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RESEARCH ARTICLE

Evaluation of Anti-arthritic Potential of Genistein in CFA Induced Rat Model

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Abstract: Rheumatoid arthritis (RA) is a debilitating chronic autoimmune pathology characterized by persistent synovitis, systemic inflammation, and progressive articular destruction. While non-steroidal anti-inflammatory drugs (NSAIDs) such as celecoxib remain the cornerstone of symptomatic management, their long-term administration is frequently associated with adverse cardiovascular and gastrointestinal events, necessitating the exploration of safer phytochemical alternatives. The present research work evaluates the anti-arthritic efficacy of genistein, a soy-derived isoflavone with known immunomodulatory properties, in comparison to celecoxib within a Complete Freund's Adjuvant (CFA)-induced rat model. Thirty Wistar rats were stratified into five experimental cohorts: normal control, CFA control, standard treatment (celecoxib 50 mg/kg), and two test groups administered genistein at 10 mg/kg and 20 mg/kg, respectively. Arthritis was induced via sub-plantar injection of CFA, and therapeutic interventions were administered orally for 28 days. Assessment of hind paw edema was conducted at weekly intervals to quantify the anti-inflammatory response. Results indicated that CFA induction precipitated significant paw swelling and inflammation. However, administration of genistein elicited a marked, dose-dependent reduction in paw volume. Notably, the 20 mg/kg genistein dosage showed an anti-inflammatory profile statistically comparable to that of celecoxib, effectively mitigating the acute and chronic phases of CFA-induced inflammation. These results indicate that genistein possesses potent anti-arthritic activity, likely mediated through the modulation of inflammatory cascades, and requires further pharmacological evaluation as a viable therapeutic adjunct or alternative to synthetic COX-2 inhibitors in the management of rheumatoid arthritis.

Keywords: Rheumatoid arthritis; Genistein; Celecoxib; Complete Freund's Adjuvant; Phytochemicals

1. Introduction

Rheumatoid arthritis (RA) represents a complex, systemic autoimmune disorder manifested by chronic inflammation of the synovial joints, leading to cartilage degradation, bone erosion, and significant functional impairment [1]. The global prevalence of RA is estimated between 0.5% and 1%, imposing a substantial burden on healthcare systems and diminishing the quality of life for affected individuals [2]. The pathogenesis of RA is multifactorial, involving a dysregulated immune response where pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), drive the proliferation of synovial fibroblasts and the recruitment of inflammatory leukocytes [3].

Current pharmacotherapeutic management primarily focus on symptomatic relief and disease modification. Disease-modifying anti-rheumatic drugs (DMARDs) and biological agents target specific immune pathways but are often limited by high costs and the potential for severe immunosuppression. Concurrently, non-steroidal anti-inflammatory drugs (NSAIDs), particularly selective cyclooxygenase-2 (COX-2) inhibitors like celecoxib, are extensively utilized to mitigate pain and inflammation by inhibiting prostaglandin synthesis [4]. Despite their efficacy, the chronic utility of COX-2 inhibitors is restricted by associated risks of cardiovascular thrombotic events, renal toxicity, and gastrointestinal ulceration. Consequently, there is a critical imperative to identify novel therapeutic agents derived from natural sources that offer comparable efficacy with an improved safety profile. Genistein (4',5,7-trihydroxyisoflavone), a predominant isoflavone found in Glycine max (soybean) and other legumes, has garnered significant scientific interest due to its diverse biological activities [5]. Structurally analogous to 17β-estradiol, genistein functions as a

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phytoestrogen, interacting with estrogen receptors to modulate immune responses. Apart from its estrogenic activity, genistein exhibits potent anti-inflammatory and antioxidant properties. Mechanistic studies suggest that genistein inhibits tyrosine kinase activity and suppresses the activation of nuclear factor-kappa B (NF-ϰB), a pivotal transcription factor regulating the expression of pro-inflammatory cytokines [6]. Moreover, genistein has been shown to scavenge reactive oxygen species (ROS), thereby attenuating the oxidative stress that contributes to articular damage in RA [7].

The pleiotropic effects of genistein ranging from antioxidant capacity to the modulation of inflammatory signaling pathways position it as a promising candidate for the management of chronic inflammatory diseases. Epidemiological data linking soy-rich diets to a reduced incidence of chronic inflammatory conditions further supports the therapeutic potential of dietary isoflavones [8]. However, direct comparative analyses of genistein against established pharmacological standards in specific arthritic models remain limited.

This research aims to rigorously evaluate the anti-arthritic potential of genistein using the Complete Freund's Adjuvant (CFA)-induced arthritis model in Wistar rats, a widely accepted experimental paradigm that mimics the pathological features of human RA. By juxtaposing the efficacy of genistein with celecoxib, this study seeks to elucidate the dose-dependent effects of this isoflavone on paw edema and inflammation, thereby contributing to the development of alternative therapeutic strategies for rheumatoid arthritis [9].

2. Materials and Methods

2.1. Chemicals and Reagents

Complete Freund's Adjuvant (CFA), consisting of heat-killed Mycobacterium tuberculosis suspended in paraffin oil, was procured from Sigma-Aldrich (Mumbai, India) and utilized for the induction of arthritis. The standard reference drug, Celecoxib, was obtained from Cipla Ltd. (Mumbai, India). Genistein (>98% purity) was purchased from Otto Chemie Pvt. Ltd. (Mumbai, India). All other chemicals and solvents used were of analytical grade.

2.2. Experimental Animals

Healthy adult Wistar albino rats, weighing between 150 and 200 g, were selected for the study. The animals were housed in polypropylene cages under standard laboratory conditions, maintained at a temperature of 22 ± 2 °C with a relative humidity of 50–60%, and subjected to a 12-hour light/dark cycle. The rats were acclimatized to laboratory conditions for one week prior to experimentation and provided with a standard pellet diet and water ad libitum [10]. All experimental procedures and protocols were conducted in strict adherence to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and received approval from the Institutional Animal Ethical Committee (IAEC Approval No: SSPU/KIPS/IAEC/2024/007).

2.3. Experimental Design and Administration of Drug

The animals were randomly allocated into five experimental groups, consisting of six rats per group (n=6). The treatment protocol was designed as follows:

- Group I (Normal Control): Administered physiological saline (10 ml/kg/day, p.o.) without arthritis induction.
- Group II (CFA Control): Arthritis was induced via intradermal injection of 0.1 ml CFA into the sub-plantar region of the right hind paw. This group received vehicle treatment.
- Group III (Standard): Arthritis was induced as per Group II. Animals received celecoxib (50 mg/kg/day, p.o.) suspended in vehicle.
- Group IV (Genistein Low Dose): Arthritis was induced as per Group II. Animals received genistein (10 mg/kg/day, p.o.).
- Group V (Genistein High Dose): Arthritis was induced as per Group II. Animals received genistein (20 mg/kg/day, p.o.).

Therapeutic interventions were initiated on the day of arthritis induction (Day 0) and continued once daily for 28 consecutive days.

2.4. Induction and Assessment of Arthritis

Arthritis was induced by a single sub-plantar injection of 0.1 ml CFA into the right hind paw of rats in Groups II through V. The severity of arthritis was quantified by measuring the change in paw volume, which serves as a primary index of edema and inflammation. Paw volume (mL) was measured using the liquid displacement method (plethysmometry) or vernier calipers calibrated

for volume estimation at baseline (Day 0) and subsequently on days 7, 14, 21, and 28 post-induction. The progression of arthritis was monitored by observing signs of erythema and swelling.

2.5. Statistical Analysis

Data are presented as mean ± Standard Error of the Mean (SEM). Statistical evaluation was performed using GraphPad Prism software. The significance of differences between groups and across time points was analyzed using two-way Analysis of Variance (ANOVA), followed by Bonferroni's post-hoc test for multiple comparisons. A probability value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of Genistein on Paw Edema

The sub-plantar injection of CFA successfully established the arthritis model, evidenced by a marked and progressive increase in paw volume in the CFA control group compared to the normal control. At baseline (Day 0), paw volumes were homogenous across all experimental groups, with no significant differences observed (Table 1).

Table 1. Effect of Genistein and Celecoxib on Paw Volume in CFA-Induced Arthritic Rats

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Normal Control	0.88 ± 0.16	0.91 ± 0.14	0.95 ± 0.13	0.88 ± 0.16	0.85 ± 0.18
CFA Control	0.93 ± 0.11	1.46 ± 0.14*	1.73 ± 0.15*	1.81 ± 0.15*	1.71 ± 0.20*
Celecoxib (50 mg/kg)	0.91 ± 0.11	0.95 ± 0.09#	1.05 ± 0.09#	0.98 ± 0.11#	0.90 ± 0.12#
Genistein (10 mg/kg)	0.96 ± 0.11	1.30 ± 0.10#	1.50 ± 0.13#	1.21 ± 0.11#	1.15 ± 0.12#
Genistein (20 mg/kg)	0.93 ± 0.10	1.00 ± 0.12#	1.33 ± 0.11#	1.01 ± 0.10#	0.96 ± 0.11#

Values expressed as Mean ± SEM (n=6). * $p < 0.05$ vs Normal Control; # $p < 0.05$ vs CFA Control.

In the CFA control group (Group II), paw swelling increased significantly, representing the acute phase of inflammation, measuring 1.26 ± 0.23 mL on Day 7. The inflammation peaked by Day 14 (1.70 ± 0.18 mL) and remained elevated throughout the 28-day observation period, confirming the chronic nature of the induced pathology. Conversely, the standard treatment group receiving celecoxib (Group III) exhibited a significant attenuation of paw edema. While a slight increase was noted on Day 7 (0.95 ± 0.08 mL), continuous treatment resulted in a progressive reduction in swelling, with paw volumes returning to near-normal levels (0.93 ± 0.16 mL) by Day 28.

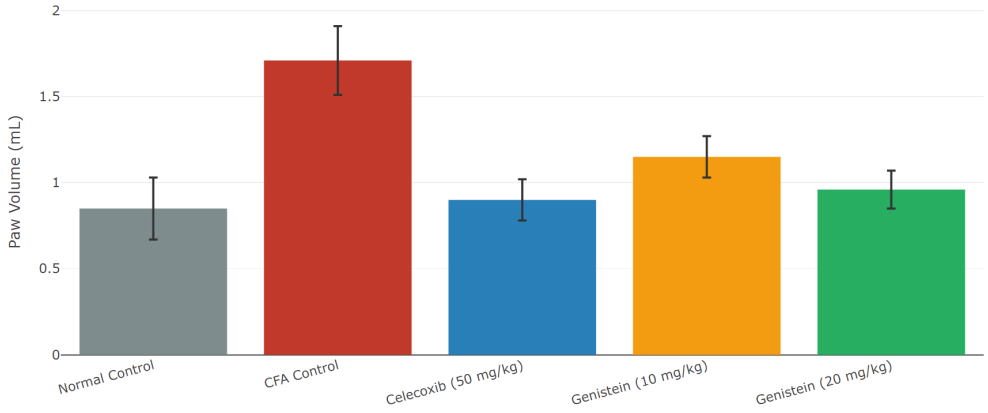

Figure 1. Comparison of Paw Volume Reduction Across All Experimental Groups at the End of the Treatment Period

Treatment with genistein showed a dose-dependent anti-arthritic effect. The low-dose genistein group (10 mg/kg) showed moderate inhibition of paw swelling, with volumes significantly lower than the CFA control but higher than the standard group. However, the high-dose genistein group (20 mg/kg) exhibited a robust reduction in paw volume. By Day 28, the paw volume in Group V was 0.96 ± 0.11 mL, which was statistically comparable to the celecoxib-treated group (0.90 ± 0.12 mL).

3.2. Statistical Comparisons

Two-way ANOVA revealed significant interactions between treatment and time ($p < 0.05$). Post-hoc analysis using Bonferroni's test confirmed that both celecoxib and genistein (20 mg/kg) treatments significantly reduced paw volume compared to the CFA control group at days 14, 21, and 28. Notably, there was no statistically significant difference between the therapeutic efficacy of 20 mg/kg genistein and 50 mg/kg celecoxib at the study endpoint, suggesting that the phytochemical at this dosage offers anti-inflammatory potency equivalent to the synthetic COX-2 inhibitor in this model.

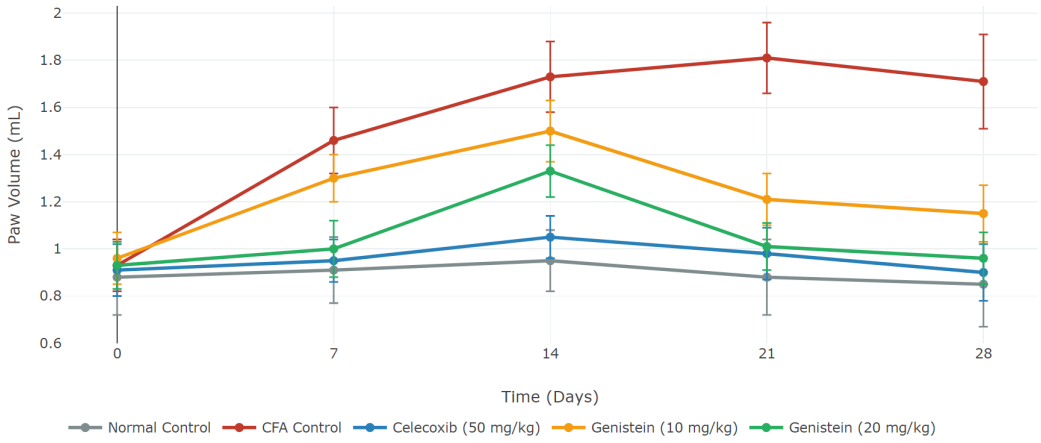

Figure 2. Effect of Genistein (10 and 20 mg/kg) and Celecoxib (50 mg/kg) on Paw Volume in CFA-induced Arthritic Rats over 28 days. Values are expressed as Mean ± SEM (n=6).

4. Discussion

The present study investigated the anti-arthritic properties of genistein in a CFA-induced rat model, a widely validated paradigm for preclinical evaluation of anti-rheumatic agents. The CFA model effectively recapitulates the hallmark pathological features of human RA, including synovial hyperplasia, chronic inflammation, and cartilage destruction, driven by a T-cell-mediated autoimmune response to mycobacterial antigens [11]. The significant increase in paw volume observed in the control group confirms the successful induction of polyarthritis, characterized by an initial acute inflammatory phase followed by a chronic immunologic phase.

Our findings show that oral administration of genistein significantly attenuates paw edema in a dose-dependent manner. The efficacy of high-dose genistein (20 mg/kg) was found to be statistically equivalent to that of celecoxib, a selective COX-2 inhibitor widely prescribed for RA management. While celecoxib effectively reduces inflammation by inhibiting prostaglandin E2 (PGE2) synthesis, its long-term use is associated with cardiovascular and renal toxicity [12]. The ability of genistein to achieve comparable edema reduction suggests its potential as a safer alternative or adjunct therapy. This aligns with previous research indicating that flavonoids can modulate inflammatory pathways with a superior safety profile compared to synthetic NSAIDs [13].

The anti-arthritic activity of genistein observed in this study can be attributed to its pleiotropic mechanisms of action. As a phytoestrogen, genistein interacts with estrogen receptors (ERs), particularly ERβ, which is known to exert anti-inflammatory effects on immune cells [14]. Moreover, genistein acts as a protein tyrosine kinase inhibitor, a critical pathway in signal transduction for T-cell activation and cytokine production.

It is well-established that the pathogenesis of RA involves the overproduction of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, which drive synovitis and osteoclastogenesis. Genistein has been reported to downregulate the activation of NF-κB, the master regulator of these cytokines [15]. Additionally, the antioxidant properties of genistein likely contribute to its therapeutic efficacy. Oxidative stress, characterized by elevated levels of reactive oxygen species (ROS) and lipid peroxidation, plays a crucial role in the tissue damage associated with RA. Genistein's capacity to scavenge free radicals and enhance endogenous antioxidant enzyme activity may protect synovial tissues from oxidative injury, a mechanism distinct from the purely cyclooxygenase-inhibitory action of celecoxib [16].

The study showed a clear dose-response relationship, where 10 mg/kg of genistein provided moderate relief, while 20 mg/kg offered maximal protection. This suggests a threshold effect for bioavailability or receptor saturation, implying that therapeutic dosing must be optimized to achieve clinical efficacy. These results corroborate findings from other inflammatory models where isoflavones

exhibited dose-dependent suppression of edema and cytokine release [17]. While paw volume serves as a robust macroscopic marker of inflammation, this study is limited by the absence of histopathological and biochemical analyses. Further research should include histological examination of joint architecture to assess synovial hyperplasia and bone erosion directly. Additionally, quantification of serum cytokine levels (TNF-α, IL-6) and oxidative stress markers would provide deeper molecular validation of the observed macroscopic effects.

5. Conclusion

In conclusion, this study provides compelling evidence that genistein possesses significant anti-arthritic activity in the CFA-induced rat model. The administration of genistein, particularly at a dose of 20 mg/kg, resulted in a reduction of paw edema comparable to the standard drug celecoxib. The therapeutic efficacy of genistein is likely mediated through a multi-targeted approach involving the modulation of inflammatory signaling and oxidative stress, distinguishing it from the single-target mechanism of NSAIDs. These findings support the potential of genistein as a potent, naturally derived therapeutic agent for the management of rheumatoid arthritis, warranting further translation into clinical evaluation.

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