSF values of Psoralen-NLRP3 were relatively high, suggesting significant flexibility in the regions responsible for inflammasome assembly, indicating the dynamic nature of the NLRP3 receptor and its interactions with Psoralen. These insights from CABS-Flex simulations are valuable for understanding the stability and binding mechanisms of Psoralen with these targets.

Psoralen Inhibited IL-1β and IL-18 Cytokines

Psoralen significantly inhibited these cytokine secretion in THP-1 cells in a concentration-dependent manner (Figure 5A and Figure 5B). Interestingly, treatment with LPS, nigericin, or psoralen alone did not alter cytokine levels. However, LPS and nigericin together triggered a significant increase in cytokine secretion. Treatment of psoralen following LPS and nigericin exposure significantly dampened these elevations, showing its potential to modulate the production of inflammatory-cytokines. These results show that psoralen is a very potent anti-inflammatory agent, especially in its ability to suppress the activation of inflammatory pathways by LPS and nigericin.

Psoralen Inhibited NLRP3 Inflammasome Activation

Western blotting analysis indicated that psoralen could indeed inhibit the NLRP3 inflammasome in THP-1 cells. The levels of expression of NLRP3 protein were inversely related to the concentration of psoralen used, hence indicating a dose-dependent manner of suppression of inflammasome activation (Figures 6A and 6B). Loading control was performed using beta-actin to guarantee equal quantification of the proteins. This reduction of NLRP3 level is consistent with the reported decrease in interleukins, thus further confirming the activity of psoralen against inflammasome activation. These results support

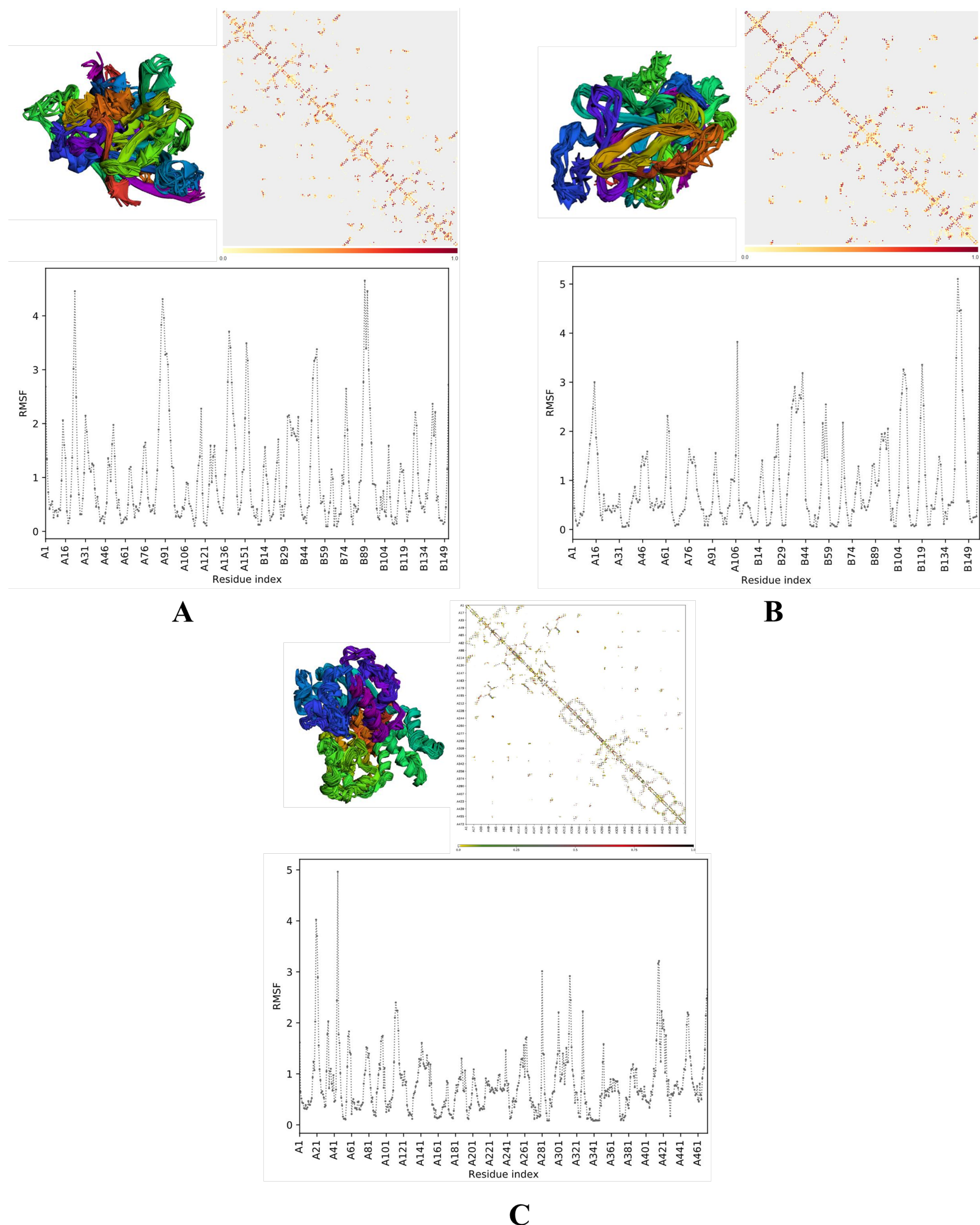

Figure 4: CABS-Flex simulation results showing RMSF values for psoralen-protein complexes. (A) Psoralen-IL-1β showed stable binding regions with moderate flexibility at peripheral sites. (B) Psoralen-IL-18 exhibited higher RMSF values, suggesting adaptable binding sites. (C) Psoralen-NLRP3 revealed high RMSF, emphasizing the receptor's dynamic binding behavior critical for inflammasome modulation.

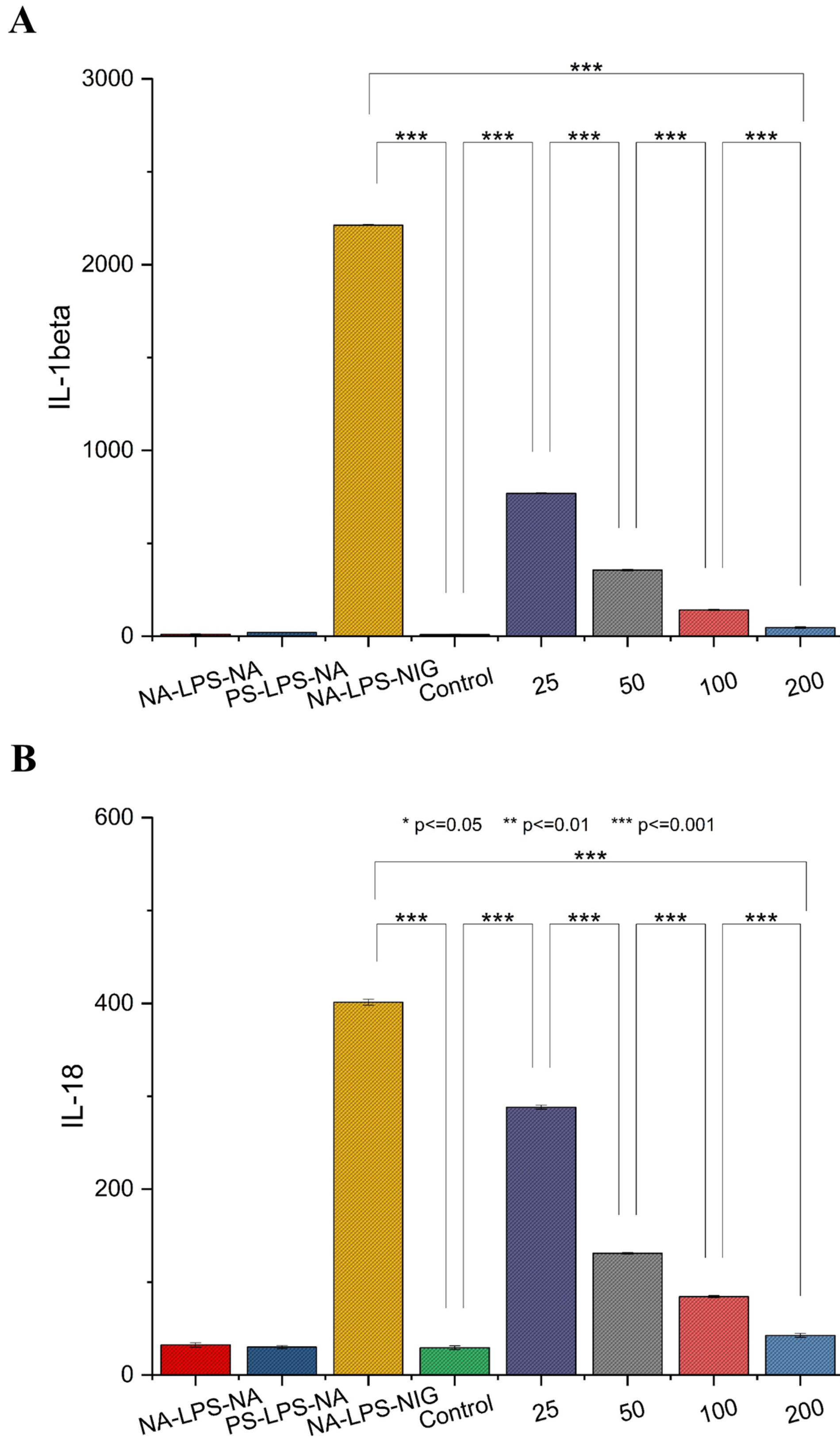

Figure 5: Psoralen inhibits interleukins secretion in THP-1 cells. Cytokine levels significantly increased upon LPS and nigericin treatment but were markedly reduced with psoralen treatment in a concentration-dependent manner, confirming its potent anti-inflammatory effects.

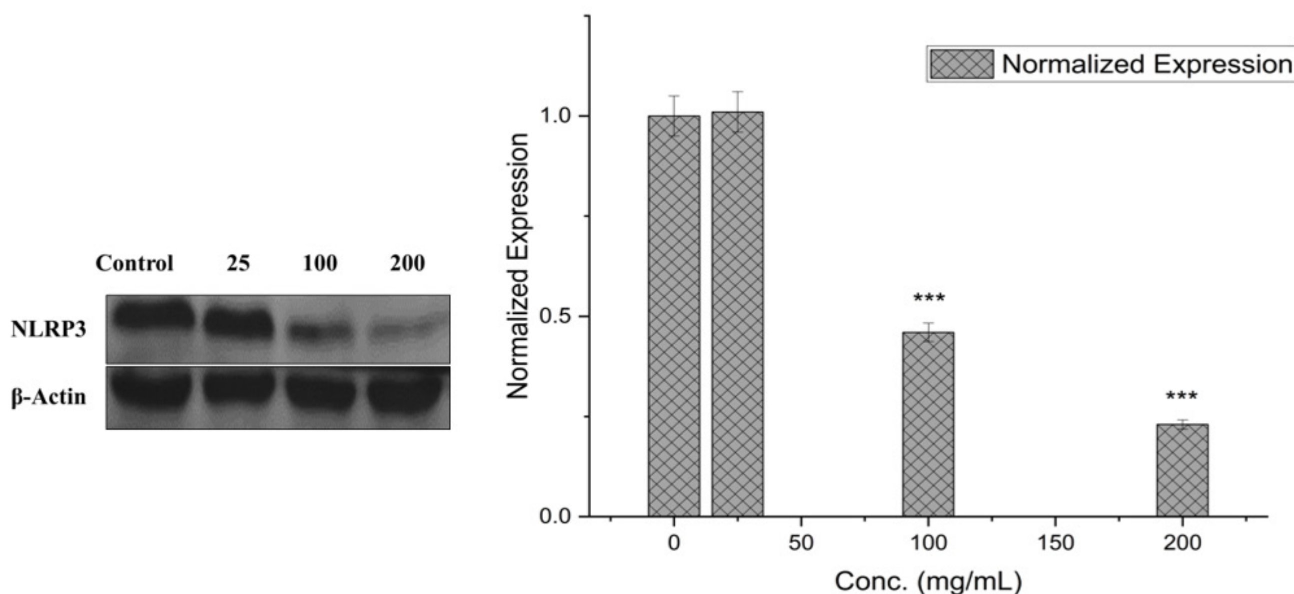

Figure 6: Western blot analysis of NLRP3 protein levels in THP-1 cells treated with psoralen. Psoralen exhibited dose-dependent suppression of NLRP3 expression, aligning with reduced cytokine levels. The β-actin as a loading control validating psoralen's efficacy against NLRP3 inflammasome activation.

the therapeutic value of psoralen against inflammasome-driven inflammatory diseases.

DISCUSSION

The core of RA's inflammatory processes is cytokine dysregulation which plays a vital role in the persistence of inflammation. These cytokines along with the NLRP3 inflammasome, are at the center of the disease's progression, making them essential targets for therapeutic intervention.

Psoralen has exhibited several biological activities, which include antioxidant, anti-proliferative, and anti-inflammatory activities.[18] New studies have further revealed the modulation of signaling pathways by psoralen, that is, key pathways associated in the modulation of immune responses. This includes NF-κB and MAPK pathways, which are central to the activation of inflammatory responses.[14] Of special interest has been its ability to inhibit the NLRP3 inflammasome because this particular inflammasome has been pivotal in RA and other inflammatory diseases.[19]

Psoralen significantly caused anti-inflammatory activity with concentration-dependent effect modulations through IL-1β and IL-18 in a monocytic THP-1 cell line commonly employed for the purpose of in vitro inflammatory response models.[20] Therefore, treatment with psoralen - together with LPS plus nigericin which is very popular among inflammation agents - resulted to marked lower concentration of IL-1β and IL-18 compared to the inflammasome activator-exposed controls, with these implying that psoralen possesses an inhibiting potential upon inflammasome formation. Neither LPS nor nigericin alone exerted a significant effect on cytokine production, implying inhibitory effects of psoralen due to its ability to suppress inflammasome activation rather than the mere decrease in the expression of inflammatory markers. These results support previous findings that showed that psoralen could regulate immune responses and, therefore, serves as a promising pharmacological candidate for inflammatory diseases such as RA.

Moreover, in line with its denaturation of proteins and inhibition of cell proliferation, psoralen also possesses the potential to modulate the IL-1β/IL-18/NLRP3 inflammasome axis. In our proliferation assays, psoralen inhibited growth across both SW-982 and THP-1 cell lines. The anti-proliferative activity likely ties into reduced pro-inflammatory cytokines, which are themselves known to promote proliferation and survival in RA.[21] In RA, increased cytokines promote the proliferation of synovial fibroblasts and immune cells, which lead to inflammation and damage of the joint.[22] The fact that Psoralen suppresses this proliferative response suggests a potential for modulating the immune cell dynamics and tissue remodeling that takes place in RA.

Moreover, protein denaturation is a factor involved in inflammatory diseases such as RA. In RA, prolonged inflammation driven by the pro-inflammatory cytokines leads to destabilization and misfolding of cellular proteins.[23] Misfolding can result to cellular dysfunction, tissue damage, and disease progression. Effects of psoralen on reducing protein denaturation are significant as it implies that psoralen might be useful in maintaining the stability and homeostasis of proteins during inflammation.[24] By targeting the IL-1β/IL-18/NLRP3 inflammasome axis and reducing cytokine production, psoralen may prevent the cellular stress that leads to protein denaturation, providing an additional layer of protection in inflammatory diseases like RA.

In addition to its anti-inflammatory effects, psoralen also appears to target the NLRP3 inflammasome directly. Psoralen would thus modulate inflammasome activation and hence can downregulate IL-1β and IL-18 production; two cytokines whose imbalance causes RA. Psoralen has been proven to regulate the immune responses and reduce inflammation.[25] Through these mechanisms, psoralen provides a multi-faceted approach to the treatment of RA, targeting the underlying immune dysregulation and the subsequent tissue damage caused by excessive inflammation.

The therapeutic potential of psoralen in RA also lies in its ability to modulate key molecular pathways involved in inflammation and cell proliferation. Modulation of the IL-1β/IL-18/NLRP3 inflammasome axis by psoralen not only inhibits cytokine production but also blocks immune cell proliferation and protein denaturation, both of which are crucial to the progression of RA. These findings provide strong evidence for the use of psoralen as a potential therapeutic agent in RA, especially in a targeted approach at modulating the inflammasome and reducing the chronic inflammation that characterizes the disease.

CONCLUSION

In conclusion, the findings of this study are suggestive of potential role of psoralen in modulating the IL-1β/IL-18/NLRP3 inflammasome axis, with subsequent effects on protein denaturation and cell proliferation. Psoralen, with its anti-inflammatory and anti-proliferative properties along with the inhibition of activation of inflammasomes, is a promising agent that needs further exploration in autoimmune and inflammatory diseases, such as rheumatoid arthritis. Further studies are warranted to understand the molecular mechanism underlying these effects and their clinical potential.

ABBREVIATIONS

RA: Rheumatoid Arthritis; **IL-1β:** Interleukin-1β; **IL-18:** Interleukin-18; **ELISA:** Enzyme-Linked Immunosorbent Assay; **BSA:** Bovine Serum Albumin; **NF-κB:** Nuclear factor kappa-light-chain-enhancer of activated B cells; **MAPK:** Mitogen-activated protein kinase; **RPMI:** Roswell Park Memorial Institute; **CCK-8:** Cell Counting Kit-8; **RCSB:** Research Collaboratory for Structural Bioinformatics; **RMSF:** Root Mean Square Fluctuation; **SDS-PAGE:** Sodium Dodecyl-Sulfate Polyacrylamide Gel Electrophoresis; **PVDF:** Polyvinylidene fluoride; **ANOVA:** Analysis of Variance; **LPS:** lipopolysaccharide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Psoralen exhibits significant anti-inflammatory and therapeutic potential against rheumatoid arthritis through multiple mechanisms. The BSA and egg albumin assays demonstrated dose-dependent inhibition of inflammation by psoralen, achieving superior efficacy compared to conventional drugs like aspirin and diclofenac at higher concentrations (200 mg/mL). Psoralen reduced protein denaturation and inhibited inflammatory cell proliferation, particularly in SW-982 synovial cells, suggesting its targeted action against joint inflammation. Molecular docking studies revealed strong interactions between psoralen and inflammatory mediators, including NLRP3, IL-1β, and IL-18. Psoralen showed the highest binding affinity for NLRP3 (-8.953) and significant electrostatic and van der Waals interactions, indicating its role in modulating inflammasome activation. Molecular dynamics simulations confirmed the stability and flexibility of these complexes, highlighting psoralen's adaptability in binding to dynamic inflammatory targets. In cellular assays, psoralen suppressed the secretion of IL-1β and IL-18 cytokines in a concentration-dependent manner and effectively inhibited NLRP3 inflammasome activation. Western blot analysis confirmed reduced NLRP3 protein expression, aligning with decreased cytokine levels. These findings display potent anti-inflammatory properties of psoralen, highlighting its therapeutic potential for managing inflammasome-driven diseases like rheumatoid arthritis through targeted modulation of inflammatory pathways.

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