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Genipin Ameliorates Experimental Rheumatoid Arthritis Through Modulation of Inflammatory Cytokines: Integrated *In Vivo*, Molecular Docking, and ADMET Studies

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Abstract

Rheumatoid arthritis (RA), a chronic inflammatory condition, affects $\approx 1\%$ of population. Existing treatments are costly and associated with adverse effects, highlighting the need for safer, cost-effective alternatives. This study investigated the anti-arthritic potential of genipin using a complete Freund's adjuvant (CFA)-induced RA-model. Thirty-six female albino rats were randomly divided to six groups. Arthritis was induced in the right hind paw, and genipin was administered from day 8 through day 28. Disease progression was monitored via arthritic scoring and plethysmometer on days 7, 14, 21, and 28. On completion, blood was collected for hematological and cytokine expression assessment. Spleen and thymus index were evaluated. Ankle joints were subject to radiographic and histopathological examination. Genipin dose-dependently retarded RA progression and normalized hematological parameters red blood cells, white blood cells, hemoglobin, platelets and erythrocyte sedimentation rate to 7.117 ± 0.1537 , 8.542 ± 0.1763 , 12.17 ± 0.2108 , 721.3 ± 36.96 and 13.00 ± 0.5774 along with spleen and thymus indices to 4.250 ± 0.0562 and 2.133 ± 0.0714 respectively. RT-PCR revealed reduced mRNA expression of pro-inflammatory markers COX-2, IL-6, TNF- α , and NF- κ B to 27.47 ± 0.5363 , 28.17 ± 0.4620 , 28.45 ± 0.4816 and 27.94 ± 0.3848 respectively. While the anti-inflammatory marker IL-4 showed increased expression to 31.70 ± 0.6811 . Radiographic and histopathological findings were similarly improved. Molecular docking studies demonstrated strong binding affinity of genipin for COX-2 and TNF- α . ADMET analysis confirmed favorable pharmacokinetic and safety profiles. Collectively, these findings position it as a promising candidate for further development as an RA therapy.

Keywords: Rheumatoid arthritis, genipin, complete freund's adjuvant, in-silico studies

1. Introduction

Rheumatoid arthritis (RA) is a prevalent inflammatory ailment, characterized by pain, stiffness and inflammation (Brown *et al.*, 2024). It is an auto immune disease caused by the

dysfunction of immune system in which anti bodies are produced by immune system against healthy cells, tissues and organs (Zeng *et al.*, 2022). The prevalence of RA has been estimated to be between 0.5% and 1.0% in Western Europe and North America, while it seems to be lower in emerging nations (Venetsanopoulou *et al.*, 2022). Joint pain, swelling, and stiffness are the initial symptoms of rheumatoid arthritis (RA), which eventually leads to edema, pannus development, and bone degradation, mostly in smaller joints. When aberrant cell development reaches the synovium, it triggers bone degradation, fibrosis, inflammation, and the release of inflammatory markers such TNF- α and IL-6 (Michaud *et al.*, 2021). It is reported that 40 among 1000000 people suffer from RA (Dar *et al.*, 2023). Conventionally, the treatment of RA involved the use of non-steroidal anti-inflammatory drugs (NSAIDs) and steroids, but these drugs only elevate symptoms without retarding the progression of the disease (Padjen *et al.*, 2020). These drugs are associated with several unwanted effects including GI ulcers, renal failure, hypertension, serious CVS effects and may worsen the preexisting heart failure (Afreen *et al.*, 2024). Glucocorticoids administration in patients can result in hyperglycemia especially in diabetic patients. Other adverse effects of glucocorticoids include skin rash, compromised immunity, hair fall and erythema (Stone *et al.*, 2021). Another category of compounds used for the relief of inflammation are disease-modifying anti rheumatic drugs (DMARDs), which include conventional DMARDs, biological DMARDs (bDMARDs), targeted DMARDs (tsDMARDs) (Ding *et al.*, 2023, Cronstein *et al.*, 2020). The use of DMARDs is associated with increased risk of infection including respiratory tract, joint, soft tissue infection and cancer (Li *et al.*, 2021). Because of these unfavorable effects, current treatment options are not preferred for RA. Scientists are exploring new phyto-chemicals for the treatment of various ailments on account of their low toxicity and low cost (Shirsath & Goswami., 2020). Plants produce two types of phytochemicals, primary metabolites, that are essential for normal growth and development of plants, and secondary metabolites, which help plants adapt to their environment and defend against threats. Secondary metabolites are particularly significant due to their diverse roles in plant physiology and biochemistry (Bitwell *et al.*, 2023). Glycosides, terpenoids, phenolic compounds and iridoid glycosied are examples of secondary metabolites produced by plants (Elshafie *et al.*, 2023). Iridoid glycosides are monoterpenoids, produced by various species of plant kingdom as secondary metabolites. They processes various pharmacological activities. Major compounds belonging to this class includes genipin, geniposide, loganin and brasoside (Wang *et al.*, 2020). The iridoid derivative genipin (GN) is primarily extracted from the fruits of *Gardenia jasminoides* and *Genipa americana*, that are ever living flowering plants

belonging to Rubiaceae family (Wahab, 2024, Chen *et al.*, 2020). GN is a white, crystalline powder that dissolves in water. It has an ester group and a dihydropyran ring in its structure (Ahmad *et al.*, 2024).

Various pharmacological characteristics of genipin have been extensively studied. Its preventive benefits against stomach injuries have been shown in earlier research (Zhao *et al.*, 2020). Additionally, Anticancer activity and antiviral activity were further demonstrated (Kim *et al.*, 2020). The aim of the study was to explore the anti-rheumatoid arthritis activity of genipin through experimental and computational approaches.

2. Material and Methods

2.1. Chemicals

Genipin, CAS no. 6902-77-8, Lot no. C17477896 was purchased from Shanghai Macklin Biochemical Technology Co., Ltd, Complete Freund's Adjuvant (CFA), Piroxicam, DMSO-4, Chloroform and Distilled water was purchased from Sigma-Aldrich.

2.2. Complete Freund's Adjuvant Induced Rheumatoid Arthritis Model

Thirty-six female albino rats 6 to 8 weeks old and weighing between 200g to 250g were used in the study. Rats were given feed 14 and freshwater *ad libitum* and were housed at standard laboratory conditions with 22° C–25° C temperature and a 12:12 hr light-dark cycle (Pardhan *et al.*, 2021). Experiment was performed after receiving ethical consideration from ethical committee of Minhaj University Lahore. Animals were randomly divided into 6 groups, each with 6 rats (n=6) (Dar *et al.*, 2023).

Table 1: Animal Groups

Group 1	Normal group	Distilled water was administered.
Group 2	Negative control group (Diseased)	CFA (without treatment)
Group 3	Positive control group (Standard)	CFA + piroxicam (10mg/kg)
Group 4	Treatment group I	CFA + genipin (50mg/kg)
Group 5	Treatment group II	CFA + genipin (75mg/kg)
Group 6	Treatment group III	CFA + genipin (100mg/kg)

Arthritis was induced in all groups on day 0, except normal group, by injecting 0.15ml of CFA in the left hind paw of rats and left for 7 days to induce arthritis. Treatment started on day 8 till day 28 (Dar *et al.*, 2023).

Paw edema was measured on day 7, 14, 21 and 28 by using digital water displacement plethysmometer (Alshehri *et al.*, 2023).

After 28 days, animals were sacrificed and blood was collected by cardiac puncture. Hematological parameters including Hb, RBC's, WBC's, ESR and platelets were evaluated by using hematology analyzer (Nazir *et al.*, 2024). Ankle joints were removed and subject to histopathological analysis for pannus formation and bone erosion (Alzarea *et al.*, 2022). Ankle joints were also subject to radiographic analysis for determination of joint deformation (Dar *et al.*, 2023). Spleen and thymus were removed for spleen and thymus index. Total RNA was extracted from collected blood samples and RT PCR was conducted (Nawaz *et al.*, 2023).

2.3. *In Silico* Analysis

Genipin was subject to molecular docking against human COX-2 (PDB ID: 5KIR) and TNF- α (PDB ID: 2AZ5) using biovia discovery studio 2025. Ligands were prepared using autodock vina 1.5.7 and the receptor grid was generated around the active site (Saleem *et al.*, 2026).

2.4. ADMET Studies

To evaluate the pharmacokinetic and toxicity profile of genipin ADMET studies were performed using SwissADME and Protox 3.0 (Arshad *et al.*, 2025).

2.5. Statistical Analysis

Graph Pad Prism 10 was opted for the analysis of data. Data was presented as mean $\pm$ S.E.M (standard error of mean). Data were analyzed by one way or two-way ANOVA followed by Tukey's post-hoc test. Level of significance $P < 0.05$ was used.

3. Results

Complete Freund's Adjuvant-Induced Rheumatoid Arthritis Model

3.1. Evaluation of Arthritic Progression

Inflammation, redness and erythema were observed by macroscopic examination and scored by scoring system explained in table 2 (Nazir *et al.*, 2024).

Table 2: Scoring System to Evaluate Severity of Disease

Score	Evaluation
0	No edema and erythema
1	Edema and erythema of the paw
2	Mild edema and erythema of the paw
3	Gross edema and erythema of the paw
4	Serious abnormality and limb immobility

Significant difference in the arthritic score of negative control and treatment groups were observed. Gradual and dose dependent fall in arthritic score was observed in treatment groups. In contrast, gradual raise in arthritic score was observed in negative control Table 3.

Table 3: Effect of genipin on arthritic score

Treatment Groups					
Days	Negative Control	Positive Control	Treatment Group I	Treatment Group II	Treatment Group III
Mean $\pm$ S.E.M					
Day 7	2.167 $\pm$ 0.1667	2.333 $\pm$ 0.2108	2.167 $\pm$ 0.1667	2.333 $\pm$ 0.2108	2.333 $\pm$ 0.2108
Day 14	3.500 $\pm$ 0.2236 ^{###}	3.333 $\pm$ 0.2108	3.500 $\pm$ 0.2236 ^{***}	3.333 $\pm$ 0.2108 ^{***}	3.167 $\pm$ 0.1667 ^{***}
Day 21	3.667 $\pm$ 0.2108 ^{###}	2.833 $\pm$ 0.3073	3.333 $\pm$ 0.2108 ^{***}	3.000 $\pm$ 0.2582 ^{***}	2.667 $\pm$ 0.2108 ^{***}
Day 28	3.833 $\pm$ 0.1667 ^{###}	2.500 $\pm$ 0.2236	2.667 $\pm$ 0.2108 ^{***}	2.500 $\pm$ 0.3416 ^{***}	2.167 $\pm$ 0.1667 ^{***}

Disease control is represented by ^{###} while treatment controls are represented by ^{***}

3.2. Evaluation of Paw Volume. More paw edema was observed in negative control as compared to treatment groups. The gradual and dose dependent reduction in paw edema in treatment groups confirms the anti arthritic activity of the compound (Table 4).

Table 4: Effect of genipin on paw edema in CFA induced model

Days	Negative Control	Normal Group	Positive Control	Treatment Group I	Treatment Group II	Treatment Group III
Mean $\pm$ S.E.M						
Day 7	0.8617 $\pm$ 0.04438	0.3367 $\pm$ 0.01892	0.8750 $\pm$ 0.03704	0.8700 $\pm$ 0.04250	0.8850 $\pm$ 0.04288	0.9083 $\pm$ 0.03390
Day 14	1.422 $\pm$ 0.03439 ^{###}	0.3717 $\pm$ 0.01815	0.9033 $\pm$ 0.01726	1.133 $\pm$ 0.04984 ^{***}	1.048 $\pm$ 0.05425 [*] ^{**}	0.8800 $\pm$ 0.01549 ^{***}
Day 21	1.858 $\pm$ 0.03280 ^{###}	0.3617 $\pm$ 0.01682	0.8650 $\pm$ 0.02446	1.0830 $\pm$ 0.01174 ^{**} [*]	0.9717 $\pm$ 0.01778 ^{***}	0.8383 $\pm$ 0.01078 ^{***}
Day 28	2.010 $\pm$ 0.03033 ^{###}	0.3750 $\pm$ 0.01478	0.7783 $\pm$ 0.02713	0.9033 $\pm$ 0.01256 ^{**}	0.8433 $\pm$ 0.01520	0.7433 $\pm$ 0.012

28				*	***	56***
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In this table disease control is represented by ### while treatment controls are represented by *** .

3.3.Hematological Parameters

The Findings of analysis showed higher WBC, platelets and ESR in negative control as compared to treatment groups. In contrast, RBC and Hb were significantly lowered in negative control as compared to treatment groups. Data suggests the improvement in hematological parameters in rats treated with genipin bringing all parameters to normal levels (Table 5).

Table 5: Evaluation of hematological parameter in arthritic rats

Parameters	Normal Group	Negative Control	Positive Control (Piroxicam 10mg/kg)	Treatment Group I (GN 50mg/kg)	Treatment group II (GN 75mg/kg)	Treatment Group III (GN 100mg/kg)
Mean ± S.E.M						
RBC's (10 ⁶ /UI)	7.450 ± 0.1945	5.233 ± 0.2140 ###	6.600 ± 0.1966	6.267 ± 0.2171 ***	6.467 ± 0.2603 ***	7.117 ± 0.1537 ***
Hb (g/dl)	12.92 ± 0.3351	8.683 ± 0.4505 ###	10.43 ± 0.1820	10.03 ± 0.2848 ***	10.45 ± 0.2778 ***	12.17 ± 0.2108 ***
WBC's 10 ³ /UI)	8.130 ± 0.1810	10.57 ± 0.1838 ###	8.533 ± 0.2591	9.022 ± 0.2577 ***	8.667 ± 0.2789 ***	8.542 ± 0.1763 ***
Platelets (10 ³ /UI)	574.0± 47.48	997.00 ± 36.94 ###	795.8 ± 27.57	892.9 ± 22.26 ***	799.2 ± 29.16 ***	721.3 ± 36.96 ***
ESR	11.33 ± 0.4944	19.50 ± 0.5578 ###	13.00 ± 0.3651	14.67 ± 0.6146 ***	14.17 ± 0.6009 ***	13.00 ± 0.5774 ***

In this table disease control is represented by ### while treatment controls are represented by *** .

3.4. Histopathological Analysis

After completion of experiment on day 28, rat’s paw was subject to histopathological analysis. Negative control represented the progression of disease showing signs of RA including pannus formation, cell infiltration and zone of inflammation. Positive control

showed moderate pannus formation. Findings from treatment control specify improvement in arthritic symptoms. Treatment groups shown significant improvement in the disease (Figure 1).

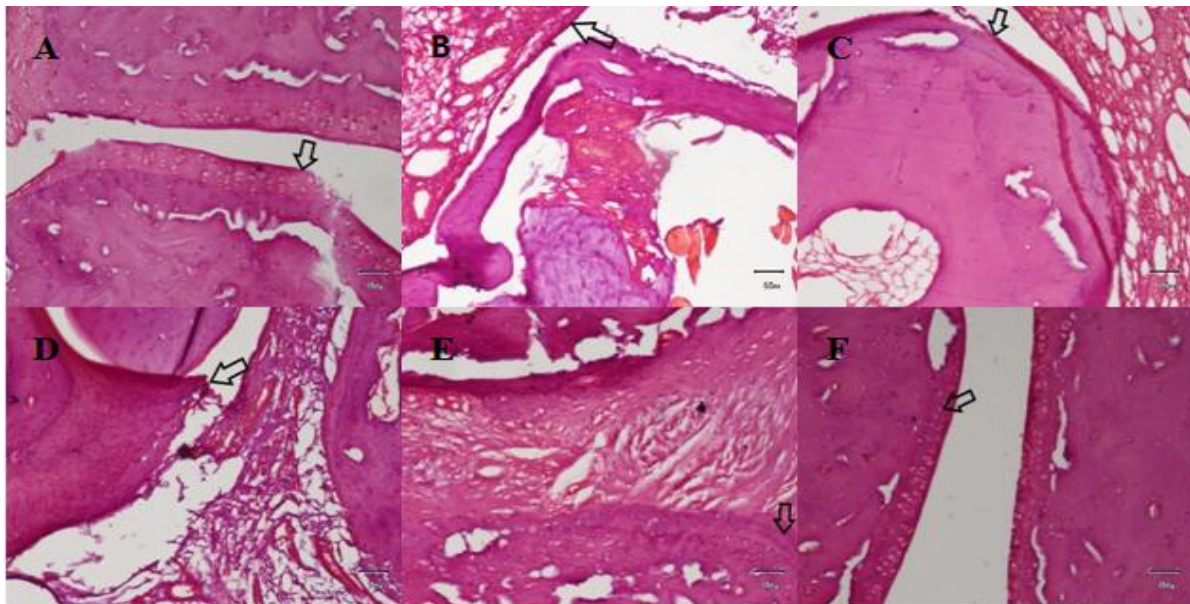


Figure 1: Hematoxylin and eosin staining of the rat's paw (A) Normal group (B) Negative control (C) Positive control (PXM 10 mg/kg) (D) Treatment group I (GN 50 mg/kg) (E) Treatment group II (GN 75 mg/kg) (F) Treatment group III (GN 100 mg/kg).

3.5. Radiographic Analysis

Analysis of X ray images revealed deformity and severity of the disease in negative control while in treatment groups genipin dose dependently preserved the joints from deformity and damages. Genipin at high dose clearly retarded the progression of the disease and protected the bones and joints (Figure 2).

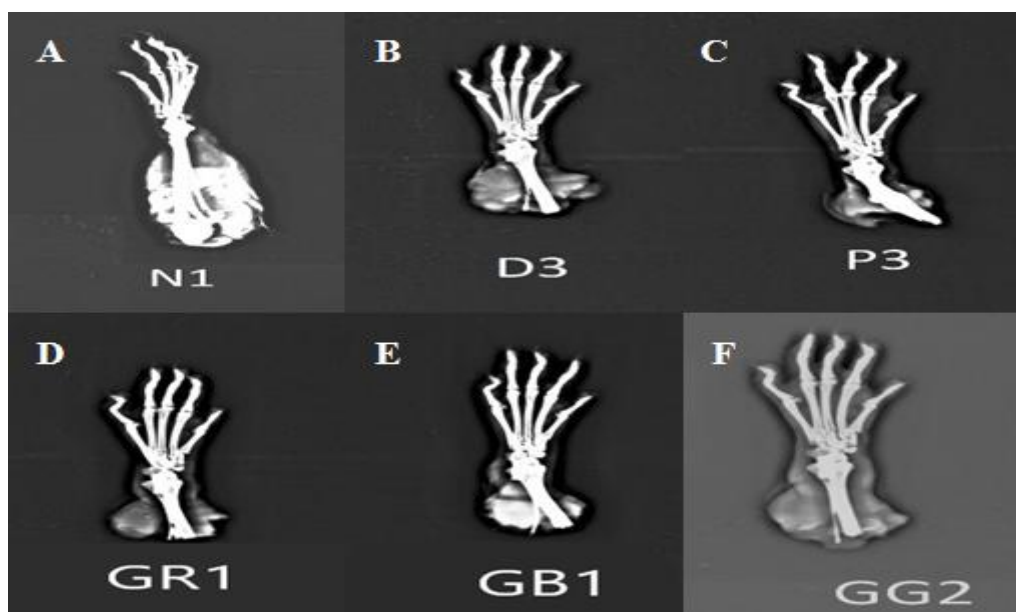


Figure 2: Radiographs of the ankle joints, (A) Normal group (B) Negative control (C) Positive control (PXM 10 mg/kg) (D) Treatment group I (GN 50 mg/kg) (E) Treatment group II (GN 75 mg/kg) (F) Treatment group III (GN 100 mg/kg)

3.6. Analysis of Spleen and Thymus Index

High spleen and thymus index were observed in negative control showing the active immune response in rats. Treatment groups, in contrast, lowered the spleen and thymus index, representing the immunomodulatory activity of the compound (Table 6).

Table 6: Evaluation of spleen and thymus index in arthritic rat

Parameters	Normal Group	Negative Control	Positive Control (Piroxicam 10mg/kg)	Treatment Group I (GN 50mg/kg)	Treatment group II (GN 75mg/kg)	Treatment Group III (GN 100mg/kg)
Mean ± S.E.M						
Spleen Index	3.465 ± 0.04822	5.750 ± 0.09574 ###	4.200 ± 0.1390	5.200±0.1065***	4.813 ± 0.1495***	4.250±0.05627***
Thymus Index	1.820 ± 0.02921	2.858 ± 0.07120###	2.100 ± 0.08944	2.567±0.04944***	2.433 ± 0.09888***	2.133±0.07149***

In this table disease control is represented by ### while treatment controls are represented by ***.

3.7. Assessment of Inflammatory Cytokines.

Expression levels of pro-inflammatory cytokines, COX-2, TNF- α , IL-6 and NF- κ B, were found higher in negative control as compared to treatment groups. The expression level of anti-inflammatory cytokine, IL-4, was found high in treatment groups while there was significantly low level in negative control. Genipin, at significantly high dose (100mg/kg) regulated the inflammatory cytokines by down regulating the pro-inflammatory cytokines and up regulating the anti-inflammatory cytokines (Figure 3).

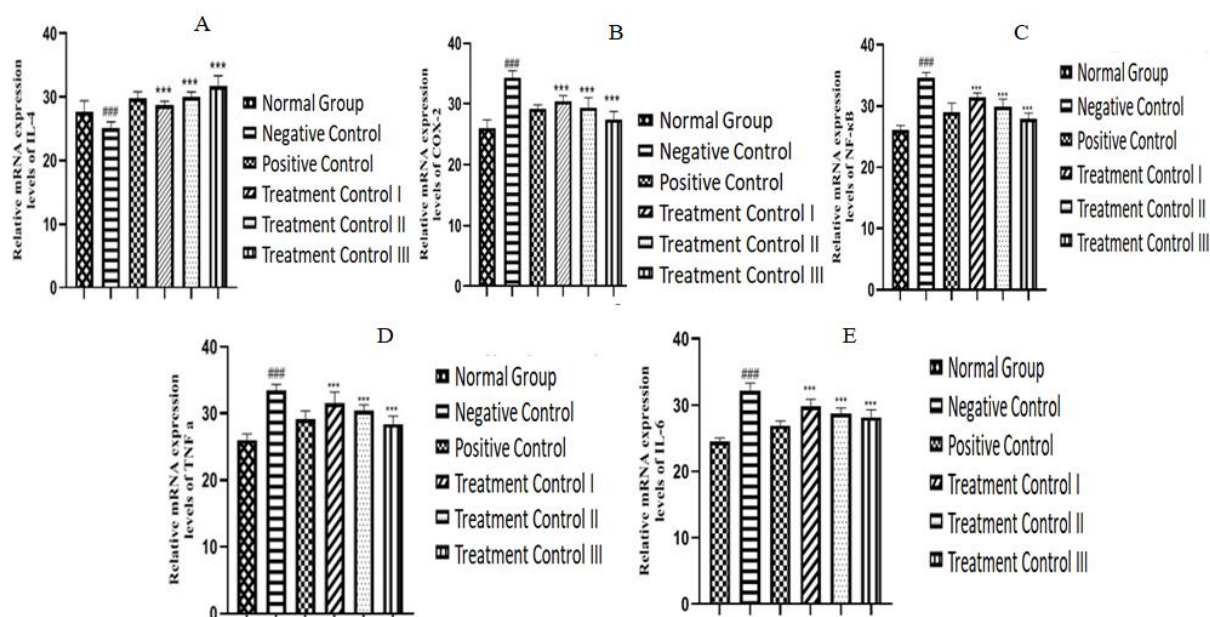


Figure 3: Effect of genipin on cytokines level (serum) of CFA induced rat (A) IL-4, (B) COX-2, (C) NF-κB, (D) TNF-α, (E) IL-6. All data is represented as $\pm$ S.E.M. Statistical analysis was performed via one way ANOVA followed by post hoc tukey test. Negative control is represented by ### while treatment controls are represented by ***

3.8.Molecular Docking

Molecular docking studies of genipin were performed on human COX-2 (PDB ID: 5KIR) and TNF-α (PDB ID: 2AZ5). These are major inflammatory cytokines involved in inflammatory process. COX 2 is responsible for production of prostaglandins that results in pain and inflammation (Ruslin *et al.*, 2022). TNF-α is a pro inflammatory cytokine produces by macrophages, T cells and mast cells. It is one of the major cytokine involved in chronic inflammation (Jang *et al.*, 2021).

Piroxicam exhibited binding energy of -9.1 kcal/mol with COX-2 and genipin exhibited binding energies of -7.5 kcal/mol with COX-2. Similarly, piroxicam exhibited binding energy of -9.4 kcal/mol with TNF-α and genipin exhibited binding energies of -7.3 kcal/mol with TNF-α. (Table:7)

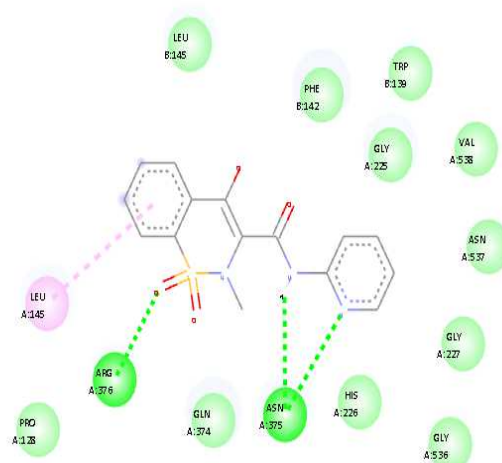
Table 7: Molecular Docking analysis of genipin and piroxicam

Binding Energies kcal/mol		
Receptor	Genipin	Piroxicam
COX-2	-7.5	-9.1
TNF- α	-7.3	-9.4

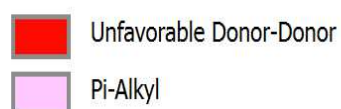
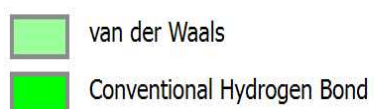
Hydrogen bonding revealed that Piroxicam formed five conventional hydrogen bonds with the COX-2 receptor, involving residues ASN37 (3.05 Å), ARG37 (2.15 Å), ARG37 (2.23 Å),

ASN375 (3.03 Å), and ASN375 (1.92 Å), reflecting strong and stable polar interactions within the active site. Cross-referencing with the 2D interaction diagram further confirmed the involvement of key residues ARG376, ASN375, GLN574, and HIS226 in hydrogen bonding interactions with the ligand. A hydrophobic pi-alkyl interaction additionally stabilized Piroxicam within the binding pocket, involving residue LEU145 at a distance of 4.48 Å. Van der Waals interactions with surrounding residues, including LEU145 (B:145), PHE142, TRP139, GLY225, VAL538, ASN537, HIS226, GLY227, GLY536, PRO128, and GLN574, further contributed to the overall stability of the Piroxicam–COX-2 complex. Similarly, genipin formed two conventional hydrogen bonds with the COX-2 receptor, involving residues CYS47 and GLN461, at distances consistent with strong hydrogen bond interactions. Additionally, one carbon-hydrogen bond was observed with residue CYS47, further contributing to the ligand's stability within the binding site. Unfavorable acceptor-acceptor interactions were also detected with residues CYS41 and GLN461, as indicated by the red dashed lines in the 2D interaction diagram. An alkyl interaction was observed with residue PRO153, providing additional hydrophobic stabilization of the ligand within the binding pocket. Van der Waals interactions further supported the binding, involving surrounding residues including VAL46, GLN42, ASN43, ARG44, GLY45, CYS36, HIS39, LEU152, and GLU465, collectively contributing to the overall stability of the Genipin–COX-2 complex (Figure 4).

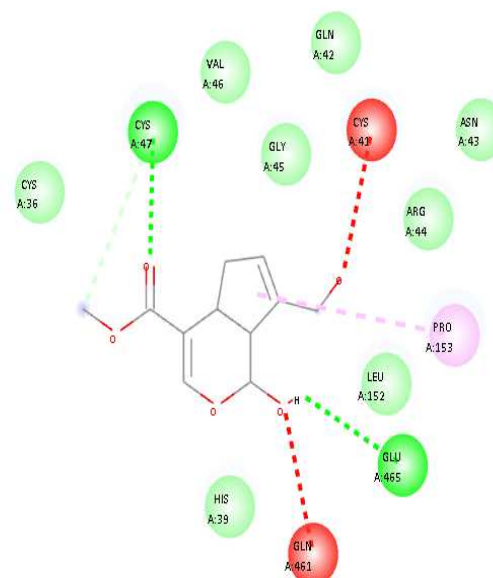
A



Interactions



B



Interactions

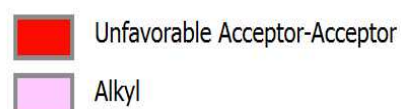
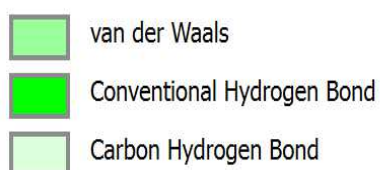


Figure 4: 2-D Molecular Docking analysis of (A) Piroxicam with COX-2 (B) Genipin with COX-2

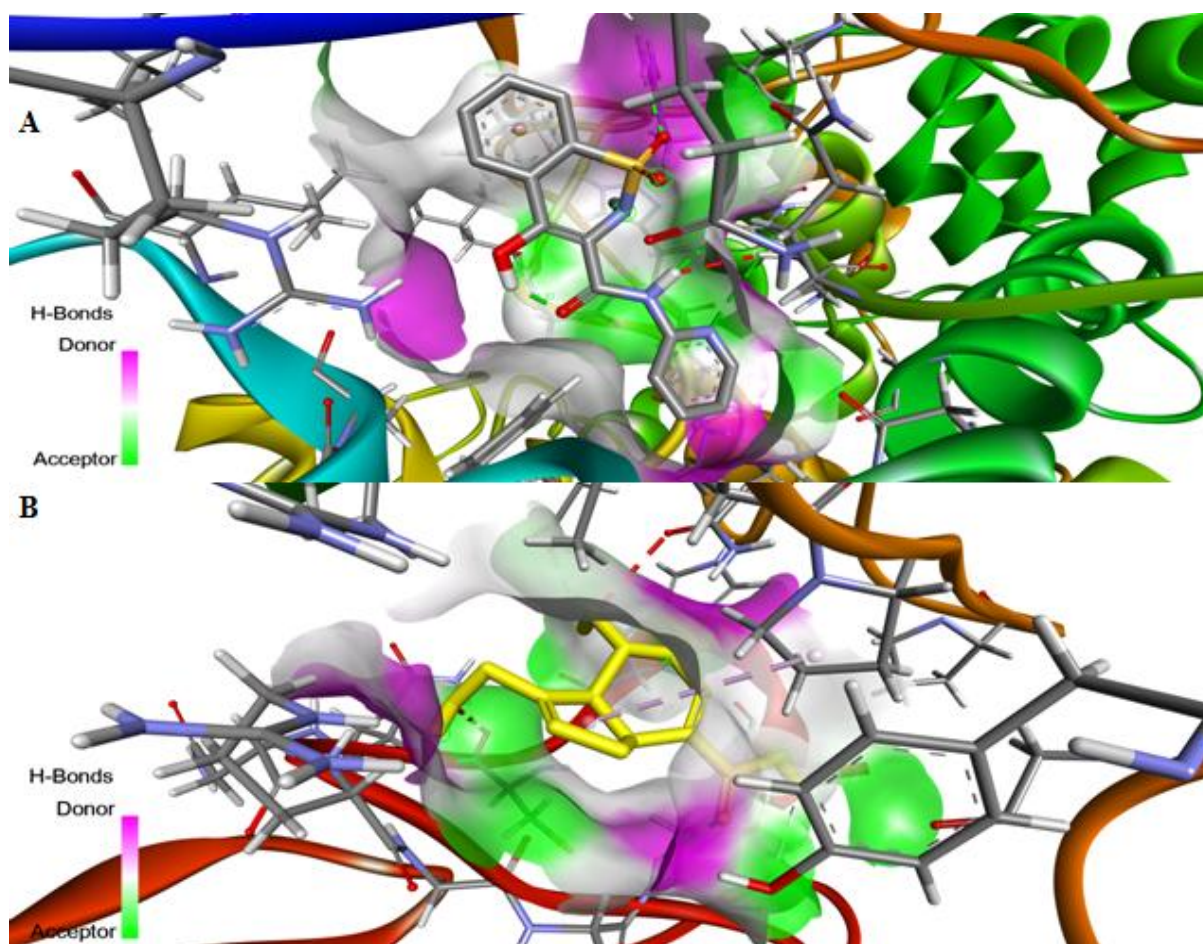
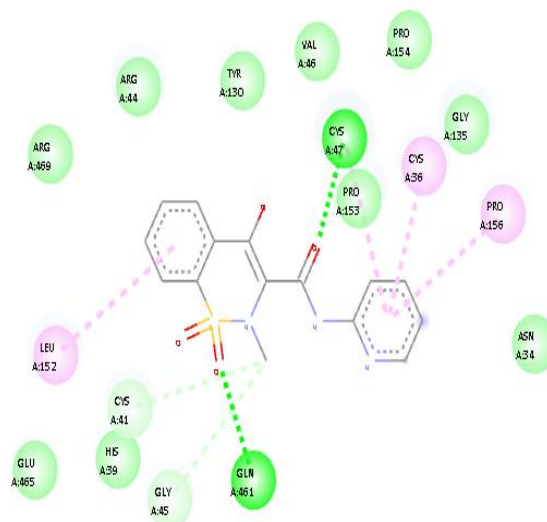


Figure 5: 3-D Molecular Docking analysis of (A) Genipin with COX-2 (B) Piroxicam with COX-2

Hydrogen bonding analysis revealed that Piroxicam formed two conventional hydrogen bonds with the TNF receptor, involving residues CYS47 and GLN461 at distances of 2.43 Å and 2.14 Å, respectively. Additionally, two carbon-hydrogen bonds were observed with residues CYS41 and GLY45, at distances of 3.74 Å and 3.35 Å. Hydrophobic pi-alkyl interactions further stabilized the ligand within the binding pocket, involving residues LEU152 (5.07 Å), CYS36 (4.58 Å), CYS47 (5.38 Å), and PRO156 (4.81 Å). Likewise, analysis of genipin with TNF- α revealed that genipin formed a conventional hydrogen bond at a distance of 2.05 Å, indicating a strong polar interaction with the receptor binding pocket. In addition, hydrophobic interactions were observed, including an alkyl interaction with residue VAL523 at a distance of 5.35 Å and a pi-alkyl interaction with residue PHE518 at a distance of 5.11 Å, further stabilizing the ligand within the binding site and supporting its potential anti-TNF activity (Figure 6).

A



Interactions



van der Waals



Conventional Hydrogen Bond

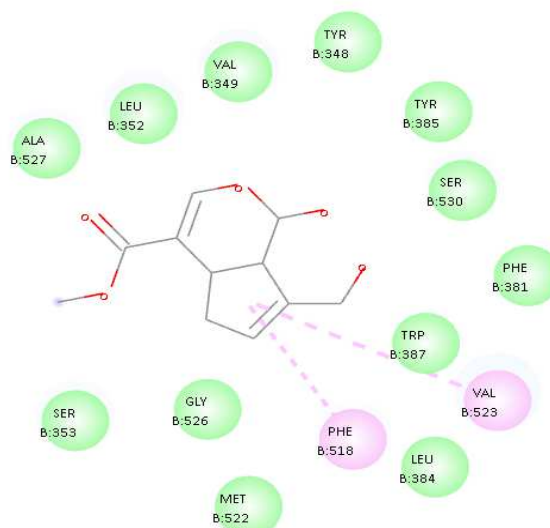


Carbon Hydrogen Bond



Pi-Alkyl

B



Interactions



van der Waals



Alkyl



Pi-Alkyl

Figure 6: 2-D Molecular Docking analysis of (A) Piroxicam with TNF- α (B) Genipin with TNF- α

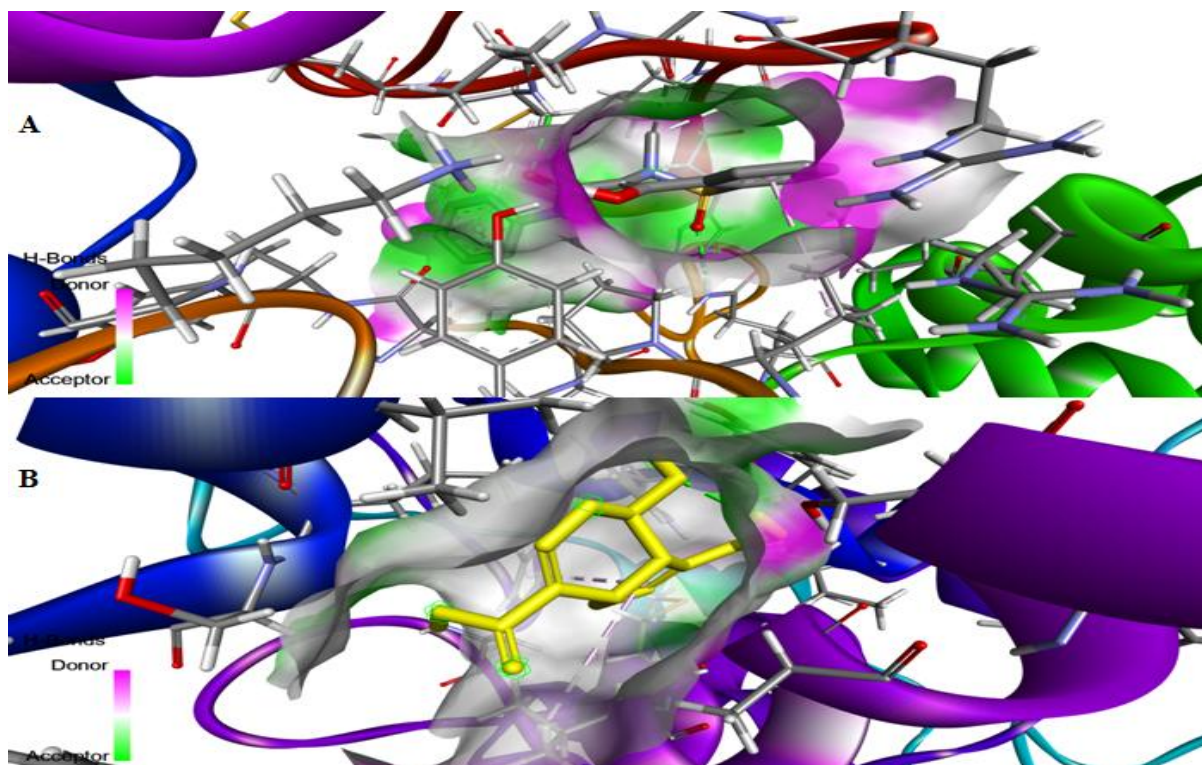


Figure 7: 3-D Molecular Docking analysis of (A) Genipin with TNF- α (B) Piroxicam with TNF- α

Docking results revealed that genipin showed favorable affinity towards TNF- α and COX-2 and results are comparable with standard piroxicam.

3.9.ADMET Analysis

Genipin has a molecular formula of $C_{11}H_{14}O_5$ with a molecular weight of 226.23 g/mol. It contains 16 heavy atoms, none of which are aromatic, and has a fraction Csp³ of 0.55, indicating a moderate degree of sp³ character. It possesses 3 rotatable bonds, 5 hydrogen bond acceptors, and 2 hydrogen bond donors. Its molar refractivity is 54.51, and the topological polar surface area (TPSA) is 75.99 Å².

In terms of lipophilicity, it is overall hydrophilic. XLOGP3 predicts a Log Po/w of -0.75, WLOGP predicts -0.05, MLOGP predicts 0.12, and SILICOS-IT predicts 0.11. The consensus Log Po/w is -0.14, collectively indicating that the compound has low lipophilicity and favors the aqueous phase.

Genipin shows high water solubility across predictive models. The ESOL model predicts a Log S of -0.57 (6.06×10^1 mg/ml; 2.68×10^{-1} mol/l), classifying it as very soluble. The Ali model predicts a Log S of -0.37 (9.68×10^1 mg/ml; 4.28×10^{-1} mol/l), also very soluble. The SILICOS-IT model predicts a Log S of 0.01 (2.33×10^2 mg/ml; 1.03×10^0 mol/l), classifying it as soluble. Similarly, genipin shows good pharmacokinetics, showing high GI absorption, does not permeate the blood-brain barrier, and is not a P-gp substrate. CYP1A2, CYP2C19,

CYP2C9, CYP2D6, and CYP3A4 inhibition data are temporarily unavailable. The predicted skin permeation (Log K_p) is -8.21 cm/s.

Physicochemical Properties		Water Solubility	
Formula	C ₁₁ H ₁₄ O ₅	Log S (ESOL)	-0.57
Molecular weight	226.23 g/mol	Solubility	6.06e+01 mg/ml ; 2.68e-01 mol/l
Num. heavy atoms	16	Class	Very soluble
Num. arom. heavy atoms	0	Log S (All)	-0.37
Fraction Csp ³	0.55	Solubility	9.68e+01 mg/ml ; 4.28e-01 mol/l
Num. rotatable bonds	3	Class	Very soluble
Num. H-bond acceptors	5	Log S (SILICOS-IT)	0.01
Num. H-bond donors	2	Solubility	2.33e+02 mg/ml ; 1.03e+00 mol/l
Molar Refractivity	54.51	Class	Soluble
TPSA	75.99 Å ²	Pharmacokinetics	
Lipophilicity		GI absorption	High
Log P _{ow} (iLOGP)	Temporarily unavailable	BBB permeant	No
Log P _{ow} (XLOGP3)	-0.75	P-gp substrate	No
Log P _{ow} (WLOGP)	-0.05	CYP1A2 inhibitor	Temporarily unavailable
Log P _{ow} (MLOGP)	0.12	CYP2C19 inhibitor	Temporarily unavailable
Log P _{ow} (SILICOS-IT)	0.11	CYP2C9 inhibitor	Temporarily unavailable
Consensus Log P _{ow}	-0.14	CYP2D6 inhibitor	Temporarily unavailable
		CYP3A4 inhibitor	Temporarily unavailable
		Log K _p (skin permeation)	-8.21 cm/s

Figure 8: Physiochemical and Pharmacokinetic profile of Genipin

Genipin satisfies all major drug-likeness criteria. It complies with Lipinski's rule of five with zero violations, and also passes the Ghose, Veber, Egan, and Muegge filters. The oral bioavailability score is 0.56, suggesting moderate predicted bioavailability.

From a medicinal chemistry perspective, the compound raises no PAINS alerts, indicating the absence of problematic substructures commonly associated with false positives in biological assays.

Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.56
Medicinal Chemistry	
PAINS	0 alert
Brenk	1 alert: isolated_alkene
Leadlikeness	No; 1 violation: MW<250
Synthetic accessibility	4.48

Figure 9: Drug likeness of Genipin

3.10. Toxicology Studies

The oral toxicity profile of Genipin was evaluated using an in Protox3.0 tool. The compound exhibited a predicted LD50 value of 237 mg/kg, classifying it under toxicity Class 3, which corresponds to a moderately toxic category. The prediction was supported by an average similarity of 100% and a prediction accuracy of 100%, indicating high confidence in the results. The octanol/water partition coefficient (logP) was recorded as -0.05 , suggesting that Genipin is hydrophilic in nature with balanced lipophilicity, which may favorably influence its absorption and bioavailability profile.

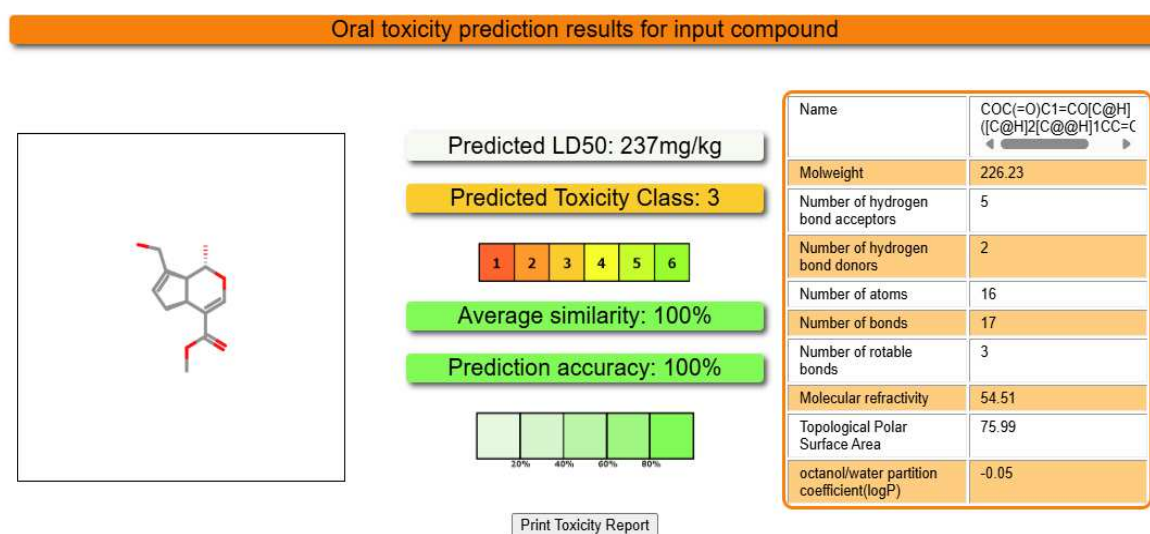


Figure 10: Toxicity prediction of genipin

Table 8: Organ toxicity studies of genipin

Classification	Target	Prediction	Probability
Organ Toxicity	Hepatotoxicity	Inactive	0.82
Organ Toxicity	Neurotoxicity	Inactive	0.81
Organ Toxicity	Nephrotoxicity	Active	0.65
Organ Toxicity	Cardiotoxicity	Inactive	0.57
Organ Toxicity	Respiratory Toxicity	Active	0.58
Toxicity end points	Carcinogenicity	Inactive	0.61

4. Discussion

Scientists around the world are exploring herbal remedies and phytochemicals, which are more economical and less toxic, for the management of rheumatoid arthritis. Likewise, in this study we tried to explore the anti-rheumatic activity of genipin which is iridoid glycoside herbal phytochemical. It is additionally believed to have anti-inflammatory, anti-angiogenic,

and neurological benefits, including shielding neurons from the toxicity associated with Alzheimer's disease (Ahmad *et al.*, 2024). To determine the effectiveness of genipin in rheumatoid arthritis CFA model was used on female albino rats (Ismail *et al.*, 2022). Rat paws that received an intraplate injection of CFA developed robust immunological responses, which are mediated by monocytes and macrophages that activate T-lymphocytes. These then interact with dendritic cells, causing the synovial membrane to release pro-inflammatory cytokines such TNF- α , IL-1 β , and IL-6. Bone, cartilage, and synovial inflammation are brought on by the overproduction of these inflammatory mediators that contribute to RA-related chronic inflammatory pain (Sial *et al.*, 2024). Edema is one of the main clinical features of RA as documented (Jeyabalan *et al.*, 2024). Paw edema of each rat was measured, by using digital water displacement plethysmometer, on day 7, day 14, day 21 and day 28. Findings from the plethysmometer showed a gradual and dose dependent reduction of edema in treatment groups as compared to disease control which showed worsening of arthritis over the time. Inflammation redness and erythema was assessed on day 7, 14, 21 and 28, by arthritic score which was given by macroscopic examination of each rat paw. Evaluation of arthritic score suggested a decline in inflammation and redness in treatment group over time. Whereas, in negative control the data suggests an increase in inflammation, erythema and redness. The results obtained are comparable to those already described (Dar *et al.*, 2023). Rheumatoid arthritis is a chronic inflammatory disease which causes anemia due to reduction of erythropoiesis (Cheng *et al.*, 2024). Assessment of hematological parameters suggested a reduction in red blood cell count and Hb in negative control reflecting anemia. In contrast, treatment groups nearly normalized the RBC count and Hb in rat. White blood cells and platelets count were raised in negative control and in the treatment group both parameters remained nearly normalized. Raised in WBC count in disease control is a reaction of immune system arousal caused by chronic inflammation in rheumatoid arthritis. Platelets count raised in negative control by overproduction of inflammatory cytokines (Masoumi *et al.*, 2024). Similarly, ESR in negative control was also raised as compared to treatment group on account of increased production of fibrinogen, plasminogen and prothrombin proteins by liver which is sparked by chronic inflammation (Tishkowski & Zubair, 2025). COX-2 is an enzyme that is produced in the body in response to chronic inflammation. In rheumatoid arthritis the synovial tissues are flooded with COX-2 which caused the production of prostaglandins, which is a key product that causes the symptoms of pain and inflammation (Ma *et al.*, 2021). RT PCR was performed on serum of all rats which showed an increased mRNA expression level of COX-2 in negative control as compared to treatment groups. IL-6

is pro inflammatory cytokine that is found in abundance in rheumatoid arthritis. It plays a major role in the pathophysiology of RA by encouraging synovitis and joint destruction. It is also known to initiate and maintain auto immune process by maturation of B cells and TH-17 cells (Jarlborg *et al.*, 2022). mRNA expression level of IL-6 in the serum of negative control rats was found elevated as compared to the treatment groups in which the levels of IL-6 were near the normal values. Tumor Necrosis Factor alpha (TNF- α), a key pro-inflammatory cytokine in RA which stimulates immune cells and encourages the generation of other inflammatory chemicals, causing inflammation, joint destruction, and synovium hyperplasia (Farrugia and Baron, 2016). In negative control, both TNF- α and NF- κ B showed an increased mRNA expression as compared to treatment groups. Interleukin-4 (IL-4) plays a multifaceted and potentially beneficial role in rheumatoid arthritis (RA). It acts as an anti-inflammatory cytokine by preventing the synthesis of pro-inflammatory mediators such as TNF- α and IL-1, and it also promotes joint health by preventing cartilage destruction. Joint homeostasis depends on a balanced release of IL-4 and pro-inflammatory cytokines; too little IL-4 causes inflammation and joint damage, while too much IL-4 may be an attempt to reduce the inflammatory response of the disease (Dong *et al.*, 2018). Serum mRNA expression level of IL-4 was found elevated in the treatment groups as compared to the negative control showing the potential beneficial effect of the compound. To evaluate the condition of disease and effect on bones, histopathological and radiographic analysis was performed (Alzarea *et al.*, 2022, Dar *et al.*, 2023). Ankle joint pathology of negative control exhibited bone resorption and decreased bone regeneration and pannus formation as compared to the treatment group. Similarly, the radiographic analysis of negative control displayed same qualities as already documented showing deformation, changes in joint spacing and bone loss (Uttra *et al.*, 2019). To validate our in-vivo findings, molecular docking was performed. Molecular docking analysis revealed good binding energies of -7.5 kcal/mol and -7.3 kcal/mol with human COX-2 (PDB ID: 5KIR) and TNF- α (PDB ID: 2AZ5) respectively with stable binding poses comparable to that of piroxicam which likely contributed to ligand stabilization as reported (Kaloni *et al.*, 2020). Besides, the pharmacokinetic and physiochemical properties of genipin revealed its favorable drug like characteristics. It possesses high solubility and oral absorption. Genipin also satisfied all five major criteria of drug likeness with zero violation. This study has demonstrated that genipin works efficiently in treating the challenging symptoms of RA and can lower oxidative stress and improve arthritic symptoms. Genipin may be used as an alternative to RA medications

since it may decrease the progression of arthritis. According to our research, genipin alleviated RA symptoms by significantly reducing paw edema and arthritic scores.

5. Conclusion

This study demonstrated that genipin exerts potent anti rheumatic activity which is likely due to its inflammatory cytokines lowering ability as evident by our findings. Molecular docking revealed favorable binding energies of genipin with human COX-2 (PDB ID: 5KIR) and TNF- α (PDB ID: 2AZ5), supporting its role in modulating inflammatory cytokines and retarding inflammation and pain. Pharmacokinetic, physiochemical profile and its compliance with Lipinski's rule of five with zero violations makes it a good candidate to be use for rheumatoid arthritis. While the present findings provide compelling preliminary evidence for the anti-rheumatic potential of genipin, further investigations are warranted before its clinical translation can be realized. Additionally, detailed toxicological profiling, including acute and chronic toxicity assessments, will be essential to establish the safety margin of genipin at therapeutically relevant doses. Future studies should also explore optimized drug delivery systems, such as nanoparticulate formulations or targeted carriers, to enhance the bioavailability and tissue-specific distribution of genipin at inflamed synovial joints.

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Author contributions

Hafiz Hassan Ahmed Naveed: He is the main contributor of this research project.

KalimUllah: He is the main supervisor of this study and guided throughout the research project.

Sarwat Nazir: She is responsible for mentoring the whole *in-vivo* experimentation included in this project.

Aneeqa Saleem: Responsible for executing and guiding the Molecular docking and ADMET studies used in this research.

Saif-Ullah Khan: Responsible for the administration and animal handling guidelines during this experimental research project.

Rizwana Raheel: Responsible for the guidance and pathological experimentation of this research project.

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Declarations

Competing interests: The authors declare no competing interests.

Ethics approval: Ethical approval was obtained from ethical committee of Minhaj University Lahore MUL/CRD/26/53.

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