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Research Article

EVALUATION OF WOUND HEALING AND ANTI-INFLAMMATORY PROPERTY OF 5,3'DIHYDROXYFLAVONE AND GENE EXPRESSION OF JAK AND COX-2 IN LIPOPOLYSACCHARIDE-INDUCED RAW264.7 CELL LINE

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ABSTRACT

Objectives: The study aims to evaluate the wound healing potential and anti-inflammatory properties of 5,3'-dihydroxyflavone (DHF) and investigate its regulatory effects on the expression of Janus kinase (JAK) and cyclooxygenase-2 (COX-2) genes in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cell line.

Methods: Cytotoxicity was evaluated using the MTT assay. RAW 264.7 cells were treated with DHF (1–100 µg/mL) for 24 h. Absorbance was measured at 490 nm and 630 nm. Wound healing was assessed through a scratch assay. LPS-induced and DHF-treated groups (12.5, 25, 50 µg/mL) were analyzed for scratch closure at 0 and 24 h. Nitric oxide (NO) production was quantified using the Griess assay. Nitrite levels were measured at 520–550 nm. Gene expression of COX-2 and JAK1 was analyzed using quantitative polymerase chain reaction (qPCR), with β-actin as a reference gene. Relative expression was determined by the $2^{-}\Delta\Delta Ct$ method.

Results: The MTT assay confirmed DHF's non-cytotoxicity, showing ≥90% viability at 1–100 µg/mL ($p>0.05$). The scratch assay showed enhanced wound closure. At 50 µg/mL, DHF achieved ~75% closure, compared to 45% in the LPS group. NO estimation revealed a dose-dependent reduction. At 50 µg/mL, DHF significantly lowered NO levels ($p<0.01$). qPCR analysis demonstrated downregulation of COX-2 (45%) and JAK1 (50%) at 50 µg/mL ($p<0.001$). These findings confirm DHF's anti-inflammatory and wound-healing potential.

Conclusion: This study demonstrates that DHF enhances wound healing in RAW 264.7 cells by downregulating JAK and COX-2 expression, highlighting its anti-inflammatory potential.

Keywords: 5,3'-Dihydroxyflavone, Raw264.7 cell line, MTT, Scratch assay, Nitric oxide assay, Gene expression, Wound healing.

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INTRODUCTION

Wound healing represents a highly regulated biological process that includes multiple overlapping phases such as hemostasis, inflammation, proliferation, and remodeling. Any disruptions in these stages, particularly persistent inflammation, can delay healing and contribute to the development of chronic wounds, which are a major global health concern [1].

Increased oxidative stress, impaired collagen production, diminished angiogenesis, poor epithelialization, altered glucose metabolism, and malfunction of fibroblasts and endothelial cells are important pathophysiological factors that contribute to delayed wound healing [2]. Complexity and surface area of the inflicted wound determine the duration of wound healing [3]. Acute wounds usually heal within 3–4 weeks. In contrast, chronic wounds, if not properly treated, may continue for months or even years [4]. It is estimated that over 13 million people worldwide suffer from chronic wounds annually, due to trauma, diabetes, vascular disease, infection, or pressure. These numbers are expected to rise owing to an ageing population. In India, epidemiological data report a prevalence of chronic wounds at 4.5/1,000 population. Acute wounds are reported at 10.5/1,000 [5].

The management of chronic wounds imposes a heavy socioeconomic burden. The global chronic wound care market was valued at approximately USD 14.24 billion in 2024 and is expected to rise to USD 25.22 billion by 2032 [6]. In India, the wound care market is projected to expand from USD 983.43 million in 2024 to USD 1,795.64 million by 2033, indicating a CAGR of 6.37% [7]. Such data emphasize the importance of affordable and effective therapeutic strategies in low- and middle-income regions.

Although skin wounds generally heal naturally, uncoordinated or prolonged inflammation can disrupt this process. Conventional treatments, such as antibiotics, trypsin, and silver sulfadiazine, are often effective, but may be expensive and associated with adverse effects, including rashes, dryness, irritation, or even severe skin reactions in sensitive individuals [8]. Later, the use of antimicrobials in the medical world has started to cause debates due to the significant risk of bacterial resistance event [9]. This underscores the importance of developing safer, low-cost therapeutic alternatives [10].

Flavonoids, are plant-derived secondary metabolites, recognized for their broad pharmacological activities including antioxidant, antimicrobial, and anti-inflammatory effects. Their therapeutic actions are mediated, primarily through the modulation of significant inflammatory enzymes such as cyclooxygenase (COX), lipoxygenase, and phosphoinositide 3-kinase [11]. Dysregulated COX-2 expression in macrophages leads to overproduction of PGE_2, which sustains inflammation and delays the change from the inflammatory to the proliferative phase of wound healing, thereby hindering the regeneration of tissue [12]. By regulating such inflammatory mediators, flavonoids are being widely used in both conventional medicine and modern pharmacology.

Among inflammatory mediators, Janus kinase 1 (JAK 1) is essential for signal transduction of pro-inflammatory cytokines (such as interleukin-6

[IL-6] and interferon-gamma) and γc family cytokines (such as IL-2 and IL-4), especially in macrophages [13]. The JAK-STAT pathway regulates multiple elements of wound healing, including inflammation, angiogenesis, cell proliferation, migration, and extracellular matrix synthesis, while aberrant activation in macrophages sustains a pro-inflammatory M1 phenotype that impairs fibroblast proliferation, angiogenesis, and tissue remodeling; thus, proper modulation of this pathway is essential for timely wound resolution [14]. Previous studies have shown that 5,3'-dihydroxyflavone (DHF) exerts a dose-dependent anti-inflammatory activity in animal models, including carrageenan-induced paw edema, comparable to diclofenac [15].

Furthermore, studies on structurally comparable flavones, such as 7,8-dihydroxyflavone, have shown that suppression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways inhibits the production of COX-2, inducible nitric oxide (NO) synthase (iNOS), and IL-1β in RAW264.7 cells stimulated by lipopolysaccharide (LPS) [16]. Abnormal activation of the JAK-STAT signaling system has also been connected to the pathogenesis of several inflammatory and autoimmune diseases [17].

The current study was conducted in light of these results to examine the anti-inflammatory and wound-healing properties of DHF in an LPS-induced RAW264.7 macrophage cell line model. Our specific goal was to use quantitative polymerase chain reaction (qPCR) analysis to evaluate the impact of DHF on JAK1 and COX-2 gene expression, scratch wound closure, NO generation, and cell survival. By targeting key inflammatory mediators and cellular functions, this study seeks to investigate the curative benefits of DHF as a natural compound for promoting wound healing and controlling inflammation.

METHODS

The study was approved by the Institutional Research Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (Deemed University), Chennai (Approval No: CSP–MED/23/NOV/96/295).

Inclusion and exclusion criteria

Only viable RAW 264.7 macrophage cells with >95% confluence and normal morphology were included for experiments. Wells showing contamination, uneven growth, or detachment were excluded from the analysis. For each assay, data were accepted only if the control group showed consistent baseline viability (≥95%) and reproducible readings across triplicates. Outlier values exceeding±2 standard deviation (SD) from the group mean were excluded before statistical analysis.

Cell culture

The RAW 264.7 cell line was acquired from NCCS in Pune. Dulbecco's Modified Eagle Medium containing 10% Fetal Bovine Serum and 1% penicillin/streptomycin antibiotic was used to sustain and subculture the cells. Subculturing was performed on reaching approximately 70% confluence.

MTT assay

According to Mosmann *et al*. [18], in 1983, mitochondrial function was examined using the MTT test. In 96-well plates, 1×10^4 cells/well were seeded, and for 24 h, they were subjected to varying doses of DHF, ranging from 1 to 100 µg/mL. Fresh culture media containing MTT solution (0.5 mg/mL) was added after the exposure, and the mixture was incubated at 37°C for 4 h. The resulting formazan crystals were solubilized in dimethyl sulfoxide, and absorbance was measured at 490 and 630 nm using a multi-well microplate reader. As controls, untreated sets were also operated in the same manner. Inhibition rate was estimated using the following formula:

$$\% \text{ Inhibition rate}=(1-[A_{490}-A_{630}\ (\text{Treated})/A_{490}-A_{630}\ (\text{control})])\times100)$$

$$\% \text{ Viability}=100-\% \text{ Inhibition rate}$$

(A_{490}=Absorbance at 490 nm, A_{630}=Absorbance at 630 nm, Treated=Cells exposed to test compound, Control=Untreated cells (full viability).

Scratch assay

Cells were inoculated into 24-well plates at a density of 1.5×10^5 cells/well, with 0.4 mL of medium. After 24 h of culture, the cells were grown to 100% confluence. A linear scratch was made with a sterile tip, washed with PBS, and replaced with new media containing 10% fasting blood sugar. Photographs of the wells were taken at 10× magnification using a microscope at time 0 [19]. Cells were exposed to DHF-treated groups at doses of 12.5, 25, and 50 µg/mL, the LPS-induced group (1 µg/mL), and the control group. After 24 h, photographs of the scratch wound were taken to investigate.

NO estimation

The cells were exposed to DHF at concentrations of 12.5, 25, and 50 µg/mL. The untreated set was retained as a control. Following a 24-h incubation period, the medium supernatant was extracted using the Griess Method [20] for the NO assay. Briefly, the 96-well plate was filled with 100 µL of a 100 µm nitrite solution for the Nitrite Standard reference curve. The remaining wells received 50 µL of the suitable matrix or buffer. 50 µL of the standard was serially diluted by dispensing it in the subsequent wells, mixing it, and removing it to the next well in accordance with the concentrations of 100, 50, 25, 12.5, 6.25, 3.13, and 1.56 µm. 50 µL was discarded from the 1.56 µm set of wells. In duplicate or triplicate, 50 µL of each experimental sample was added to wells. Both the standard and the experimental samples received 50 µL of 1% sulfanilamide in 5% phosphoric acid. Kept out of the light and incubated at room temperature for 5–10 min. After adding 50 µL of the 0.1% NED solution, the mixture was allowed to sit for 5–10 min in the dark at room temperature. A purple/magenta color will begin to develop immediately. The wavelength range used for measuring absorbance was 520–550 nm.

qPCR

From each group, total RNA was extracted using the RNAiso Plus method [21]. Total RNA was treated with DNase I before cDNA synthesis to remove genomic DNA contamination. Using the PrimeScript 1st Strand cDNA Synthesis Kit (TaKaRa, Cat. No: 6110A), the extracted total RNA was measured and converted into cDNA. Briefly, one µg of RNA, 1 µL of 50 µM oligo(dT) primer, 1 µL of 10 mm dNTP mixture, and 9 µL of RNase-free water were mixed and heated for 5 min at 65°C, then promptly cooled. Next, 10 µL of the prime mixture, 4 µL of buffer, 100 U of reverse transcriptase, and 20 U of RNase inhibitor were

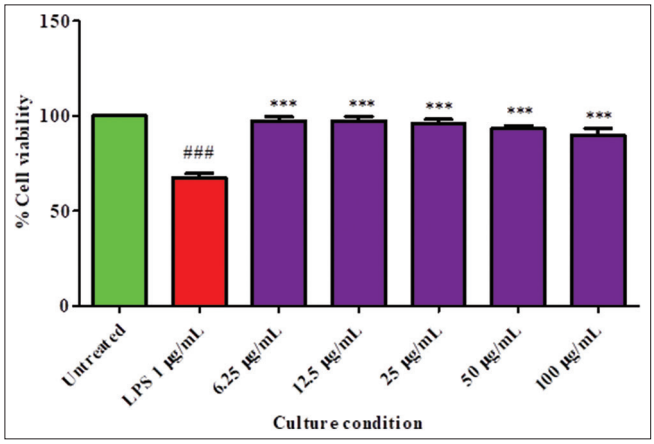

Fig. 1: MTT cytotoxicity of 5,3'-dihydroxyflavone (1–100 µg/mL) on RAW 264.7 cells. The DHF-treated group (***) showed a highly significant increase in cell viability compared with LPS-treated group, while the LPS-induced group (###) showed a highly significant reduction in cell viability compared to the untreated/control group. DHF treatment at concentrations of 1–100 µg/mL showed ≥90% cell viability with no significant difference compared to the untreated control (p>0.05), indicating non-toxicity. In contrast, LPS treatment significantly reduced cell viability compared to the untreated/control (p<0.05), confirming its cytotoxic effect

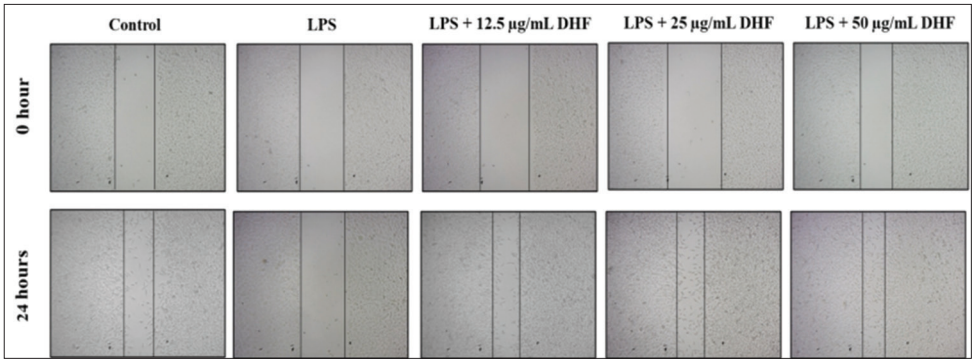

Fig. 2: Scratch assay wound images at 0 h and 24 h showing that lipopolysaccharide inhibits wound closure, while 5,3'-dihydroxyflavone (12.5–50 µg/mL) restores cell migration in a dose-dependent manner. The scratch-wound assay showed that DHF markedly improved cell migration in RAW 264.7 cells. The LPS-treated group exhibited almost no wound closure (<1% at 24 h), whereas DHF restored migration in a clear dose-dependent manner, achieving ~48–57% closure. One-way ANOVA showed significant differences among groups, and Tukey's *post hoc* test confirmed that all DHF concentrations significantly increased wound closure compared with LPS (**p<0.0001). Overall, these findings indicate that DHF effectively counteracts LPS-induced inhibition of wound healing**

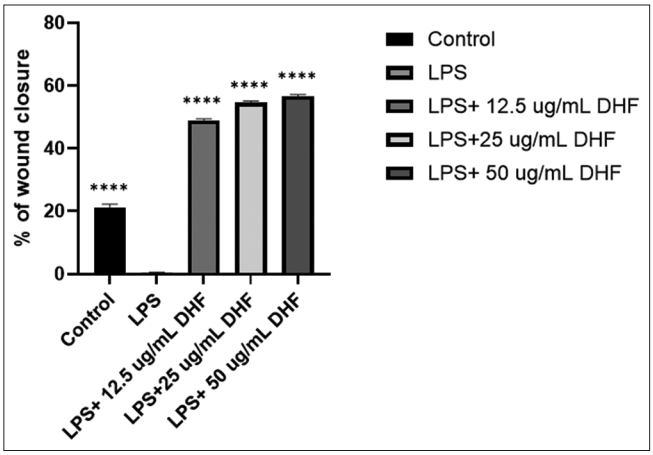

Fig. 3: Effect of 5,3'-dihydroxyflavone (DHF) on lipopolysaccharide (LPS)-induced reduction in wound closure. LPS significantly decreased wound closure, while DHF (12.5–50 µg/mL) dose-dependently restored migration. Values are mean±standard deviation. ****p<0.0001 versus LPS.

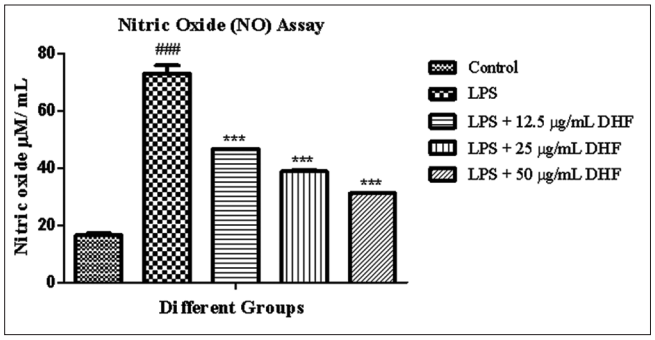

Fig. 4: Effect of 5,3'-dihydroxyflavone on nitric oxide production in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages. ###p<0.001 versus control; ***p<0.001 versus LPS-treated group. Nitric oxide (NO) production was quantified in untreated (control), LPS-induced, and DHF-treated RAW 264.7 macrophages, as shown in Fig. 4. The control group showed a basal NO level of 15.62 µm/mL, while exposure to LPS significantly elevated NO production to 73.05 µM/mL (###p<0.001 vs. control), indicating successful induction of inflammation. Treatment with DHF (12.5–50 µg/mL) significantly and dose-dependently reduced NO levels compared with the LPS group (p<0.001, * vs. LPS). The highest inhibition was observed at 50 µg/mL DHF, suggesting a potent anti-inflammatory effect. These findings indicate that DHF effectively suppresses LPS-induced NO synthesis in macrophages without cytotoxic effects**

added, and the reaction was incubated at 43°C for 45 min. qPCR was performed using the TB Green® Premix Ex Taq II kit (TaKaRa, Cat. No: RR820A) with 2 µL of synthesized cDNA. A total volume of 20 µL was prepared containing 10 µL of TB Green Premix Ex Taq II, 0.4 µL of ROX Reference Dye, 1 µL each of 10 µM forward and reverse primers, and 5.6 µL of sterile distilled water. Amplification was performed on a Bio-Rad instrument. The pro-inflammatory genes COX-2 and JAK1 were analyzed, with β-actin as the reference gene. Gene-specific primers were designed to span exon–exon junctions to ensure cDNA specificity. The Primer sequences are listed in Table 1. The qPCR cycling conditions were: initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 15 s, 58°C for 30 s, and 72°C for 30 s. Relative gene expression was calculated using the $2^{-}\Delta\Delta Ct$ method.

Table 1: Primer sequences used for qPCR

Primer	Forward sequence	Reverse sequence
JAK 1	ATGGAAGACGGAGGCAATGGT	GGAACTTTAGAGGCAGAATAC
COX-2	ATGCTCCTGCTTGAGTATGT	CACTACATCCTGACCCACTT
Beta actin	GCAGATGTGGATCAGCAAGC	GCAGCTCAGTAACAGTCCGC

JAK 1: Janus kinase 1, COX-2: Cyclooxygenase-2, qPCR: Quantitative polymerase chain reaction

Statistical analysis

Graphpad Prism 5.1 was used to analyze every experiment. All experiments were duplicated, and the results are shown as Mean±SD. One-way analysis of variance (ANOVA) was used, and the group comparison was analyzed using Tukey's multiple comparison test. p<0.001 are considered as statistically significant.

Instruments/equipment/apparatus

All instruments used in this study were of analytical or cell-culture grade. A CO_2 incubator (Thermo Fisher Scientific, Series 8000, USA) was used to maintain cell cultures. Absorbance for MTT and NO assays was measured using a Microplate Reader (Bio-Rad Model 680). Scratch wound images were captured using an inverted phase-contrast microscope (Olympus CKX53). Quantitative PCR was performed on a real-time PCR detection system (Bio-Rad CFX96). Data were analyzed using GraphPad Prism software version 5.1 (GraphPad Software).

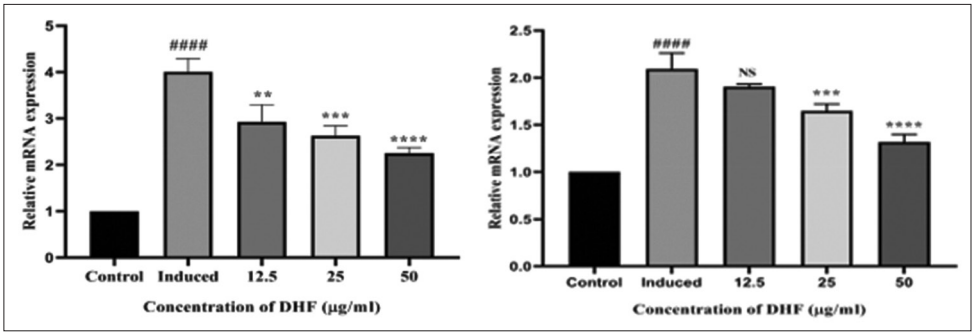

Fig. 5: Effect of 5,3'-dihydroxyflavone on Janus kinase 1 and cyclooxygenase-2 mRNA expression in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages. ###p<0.001 versus control; ***p<0.001 versus LPS. The relative mRNA expression levels of COX-2 and JAK1 in control, LPS-induced, and DHF-treated RAW 264.7 macrophages. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test, showing a highly significant overall difference (p<0.0001). The LPS-induced group displayed a marked upregulation of both COX-2 and JAK1 compared to the control (####p<0.0001 vs. control), confirming successful inflammatory induction. Treatment with DHF at 12.5, 25, and 50 µg/mL significantly downregulated the expression of both genes in a dose-dependent manner (**p<0.0001 vs. LPS**). These results demonstrate that DHF effectively suppresses JAK1 and COX-2 expression, confirming its strong anti-inflammatory potential in LPS-stimulated RAW 264.7 macrophages**

RESULTS

MTT assay

The MTT assay demonstrated that 5,3'-dihydroxyflavone (DHF) was non-cytotoxic to RAW 264.7 cells over the concentration range of 1–100 µg/mL, with cell viability remaining ≥90% compared to the untreated control . In contrast, lipopolysaccharide (LPS) treatment significantly reduced cell viability, confirming its successful induction of cellular stress (Fig. 1).

Scratch assay

The scratch wound assay revealed that LPS markedly inhibited cell migration in RAW 264.7 macrophages. Treatment with DHF significantly restored wound closure in a dose-dependent manner, with the highest concentration (50 µg/mL) showing maximum enhancement of cell migration at 24 h compared to the LPS-treated group (Figs. 2 and 3).

NO assay

NO estimation showed a significant increase in NO production following LPS stimulation compared to the control group ($p<0.001$). DHF treatment significantly and dose-dependently reduced NO levels, with the greatest inhibition observed at 50 µg/mL, indicating a strong anti-inflammatory effect (Fig. 4).

Gene expression

Quantitative PCR analysis demonstrated that LPS significantly up-regulated COX-2 and JAK1 mRNA expression in RAW 264.7 cells. Treatment with DHF resulted in a significant, dose-dependent down-regulation of both COX-2 and JAK1 expression, with maximum suppression observed at 50 µg/mL (Fig. 5).

DISCUSSION

The current study evaluated wound healing and the anti-inflammatory property of DHF in an *in vitro* model of LPS-induced inflammation using RAW264.7 macrophages. The results demonstrated DHF is non-toxic over a broad concentration range, increases cell migration in scratch tests, decreases the production of NO, and significantly downregulates the expression of pro-inflammatory genes JAK1 and COX-2.

With treated groups retaining more than 90% cell viability, the MTT assay verified that DHF did not cause cytotoxicity up to 100 µg/mL. In the scratch assay, DHF treatment enhanced wound closure compared to LPS-only groups, indicating that DHF promotes cellular migration and regeneration–key processes in wound healing. This aligns with established evidence that flavonoids enhance wound repair by modulating inflammation, promoting angiogenesis, and supporting re-epithelialization [22].

In terms of inflammatory modulation, LPS exposure markedly elevated NO release, which is consistent with the activation of macrophages during inflammatory responses. Treatment with DHF significantly reduced NO levels in a dose-dependent way, supporting its potential in reducing inflammation. The results are in agreement with previous studies, which demonstrate that dihydroxyflavones suppress iNOS activity, thereby reducing NO production in activated macrophages. The Griess assay, employed in this study, remains the gold standard for NO estimation in anti-inflammatory research [23].

At the molecular level, gene expression analysis revealed that LPS exposure significantly upregulated COX-2 and JAK1, both of which were effectively downregulated by DHF treatment. COX-2, a key mediator of prostaglandin synthesis, plays a central role in inflammatory processes and is a validated therapeutic target in inflammatory disorders [24]. During wound healing, the NF-κB, COX-2, and JAK/STAT pathways act as key interconnected regulators of inflammation and repair. The activation of NF-κB following tissue injury induces cytokines (TNF-α, IL-1β, IL-6) and inflammatory enzymes such as COX-2, initiating immune cell recruitment and early inflammatory responses. Sustained NF-κB activation, however, prolongs inflammation and delays tissue repair. COX-2, a downstream effector of NF-κB, drives prostaglandin production that amplifies inflammation, while its controlled modulation promotes wound closure. Concurrently, cytokines and growth factors activate the JAK/STAT pathway to stimulate cell proliferation and differentiation during the proliferative phase; disturbances in its regulation contribute to chronic wounds. Hence, coordinated activation and timely resolution of NF-κB, COX-2, and JAK/STAT signaling are essential for efficient wound progression and healing [25]. Similarly, the pathophysiology of autoimmune and chronic inflammatory disorders is linked to JAK1, a key kinase in the JAK–STAT signaling cascade. Its inhibition by DHF suggests modulation of upstream cytokine signaling pathways [26]. Similar results have been documented for 7,8-dihydroxyflavone, which inhibits the production of iNOS, IL-1β, and COX-2 in macrophages activated by LPS through the modulation of NF-κB and MAPK pathways [27]. Similarly, exposure to PT enhanced ROS levels in SH-SY5Y cells; however, 7,3'-dihydroxyflavone (7,3'-DHF) therapy decreased ROS buildup and decreased high IL-6 and TNF-α levels, brought about by PT toxicity [28]. Apigenin has also been shown to have protective anti-inflammatory properties by inhibiting NF-κB activation, which in turn suppresses the production of pro-inflammatory cytokines (IL-1β, TNF-α) in macrophages generated by LPS [29]. In addition, Luteolin reduces COX-2 and iNOS expression in activated macrophages, through the inhibition of MAPK and STAT3 signaling, confirming its strong anti-inflammatory activity [30]. Numerous studies have demonstrated the ability of Acacia catechu bark extracts to scavenge-free radicals and

have anti-inflammatory qualities, which make them useful for treating microbial infections and accelerating wound healing [31].

These findings collectively support the hypothesis that DHF exerts anti-inflammatory effects through transcriptional regulation of pro-inflammatory genes, potentially through similar upstream signaling mechanisms. Furthermore, *in vivo* experiments have shown that flavonoids possess significant wound-healing and anti-inflammatory qualities, with effects comparable to conventional non-steroidal anti-inflammatory drugs [32]. When combined with the current *in vivo* findings, this strengthens the argument for DHF as a viable natural treatment option for inflammatory and wound-associated conditions.

Overall, this research supports the therapeutic relevance of DHF in controlling inflammation and accelerating wound healing. Future studies should specifically assess the signaling cascade involved in DHF's action. The measurement of phosphorylated JAK1 and STAT3 proteins by Western blot can confirm inhibition of the JAK/STAT pathway. In addition, NF-κB p65 nuclear translocation may be examined by immunofluorescence to determine upstream regulation. Evaluating these markers will clarify whether DHF acts directly on JAK/STAT or through NF-κB/MAPK pathways. Such targeted experiments will help establish the precise molecular mechanism underlying DHF's anti-inflammatory and wound-healing effects.

CONCLUSION

The research demonstrates that DHF exhibits significant anti-inflammatory and wound-healing activity *in vitro*. It enhanced cell migration in scratch assays, reduced NO generation, and significantly downregulated pro-inflammatory gene expression of COX-2 and JAK1 in the LPS-stimulated RAW264.7 cell line. These indicate that 5,3′-DHF might work by altering key inflammatory signaling pathways. Given its non-toxic profile and potent bioactivity, 5,3′-DHF represents a potential natural therapeutic agent for the treatment of inflammatory conditions and supporting wound healing. Further *in vivo* studies are warranted to validate its effectiveness and explore underlying molecular mechanisms

Limitations of the study

A limitation of the present study is the absence of a positive control in the scratch wound assay. While the comparison between LPS-induced and DHF-treated macrophages demonstrated a dose-dependent improvement in cell migration, inclusion of a known pro-migratory standard, such as epidermal growth factor or transforming growth factor-β, would have provided a benchmark to validate the assay's responsiveness and to more accurately compare the efficacy of DHF. Future studies will include a known pro-migratory agent as a positive control to benchmark DHF's wound-healing efficacy.

ETHICS STATEMENT

This study was conducted, following ethical guidelines, with no involvement of human or animal subjects. All experiments utilized commercially available cell lines and reagents. A waiver for ethical review was obtained from the institutional research ethics committee.

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AUTHORS CONTRIBUTION

Each author made an equal contribution to this work. The following contributions to the work are confirmed by the authors: NATASHA GLADGE VARGHESE conceptualized and designed the study; KAVITHA RAMASAMY analyzed the data and interpreted the findings; and KRANTHI KARUNAI KADAL Guided and prepared the draft manuscript. After reviewing the findings, each author gave their approval to the manuscript's final draft.

CONFLICTS OF INTEREST

No conflicts of interest are disclosed by the authors.

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Nil.

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