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Daidzein as an IKK-β inhibitor for the management of arthritis: In-vitro and in-vivo approach

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Abstract

Rheumatoid arthritis is an autoimmune disorder where the immune system mistakenly attacks the synovium (the lining of the membranes that surround the joints). Chronic inflammation leads to joint damage, cartilage loss, and bone erosion. The exact cause of RA is not fully understood. Nuclear factor kappa β (NF-κβ) functions as a transcription factor for a variety of cytokines, cell adhesion molecules, and enzymes that are involved in the destructive mechanisms of rheumatoid arthritis. The purpose of this study is to characterise the efficacy of "Daidzein" as an IkappaB kinase-beta (IKK-β) inhibitor in collagen-induced arthritis (CIA) model in mice. Arthritis was induced in Balb/C male mice through subcutaneous immunisation with bovine type II collagen on days 0 and 21. Daidzein was administered orally every day after the onset of the disease. The incidence and severity of the disease were clinically assessed throughout the study, and biochemical testing was performed at the conclusion (Day 42). In-vitro findings of the study demonstrated Daidzein as potent inhibitor of IKK-β with IC50 value of 2.32 µM and 3.77 µM in IKK-β and NF-κ β translocation assay. Furthermore, Daidzein (dose range of 10-100 mg/kg, p.o.) was effective in reducing disease incidence and clinical signs in a dose-dependent manner, with an ED_{50} value of 83.52 mg/kg. The finding of the present study demonstrate dose-dependent efficacy in terms of both disease severity (clinical scoring) and inflammatory markers (biochemical evaluation of the serum and joints). IKK-β has been reported to play an important role in the pathogenesis of arthritis, and inhibitors of this enzyme represent a promising target for the development of novel treatments for arthritis and other inflammatory conditions. Finding of the present study suggests "Daidzein" represents an novel inhibitor of IKK-β with promising anti-inflammatory activity

Introduction

Arthritis is a general term for conditions that affect the joints and cause pain, swelling, stiffness, and decreased joint mobility [1.2]. Several signaling pathways play a role in the development and progression of arthritis. Some key signaling pathways associated with arthritis are such as NF-κB (Nuclear Factor-kappa B) pathway, MAPK (Mitogen-Activated Protein Kinase) pathway, JAK-STAT (Janus Kinase - Signal Transducer and Activator of Transcription) pathway, TGF-β (Transforming Growth Factor-beta) pathway, Wnt (Wingless-related integration site) pathway, PI3K-Akt (Phosphoinositide 3-Kinase - Protein Kinase B) pathway etc [3-5]. Among these multiple pathways,

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IKK pathway has drawn the attention of different researchers due to multiple facets. IKK (IκB kinase) signalling is a pivotal cellular pathway involved in the regulation of various cellular processes, primarily centered around the control of the nuclear factor-kappa B (NF-κ β) transcription factor [6-9]. IKK is a complex comprising two catalytic subunits, IKKα and IKKβ, and a regulatory subunit, NEMO (NF-κB essential modulator). Activation of the IKK complex occurs in response to various extracellular signals, including proinflammatory cytokines, pathogen-associated molecular patterns (PAMPs), and stressors [10-13]. In arthritis IKK pathway is activated in synovial cells, particularly macrophages and fibroblast-like synoviocytes, within the inflamed joint [14-17]. This activation is driven by inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1). These molecules contribute to the perpetuation of the inflammatory response and the recruitment of immune cells. The effectiveness of novel treatments (such as anakinra) that target interleukin-1 (IL-1) or tumour necrosis factor (TNF-α) underlines the critical part that cytokines play in the etiology of RA [18-21].

Daidzein is a natural compound classified as an isoflavone, which is a type of flavonoid. It is commonly found in various plant-based sources, particularly in legumes such as soybeans, lentils, and chickpeas. Soy-based products, like tofu and soy milk, contain significant amounts of daidzein. Daidzein has been studied for its potential health benefits and is known for its role as a phytoestrogen [22-25]. Chemically Daidzein has a characteristic isoflavone structure, consisting of two benzene rings (A and B) connected by a three-carbon chain forming an oxygen-containing heterocycle. This structure is shared with other isoflavones and is responsible for its biological activity [26,27]. Daidzein is classified as a phytoestrogen, meaning it has a structure similar to the hormone estrogen. As a result, it can interact with estrogen receptors in the body, leading to estrogenic or anti-estrogenic effects. These interactions can influence hormonal balance. Daidzein may influence specific signalling pathways such as, Estrogen Receptor (ER) signalling, Anti-Inflammatory signalling etc [28-31]. Daidzein's interactions with signalling pathways are not limited to estrogen receptors. It has also been studied for its anti-inflammatory effects. Daidzein may inhibit the activation of NF-κ β (nuclear factor-kappa β), a key transcription factor involved in inflammation. By doing so, it can reduce the expression of pro-inflammatory genes and decrease the inflammatory response. In fact, RA synoviocytes has been reported to block NF-κB-dependent transcription when dominant-negative IKK-β is used, but not dominant-negative IKK-α [32]. Many scientists are interested in the development of selective IKK-I inhibitors since NF-κ β plays a crucial role in controlling inflammatory cascades. Daidzein, is well absorbed, distributed, metabolized, and eliminated by the body. The bioavailability of daidzein can vary widely among individuals due to differences in gut microbiota composition, which influences its conversion to equol [38]. So in an attempt to evaluate therapeutic potential of Daidzein, the study was designed to test the proposed the IKK/NF-κ β -mediated anti-inflammatory response in collagen-induced arthritis mice model.

Materials and Methods

Daidzein**:** Daidzein has been studied for its potential health benefits and is known for its role as a phytoestrogen. Chemically Daidzein has a characteristic isoflavone structure, consisting of two benzene rings (A and B) connected by a three-carbon chain forming an oxygen-containing heterocycle. This structure is shared with other isoflavones and is responsible for its biological activity [28-31]. Daidzein is classified as a phytoestrogen, meaning it has a structure similar to the hormone estrogen [32-34]. As a result, it can interact with estrogen receptors in the body, leading to estrogenic or anti-estrogenic effects [35-38].

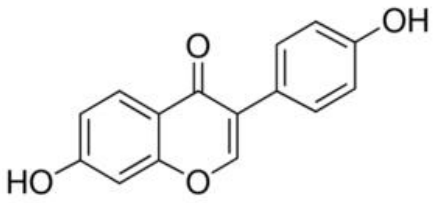

Fig 1. Structure of Daidzein (adopted from https://pubchem.ncbi.nlm.nih.gov)

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In-vitro assays

IKK-β inhibitory activity: IKK-activity was measured using an ELISA-based IKK-activity assay (Calbiochem) under the manufacturer's recommended conditions. In brief, GSTIB 50-amino acid peptide (with Ser32 and Ser36 IKK- phosphorylation sites) is used as a substrate reagent, at 30°C incubation time for 30 minutes with human recombinant IKK-β in glutathione-coated plate (96-well) performing substrate phosphorylation and capture simultaneously. Following that, the phosphorylated GST-IB substrate was detected using an anti-phospho IB antibody, followed by an HRP-conjugated secondary antibody. The colour development of the HRP substrate was monitored at 450 nm using a Tecan ELISA plate reader, and the absorbance intensity was used to calculate IKK-β activity [39].

NF-κB transactivation activity: HEK293 cells stably transfected with an NF- β luciferase reporter (293/NFB-luc cells, P., RC0014) were seeded at a density of 6×106 cells/10 cm dish in DMEM (Dulbecco's Modified Eagle Medium), incubated for 18 h to allow adherence, and transfected with 5 g pEGFP-C1 (Clontech, France). The cells were harvested and re-seeded in serum-free DMEM in a 96-well plate, then incubated overnight at 37°C and 5% CO_2. On the experimental day, the cells were treated for 30 minutes with the respective test compounds before being stimulated for 6 hours with 2 ng/ml human recombinant TNF-α. After lysis, the luciferase luminescence and EGFP fluorescence were measured using a Genios Pro plate reader (Tecan, Austria). To account for differences in cell number and/or transfection efficiency, the luciferase signals derived from the NF-κβ reporter were normalised by the EGFP-derived fluorescence [40].

In-Vivo

Murine CIA model: On day 0 and day 21, male Balb/C were immunised subcutaneously with 100 g of bovine type II collagen (Elastin Products, Sigma Aldrich, USA) in 0.1 ml of Adjuvant System (Sigma Aldrich, USA) with monophosphoryl lipid A. Following the booster injection on day 21, the mice were randomly divided into seven groups and regularly monitored for the development and severity of paw inflammation. All animal procedures were approved by the Institutional Animal Care and Use Ethics Committee. The various groups are as follows in Table 1:

Table1: Different treatment groups in the study

Group	Treatment (mg/kg)	Route of Administration	Animals/Group
Group-I	Vehicle (0.25% CMC)	*Per oral*	6
Group-I	Type II Collagen Control	*Intraperitoneal*	6
Group-III-VI	Daidzein (3, 10, 30 & 100, b.i.d) for 14 days	*Per oral*	6
Group-VII	Dexamethasone (1 mg/kg)	*Per oral*	6

In this model, Daidzein administration began only after a paw of an animal scored 2 (typically 1 week after the second collagen immunisation), at which point the animal was randomly assigned to a treatment group. Following that, treatment was continued daily for 14 days, and the severity of inflammation in all four paws was monitored daily, as described above. Statistical significance was determined using an analysis of variance, unless otherwise stated (ANOVA) (Graph Pad prism 5).

Clinical Scoring

Each paw was scored individually as follows: 0 normal; 1 one (or more) joints swollen on digits; 2 plantar surface of paw inflamed and paw thickness increased; 3 ankylosis (significantly reduced flexion/extension hock joint motion). The scores for each mouse's four paws were added up, and the mean score for each treatment group was computed. Each paw was scored individually as follows: 0 means normal; 1 means one or more digit joints are swollen; 2 means the plantar surface of the paw is inflamed and the paw thickness has increased; 3 ankylosis (significantly reduced flexion/extension hock joint motion). The scores for each mouse's four paws were added up, and the mean score for each treatment group was computed [41].

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Anti-collagen IgG enzyme-linked immunosorbent assay (ELISA): Overnight at 4°C, flat-bottomed 96-well ELISA plates were coated with bovine type II collagen (1 g/ml). After blocking the plates with milk diluent, duplicate wells were filled with 3-fold serial dilutions of mouse sera and incubated for 2 hours at room temperature. Plates were washed before adding horseradish peroxidase-conjugated goat anti-mouse IgG detection antibody (Southern Biotechnology, Birmingham, AL). The plates were developed with TMB (tetramethylbenzidine) substrate (Kirkegaard & Perry, Gaithersburg, MD) and read at 450 nm after a final washing step. Titers of anticollagen were calculated as the reciprocal of the serum dilution that produced an optical density of 1.0. (within the linear portion of the dilution curve).

Real-time PCR

The RNeasy Plus Mini kit (Qiagen, Hilden, Germany) was used to prepare total RNA from cell lysates. After sacrificing the mice, total mRNA was extracted from the joint using TRIZOL reagent (Invitrogen) according to the manufacturer's instructions. Real-time PCR was used to measure the expression levels of IL-34, IL-1, and IL-17A mRNA, which were normalised to GAPDH mRNA. The primers used were: 5′-CTGGAGCCCACCAAGAACGA-3′ (forward) and 5′-GCCTCCGACTTGTGAAGTGGT-3′(reverse) for IL-34 mRNA, 5′- TCCAGGATGAGGACATGAGCAC-3′ (forward) and 5′-GAACGTCACACACCAGCAGGTTA-3′ (reverse) for IL-1β mRNA, 5′- CAC CTC ACA CGA GGC ACA AG-3′ (forward), and 5′-GCA GCA ACA GCA TCA GAG ACA-3′ (reverse) for IL-17A, 5′-GTG TGC GAC ATA CTC AAG CAG GA-3′ (forward), and 5′-TGA AGT GGT AAC CGC TCA GGT G-3′ (reverse) for GAPDH mRNA.

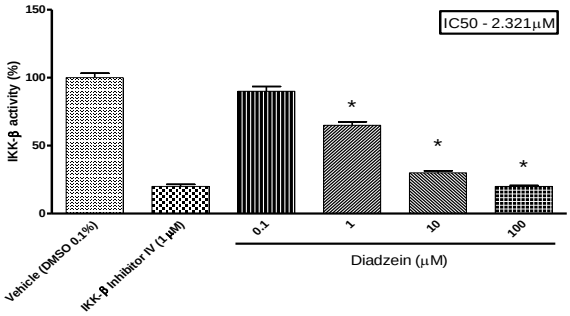
Statistical analysis

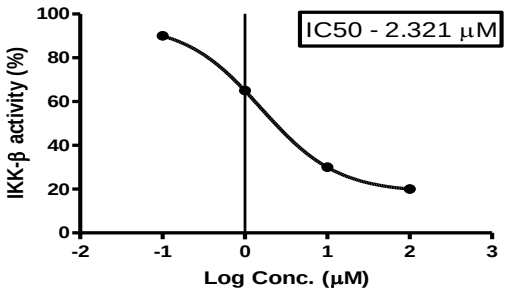
The data were analysed using one-way ANOVA, followed by the Tukey's test (Graph pad prism 5.0). The values are all expressed as mean ± SEM. The criterion for statistical significance in all tests was $P < 0.05$.

Result

Daidzein's effect on IKK-activity

Daidzein demonstrated IC_{50} value of 2.321 µM in *In-Vitro* IKK-β inhibitory assay (Fig 2a, 2b).





(a) IKK-β inhibition activity **(b) IKK-β inhibition activity: IC_{50}**

Fig 2. Effect of Daidzein on IKK-β activity, (a) IKK-β inhibition activity, (b) IC_{50}. The data is shown as (mean ± SD, n=6 well/treatment). #P <0.05 as compared with the DMSO treatment, *P<0.05 as compared with DMSO control group, (one-way ANOVA followed by Tukey's test).

Effect of Daidzein on NF-κB transactivation activity

Daidzein demonstrated IC50 value of 3.774 µM in *In-Vitro* NF-κB transactivation activity assay (Fig 3).

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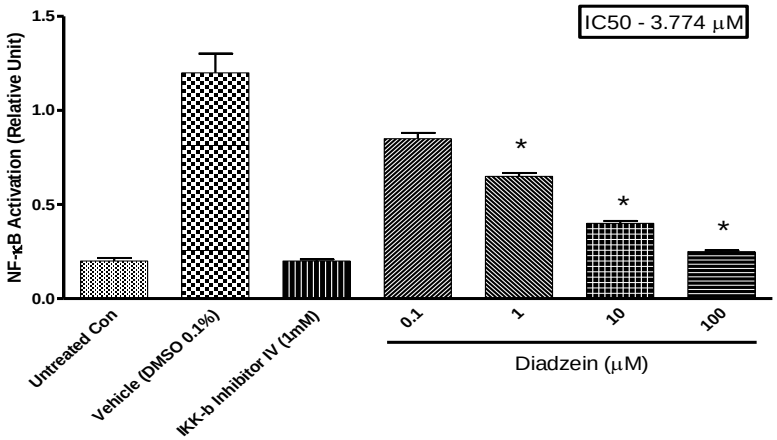

Fig 3. Daidzein influence on NF-κβ transactivation activity (IC_{50}). The data is shown as (mean ± SD, n=6 well/treatment). #P <0.05 as compared with the DMSO treatment, *P<0.05 as compared with DMSO control group, (one-way ANOVA followed by Tukey's test).

Daidzein effect on paw thickness and clinical scoring

Animals exposed to Collagen Type-II control animals showed a significant increase in paw thickness and clinical scoring on the final day when compared with naive animals, as shown in Fig 4. (a, b). Daidzein treatment at 3 mg/kg p.o. was unable to reduce increased paw thickness and clinical scoring when compared with the Collagen Type-II control group. While Daidzein (10, 30, and 100 mg/kg) and dexamethasone (1 mg/kg) treatments for 14 days significantly reduced paw thickness, [one-way ANOVA, $p < 0.05$, (DF = 6, 35; DFd = 41; F = 265.1)] and clinical scoring [one-way ANOVA, $p < 0.05$, (DF = 6, 35; DFd = 41; F =138.7] as compared with the control group (fig 4 a, b). Daidzein demonstrated ED_{50} value of 83.521 mg/kg for clinical scoring

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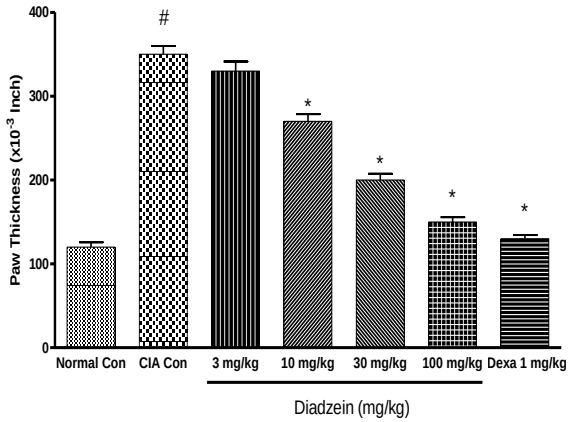

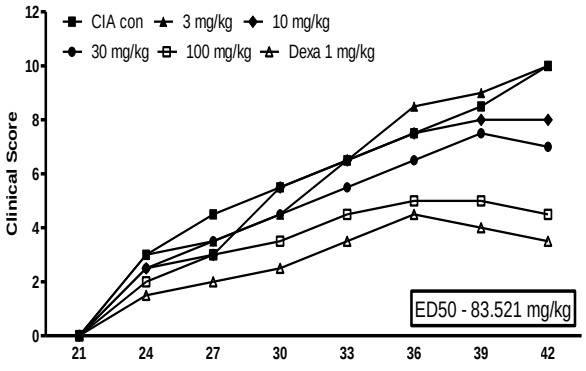

(a) Paw Thickness **(b) Clinical Scoring**

Fig 4. Effect of Daidzein on (a) paw thickness and (b) clinical scoring. The data is shown as (mean ± SD, n=6 mice/group). #P <0.05 as compared with the vehicle-treated group, *P<0.05 as compared with Collagen Type-II treated group, (one-way ANOVA followed by Tukey's test).

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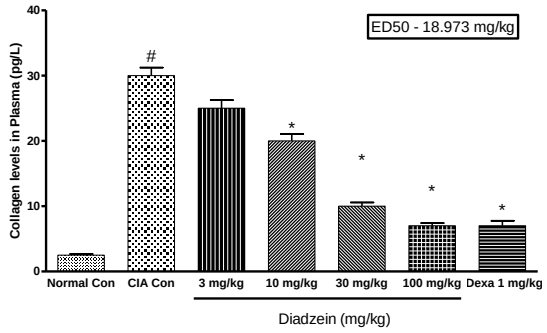
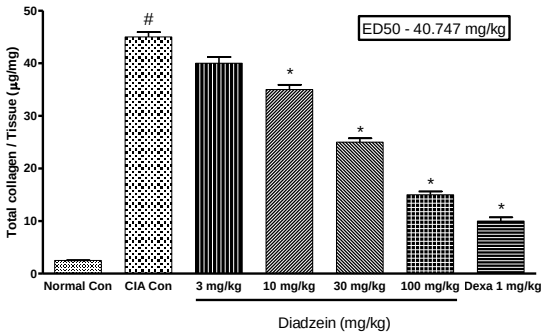
Daidzein's effect on collagen levels

Animals exposed to Collagen Type-II control animals showed a significant increase in collagen levels in plasma and tissue on the last day, as shown in Fig 5 (a, b), compared with naïve. Daidzein treatment at 3 mg/kg p.o. was unable to reduce the increased collagen levels when compared with the Collagen Type II control group. While Daidzein (10, 30, and 100 mg/kg) and dexamethasone (1 mg/kg) treatment for 14 days significantly reduced the increased collagen levels in plasma and skin [one-way ANOVA, $p < 0.05$, (DF = 6, 35; DFd = 41; F = 255.1)] and tissue (paw) [one-way ANOVA, $p < 0.05$, (DF = 6, 35; DFd = 41; F =148.8] as compared with the control group (fig 5 a, b). Daidzein demonstrated ED_{50} value of 26.70 and 25.38 mg/kg for collagen levels in plasma and joint tissue.



(a) Collagen level in Plasma

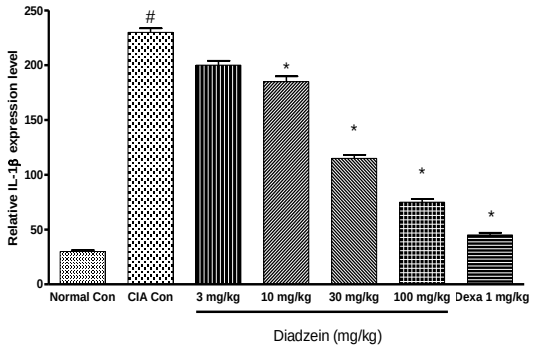


(b) Collagen level in Tissue (Paw)

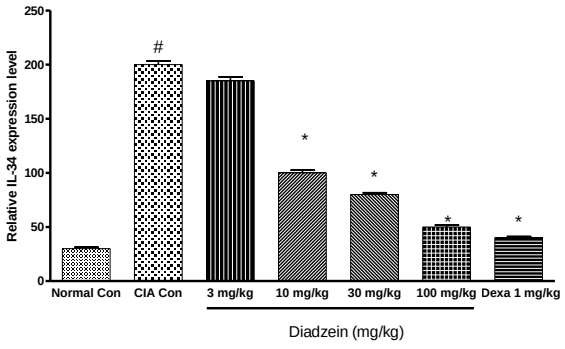
Fig 5. Effect of Daidzein on Collagen level (a) in plasma and (b) in Tissue (Paw). Statistics are displayed as (mean ± SD, n=6 mice/group). #P <0.05 as compared with the vehicle-treated group, *P<0.05 as compared with Collagen Type-II treated group, (one-way ANOVA followed by Tukey's test).

Effect of Daidzein on inflammatory cytokines (mRNA levels)

In contrast to naive animals, animals exposed to Collagen Type-II control animals on the final day displayed a significant rise in IL-1β, IL-34, and IL-17A mRNA levels (Fig. 6) (a, b and c). The increased IL-1, IL-34, and IL-17A levels relative to the Collagen Type-II control group could not be reduced by Daidzein at 3 mg/kg p.o.. While a 14-day treatment with Daidzein (10, 30 and 100 mg/kg) and dexamethasone (1 mg/kg) substantially reversed the elevated IL-1β (one-way ANOVA, $p < 0.05$, (DF = 6, 35; DFd = 41; F = 261.1), IL-34 (one-way ANOVA, $p < 0.05$, (DF = 6, 35; DFd = 41; F =127.8) and IL-17A (one-way ANOVA, $p < 0.05$, (DF = 6, 35; DFd = 41; F =166.8) mRNA level as compared with the control group (fig 6 (a, b and c) respectively for IL-1β, IL-34 and IL-17A.



(a)



(b)

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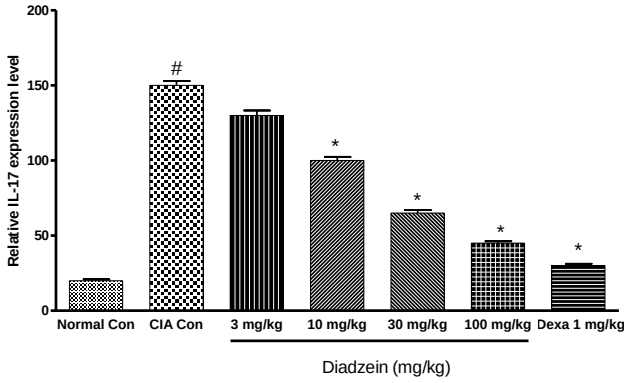
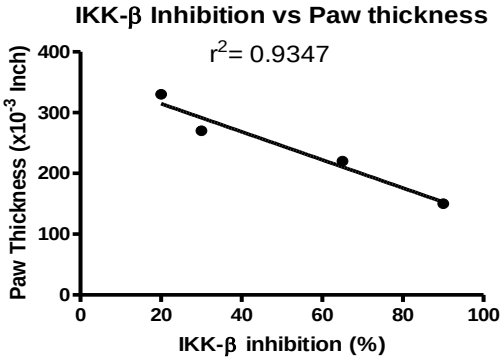


(c)

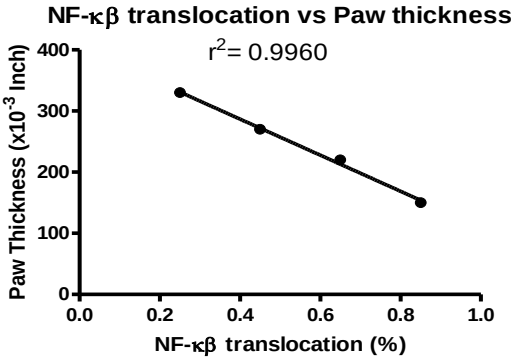
Figure 6. **Effect of Daidzein on pro-inflammatory cytokines at mRNA level (a) IL-1β, (b) IL-34, (c) IL-17A.** The data is shown as mean ± SD, n=6 mice/group. #P <0.05 as compared with the vehicle-treated group, *P<0.05 as compared with Collagen Type-II treated group, (one-way ANOVA followed by Tukey's test).

***In vitro-In vivo* correlation of IKK-β inhibition potential, NF-κβ transactivation activities with paw thickness**

The finding of the present study demonstrates a linear correlation between IKK-β inhibition potential, NF-κβ transactivation activities with paw thickness in rat CIA model. Both, IKK-β inhibition potential, NF-κβ transactivation activity demonstrate a negative correlation with correlation coefficient of 0.93 ($p < 0.05$) and correlation coefficient of 0.99 ($p < 0.05$) respectively for IKK-β inhibition potential, NF-κβ transactivation activity (Fig. 7). These findings provide a direct evidence for the involvement of IKK signalling in the pathogenesis of arthritis.



(a)



(b)

Figure 7. ***In vitro-In vivo* correlation of IKK-β inhibition potential, NF-κβ transactivation activities with paw thickness (a) IKK-β inhibition potential, (b) NF-κβ translocation.**

DISCUSSION

As per published reports, elevated pro-inflammatory cytokine are strongly linked to the onset and progression of rheumatoid arthritis (RA) [42]. Though role of TNF-α has been extensively researched and well established with various inflammatory disease conditions, yet several other prominent pro-inflammatory cytokines role needs to be investigated further. IL-1 has recently attracted attention from researchers as a potential target for the treatment of various inflammatory conditions. IL-1 is an important mediator for the deterioration of bone and cartilage in RA [43-45]. In addition to directly inhibiting IL-1/TNF-α, IKK-β, an intermediary enzyme that NF-κβ is downstream of, can also be targeted. Both RA and CIA depend on these cytokines to generate the inflammation and joint degradation, as demonstrated by the efficiency of protein-based treatment drugs targeted against these

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cytokines that has been established in both rodents and humans [46-48]. This activation of the IKK tripartite complex results in the phosphorylation-induced degradation of IκB. This pathway is activated by some TNF-α family cytokines through the selective activation of IKK-β homodimers by the upstream kinase. Numerous immune receptors rely on the traditional IKK-driven pathway's antiapoptotic effect, including T and B cell receptors, TLR4 and TNF- receptor type 1 (TNFR1), which all produce pro-survival and pro-death signals after ligation [49,50]. The majority of the time, survival signals take precedence, but when IKK-β or NF-κβ functions are compromised, receptor activation causes cell death. Since RA and CIA share a lot of the same underlying immunological pathogenic pathways, the mouse CIA model is extensively used to study the origins of the disease and prospective treatments for RA.

The findings of the present study demonstrated that Daidzein is a potent IKK-β inhibitor with anti-inflammatory potential and have therapeutic efficacy in the CIA model. IKK-β inhibitor efficacy in terms of clinical and histologic end points (markers) in the murine CIA model is consistent with previous research findings demonstrating anti-inflammatory efficacy in different disease models. Daidzein has been shown to inhibit the production of pro-inflammatory cytokines, such as interleukin-1 beta (IL-1β), IL-17 and IL-34 [51-55]. By reducing the levels of these inflammatory mediators, daidzein may help mitigate inflammation in different tissues. Daidzein may modulate signaling pathways associated with inflammation. As per findings of the present study diadzein demonstrated to inhibit the NF-κB signaling pathway in concentration dependent manner in NF-κβ translocation (IC_{50} value of 3.774 µM) assay. The therapeutic potential of IKK-β inhibitor (Daidzein) dose-dependently (3-100 mg/kg dosing range) suppressed NF-κβ dependent cytokine production in vivo in BALB/c mice according to a prior researchs [56-60]. NF-κ β is a transcription factor that plays a central role in regulating the expression of genes such as IL-1β, IL-17 and IL-34 involved in inflammation.

Daidzein may inhibit enzymes involved in the inflammatory process, such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). COX-2 is responsible for the production of prostaglandins, which are involved in the inflammatory response. Daidzein may modulate immune responses by affecting the activity of immune cells. It has reported to influence the balance between pro-inflammatory and anti-inflammatory immune responses [61-64]. Further as per several reports daidzein may affect the expression of adhesion molecules, which play a role in the migration of immune cells to sites of inflammation. Additionally daidzein has been reported to inhibit the activation of inflammasomes, which are multi-protein complexes involved in the regulation of inflammation [65-67]. Inflammasomes has been reported to contribute toward the maturation and release of pro-inflammatory cytokines. It's important to note that while these findings suggest potential anti-inflammatory effects of daidzein, the degree of effectiveness can vary, and not all studies report consistent results. Moreover, the translation of findings from laboratory studies to clinical applications in humans requires further investigation and clinical trials [68].

The effectiveness observed in this model is most likely due to the pleiotropic effects of IKK-β inhibition on the transcription of a number of proteins involved in the inflammatory and destructive aspects of disease, despite the fact that Daidzein appears to inhibit the transcription of IL-1β in the joints of these arthritic mice. These proteins all rely on NF-κβ to support their transcription, together with IL-1, TNF-α, IL-6, VCAM-1, ICAM-1, MMP-1, MMP-3, and NOS. According to the findings of the current study, Daidzein is a potent inhibitor of the catalytic subunit of IKK-β that also exhibits moderate selectivity for IKK-β over IKK-α and significant selectivity over other kinases [69,70]. In IKK-β *in-vitro* enzymatic inhibition assay Daidzein demonstrated IC50 value of 2.321 µM. Both in vitro and in vivo, the drug prevents the transcription of pro-inflammatory cytokines by attaching to an undisclosed allosteric binding site on IKK-β catalytic subunits. Due to the drug's good pharmacokinetics (oral bioavailability of 80% and intravenous half-life of approximately 3.2 hours), studies evaluating the efficacy of IKK-inhibitors in disease models are particularly well suited to employ it [71].

In the murine CIA model, we report that oral treatment of Daidzein was effective. The findings imply that IKK-β inhibitors could be very effective pharmacologic agents for the treatment of RA and other chronic

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inflammatory diseases. NF-κ β controls adaptive immune responses in addition to regulating the synthesis of inducible cytokines. In fact, measurements of the animals used in this experiment revealed that Daidzein significantly inhibited serum anti-collagen antibody titers at doses of 30 and 100 mg/kg, which may have contributed to the Daidzein's impressive efficacy and IC values of 18.973 and 40.747 mg/kg in plasma and joint tissue.

Dexamethasone and Daidzein's effectiveness are comparable (which is used in RA). Instead, the illness resolution shown with Daidzein at doses of 30 and 100 mg/kg is comparable to that of dexamethasone. Daidzein demonstrated ED_{50} value of 83.521 mg/kg for clinical scoring. Dexamethasone's anti-inflammatory properties are thought to be due to its interaction with the glucocorticoid receptor, which prevents NF-κB from translocating, whereas the results of the current study show that IKK-β enzyme inhibition and downstream signalling mediators (NF-κβ translocation) and effector molecules (IL-1b, IL-34, and IL-17) are inhibiting IKK-β expression [72].

In the current investigation, we show that Daidzein therapy slows the progression of CIA symptoms. This is consistent with research showing that inflammation and tissue degradation can occasionally be independent processes in both human and animal models of RA. It is quite significant that, despite the presence of inflammation, Daidzein administration was found to significantly reduce inflammatory cytokines including IL-17, IL-34, and IL-1β [73,74]. Moreover, there was a favourable association between the disease severity and the decreased serum and tissue collagen levels. Since many of dexamethasone's toxicities are thought to be caused by glucocorticoid receptor-dependent transactivation, an IKK-β inhibitor may potentially be effective as a dexamethasone substitute.

To sum up, Daidzein's IKK-β inhibitory activity is responsible for its therapeutic potential. The results of the current investigation showed that Daidzeins are similarly effective against joint inflammation in CIA in mice. The findings imply that IKK-β inhibitors could be very effective pharmacologic agents for the treatment of RA and other chronic inflammatory diseases.

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