JOURNAL OF PHARMA INSIGHTS AND RESEARCH ISSN NO. 3048-5428

RESEARCH ARTICLE

Evaluation of the Topical Therapeutic Efficacy of Daidzein in Mitigating Inflammation and Rheumatoid Arthritis

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Publication history: Received on 21st October 2025; Revised on 5th November 2025; Accepted on 9th November 2025

Article DOI: 10.69613/2pe8r168

Abstract: Rheumatoid arthritis (RA) is a debilitating autoimmune pathology characterized by chronic synovial inflammation and articular destruction. Current therapeutic modalities often entail significant adverse effects, necessitating the exploration of phytochemical alternatives with favorable safety profiles. Daidzein, a prominent soy-derived isoflavone, exhibits documented anti-inflammatory and antioxidant properties; however, its topical efficacy in arthritic models remains understudied. The present investigation elucidates the therapeutic potential of daidzein gel formulations (2% and 5% w/v) in mitigating acute and chronic inflammation using *in vivo* Wistar rat models. Antioxidant capacity was initially quantified via 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide (H_2O_2) scavenging assays, revealing substantial free radical neutralization with IC50 values of 103.55 ± 1.62 µg/mL and 67.28 ± 0.14 µg/mL, respectively. Subsequently, anti-inflammatory efficacy was assessed through carrageenan-induced paw edema, while anti-arthritic potential was evaluated using the Complete Freund's Adjuvant (CFA)-induced model over a 28-day period. Topical application of 5% daidzein gel showed a marked reduction in paw edema, exhibiting statistical parity with the standard non-steroidal anti-inflammatory drug, diclofenac. Specifically, the high-dose formulation significantly curtailed inflammatory progression in the chronic phase, normalizing paw volume measurements by day 28. These results substantiate the utility of daidzein as a potent bioactive agent capable of modulating oxidative stress and inflammatory cascades, thereby offering a viable adjunct strategy for the management of rheumatoid arthritis via topical administration.

Keywords: Daidzein; Rheumatoid Arthritis; Oxidative Stress; Carrageenan; Complete Freund's Adjuvant.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder that affects approximately 0.5% to 1% of the global population, manifesting primarily as persistent synovial inflammation [1]. The pathology is characterized by synovial hyperplasia, wherein the rapid proliferation of synovial cells and the infiltration of immune effectors leads to the formation of pannus tissue. This inflammatory cascade results in the progressive degradation of articular cartilage and subchondral bone, culminating in joint deformity, functional impairment, and significant pain [2]. While the disease predominantly targets the small joints of the hands and feet, its systemic nature implies potential extra-articular involvement, affecting organs such as the cardiovascular system and lungs, which complicates clinical management [3]. Current pharmacological interventions include non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, disease-modifying anti-rheumatic drugs (DMARDs), and biological response modifiers. Although advanced therapies like Janus kinase (JAK) inhibitors and anti-tumor necrosis factor (TNF) biologics have improved prognosis, their long-term use is often limited by high costs and adverse effects, including susceptibility to infections and gastrointestinal toxicity [4].

In light of these challenges, recent pharmaceutical research has pivoted towards phytoconstituents, particularly polyphenols, as safer adjunctive therapies. Isoflavonoids, a subclass of flavonoids, have garnered attention for their pleiotropic pharmacological activities. Daidzein (4',7-dihydroxyisoflavone), a naturally occurring phytoestrogen found abundantly in metabolic byproducts of soy, possesses documented antioxidant and anti-inflammatory capabilities [5]. Despite its therapeutic promise, the clinical utility of daidzein is frequently hampered by poor aqueous solubility and low oral bioavailability, which often necessitates high oral dosages to achieve therapeutic concentrations [6]. Topical delivery systems offer a strategic advantage by allowing direct application to the affected joint, potentially bypassing first-pass metabolism and minimizing systemic side effects.

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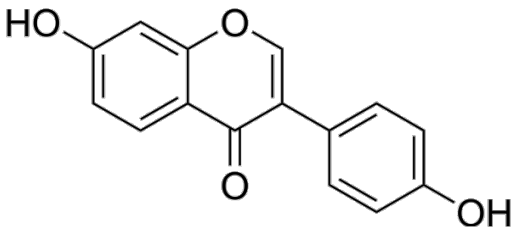

Figure 1. Chemical Structure of Diadzein

Consequently, this study aims to formulate and evaluate the efficacy of daidzein-based topical gels. We hypothesize that daidzein can effectively mitigate oxidative stress and inflammatory responses in Wistar rats subjected to carrageenan-induced acute inflammation and Complete Freund's Adjuvant (CFA)-induced chronic arthritis.

2. Materials and Methods

2.1. Chemicals and Reagents

High-purity Daidzein (98% via HPLC) was obtained from NAC Chemicals (India) Pvt. Ltd. The reagents required for antioxidant assays, including hydrogen peroxide (H_2O_2) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH), as well as the inflammatory agent carrageenan, were procured from Hi-Media Laboratories. All solvents, including methanol, and other reagents utilized in the experimental protocols were of analytical grade to ensure the precision of the biochemical analyses.

2.2. Formulation of Topical Gel

The topical gel formulations were prepared using a direct dispersion method. Accurately weighed Carbopol 934 was dispersed in a portion of distilled water under continuous stirring and allowed to hydrate for 24 hours to ensure complete swelling and absence of lumps. Separately, the required amount of Daidzein was dissolved in a mixture of ethanol and propylene glycol; methyl paraben and propyl paraben were subsequently added to this solution. This drug-containing organic phase was slowly incorporated into the hydrated Carbopol base with gentle agitation to prevent air entrapment. Finally, triethanolamine was added dropwise to the mixture to neutralize the polymer, adjusting the pH to the range of 6.4–6.8 and achieving the desired viscous gel consistency. The final weight was adjusted with the remaining distilled water, and the gel was stored in air-tight containers for further evaluation

Table 1. Composition of Daidzein Gel Formulations

Ingredient	Function	Quantity	
		2% Formulation	5% Formulation
Daidzein	Active Pharmaceutical Ingredient	2.0	5.0
Carbopol 934	Gelling Agent	1.0	1.0
Propylene Glycol	Co-solvent / Penetration Enhancer	15.0	15.0
Ethanol (95%)	Co-solvent	10.0	10.0
Methyl Paraben	Preservative	0.18	0.18
Propyl Paraben	Preservative	0.02	0.02
Triethanolamine	pH Adjuster / Neutralizer	q.s. to pH 6.4–6.8	q.s. to pH 6.4–6.8
Distilled Water	Vehicle	q.s. to 100	q.s. to 100

2.3. Experimental Animals

The *in vivo* studies employed Albino Wistar rats of either sex, weighing between 150–200 g and aged 15–18 weeks. The animals were sourced from the central animal facility of Shri Shankaracharya Professional University. They were housed in polypropylene cages under standard laboratory conditions, maintained at a temperature of 25 ± 2 °C with a relative humidity of 60–70%, and subjected to a 12-hour light/dark cycle. The animals were provided with *ad libitum* access to a standard pellet diet and water. All experimental procedures were conducted in strict adherence to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA). The study protocol received ethical clearance from the Institutional Animal Ethical Committee (Approval No. SSPU/KIPS/2024-25/003), ensuring the humane treatment of animals throughout the investigation.

2.4. *In Vitro* Antioxidant Assays

2.4.1. Hydrogen Peroxide Scavenging Assay

The scavenging capacity of daidzein against hydrogen peroxide, a reactive oxygen species (ROS) capable of inducing cellular oxidative damage, was evaluated spectrophotometrically. Daidzein solutions were prepared at varying concentrations ranging from 20 to 100 µg/mL. The assay operated on the principle that antioxidants donate electrons to neutralize H_2O_2, reducing the absorbance measured at 230 nm [7]. The tests were performed in triplicate to ensure reproducibility, and the percentage inhibition was calculated to determine the concentration required to scavenge 50% of the radical (IC50).

2.4.2. 2DPPH Radical Scavenging Assay

The free radical scavenging activity was further assessed using the stable radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH). This colorimetric assay relies on the reduction of the purple DPPH radical to a yellow-colored diphenylpicrylhydrazine in the presence of a hydrogen-donating antioxidant [8]. A 0.1 mM methanolic solution of DPPH was mixed with daidzein at concentrations of 20, 40, 60, 80, and 100 µg/mL. The reaction mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was subsequently measured at 517 nm against a methanol blank. A decrease in absorbance indicated increased radical scavenging activity, and the percentage inhibition was quantified relative to the DPPH control [9].

2.5. *In Vivo* Anti-inflammatory Activity

Acute inflammation was evaluated using the carrageenan-induced paw edema model. Wistar rats were randomized into five groups (n=5). Group I served as the normal control, receiving a subplantar injection of 0.1 mL saline. Groups II through V were subjected to inflammation induction via a subplantar injection of 0.1 mL carrageenan (1% w/v in saline) into the left hind paw. Topical treatments were applied 30 minutes prior to the carrageenan challenge. Group II (Disease Control) received a placebo gel; Group III (Standard) received 1% w/w diclofenac gel; Group IV received 2% w/w daidzein gel; and Group V received 5% w/w daidzein gel. Paw thickness was measured using a vernier caliper immediately before injection (0 h) and at 1, 2, 3, 4, and 24 hours post-injection [10]. The anti-inflammatory efficacy was determined by calculating the percentage inhibition of paw edema relative to the control group.

2.6. *In Vivo* Anti-arthritic Activity

Chronic inflammation and arthritic potential were assessed using the Complete Freund's Adjuvant (CFA)-induced arthritis model. Rats were divided into five groups (n=6). Arthritis was induced in Groups II through V by a single intradermal injection of 0.1 mL CFA into the left hind paw, while Group I received saline. Following induction, treatments (Placebo, Diclofenac 1%, Daidzein 2%, and Daidzein 5%) were applied topically once daily for 28 days. Paw edema was measured on days 0, 7, 14, 21, and 28 to monitor the progression of the disease and the therapeutic response [11]. The percentage induction and inhibition of paw edema were calculated to quantify the therapeutic effect using the following formulae:

$$\%\ induction\ of\ paw\ oedema = \frac{Vc - Vn}{Vn} \times 100$$

$$\%\ inhibition\ of\ paw\ oedema = \frac{Vc - Vt}{Vc} \times 100$$

Where *Vc* is the paw oedema of the control group, *Vn* is the paw oedema of the normal control group, and Vt is the paw oedema of drug drug-treated group.

3. Results and Discussion

3.1. Antioxidant Activity

The oxidative stress associated with inflammatory joint diseases necessitates therapeutic agents with strong free radical scavenging capabilities. The antioxidant profile of daidzein was characterized using DPPH and H_2O_2 assays, revealing a concentration-dependent increase in scavenging activity. In the H_2O_2 assay, daidzein demonstrated robust activity with an IC50 value of 67.28 ± 0.14 µg/mL. At the highest concentration tested (100 µg/mL), the inhibition reached 80.86 ± 0.37%. Similarly, in the DPPH assay, daidzein exhibited significant electron-donating capacity with an IC50 of 103.55 ± 1.62 µg/mL and a maximal inhibition of 49.37

± 0.80% at 100 µg/mL. These results indicate that daidzein effectively mitigates oxidative burden by neutralizing reactive oxygen species, a mechanism critical for attenuating the tissue damage observed in rheumatoid arthritis [12].

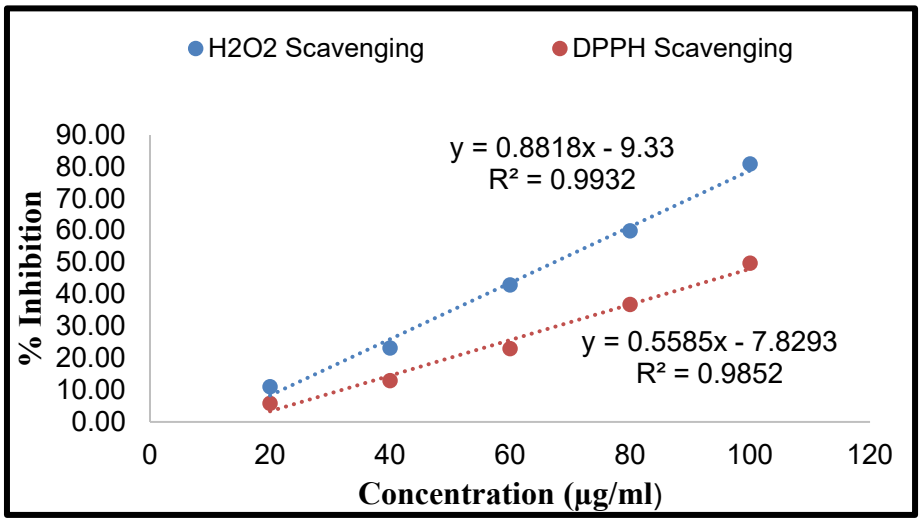

Figure 2. Free radical scavenging activity of daidzein on H_2O_2 assay and DPPH assay

Table 2. Antioxidant activity parameters and IC50 value of daidzein

Parameter	DPPH Assay	H_2O_2 Assay
Concentration range (µg/mL)	10–100	10–100
Incubation time	30 min @ room temps	10 min @ 37 °C
Detection wavelength	517 nm	230 nm
Daidzein IC_{50} value (µg/mL)	103.55 ± 1.62	67.28 ± 0.14

3.2. Acute Anti-inflammatory Efficacy

The carrageenan-induced paw edema model is a standard protocol for assessing anti-inflammatory agents, particularly those targeting the acute phase of inflammation mediated by histamine, serotonin, and prostaglandins [13]. Visual assessment of the paws revealed significant erythema and swelling in the control group following carrageenan injection, whereas treatment groups showed visible reduction in inflammation.

Quantitative analysis indicated that paw volume in the control group increased progressively, peaking at 4 hours with a 234.62% induction compared to normal rats. Topical application of daidzein significantly attenuated this response.

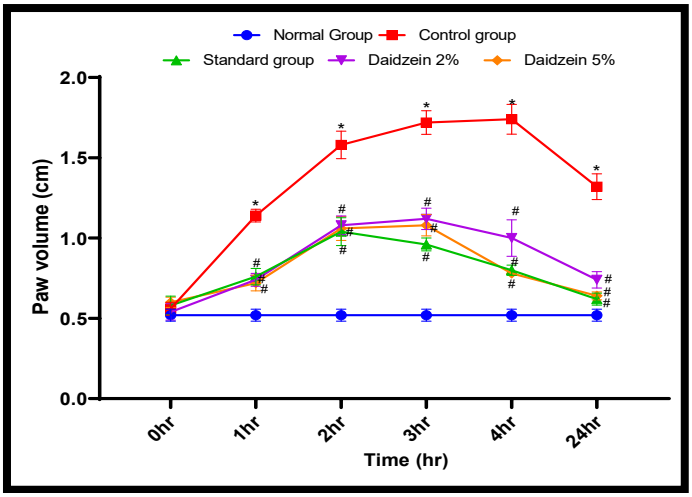

Figure 3. Anti-inflammatory effect of daidzein on carrageenan-induced paw edema

The standard treatment (diclofenac) exhibited a 54.02% reduction in edema at 4 hours. Notably, the 5% daidzein formulation demonstrated comparable efficacy, reducing edema by 55.57% at the same time point. The 2% daidzein formulation showed a moderate but significant anti-inflammatory effect, with a 42.53% reduction at 4 hours. Statistical analysis confirmed that the 5% daidzein gel was equipotent to the standard diclofenac gel ($P > 0.05$), suggesting that higher concentrations of daidzein can effectively modulate the acute inflammatory mediators released following carrageenan injury. The detailed changes in paw volume and percentage inhibition are shown below.

Table 3. Effect of daidzein on paw volume in carrageenan-induced paw edema

Time (Min)	Normal Group	Paw volume (cm)			
		Control Group	Standard Group	Daidzein 2%	Daidzein 5%
0 hr	0.52 ± 0.03	0.56 ± 0.04	0.58 ± 0.05	0.54 ± 0.05	0.6 ± 0.03
1 hr	0.52 ± 0.03	1.14 ± 0.04	0.76 ± 0.05	0.74 ± 0.04	0.60 ± 0.04
2 hr	0.52 ± 0.03	1.58 ± 0.08	1.04 ± 0.08	1.08 ± 0.05	1.10 ± 0.07
3 hr	0.52 ± 0.03	1.72 ± 0.07	0.96 ± 0.04	1.12 ± 0.06	0.90 ± 0.06
4 hr	0.52 ± 0.03	1.74 ± 0.09	0.80 ± 0.03	1.00 ± 0.11	0.80 ± 0.02
24 hr	0.52 ± 0.03	1.32 ± 0.08	0.62 ± 0.03	0.74 ± 0.05	0.70 ± 0.02

*All the data were expressed as mean ± SEM (n = 5 animals). Two-way ANOVA analysis revealed significant differences between multiple groups [column factor, $F(4, 20) = 97.34$, $P < 0.0001$] with Bonferroni's post hoc test. *P<0.05 as compared to the normal group; #P<0.05 as compared to control group.*

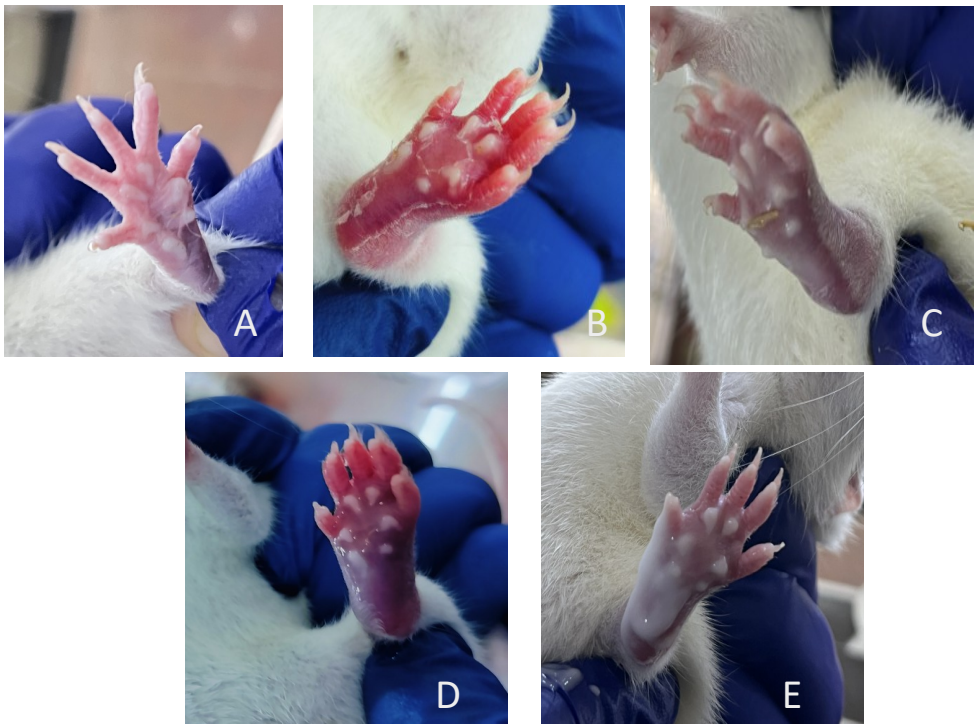

Figure 4. Anti-inflammatory effect of daidzein on carrageenan-induced paw edema
(Visual examination: A=Normal, B=Control, C=Standard, D=Daidzein 2%, E=Daidzein 5%)

Table 4. Percentage change in paw oedema after treatment of daidzein on carrageenan-induced paw edema

Sl No.	Time (h)	% Changes in paw volume			
		Control group	Standard group	Daidzein 2%	Daidzein 5%
1	0 h	7.69	3.57	-3.57	7.14
2	1 h	+119.23	-33.33	-35.09	-36.84
3	2 h	+203.85	-34.18	-31.65	-32.91
4	3 h	+230.77	-44.19	-34.88	-37.21
5	4 h	+234.62	-54.02	-42.53	-55.17
6	24 h	+153.85	-53.03	-43.94	-51.52

3.3. Chronic Anti-arthritic Efficacy

To evaluate the long-term therapeutic potential, the CFA-induced arthritis model was employed. This model mimics the chronic, immunological, and cellular features of human RA. Following CFA injection, the control group displayed a sustained increase in paw volume, which peaked at day 14 (1.61 ± 0.08 cm) and remained elevated through day 28, confirming the establishment of chronic arthritis. Treatment with daidzein gel resulted in a significant, dose-dependent reduction in paw volume over the 28-day period.

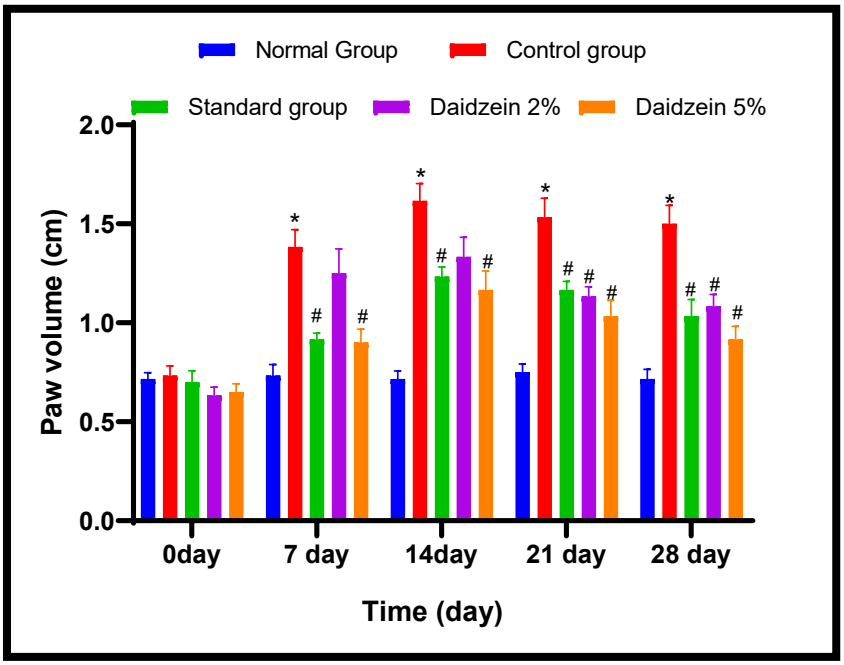

Figure 5. Effect of daidzein on the paw volume in CFA-induced paw edema in rats

By day 28, the control group exhibited a 109.30% increase in paw volume relative to the normal group. In contrast, the group treated with 5% daidzein showed a marked inhibition of edema, with a 38.89% reduction compared to the control group. This effect surpassed the reduction observed in the 2% daidzein group (27.78%) and was numerically superior to the diclofenac group (31.11%), although statistically comparable. The ability of daidzein to suppress edema in the later stages of the study (days 14–28) indicates its potential efficacy against the chronic proliferative phase of arthritis, likely by inhibiting the production of pro-inflammatory cytokines such as TNF-α and IL-6, which are drivers of synovial inflammation [14].

Table 5. Effect of daidzein on the paw volume in CFA-induced paw edema in rats

Sl No.	Time (h)	Paw volume (cm)			
		Control Group	Standard Group	Daidzein 2%	Daidzein 5%
0 day	0.71 ± 0.03	0.73 ± 0.04	0.70 ± 0.05	0.63 ± 0.04	0.65 ± 0.04
7 days	0.73 ± 0.05	1.38 ± 0.08	0.91 ± 0.03	1.25 ± 0.12	0.90 ± 0.06
14 days	0.71 ± 0.04	1.61 ± 0.08	1.23 ± 0.04	1.33 ± 0.09	1.16 ± 0.09
21 days	0.75 ± 0.04	1.53 ± 0.09	1.16 ± 0.04	1.13 ± 0.04	1.03 ± 0.08
28 days	0.71 ± 0.04	1.50 ± 0.09	1.03 ± 0.08	1.08 ± 0.06	0.91 ± 0.06

*All the data were expressed as mean ± SEM (n = 6 animals). Two-way ANOVA analysis revealed significant differences between multiple groups [column factor, F (4, 25) = 41.48, P < 0.0001] with Bonferroni's post hoc test. *P<0.05 as compared to the normal group; #P<0.05 as compared to control group.*

Table 6. Percentage change in paw oedema after treatment of daidzein on CFA-induced paw edema

S No.	Time (h)	% Changes in paw volume			
		Control group	Standard group	Daidzein 2%	Daidzein 5%
1	0 day	+3.29	-4.55	-14.09	-11.36
2	7 days	+88.64	-33.73	-9.64	-34.94
3	14 days	+125.58	-23.71	-17.53	-27.84
4	21 days	+104.44	-23.91	-26.09	-32.61
5	28 days	+109.30	-31.11	-27.78	-38.89

+ percent induction compared to the normal control group; -percent inhibition compared to the carrageenin control group

4. Conclusion

The present study provides compelling evidence supporting the therapeutic versatility of daidzein in the management of inflammation and rheumatoid arthritis. Our findings demonstrate that daidzein possesses potent antioxidant activity, effectively scavenging H_2O_2 and DPPH radicals, thereby addressing the oxidative stress component of articular diseases. *In vivo* evaluations confirmed that topical application of daidzein gel significantly ameliorates both acute edema induced by carrageenan and chronic arthritic inflammation induced by CFA. Notably, the 5% daidzein formulation exhibited therapeutic efficacy comparable to the standard NSAID, diclofenac, particularly in the chronic phase of the disease. These results show the potential of daidzein as a safe and effective phytochemical alternative for RA management.

Compliance with ethical standards

Acknowledgements

The authors are grateful to the management and central animal facility of Shri Shankaracharya Professional University for providing the necessary facilities and support to carry out this research work.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Statement of ethical approval

All experimental procedures involving animals were conducted in strict adherence to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA). The study protocol was approved by the Institutional Animal Ethical Committee of Shri Shankaracharya Professional University (Approval No. SSPU/KIPS/2024-25/003).

Statement of informed consent

Not applicable. The present research work does not contain any studies performed on human subjects.

References

[1] Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet. 2016 Oct 22;388(10055):2023-38.

[2] McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011 Dec 8;365(23):2205-19.

[3] Cojocaru M, Cojocaru IM, Silosi I, Vrabie CD, Tanasescu R. Extra-articular manifestations in rheumatoid arthritis. Maedica (Bucur). 2010 Dec;5(4):286-91.

[4] Burmester GR, Pope JE. Novel treatment strategies in rheumatoid arthritis. Lancet. 2017 Jun 10;389(10086):2338-48.

[5] Vitale DC, Piazza C, Melilli B, Drago F, Salomone S. Isoflavones: estrogenic activity, biological effect and bioavailability. Eur J Drug Metab Pharmacokinet. 2013 Mar;38(1):15-25.

[6] Huang Y, Zhao X, Zu Y, Wang L, Deng Y, Wu M. Enhanced solubility and bioavailability of daidzein by a novel nanosuspension formulation. Int J Nanomedicine. 2010 Oct 21;5:899-906.

[7] Fernando CD, Soysa P. Optimized enzymatic colorimetric assay for determination of hydrogen peroxide (H_2O_2) scavenging activity of plant extracts. MethodsX. 2015;2:283-91.

[8] Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958 Apr 26;181(4617):1199-200.

[9] Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. Molecules. 2022 Feb 16;27(4):1326.

[10] Winter CA, Risley EA, Nuss GW. Carrageenin induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proc Soc Exp Biol Med. 1962 Dec;111:544-7.

[11] Newbould BB. Chemotherapy of arthritis induced in rats by Mycobacteria adjuvant. Br J Pharmacol Chemother. 1963 Aug;21(1):127-36.

[12] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44-84.

[13] Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan edema in rats. J Pharmacol Exp Ther. 1969 Apr;166(1):96-103.

[14] McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol. 2007 Jun;7(6):429-42.