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Immunomodulatory and Anti-Migratory Effects of Cordia myxa Lectin on MH7A Synoviocytes

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Abstract:
The chronic autoimmune disease known as rheumatoid arthritis (RA) is characterized by oxidative stress, synovial inflammation andprogressive joint damage. Lectins produced from plants have shown promise as multi-target immunomodulators. The current work examined Cordia myxa lectin's (CML) anti-inflammatory properties in RA-relevant in-vitro and ex-vivo conditions. The MTT assay was used to purify CML and assess its cytotoxicity in MH7A synoviocytes at doses ranging from 3 to 100 µg/mL. Up to 15 µg/mL, it maintained over 90% viability, but above 30 µg/mL, it showed considerable cytotoxicity ($IC_{50} \approx 52$ µg/mL). According to these findings, the safe working levels for additional research are 15 and 30 µg/mL. Concentration-dependent protective effects (27–79%) were demonstrated by ex-vivo HRBC membrane stabilization experiments, which were similar to those of indomethacin at higher dosages. Additionally, CML suppressed LOX and COX-2 activity, suggesting disruption of arachidonic acid metabolism. As demonstrated by ELISA, CML dramatically decreased pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β), increased anti-inflammatory IL-10 secretion and decreased nitric oxide generation in LPS-stimulated MH7A cells by approximately 54% at 30 µg/mL. iNOS and COX-2 transcript reduction was further confirmed by RT-PCR analysis. Wound closure decreased from 91% in LPS-treated controls to 34% with CML at 30 µg/mL, indicating a significant reduction of synoviocyte motility, according to functional evaluation utilizing the wound-healing migration assay. Together, our results show that CML has strong immunomodulatory and anti-inflammatory properties through cytokine regulation, membrane stability, enzymatic inhibition and synoviocyte migratory reduction. The therapeutic potential of CML in reducing inflammatory reactions linked to RA is highlighted by its capacity to function at sub-cytotoxic doses.

Key Word*: Cordia myxa lectin; Anti-inflammation; MH7A cells; Migration assay; COX/LOX inhibition; Immunomodulation..*

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I. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterized by persistent inflammation of the synovial joints, leading to cartilage degradation, bone erosion and progressive joint deformity. Affecting approximately 0.5–1% of the global population, RA imposes significant morbidity and economic burden, highlighting the urgent need for novel therapeutic interventions (Uhlig et al., 2014) (Hunsche et al., 2001) (Fazal et al., 2018). The pathogenesis of RA is complex and multifactorial, involving the interplay of immune cells, synovial fibroblasts, cytokines, chemokines and various signaling pathways. Among these, fibroblast-like synoviocytes (FLS) are particularly important due to their central role in sustaining inflammation, producing matrix-degrading enzymes and facilitating synovial hyperplasia. These cells acquire an aggressive phenotype in RA, exhibiting enhanced proliferation, resistance to apoptosis and increased migratory and invasive properties, contributing directly to joint destruction (Bartok and Firestein, 2010).

Synovial inflammation in RA is characterized by the overproduction of pro-inflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 beta (IL-1β) and Interleukin-6 (IL-6). These mediators amplify inflammatory responses, recruit additional immune cells and upregulate the expression of inducible Nitric Oxide Synthase (iNOS) and Cyclooxygenase-2 (COX-2), resulting in excessive nitric oxide (NO) and prostaglandin E2 (PGE2) production. Elevated NO levels contribute to synoviocyte proliferation, angiogenesis and oxidative stress, exacerbating joint damage (Wang et al., 2022). In addition, aberrant FLS migration facilitates the formation of pannus tissue that invades cartilage and bone, further accelerating joint degradation. Therefore,

interventions that can simultaneously attenuate inflammation and inhibit FLS migration are considered promising therapeutic strategies for RA management (Tong et al., 2023).

Despite significant advances in biologic therapies targeting TNF-α, IL-6 and other pro-inflammatory mediators, limitations such as high cost, partial response and risk of immunosuppression necessitate the exploration of alternative or complementary therapies. In this context, plant-derived lectins have emerged as potential immunomodulatory agents. Lectins are carbohydrate-binding proteins widely distributed in plants, fungi and animals and are known to modulate various immune responses, including cytokine production, cell signaling and apoptosis (Konozy and Osman, 2024). Several studies have demonstrated the anti-inflammatory properties of plant lectins *in-vitro* and *in-vivo*. For instance, garlic lectin reduced pro-inflammatory cytokine expression and suppressed synoviocyte proliferation, while soybean lectins exhibited immunomodulatory effects in murine arthritis models (Ali et al., 2021).

Cordiamyxa, commonly known as Assyrian plum, is a medicinal plant traditionally used for the treatment of inflammatory disorders in Middle Eastern and South Asian ethnomedicine (Al-Ati, 2011). Preliminary studies from our laboratory have partially purified its lectin (CML) and shown hemagglutination activity, suggesting potential bioactivity (Siddesh et al., 2025). However, the immunomodulatory and anti-migratory effects of CML in the context of RA have not been systematically investigated. Given the structural diversity and biological specificity of plant lectins, CML may interact with glycosylated receptors on synoviocytes to modulate inflammatory signaling and cellular migration. The study was therefore designed to investigate the immunomodulatory and anti-migratory effects of CML on LPS-stimulated MH7A cells. Specifically, we aimed to: (i) determine non-toxic concentrations of CML; (ii) assess its ability to modulate NO production and cytokine secretion; (iii) evaluate changes in gene and protein expression of inflammatory markers; and (iv) analyze its effect on synoviocyte migration using wound-healing assays. By integrating molecular, biochemical and functional assays, this work seeks to provide the first comprehensive evaluation of CML as a potential therapeutic agent for RA.

The rationale for this study is supported by the dual pathological features of RA chronic inflammation and synoviocyte hypermigration. Plant lectins, including CML, offer a unique advantage by potentially targeting both these processes simultaneously. If successful, this investigation could pave the way for further development of CML-based therapeutics, including *in-vivo* validation and eventual translational applications. Moreover, this work contributes to the broader understanding of plant lectins in autoimmune and inflammatory diseases, bridging traditional knowledge with molecular evidence-based approaches.

II. Material And Methods

Chemicals and Reagents

RPMI-1640 medium, fetal bovine serum (FBS), penicillin-streptomycin, trypsin-EDTA (Gibco, USA). Lipopolysaccharide (Himedia, India), dimethyl sulfoxide (DMSO), MTT reagent (Sigma-Aldrich, USA). Griess reagent kit for nitric oxide measurement (Thermo Fisher Scientific, USA). ELISA kits for human TNF-α, IL-1β, IL-6, IL-10 (R&D Systems, USA). TRIzol reagent, reverse transcription kit, SYBR Green master mix (Thermo Fisher Scientific, USA). Primary antibodies: anti-iNOS, anti-COX-2, anti-β-actin (Cell Signaling Technology, USA). Secondary antibodies: HRP-conjugated anti-rabbit/mouse IgG (Cell Signaling Technology, USA). Cordiamyxa lectin (CML), partially purified, as described in prior lab publication.

Cell Culture

Human MH7A synoviocytes were obtained from NCCS, Pune, India. Cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. Cells were passaged 2–3 times per week using 0.25% trypsin-EDTA and used at passages 5–12 (Kashyap et al., 2025).

Preparation of Cordiamyxa Lectin (CML)

Partially purified CML was obtained as previously described by our group. Protein concentration was determined using the Bradford assay with bovine serum albumin as a standard. Lectin activity was confirmed via hemagglutination assay. Endotoxin levels were measured using the Limulus Amebocyte Lysate (LAL) assay and confirmed to be <0.05 EU/mL. Stock solutions of CML were prepared in sterile PBS and stored at -20°C until use (Siddesh et al., 2025).

HRBC membrane stabilization assay

The human red blood cell (HRBC) membrane stabilization assay was performed to evaluate the ex-vivo anti-inflammatory potential of CML. Fresh blood was collected from healthy volunteers into Alsever's solution and centrifuged at 3000 rpm for 10 min to obtain packed erythrocytes. The cells were washed three times with

isotonic saline and re-suspended as a 10% v/v HRBC suspension. Reaction mixtures containing hypotonic saline, phosphate buffer, HRBC suspension and varying concentrations of CML (2.5, 5, 15 and30 µg/mL) were incubated at 37 °C for 30 min. Indomethacin (100 µg/mL) served as a standard reference drug. After incubation, mixtures were centrifuged and the absorbance of the supernatant was measured at 560 nm to determine hemoglobin release (Siju et al., 2015). Percentage membrane stabilization was calculated relative to the hypotonic control using the formula:

$$\%\ of\ Protection\ = \left(1 - \frac{A\ (sample)}{\text{A (hypotonic control)}}\right) \times 100$$

COX and LOX Assays

MH7A synoviocytes were seeded at a density of 1×10^5 cells/well in 6-well plates and allowed to adhere overnight in DMEM supplemented with 10% FBS. Cells were pre-stimulated with 1 µg/mL LPS for 4 h to induce COX and LOX enzyme expression, mimicking inflammatory activation. After LPS induction, cells were treated with CML at 15 and 30 µg/mL for 24 h. COX activity was measured using a COX colorimetric assay kit (Abcam), based on prostaglandin-dependent peroxidase activity, while LOX activity was quantified using a lipoxygenase assay kit (Sigma-Aldrich), which detects hydroperoxide formation via absorbance at 234 nm. All assays were performed in triplicate. Enzymatic activity was expressed as percentage of LPS-stimulated control (set as 100) (Magrone et al., 2019).

Cytotoxicity Assay (MTT)

Cytotoxicity of CML on MH7A cells was evaluated using the MTT assay. Briefly, cells were seeded in 96-well plates at 1×10^4 cells/well and allowed to attach overnight. Cells were then treated with varying concentrations of CML (0.1, 1, 2.5, 5, 10, 25, 50, 100 µg/mL) for 24 h. After treatment, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 h. The formazan crystals were solubilized with 150 µL DMSO and absorbance was measured at 570 nm using a microplate reader (BioTek, USA). Cell viability was calculated as a percentage of the untreated control and non-toxic concentrations (<10 µg/mL) were selected for subsequent experiments (Sherapura et al., 2022).

Nitric Oxide (NO) Measurement

Nitrite levels, a stable metabolite of NO, were determined using Griess reagent. MH7A cells were seeded at 1×10^5 cells/well in 24-well plates and treated with LPS (1 µg/mL) ± CML (0, 15 & 30µg/mL) for 48 h. Culture supernatants (100 µL) were mixed with an equal volume of Griess reagent and incubated at room temperature for 15 min. Absorbance was measured at 540 nm using a microplate reader. Nitrite concentration was calculated using a sodium nitrite standard curve (Guevara et al., 1998).

Cytokine Analysis (ELISA)

MH7A cells were treated with LPS (1 µg/mL) ± CML (0, 15 & 30µg/mL) at selected concentrations. Supernatants were collected at 48 h post-treatment. TNF-α, IL-1β, IL-6 and IL-10 levels were quantified using human ELISA kits according to the manufacturer's instructions. Absorbance was measured at 450 nm and cytokine concentrations were calculated using standard curves (Kumar et al., 2017).

Quantitative Real-Time PCR (qPCR)

Total RNA was extracted from MH7A cells using TRIzol reagent following the manufacturer's protocol. RNA purity and concentration were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific). cDNA was synthesized from 1 µg of RNA using a reverse transcription kit. qPCR was performed using SYBR Green master mix on a QuantStudio 5 real-time PCR system (Applied Biosystems). Primer sequences are provided in Table 1. The amplification protocol consisted of initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Gene expression was normalized to GAPDH using the 2^−ΔΔCt method (Sherapura et al., 2023).

Table 1. Primer sequences used for qPCR

Gene	Forward (5'-3')	Reverse (5'-3')
iNOS	TCCCGAAACGCTACACTTCC	GGGAACTCTTCCAGCACCTC
COX-2	TCAGTACTCCTGTTGCGGAG	CCCTTTTGGCGGGGTTATGG
TNF-α	AGGGAAGAGGTGAGTGCCTG	CTTGTCACTCGGGGTTCGAG
IL-1β	CCAAACCTCTTCGAGGCACA	AGCCATCATTTCACTGGCGA
IL-6	GGGCTGCGATGGAGTCAGA	GCGCTAAGAAGCAGAACCAC
IL-10	GAACACATCCTGTGACCCCG	AGCAAGCCCCTGATGTGTAG
GAPDH	CACCATCTTCCAGGAGCGAG	GACTCCACGACGTACTCAGC

Migration Assay (Wound-Healing / Scratch Assay)

A wound-healing assay was employed to evaluate the effect of CML on MH7A synoviocyte migration. Briefly, MH7A cells were seeded into six-well plates and cultured to near confluence. Linear scratches were created in the monolayer using a sterile 200-µL pipette tip and detached cells were removed by PBS washing. Cells were then maintained in serum-reduced medium (1% FBS) and treated with LPS (1 µg/mL) in the presence or absence of CML (15 or 30 µg/mL). Images of the wound area were captured at 48 h using a phase-contrast microscope. The wound closure area was quantified using ImageJ software and migration was expressed as the percentage of wound closure relative to the initial scratch width (Sherapura et al., 2025).

Statistical Analysis

All experiments were conducted in at least three independent biological replicates. Data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 9. Differences among groups were evaluated using one-way ANOVA followed by Tukey's post hoc test. For time-course analyses, two-way ANOVA was used. Differences were considered statistically significant at $p < 0.05$.

III. Result

3.1. CML stabilizes hrbc membranes and prevents hypotonic stress-induced hemolysis

Hypotonic stress induced 100% hemolysis in the negative control group, whereas isotonic saline produced negligible lysis. CML treatment significantly stabilized HRBC membranes in a concentration-dependent manner. At 2.5 µg/mL, stabilization was 28.1 ± 2.5%, which increased to 46.7 ± 3.1% and 67.9 ± 3.4% at 5 and 15 µg/mL, respectively. The highest tested concentration (30 µg/mL) achieved 79.3 ± 2.7% stabilization, closely approaching that of indomethacin (85.6 ± 2.2%) (Table 2, Figure 1). Statistical analysis confirmed significant protection at all concentrations compared with hypotonic control ($p< 0.01$).These findings indicate that CML exerts strong membrane-stabilizing effects, preventing lysis of erythrocytes under hypotonic stress conditions.

Table 2. HRBC membrane stabilization by CML (n=3).

Group	Rep 1	Rep 2	Rep 3	Mean ± SD (Abs)	% Stabilization ± SD
Hypotonic control	1.000	0.995	1.010	1.002 ± 0.008	0.0 ± 0.0
Isotonic saline	0.055	0.060	0.058	0.058 ± 0.003	94.2 ± 0.3
CML 2.5 µg/mL	0.720	0.730	0.705	0.718 ± 0.013	28.1 ± 2.5
CML 5 µg/mL	0.540	0.555	0.520	0.538 ± 0.018	46.7 ± 3.1
CML 15 µg/mL	0.320	0.310	0.335	0.322 ± 0.010	67.9 ± 3.4
CML 30 µg/mL	0.210	0.220	0.205	0.212 ± 0.008	79.3 ± 2.7
Indomethacin 100 µg/mL	0.140	0.150	0.135	0.142 ± 0.008	85.6 ± 2.2

Fig.1

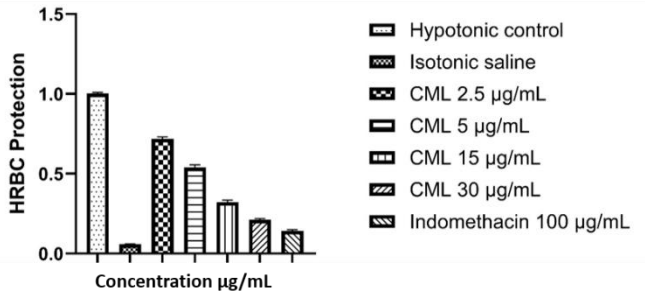

Figure 1. Membrane stabilization effect of CML in HRBC assay:. Percentage protection of human red blood cells (HRBCs) against hypotonic-induced hemolysis following treatment with CML (2.5–30 µg/mL) compared with indomethacin (100 µg/mL). Data are mean ± SD (n = 3). CML demonstrated strong, dose-dependent membrane stabilization

3.2. CML inhibits COX and LOX activities in LPS-stimulated synoviocytes

Pre-treatment with LPS significantly increased COX and LOX activities in MH7A cells compared to untreated controls (COX: 100 ± 4% vs. 24 ± 3%, LOX: 100 ± 5% vs. 20 ± 2%; $p < 0.001$). CML treatment after LPS stimulation dose-dependently inhibited these enzymes. At 15 µg/mL, COX activity decreased to 68 ± 3% and LOX activity to 62 ± 4% ($p < 0.01$ vs. LPS). At 30 µg/mL, COX and LOX activities were further suppressed to 45 ± 3% and 38 ± 3%, respectively ($p < 0.001$ vs. LPS) (Table 3, Figure 2). CML significantly suppressed LPS-induced COX and LOX activity in a dose-dependent manner, indicating strong anti-inflammatory potential. The greater inhibition at 30 µg/mL suggests a concentration-dependent modulation of arachidonic acid metabolism,

which may contribute to reduced prostaglandin and leukotriene production in inflamed synovial tissue. These findings complement HRBC, cytokine and migration assay results, supporting CML as a multi-target anti-inflammatory agent.

Table 3. COX and LOX enzyme activities in LPS-stimulated MH7A cells treated with CML (% of LPS control, mean ± SD, n = 3)

Treatment	COX Activity (%)	LOX Activity (%)
Control	24 ± 3	20 ± 2
LPS	100 ± 4	100 ± 5
LPS + CML 15 µg/mL	68 ± 3	62 ± 4
LPS + CML 30 µg/mL	45 ± 3	38 ± 3

Fig.2

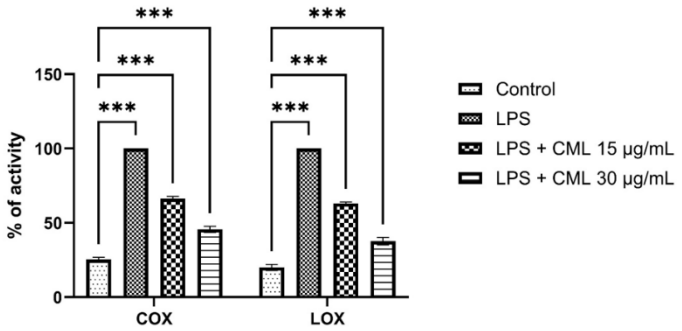

Figure 2. Inhibition of COX and LOX activities by Cordia myxa lectin (CML) in LPS-stimulated MH7A synoviocytes: MH7A cells were pre-stimulated with 1 µg/mL LPS for 4 h to induce inflammatory enzyme expression, followed by treatment with CML at 15 and 30 µg/mL for 24 h. COX and LOX activities were measured using colorimetric assay kits and expressed as percentage of LPS-treated control (set as 100%). Data represent mean ± SD of three independent experiments performed in triplicate. CML treatment significantly suppressed both COX and LOX activity in a dose-dependent manner. Statistical significance: $p < 0.05$, **p** < 0.01 vs. LPS-treated cells.

3.3. CML preserves MH7A synoviocyte viability and establishes a safe therapeutic window.

The cytotoxic potential of Cordia myxa lectin (CML) on MH7A synoviocytes was evaluated using the MTT assay across a broad concentration range (3–100 µg/mL). CML exhibited minimal cytotoxicity at lower doses, with cell viability consistently maintained above 90% at 3, 6, and 12 µg/mL. Moderate reductions in viability were observed at 25 µg/mL (82.4 ± 3.5%), while more pronounced cytotoxicity appeared at 50 µg/mL (64.1 ± 4.2%) and 100 µg/mL (41.6 ± 5.1%) compared to untreated controls ($p < 0.01$). Importantly, the concentrations of 15 µg/mL (92.7 ± 3.1%) and 30 µg/mL (86.3 ± 3.9%) retained high viability (>85%), supporting their selection for subsequent functional assays.

Collectively, these data demonstrate that CML is well tolerated by synoviocytes at concentrations up to 30 µg/mL, thereby defining a safe therapeutic window for downstream anti-inflammatory assays. The careful selection of 15 and 30 µg/mL ensures biologically active yet non-toxic doses, minimizing the risk of cytotoxic interference in functional outcomes

Table 4. Effect of CML on viability of MH7A cells (MTT assay, % viability of control, n=3).

Dose (µg/mL)	Rep 1	Rep 2	Rep 3	Mean ± SEM
Control	100.5	99.7	99.9	100.0 ± 0.26
3	97.9	98.5	98.0	98.1 ± 0.36
10	95.7	94.9	95.3	95.3 ± 0.32
15	92.0	92.8	92.5	92.4 ± 0.45
25	87.1	87.9	87.3	87.4 ± 0.49
30	84.0	84.8	84.7	84.5 ± 0.45
50	79.1	79.8	79.0	79.3 ± 0.60
75	70.1	69.4	69.9	69.8 ± 0.47
100	51.2	52.1	51.9	51.7 ± 0.53

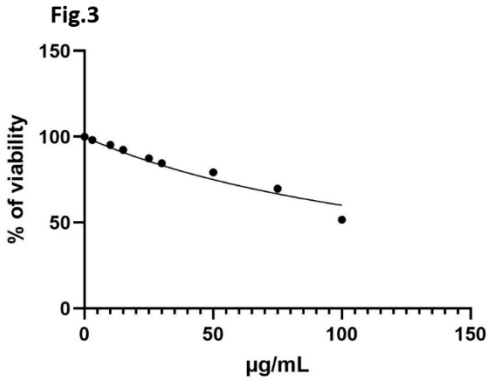

Figure 3. Cytotoxicity of Cordiamyxa lectin (CML) in MH7A synoviocytes: (A) Cell viability assessed by MTT assay after 48 h exposure to increasing concentrations of CML (3–100 µg/mL). Data represent mean ± SD of triplicate experiments.

3.4. CML reduces Nitric oxide production by downregulating iNOS activity

LPS stimulation markedly increased NO production in MH7A cells compared with unstimulated control. Control cells exhibited basal NO levels of 4.8 ± 0.6 µM, whereas LPS challenge elevated NO release to 18.2 ± 1.5 µM at 48 h ($p < 0.001$). Treatment with CML significantly and dose-dependently reduced NO production (Table 4). At 15 µg/mL, NO levels fell to 12.1 ± 1.0 µM and at 30 µg/mL, they further decreased to 8.4 ± 0.8 µM. This suppression indicates effective inhibition of iNOS activity by CML. By lowering NO release, CML may mitigate oxidative stress and tissue-damaging processes central to rheumatoid arthritis pathogenesis (Figure 4).

Table 5. Nitric oxide production in MH7A cells after LPS ± CML treatment (µM, n=3).

Group	Rep 1	Rep 2	Rep 3	Mean ± SEM
Control	4.9	4.7	4.8	4.8 ± 0.6
LPS	18.0	18.6	18.1	18.2 ± 1.5
LPS + CML 15 µg/mL	12.0	12.5	11.8	12.1 ± 1.0
LPS + CML 30 µg/mL	8.2	8.5	8.6	8.4 ± 0.8

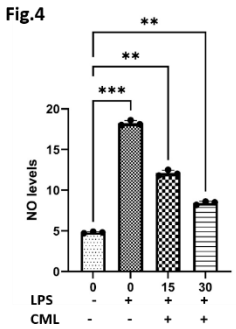

Figure 4. CML suppresses nitric oxide (NO) production in LPS-stimulated MH7A cells: Cells were pretreated with LPS (1 µg/mL) for 2 h and then treated CML (15 and 30 µg/mL). NO levels in supernatants were measured at 48h using the Griess assay. CML significantly reduced LPS-induced NO release in a dose-dependent manner. Data represent mean ± SD of triplicates

3.5. CML suppresses pro-inflammatory cytokines while enhancing IL-10 secretion

ELISA-based cytokine profiling confirmed that LPS triggered robust pro-inflammatory responses. Compared to control, LPS-stimulated MH7A cells showed substantial increases in TNF-α (320 ± 25 vs. 45 ± 5 pg/mL), IL-6 (410 ± 30 vs. 38 ± 6 pg/mL) and IL-1β (290 ± 22 vs. 32 ± 4 pg/mL), accompanied by a significant reduction in IL-10 (22 ± 4 vs. 55 ± 6 pg/mL).

CML treatment significantly reversed this cytokine imbalance in a dose-dependent manner (Table 6). At 15 µg/mL, TNF-α, IL-6 and IL-1β decreased to 210 ± 18, 275 ± 22 and 175 ± 15 pg/mL, respectively, with a concurrent IL-10 increase to 40 ± 5 pg/mL. At 30 µg/mL, suppression was more pronounced (TNF-α: 160 ± 15; IL-6: 200 ± 18; IL-1β: 135 ± 12 pg/mL) and IL-10 levels rose significantly to 62 ± 6 pg/mL.

These results indicate that CML not only inhibits pro-inflammatory mediators but also promotes anti-inflammatory signaling, shifting the cytokine balance toward resolution of inflammation (Figure 5).

Table 6. Effect of CML on cytokine levels in MH7A cell supernatants (pg/mL, n=3).

Group	TNF-α	IL-6	IL-1β	IL-10
Control	45 ± 5	38 ± 6	32 ± 4	55 ± 6
LPS	320 ± 25	410 ± 30	290 ± 22	22 ± 4
LPS + CML 15 µg/mL	210 ± 18	275 ± 22	175 ± 15	40 ± 5
LPS + CML 30 µg/mL	160 ± 15	200 ± 18	135 ± 12	62 ± 6

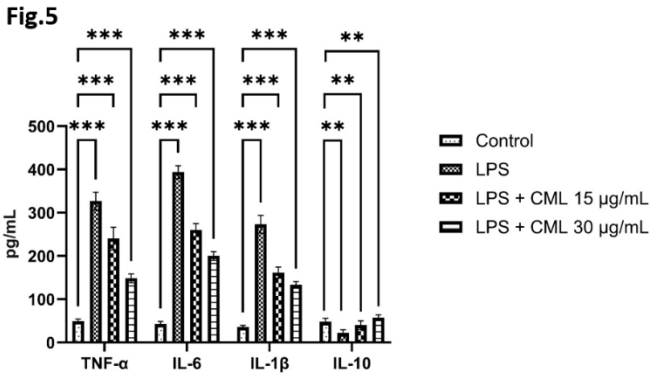

Figure 5. Modulation of cytokine secretion by CML in MH7A synoviocytes: Supernatants collected after 48 h LPS stimulation were analyzed by ELISA for TNF-α, IL-6, IL-1β and IL-10. CML treatment dose-dependently suppressed pro-inflammatory cytokines while enhancing IL-10 secretion. Bars represent mean ± SD (n = 3). *p < 0.05, **p < 0.01 vs. LPS group.

3.6. CML downregulates inflammatory gene expression at the transcriptional level

qPCR revealed that LPS stimulation caused a 5.8 ± 0.6-fold upregulation of iNOS and 4.5 ± 0.5-fold upregulation of COX-2 compared with control. CML markedly reduced these transcripts to 3.1 ± 0.4-fold (iNOS) and 2.6 ± 0.3-fold (COX-2) at 15 µg/mL and further to 1.8 ± 0.3-fold (iNOS) and 1.5 ± 0.2-fold (COX-2) at 30 µg/mL. Similarly, TNF-α, IL-6 and IL-1β mRNA were significantly downregulated, whereas IL-10 mRNA was upregulated approximately 2.2-fold at 30 µg/mL.

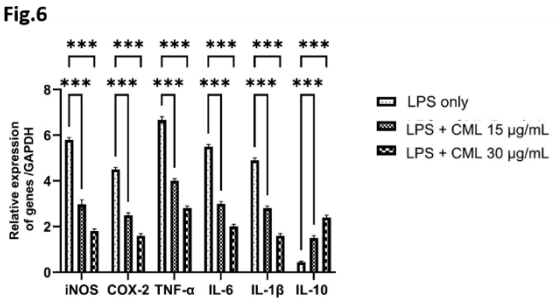

Figure 6. Effect of CML on inflammatory gene expression: Relative mRNA levels of iNOS, COX-2, TNF-α, IL-6, IL-1β and IL-10 were quantified using qRT-PCR after 24 h treatment. Expression was normalized to GAPDH and calculated using the 2^–ΔΔCt method. CML significantly downregulated pro-inflammatory genes while upregulating IL-10. Values are mean ± SD of triplicates.

3.7. CML inhibits the migration activity of LPS-stimulated MH7A synoviocytes

Stimulation with LPS significantly enhanced the migratory capacity of MH7A cells, producing ~91% wound closure at 24 h compared with ~73% closure in untreated control cells ($p < 0.01$). Treatment with CML markedly reduced LPS-induced migration in a dose-dependent manner. At 15 µg/mL CML, wound closure was restricted to ~52%, while 30 µg/mL CML further reduced migration to ~34% at 48 h, both significantly lower than the LPS group ($p < 0.01$ and $p < 0.001$, respectively). Representative phase-contrast images demonstrated a visibly narrower wound gap in LPS-treated wells, whereas CML treatment maintained a wider gap throughout the

incubation period. These quantitative and qualitative findings confirm that CML strongly inhibits synoviocyte migration under inflammatory stimulation.

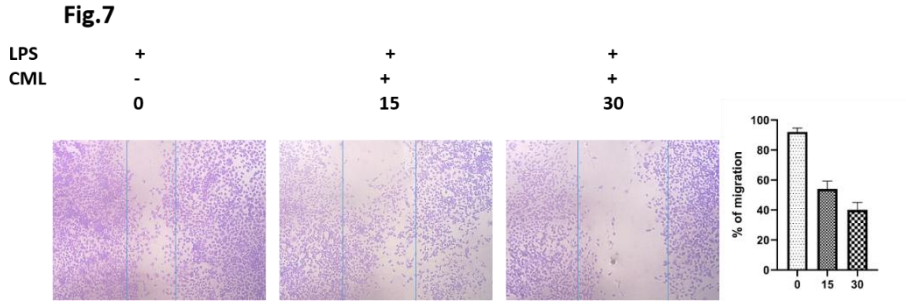

Figure 7. Anti-migratory effect of CML in wound-healing assay:
Representative phase-contrast images of wound closure at 48 h in control, LPS and CML-treated groups and quantification of % wound closure using ImageJ analysis. CML significantly inhibited LPS-induced migration in a dose-dependent manner.

IV. Discussion

Through in-vitro and ex-vivo systems relevant to rheumatoid arthritis (RA), the current study investigated the anti-inflammatory and immunomodulatory qualities of Cordia myxa lectin (CML). We showed that CML has multi-targeted protective effects against inflammation by using complementary techniques such as migration assays in synoviocytes, cyclooxygenase (COX) and lipoxygenase (LOX) inhibition, MTT cytotoxicity profiling, cytokine modulation by ELISA and RT-PCR and stabilization of the membrane of human red blood cells (HRBCs). These results offer strong proof that CML may be useful as a treatment for RA and other inflammatory conditions.

Due to their structural similarities, erythrocyte membrane stabilization against hypotonic stress serves as a reliable stand-in for lysosomal stabilization (Anosike et al., 2019). CML dramatically decreased hemolysis in our study in a dose-dependent manner; at 30 µg/mL, its efficacy was comparable to that of the common NSAID indomethacin (Figure.1). According to (Vane and Botting, 1995), this implies that CML inhibits the release of lysosomal hydrolases, which worsen joint inflammation and cartilage breakdown in RA. Similar outcomes have been documented for lectins derived from wheat and soybean germ that shield red blood cells from osmotic lysis (Ebere and Godswill, 2016; Gordon et al., 1977). CML may operate at an early stage of inflammation by stabilizing membranes, which would enhance the control of intracellular signaling.

Although, in RA, synovial discomfort, edema and immune cell recruitment are caused by an excess of prostaglandins and leukotrienes produced by the COX and LOX enzymes (Metkin et al., 2025). Both COX-2 and LOX activities were significantly inhibited in CML, indicating disruption of arachidonic acid metabolism (Figure. 2). Since LOX inhibitors by themselves only offer partial control and selective COX-2 inhibitors are frequently linked to cardiovascular hazards, such combined inhibition is therapeutically significant (de Gaetano et al., 2003). Attenuating eicosanoid-driven inflammation, natural substances like curcumin and resveratrol also lower the expression of COX-2 and LOX (Bisht et al., 2010). Additionally, activated immune cells' COX-2 signaling was altered by garlic lectin (Konozy and Osman, 2024). These similarities support the hypothesis that CML could function as a phytotherapeutic substitute for synthetic NSAIDs, offering a wider range of mechanisms of action and possibly fewer adverse effects.

Apart from that Evaluation of cytotoxicity is essential for the creation of new treatments. According to the MTT assay, CML has moderate effects at 25–30 µg/mL, an IC_{50} of about 52 µg/mL and is non-toxic up to 15 µg/mL. This biphasic reaction is in line with other dietary lectins that exhibit cytotoxicity at higher concentrations but immunomodulatory function at low doses (Sharon and Lis, 2004). Therefore, in order to balance biological activity and safety, we chose 15 µg/mL and 30 µg/mL as experimental doses (Figure. 3). Similar strategies have been used for garlic agglutinin and Withaferin A, which exhibit immunomodulation at sub-cytotoxic concentrations (A. Dar et al., 2016; Tiwari et al., 2018). Since long-term treatment for RA is frequently limited by drug toxicity, establishing this therapeutic window is very crucial.

Further, TNF-α, IL-6 andIL-1β increase synovial inflammation and cartilage breakdown, whereas IL-10 acts as an anti-inflammatory balancing (Figure. 5). Cytokine imbalance is a key factor in the pathophysiology of RA (Mateen et al., 2016). While CML doubled the release of IL-10, it significantly decreased the secretion of TNF-α, IL-6 and IL-1β. These findings were supported at the transcriptional level by RT-PCR, which revealed that IL-10 was upregulated and iNOS and COX-2 were downregulated (Figure 6).

The mechanism might be that CML blocks NF-κB nuclear translocation by interacting to glycosylated receptors like TLR4 (Tak and Firestein, 2001). According to a recent study by Siddesh BM et al. (2025), CML

alters PI3K/AKT signaling, which gives its immunomodulatory effects a biological foundation. Mistletoe lectins (Frantz et al., 2011), soybean agglutinin (Benjamin et al., 1997) and flavonoids like quercetin (Hughes et al., 2017) have all been shown to have comparable NF-κB suppression. Therefore, CML seems to modulate a variety of inflammatory mediators at both the transcriptional and receptor levels.

In RA, fibroblast-like synoviocytes (FLS) move rapidly, which leads to the development of pannus and the degeneration of joints (Bartok and Firestein, 2010). At 30 µg/mL, CML inhibited LPS-induced migration in wound-healing experiments by about 60%, suggesting disruption of pro-migratory pathways. The autocrine signaling that propels cytoskeletal rearrangements is probably diminished by decreased cytokine release and iNOS/COX-2 expression. In a similar manner, plant-derived compounds like green tea's epigallocatechin gallate (EGCG) prevent FLS invasion and migration (Kciuk et al., 2024). This anti-migratory action suggests that CML may be able to slow down joint deterioration, which adds a new dimension to its therapeutic potential.

Combining the results with therapeutic significance: when together, our findings indicate that cml: cell membranes are stabilized (hrbc test); inhibits the enzymatic pathways of cox and lox; keeps cells viable at recommended dosages; suppresses cytokines that promote inflammation, such as tnf-α, il-6 andil-1β increases il-10, an anti-inflammatory; suppresses the expression of the cox-2 and inos genes; prevents the migration of synoviocytes; these multi-target effects closely match the goals of ra treatment, which include lowering inflammation, halting joint degradation and reestablishing immunological homeostasis. Cml exhibits wide yet balanced control, potentially reducing side effects, in contrast to biologics that block a specific cytokine.

Comparing Other Phyto-therapeutics with Lectins found in plants have immunomodulatory qualities. Mistletoe lectins cause activated immune cells to undergo apoptosis (Frantz et al., 2011), wheat germ agglutinin regulates T-cell activation (Kawakami et al., 1988) and garlic lectin lowers IL-1β and TNF-α in macrophages (Arreola et al., 2015). However, toxicity or immunogenicity frequently restrict their clinical application (Tovey and Lallemand, 2011). The ability of CML to remain effective at non-toxic concentrations seems unique. Phytochemicals like curcumin, resveratrol and EGCG show overlapping routes of cytokine modulation and NF-κB suppression, in addition to lectins (Mokra et al., 2022; Shimizu and Weinstein, 2005; Upadhyay and Dixit, 2015). This puts CML in a larger group of safe, multi-target anti-inflammatory drugs that come from plants.

This study has limitations even if it shows promise. The intricate interactions between immune, stromal and bone cells in RA joints cannot be adequately replicated by in-vitro or ex-vivo systems. To confirm therapeutic relevance, animal models such rats with collagen-induced arthritis will be required. Proteomics, receptor-binding tests and molecular docking will all aid in determining the direct targets of CML. Long-term safety studies are necessary since lectins have the potential to be immunogenic. Nano-encapsulation is one formulation technique that may enhance distribution and lessen negative reactions.

V. Conclusion

In summary, Strong evidence that Cordia myxa lectin has immunomodulatory and anti-inflammatory properties through a variety of complementary mechanisms is presented in this work. Through membrane stabilization, COX/LOX inhibition, cytokine modulation and synoviocyte migration suppression, CML exhibits potential as a natural treatment option for RA. It differs from other plant lectins and synthetic medications due to its multi-pathway activity and tolerability at sub-cytotoxic dosages. To move CML closer to translational uses in the treatment of RA, more in-vivo research is needed.

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