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Original Article

Therapeutic potential of kodo millet in rheumatoid arthritis through immunomodulation and antioxidant enhancement

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Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disease, and conventional medications used to treat RA have anti-inflammatory activity as one of their primary modes of action. Since medications have unfavorable side effects, dietary therapy for arthritis is presently getting lots of attention. Kodo millet (*Paspalum scrobiculatum*) is a highly nutritious grain belonging to the Poaceae family, rich in fiber, nutrients and antioxidant content. They are the least exploited cereals and have pharmacological benefits in various conditions. The present study reveals the potential effects of kodo millet on immune regulation and antioxidant defense in collagen-induced arthritic (CIA) rats. Female Wistar rats were divided into three groups. Group I- Normal, Group II -CIA, Group III- CIA+ 10% cooked kodo millet (KM). After 60 days of experimental period and millet supplementation, organ indices, B-cell, Th2, Th17 immune responses, RANKL/NFATc pathways, cytokines and level of antioxidants were analyzed. Our findings reveal that 10% cooked kodo millet enhances B cell functionality, Th2-Th17 immunocyte balance. It attenuates inflammation by modulating RANKL/NFATc pathway, resulting in decreased proinflammatory mediators, increased levels of anti-inflammatory cytokines and improved tissue integrity. Millet inhibited ROS production and boosted antioxidant status via increasing SOD and GSH and decreasing activity of MPO, MDA and NO. The results highlight the potential of kodo millet as a functional food to manage the progression of rheumatoid arthritis.

Keywords: Kodo millet, Dietary intervention, Immunomodulation, Oxidative stress.

1. Introduction

Rheumatoid arthritis (RA) is a long-lasting autoimmune inflammatory disorder that occurs more commonly in women than in men, posing a significant global health challenge. Approximately 1% of adults worldwide, and 0.95% in India, suffer from this debilitating inflammatory condition, which ultimately results in permanent joint damage and loss of mobility [1-4]. The causes of RA are complex and involve both genetic and environmental elements [5]. This disease can impact any joint and nearly every organ in the body, presenting symptoms such as pain, swelling, tenderness, morning stiffness, synovitis, systemic inflammation, deterioration of cartilage and bone and impaired movement [6]. A significant element in progression of RA is the disruption in the balance of immune cell types, including helper T-cell (Th) Th1/Th2 and Th17/regulatory T-cell (Treg cells), which undermines immune stability. This disruption, along with the triggering of pathways like Toll-like receptor 4 (TLR4) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), results in an excessive release of pro-inflammatory cytokines. These cytokines exacerbate joint inflammation and lead to tissue damage. In addition, the activation of TLR4 and NF-κB increases oxidative stress and affects the development of osteoblasts and osteoclasts, which in turn boosts bone resorption and speeds up joint degradation. These interrelated pathways fuel immune dysfunction and tissue deterioration, making them important targets for treatment in RA [7,8]. As no proper treatments are available, the current treatment intends to minimize or alleviate symptoms using medications like analgesics, steroids, nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, disease-modifying antirheumatic drugs (DMARDs), anti-cytokines [6], etc., regardless of their side effects.

In recent years, an increasing number of studies have highlighted the significant role of diet in the risk and progression of RA [9,10]. Lack of complete cure and undesirable side effects of conventional medications lead RA patients to seek dietary modifications as a key component of disease management strategy by complementing conventional therapies. The beneficial effects of a healthy diet may include a reduced risk of disease progression, with several studies recognizing the protective role of fish,

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vegetables, and Mediterranean-style diets in combating rheumatoid arthritis [5,11].

Millets are small-seeded, drought-tolerant crops free from gluten that belong to the *Poaceae* family [12]. They are abundant in proteins, carbohydrates, fats, vitamins, minerals, phytochemicals, and antioxidants, making them a comprehensive answer to a variety of health concerns [13]. Millet has been cultivated and used all over the world for thousands of years and they were declared a nutri-cereal by the Indian government in April 2018. The United Nations General Assembly approved a resolution designating 2023 as the International Year of Millets, as proposed by India to the Food and Agriculture Organization (FAO), to recognize the significance and advantages of these grains for the global food system [14]. This research aims to explore the effects of kodo millet consumption on rheumatoid arthritis, focusing on its potential to reduce inflammation, modulate immune function, and improve joint health. By reviewing current literature and examining the underlying mechanisms, this article seeks to assess the therapeutic potential of Kodo millet (*Paspalum scrobiculatum*) as a dietary intervention for female Wistar rat models with RA, ultimately contributing to a more holistic approach to managing this debilitating condition.

2. Materials and methods

2.1. Collection of the millets

Kodo millet was purchased from ICAR – Indian Institute of Millet Research, Hyderabad.

2.2. Induction of animal model and treatment

Female Wistar rats (150±10 g, Age 1-2 months), which were bred in the Department animal house, were divided into three groups of six animals each as follows.

Group I – Normal rats

Group II- Collagen-induced arthritis (CIA) rats

Group III- CIA rats supplemented with 10% Cooked Kodo Millet (KM) in normal rat feed

Arthritis was induced in Groups II and III by immunization with 100µl Bovine type II collagen (4 mg/ml) dissolved in 0.1 M acetic acid and emulsified with an equal volume of Complete Freund's Adjuvant (CFA) at 4ºC. The animals received 2 doses of injections at 2 sites near the tail on day 0 & booster dose on day 7. The animals were kept in polypropylene cages set up in environments with regulated humidity (55-60%), temperature (28-32 °C), and light-dark cycle (12:12 hours). The rats were fed with a standard meal provided by VRK Nutritional Solutions, Maharashtra, India and were allowed unlimited access to water. Kodo millet grains were cleaned, soaked overnight (8 hours) in distilled water, cooked and mixed with laboratory animal feed to replace 10% of the total diet by weight. Food intake was monitored to ensure uniform consumption. The animals of group I and II were fed with normal rat chow and group III was supplemented with 10% Cooked Kodo Millet (KM) in normal rat feed for 45 days from day 15. After 60days, blood, spleen, cartilage, thymus, and liver were collected for various assays. All experimental procedures used were approved and strictly followed according to the guidelines of the animal ethics committee, Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (IAEC-16-KU-16/2022-BCH-AH (43)), according to the Government of India accepted principles for laboratory animal use and care.

2.3. Evaluation of body weight and paw swelling

Body weight and paw swelling of rats were measured starting from the first day of experiment and at regular intervals until the last day. A soft, non-stretchable thread was gently wrapped around the paw, marked the point and swelling was carefully measured with the ruler and represented as paw perimeter in centimeters [15]. The measurements were compared with previous measurements to assess the changes in swelling.

2.4. Immune organs index

After the experimental period, the spleen and thymus of the rats were surgically removed. The spleen and thymus index were calculated among the groups as the ratio of organs to the body weight of animal [16] to evaluate the impact of millet supplementation on the immune system. The index was calculated using the formula "Organ weight(mg)/ body weight(g)".

2.5. Biochemical estimations

Rheumatoid factor levels were measured using turbidimetric method by the RF Turbilatex kit from Euro Diagnostic system Pvt. Ltd. C-reactive protein was estimated in the Erba auto analyzer.

2.6. Assay of serum immunoglobulins

Immunoglobulin G (IgG), IgM and IgD levels in serum were quantified by competitive inhibition enzyme immunoassay technique using an ELISA kit from Cloud-Clone Corp.

2.7. Circulatory immune complex (CIC)

The level of CIC in the serum of all experimental groups was determined by the method of Seth and Srinivas using Polyethylene glycol (PEG) based turbidimetric assay. Serum was diluted in borate-buffered saline (BBS, PH 8.4) and mixed with PEG, incubated at 25°C for 60 minutes and absorbance was measured at 450nm. The result was calculated by the formula (A 450 PEG- A450 WITH BBS) x 1000, expressed as PEG index [17].

2.8. Preparation of lymphocyte samples and lymphocyte proliferation assay

After the experimental period, spleens were excised under sterile conditions and cell suspension was prepared in 10 % Fetal bovine serum (FBS) (Gibco) RPMI 1640 medium (Sigma Aldrich) by passing through a stainless-steel sieve. The medium was also supplemented with 1% antibiotic-antimycotic solution (Himedia). The cells were centrifuged at 1000rpm for 5 minutes and pellet was resuspended in 4 ml medium. The suspension was again layered on an equal volume of Ficoll solution, centrifuged at 2500 rpm for 20 minutes. A band obtained at the interface was collected, washed and resuspended in RPMI 1640 medium. Lymphocyte proliferation assay was done by the method described by Wang *et al*. [18]. 100 µl/well ($5x10^6$ cells/ml) cells were incubated in 96 96-well culture plate and lymphocyte proliferation was induced by adding Concanavalin A purchased from Sigma (10µg/ml ,100µl/well). The plates were incubated at 37°C with 5% CO2. After 44 hours, 20 µl of MTT (5mg/ml, Himedia) was added to all wells and incubated for 4 hours under the same

conditions. The supernatant was removed and dimethyl sulphoxide (DMSO) from Merck was added to solubilize formazan crystals and absorbance was measured at 470 nm. The mean absorbance value represents T-lymphocyte proliferation.

2.9. Cytokine estimation by Enzyme-Linked Immunosorbent Assay (ELISA)

For immunological studies, splenocytes were incubated for 72 hours with pre-coated Anti-CD3 monoclonal antibody and culture supernatants were collected to serve as antigen and indirect ELISA was performed. Homogenate from cartilage prepared in lysis buffer and used to analyze the markers of inflammation. Interleukin-6 (IL-6), IL-17, IL-4, IL-10 (cultured splenocytes), Receptor activator of nuclear factor kappa-B ligand (RANKL), Nuclear factor of activated T-cells (NFATc), Matrix metalloproteinase 9 (MMP-9) levels were estimated using specific antibodies. Antibodies used were purchased from Abcam and Cell Signaling Technology. The antigen on ELISA plates was pre-coated with varying amounts of cell lysate and culture supernatants. Following overnight incubation at 37°C, wells were washed with PBS. Free binding sites were blocked with 0.2% gelatin in PBS with 0.05% Tween 20. Primary antibody (1:500 in PBS-Tween 20) was incubated for 2 hours. Following a thorough PBS-Tween 20 washing, secondary antibody (1:1000) was added and incubated for 1 hour. Immune complex was produced by the O-dianisidine substrate and horseradish peroxidase (HRP) along with 30% H2O2 system that was measured spectrophotometrically using a microplate reader at 450 nm.

2.10. Protein extraction and Western blot analysis

Cartilage tissue was lysed in RIPA buffer containing protease inhibitor cocktail, followed by centrifugation at 13,000 f for 15 minutes at 4° C. Protein concentration was determined by Protein assay of the samples was done by the method of Lowry O.H. *et al.* [19] using alkaline copper reagent and Folin's reagent. The color produced was measured at 560nm. β-actin served as internal standard. All samples were mixed with 6x sample loading dye and boiled at 95 °C for 5 minutes and 40 µg of sample was loaded on SDS-PAGE gels. After the run, proteins were transferred to 0.45 µm nitrocellulose membrane in transfer buffer for 1 and half hours. The membranes were blocked with 5% skim milk powder (Fluka Analytical) for 1 hour and after washing, incubated with primary antibody (RANKL-1:1000) overnight at 4°C. HRP-conjugated secondary antibodies were incubated for 1 hour at 37°C. Bands were visualized using ECL-substrate (BIO-RAD) and quantified using Image Lab 6.1 software (Bio-Rad).

2.11. RNA preparation and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

RNA isolation was done by homogenizing 100 mg tissue in 1 ml TRI reagent (Sigma Aldrich) and using chloroform-isopropanol method and quantified at 260nm. cDNA synthesis was carried out in an Eppendorf master cycler using Verso cDNA synthesis kit containing oligo dT primer, reaction buffer, RNase inhibitor, dNTPs and reverse transcriptase enzyme following manufacturers instructions. Reverse transcription (RT) was performed with the Reverse transcription system (Eppendorf master cycler) using PCR master mix according to the protocol and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as control. The PCR products were electrophoresed on 1.5% agarose gel and stained with ethidium bromide. The bands were observed and quantified by densitometry in in Gel Documenter (BIO RAD) against internal standard GAPDH.

2.12. Oxidative stress and antioxidant markers

The level of markers of oxidative stress, i.e., Nitric oxide (NO) in serum, Malondialdehyde (MDA), glutathione (GSH) and antioxidant enzyme Superoxide dismutase (SOD) in cartilage were determined in all experimental groups. The analysis of malondialdehyde (MDA), a lipid peroxidation product, was done by TCA-TBA-HCl reagent method described by Okhawa *et al.* [20]. The absorbance was measured at 530 nm and concentration of MDA was expressed as mmol/g tissue. Superoxide dismutase (SOD) activity was assayed by the method of Kakkar *et al.* [21]. Absorbance was measured at 560nm. One Unit of enzyme activity is expressed as Units/mg protein. Glutathione (GSH) was estimated by the method of Benke *et al.* [22]. Myeloperoxidase (MPO) in serum was estimated by the method of Bradley *et al.* [23].

2.13. Dichloro-dihydro fluorescein diacetate (DCFH-DA) Assay for ROS estimation by Flowcytometry

The reactive oxygen species (ROS) levels in liver samples were estimated using DCFH-DA. 250 g of liver tissue was homogenized in PBS (PH 7.4) and processed to obtain single cell suspensions through cell strainer of 40µm. The cells were incubated with 10µM DCFH-DA at 37°C for 30 minutes in dark [24,25]. Upon oxidation by ROS, DCFH gets converted to fluorescent compound DCF and its intensity was measured using flow cytometer with excitation and emission wavelengths of 485 nm and 535 nm, respectively, as an indicator of intracellular ROS level.

2.14. Hematoxylin-Eosin staining

The spleen was fixed in 10% formalin solution, embedded in paraffin, and sectioned at 5µm thickness. The sections were then stained with hematoxylin to visualize nuclei and eosin to stain the cytoplasmic components to analyze inflammation and overall tissue architecture [26]. Stained sections were viewed under light microscope.

2.15. Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM) values. The results from all the experiments were statistically analyzed by One-way ANOVA (analysis of variance) followed by Tukey's multiple-range tests using GraphPad Prism 5 software. P-value < 0.05 was considered significant.

3. Results

3.1. Effect of Kodo millet on body weight changes and paw edema inhibition

Changes in body weight and paw perimeter were measured on days 0,7,14,21,28,45 and 60. After collagen induction, a gradual increase in paw perimeter was observed in normal rats, indicating inflammation and swelling, while the experimental groups receiving 10% Kodo millet supplementation along with normal feed showed a signi-

ficant decrease in inflammation gradually (Fig.1A & C). Additionally, CIA induction led to noticeable weight loss observed as part of disease progression. However, dietary intervention with millet feed mitigated these changes and balanced a healthy body weight (Fig.1B).

3.2. Effect of Kodo millet on markers of RA

The Kodo millet supplementation led to a significant decrease in serum arthritic markers like rheumatoid factor (RF), cyclic citrullinated protein (CCP) antibody and uric acid (Fig.1D). The RF levels in CIA rats significantly reduced ($p<0.05$) on millet supplementation. Similarly, uric acid levels were significantly reduced in millet-fed rats. The reduction in these markers indicated potential amelioration of autoantibody-mediated response and improvement in purine metabolism related to arthritis progression.

3.3. Effect of Kodo millet on spleen and thymus indices

Spleen and thymus are important immune organs and their indices serve as an important parameter for assessing immune status and organ responsiveness in animal models on supplementation of kodo millet. The results of both indices showed a significant reduction ($p<0.05$) compared to disease models after 45 days of millet supplementation (Fig.1E & F).

3.4. Histopathology of spleen

The histopathology of spleen tissue from normal group exhibited normal spleen architecture along with no signs of inflammatory infiltration. CIA groups showed significant architectural changes, such as splenic hyperplasia and immune cell infiltration, along with increased sinusoidal density with prominent germinal centers, which might be due to prolonged immune cell activation. After millet supplementation, sinusoid density, germ center size, and white pulp cellularity were decreased that supporting the improvement of disease state by reducing inflammation (Fig.2A).

3.5. Effect of Kodo millet on BAFF in serum

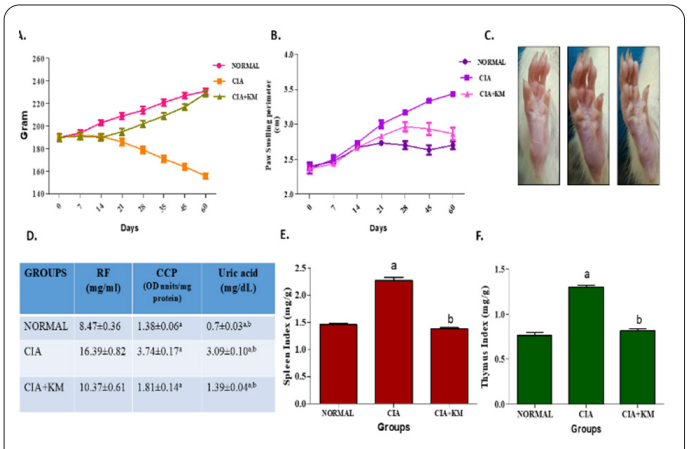
The levels of B cell activating factor (BAFF) in serum were significantly ($p<0.05$) elevated in CIA rats compared to healthy controls (Fig.2B), which promotes B cell activation and autoimmunity in CIA models and millet supplementation significantly reduced serum BAFF levels, which correlates with amelioration of disease progression.

3.6. Effect of Kodo millet on gene expression of APRIL in spleen

A proliferation-inducing ligand (APRIL) expressed by various immune and non-immune cells in the spleen and the gene expression of APRIL was upregulated in CIA rats (Fig.2C&D) compared to the normal rats, which supports B cell promotion and immunoglobulin class switching. After millet supplementation, gene expression was significantly ($p<0.05$) downregulated by which disease environment was modified.

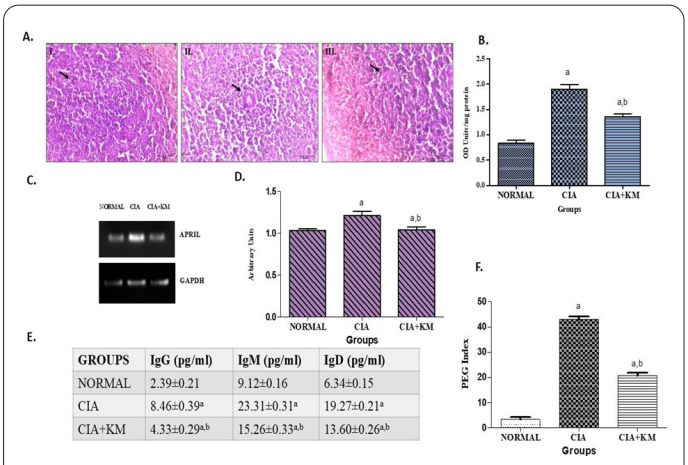
3.7. Effect of Kodo millet on Serum immunoglobulins

Serum levels of IgG, IgM and IgD were estimated by the ELISA method and their levels were significantly elevated ($p<0.05$) in the arthritic model, which is consistent with hyperactivation of humoral immunity. 45 days of Kodo millet supplementation significantly ($p< 0.05$) reduced immunoglobulin levels compared to untreated disease control (Fig.2E). This reduction indicates millet's potential to alleviate autoantibody production and humoral immunity response.



GROUPS	RF (mg/ml)	CCP (OD units/mg protein)	Uric acid (mg/dL)
NORMAL	8.47±0.36	1.38±0.06^{a}	0.7±0.03^{a,b}
CIA	16.39±0.82	3.74±0.17^{a}	3.09±0.10^{a,b}
CIA+KM	10.37±0.61	1.81±0.14^{a}	1.39±0.04^{a,b}

Fig. 1. A. Effect of kodo millet on body weight at regular intervals to assess overall physiological changes. B. Changes in paw perimeter after CIA induction to evaluate inflammation and edema. C. Photo of the hind paw at the end of experiment. D. RF (Rheumatoid factor), CCP (Cyclic citrullinated protein) are antibodies measured to evaluate changes in autoimmune responses and uric acid levels are measured to analyse metabolic function and joint inflammation. E. Spleen index and F. Thymus index were calculated as the ratio of organ weight to body weight. Group I- Normal, Group II- CIA rats, Group III- CIA+KM. Data are expressed as Mean ± SEM values (n=6, $p<0.05$). Results were statistically analysed by one-way ANOVA and Tukey's test. 'a'-significant difference compared to Normal group, 'b'- significant difference compared to CIA group.



GROUPS	IgG (pg/ml)	IgM (pg/ml)	IgD (pg/ml)
NORMAL	2.39±0.21	9.12±0.16	6.34±0.15
CIA	8.46±0.39^{a}	23.31±0.31^{a}	19.27±0.21^{a}
CIA+KM	4.33±0.29^{a,b}	15.26±0.33^{a,b}	13.60±0.26^{a,b}

Fig. 2. Effect of KM on humoral immunity. A. Histopathology of spleen tissue section stained with haematoxylin and eosin (H&E) stains. Images were captured under light microscope with 40X magnification. The black arrow represents the white pulp. B. B- cell activating factor (BAFF) levels in serum were analysed by ELISA to assess B cell survival and immune response. C. Gene expression of A-proliferation inducing ligand (APRIL) to understand its role in antibody production. D. Relative density in arbitrary units. E. Serum Immunoglobulin concentration was estimated by ELISA to analyse humoral immune modulation. F. Turbidimetric estimation of Circulating immune complex (CIC) to analyse immune-complex mediated inflammation. Group I- Normal, Group II- CIA rats, Group III- CIA+KM. All Data are expressed as Mean ± SEM values (n=6, $p<0.05$). Results were statistically analysed by one-way ANOVA and Tukey's test. 'a'-significant difference compared to Normal group, 'b'- significant difference compared to CIA group.

3.8. Effect of Kodo millet on circulating immune complex

During arthritic progression, immune complexes are formed as a result of interaction between autoantibodies and self-antigens. Fig.2F illustrates the effect of kodo millet on circulating immune complex (CIC) in serum and it is shown that it was significantly increased in sera of diseased group compared to normal group. However, 10% millet supplementation significantly ($p<0.05$) reduced CIC levels compared to arthritic rats, indicating its potential to modulate immune responses.

3.9. Effect of Kodo millets on lymphocyte proliferation

Immune activation in different groups was further confirmed and quantified by lymphocyte proliferation assay, providing a detailed assessment of cellular response. Lymphocytes have been isolated from spleen and stimulated with the mitogen concanavalin. The proliferation was estimated using MTT and changes in absorbance were measured at A470 represented in Fig.3A. A470 values of CIA groups were significantly elevated compared to normal control an indicative of enhanced immune activation and after 10% kodo millet supplementation, it is significantly ($p<0.05$) decreased.

3.10. Effect of Kodo millet on Th17 cell types

Retinoic acid receptor-related orphan nuclear receptor (RORγ) is the specific transcription factor for Th17 cells and its expression was significantly ($p<0.05$) upregulated in CIA groups. Th 17 cells produce proinflammatory cytokines IL-17A and IL-6, which were also increased in diseased group. After millet supplementation, the levels of IL-17A and IL-6 were significantly reduced along with downregulation of RORγ, depicted in Fig.3B, C&D.

3.11. Effect of Kodo millet on Th2 cell types

Th2 cells take part in immune regulation by suppressing excessive inflammation and GATA-3 is the regulatory transcription factor. Collagen induction downregulated GATA-3 expression compared to healthy rats and after millet supplementation, it was significantly upregulated ($p<0.05$) (Fig.4B&E). Th2 cells produce important anti-inflammatory cytokines IL-4 and IL-10 that suppress Th17 cells types. The levels of these cytokines declined in CIA rats and after 45 days of kodo millet supplementation, their levels were significantly ($p<0.05$) elevated (Figure 3F).

3.12. Effect of Kodo millet on RANKL-NFATc mediated osteoclastogenesis

The protein levels of RANKL were significantly ($p<0.05$) elevated in CIA groups compared to normal group by ELISA and western blot and after millet supplementation, the protein was significantly ($p<0.05$) reduced in Group III (Fig.4A, B&C). During RA progression, elevated RANKL levels produced by synovial fibroblasts lead to overactivation of NFAT (Nuclear factor of activated T-cells) and our results show increased levels of NFAT in CIA groups. After millet supplementation, the levels of NFAT significantly ($p<0.05$) reduced (Fig.4D), supporting the ameliorative effect of millet on joint damage.

3.13. Effect of Kodo millet on MMP-9

The activity of MMP9 (Gelatinase B) was significantly ($p<0.05$) elevated in CIA rats detected by qPCR (Fig.4E) and ELISA (Fig.4F), in the synovial joint might cause ECM degradation and bone damage. After 45 days of kodo millet supplementation, it markedly reduced the acti-

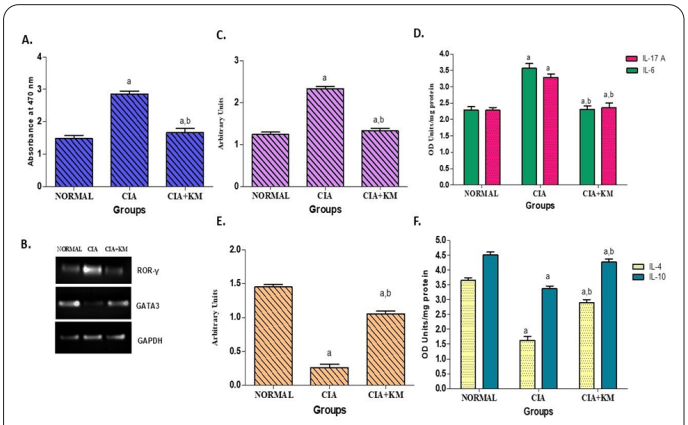


Fig. 3. Effect of KM on T helper cell subtypes. After 60 days of experimental period, splenocytes were isolated and cultured in RPMI 1640 medium, and cells and supernatants were collected. A. Lymphocyte proliferation was analysed using MTT to evaluate cell viability, which indicates immune response modulation under different conditions. B. mRNA expressions of Retinoic acid receptor-related orphan receptor γ (ROR-γ) and GATA3 on agarose gel compared to internal control GAPDH. They regulate Th17 and Th2 cell differentiations, respectively and orchestrate pro- and anti-inflammatory cytokine production. C. Relative density of ROR-γ in arbitrary units. D. Proinflammatory cytokines, IL-17A and IL-6 levels by ELISA. E. Relative density of GATA3 in arbitrary units. F. Anti-inflammatory cytokines IL-4 and IL-10 levels by ELISA. Group I- Normal, Group II- CIA rats, Group III- CIA+KM. All Data are expressed as Mean ± SEM values (n=6, $p<0.05$). Results were statistically analysed by one-way ANOVA and Tukey's test. 'a'-significant difference compared to Normal group, 'b'- significant difference compared to CIA group.

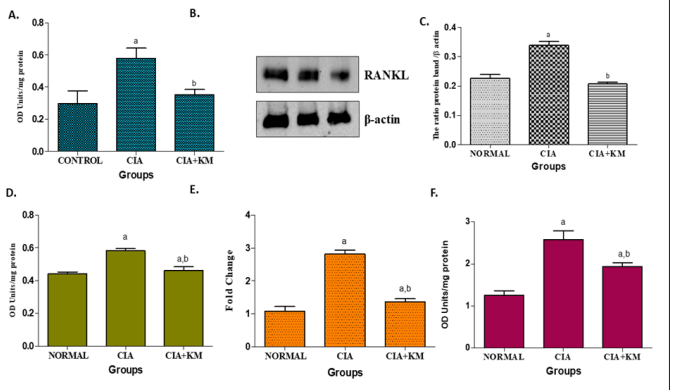


Fig. 4. Effect of KM on RANKL-mediated osteoclastogenesis in cartilage. Receptor activator of nuclear factor kappa-B ligand (RANKL) binds to the surface of osteoclast precursors in cartilage and causes downstream signalling for bone degradation. A. Estimation of protein levels of RANKL in cartilage by ELISA. B. Western blot analysis of RANKL protein and housekeeping gene β-actin. C. Relative quantification of RANKL from western blot D. Estimation of (nuclear factor of activated T-cells) NFATc by ELISA. E. Relative Matrix metalloproteinase 9 (MMP9) expression by qPCR, F. Protein levels of MMP9 by ELISA. Group I- Normal, Group II- CIA rats, Group III- CIA+KM. All Data are expressed as Mean ± SEM values (n=6, $p<0.05$). Results were statistically analysed by one-way ANOVA and Tukey's test. 'a'-significant difference compared to Normal group, 'b'- significant difference compared to CIA group.

vity of MMP9, suggesting the inhibitory activity of kodo millet on its proteolytic function.

3.14. Effect of Kodo millet on ROS generation

FACS analysis confirmed elevated ROS generation in single cell suspension of liver cells from CIA rats. 45 days of millet supplementation to collagen-induced groups significantly reduced systemic inflammation or oxidative stress, significantly reduced hepatic ROS levels, which were estimated using DCFHDA (Fig.5A).

3.15. Effect of Kodo millet on antioxidants and oxidative stress indicators

Oxidative stress indicators and lipid peroxidation products, such as MDA and NO levels, were significantly ($p<0.05$) elevated in serum of CIA rats compared to normal rats. Similarly, proinflammatory enzyme MPO was elevated and antioxidant enzymes SOD and GSH were significantly ($p<0.05$) decreased after CIA induction. After kodo millet supplementation, there was a significant decrease in MPO and MDA along with improved levels of SOD and GSH (Fig.5B-F).

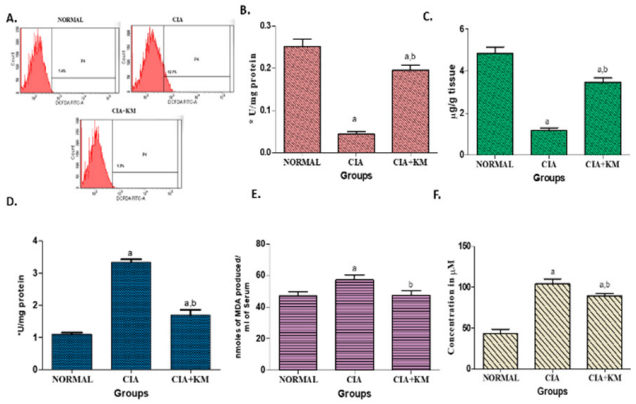


Fig. 5. Effect of Kodo millet on oxidative stress parameters. A. Effect of KM on ROS production in liver. B. The activity of Superoxide dismutase (SOD), an essential antioxidant that converts superoxide radicals to hydrogen peroxide and protects the body from oxidative stress. C. Concentration of Glutathione (GSH) in cartilage, an intracellular antioxidant, plays a protective role from oxidative damage. D. Myeloperoxidase (MPO) is a marker of nuclear infiltration and its activity is estimated in serum. E. lipid peroxidation product malondialdehyde (MDA) concentration in serum. F. Nitric oxide (NO) is a gaseous inflammatory molecule produced from L-arginine by nitric oxide synthase, which plays crucial role in oxidative stress and synovial inflammation by modulating osteoclastogenic pathways and its concentration was estimated in serum. Group I- Normal, Group II- CIA rats, Group III- CIA+KM. All Data are expressed as Mean ± SEM values (n=6, $p<0.05$). Results were statistically analysed by one-way ANOVA and Tukey's test. 'a'-significant difference compared to Normal group, 'b'- significant difference compared to CIA group.

4. Discussion

Rheumatoid arthritis is a multifactorial disease-causing persistent inflammation and progressive joint damage, affecting approximately 1% population in the world [27,28]. Most rheumatoid arthritis models exhibit similar clinical and pathological features to human rheumatoid arthritis and collagen-induced arthritis is a well-established model in rats [29]. Even though pharmacological interventions, including NSAIDs and DMARDs, are the mainstay treatment, dietary modifications and dietary therapy emerged as complementary strategy to improve overall health conditions in progression of RA. Hence, our study investigated the disease-modifying potential of kodo millet in CIA models induced in female Wistar rats, focusing on the pathway involved in immunomodulation, inflammation and oxidative stress.

The animals induced with collagen cause significant reduction in body weight, paw swelling and redness related to increased metabolism and inflammation consistent with previous studies on arthritis [1,30]. 10% kodo millet supplementation for 45 days maintained a healthy body weight, paw volume and erythema reduction and movement of rats was improved as part of mitigative effect on inflammation. Increased levels of RF, CCP and uric acid are the progressive symptoms of RA and these were significantly higher in the serum of arthritic groups. Hyperactivation of B cells and heightened antibody production are indicated by RF rats, which play a crucial role in immune complex formation. Kodo millet supplementation to arthritic rats decreased the levels of RF, which agrees with the research of Xiong, Ri-Bo *et al.* [31]. Significant reduction in uric acid levels aligns with previous studies in curcumin-treated collagen-induced arthritis [32]. CCP antibodies are specific markers of RA and are elevated in autoimmune conditions. Significant reduction in CCP was also observed in research conducted by Mathew LE *et al.* [33] who were studying about combination therapy using fluvastatin and betulinic acid in RA rats.

The spleen and thymus are vital lymphoid organs that play central roles in the immune system and a previous study with celastrol treatment reduced the increased index of organ indices in CIA rats [34]. Similarly, in our study, after millet supplementation spleen and thymus indices were significantly reduced in collagen-induced rats, which suggests effective suppression of enhanced production of the immunocytes and ability to balance immune cell homeostasis. Splenomegaly is the direct consequence of expanded immune response [35,36] and histopathological examination of CIA rats showed marked structural alterations. Kodo millet supplementation to RA rats improved hyperplasia and germinal center variations, highlighting the immunomodulatory property of millet, aligning with the findings of Chang Yan *et al.* [37], who also observed similar effects of a novel compound against autoimmune arthritis. To conduct a more comprehensive and systemic investigation, we observed modulation of B-cell activation factor (BAFF), A proliferation-inducing ligand (APRIL), immunoglobulins like IgG, IgM and IgD and CIC by various techniques.

During millet supplementation, the maintenance of the aforementioned parameters supports the potential of millets to suppress B-cell hyperactivity and inhibit the overproduction of autoantibodies, highlighting their multifaceted role in humoral immunity. Elevated levels of BAFF and APRIL in CIA rats indicate B-cell proliferation and disease progression. However, 45 days of kodo millet supplementation significantly reduced BAFF levels and downregulated APRIL expression in spleen tissue. These changes align with therapeutic modulation of BAFF and APRIL, thereby ameliorating the autoimmune condition [38]. Improved serum concentrations of IgG, IgM, and IgD following dietary modification further indicate the ability to suppress B-cell-mediated disease progression.

Lymphocyte proliferation in the spleen plays a critical role in autoimmune conditions, including antibody production; therefore, we isolated and cultured splenocytes from all experimental groups. CIA rats exhibited elevated lymphocyte proliferation and upregulation of the regulatory transcription factor ROR-γ, accompanied by increased levels of proinflammatory cytokines IL-6 and IL-17A. Simultaneously, the anti-inflammatory system was disrupted, evidenced by downregulation of GATA3 and corresponding decreases in anti-inflammatory cytokines IL-4 and IL-10. Millet supplementation restored balance between T helper (Th) cell subsets, improving homeostasis of inflammatory mediators, lymphocyte proliferation, and regulatory gene expression. These findings are consistent with the work of Peter, Jasmine *et al*. [39].

In CIA rats, immunocyte activation and modulation are primarily regulated by the spleen, with localized inflammation occurring in other organs, including the joints [40]. We investigated the effect of kodo millet on the RANKL-NFATc pathway and matrix metalloproteinase 9 (MMP9) in the CIA model. RANKL binds to osteoclast precursor surfaces in cartilage, activating the transcription factor NFATc, which drives downstream signaling to increase the expression of matrix metalloproteinases [41,42]. In our study, the increased levels of RANKL in the synovial joint were confirmed by Western blot in CIA rats compared to normal rats. NFATc is the transcription factor regulating this pathway and elevated levels of NFATc are found to be lowered after kodo millet consumption, along with normal levels of RANKL. MMP-9 is an important extracellular matrix-degrading enzyme activated by NFATc that contributes to joint destruction. In dietary modified groups, their expression was downregulated, similar to a study conducted by Pu, Jiang, *et al.* [43]. These findings highlight the role of kodo millets in targeting the RANKL/NFATc/MMP9 axis in RA.

In RA, elevated levels of reactive oxygen species have arisen due to chronic inflammation, immune cell activation and tissue damage. Proinflammatory mediators released by the immunocytes in turn increase ROS production and inhibit antioxidant system and contributing to oxidative stress. After millet supplementation, MDA and NO were reduced, along with the improved status of SOD and GSH in cartilage tissues of CIA rats. SOD is the first antioxidant system that works along with the co-substrate GSH to inhibit oxidative stress and excessive ROS production. Here, kodo millets are rich in bioactive compounds and show similar effects as observed in the case of administration of Siweixizangmaoru decoction in CIA rats [44,45]. In addition, Elevated Serum MPO activity in CIA rats was significantly reduced after kodo millet supplementation, as in Sindhu *et al.* [46]

As a result, 45 days of millet supplementation in CIA rats demonstrated its potential as a promising dietary intervention for managing inflammatory conditions, including rheumatoid arthritis. However, many people in modern society are unfamiliar with the diversity of millets and remain largely unaware of their health benefits. Furthermore, millets are often exclusively perceived as bird feed rather than valuable human nutrition [47]. Incorporating kodo millet into the diet can modulate oxidative stress, reduce inflammation, and enhance immune regulation, thereby improving overall symptoms and aiding disease management, especially in populations with limited access to other nutrient-rich foods.

Kodo millet demonstrates significant potential as a functional food for dietary therapy in a rheumatoid arthritis model, owing to its immunomodulatory properties, ability to mitigate inflammatory pathways, and capacity to improve antioxidant status, thereby protecting against tissue damage. Our study highlights kodo millet's role in restoring redox balance by modulating Th2-Th17-mediated immune responses, targeting the RANKL/NFATc signaling axis, inhibiting reactive oxygen species (ROS) production, and enhancing antioxidant enzyme activity. These findings underscore the therapeutic potential of kodo millet in slowing inflammation progression and managing rheumatoid arthritis by correcting immune system dysfunction.

Abbreviation

RA, rheumatoid arthritis; CIA, collagen-induced arthritis; KM, kodo millet; Th, helper T cell; Treg, regulatory T cells; TLR4, toll-like receptor 4; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NSAIDs, nonsteroidal anti-inflammatory drugs; DMARDs, disease-modifying antirheumatic drugs; CPCSEA, control and supervision of experiments on animals; FAO, Food and agricultural organization; RF, rheumatoid factor; CCP, cyclic citrullinated protein; BAFF, B cell activating factor; APRIL, a proliferation inducing ligand; RORγ, retinoic acid receptor related orphan nuclear receptor; CIC, circulating immune complex; PEG, Polyethylene glycol; BBS, Borate buffered saline; FBS, Fetal bovine serum; ELISA, Enzyme linked immunosorbent assay; DMSO, Dimethyl sulfoxide, RANKL, receptor activator of nuclear factor kappa-B ligand; NFATc, nuclear factor of activated T-cells; IL, interleukin; Ig, immunoglobulin; MMP9, matrix metalloproteinase 9; ROS, reactive oxygen species; SOD, superoxide dismutase; MPO, myeloperoxidase; MDA, malondialdehyde; GSH, glutathione; NO, nitric oxide

Conflict of interests

The authors declare no conflict of interest.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

All experiments were conducted as per the current institutional animal ethics committee, Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (IAEC-16-KU-16/2022-BCH-AH (43)), according to the Government of India accepted principles for laboratory animal use and care.

Informed consent

The authors declare that no patients were used in this study.

Availability of data and material

All data are included in the manuscript.

Authors' contributions

Abhirami Sunitha and Antony Helen designed, performed, analyzed the data and wrote the manuscript. Amrutha Dileep Kumar Sreeja Kumari, Mani Sebastian, Haritha Rajan and Salu Valsala Sasi Kumar contributed to the ana-

lysis of the study. Maya Gopinathan Pillai contributed to the preparation of the manuscript.

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References

1. Cui X, Wang R, Bian P, Wu Q, Seshadri VD, Liu L (2019) Evaluation of antiarthritic activity of nimbolide against Freund's adjuvant induced arthritis in rats Artif cells Nanomed Biotechnol 47:3391-8 doi: 10.1080/21691401.2019.1649269
2. Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ, Xu J (2018) Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies Bone Res 6: 15 doi: 10.1038/s41413-018-0016-9
3. Fikry EM, Gad AM, Eid AH, Arab HH (2019) Caffeic acid and ellagic acid ameliorate adjuvant-induced arthritis in rats via targeting inflammatory signals, chitinase-3-like protein-1 and angiogenesis. Biomed Pharmacother 110: 878–886 doi: 10.1016/j.biopha.2018.12.04
4. Sharma A, Khan R, Gupta N, Sharma A, Zaheer MS, Abbas M, et al. (2018) Acute phase reactant, Pentraxin 3, as a novel marker for the diagnosis of rheumatoid arthritis Clin Chim Acta 480: 65–70 doi: 10.1016/j.cca.2018.01.035
5. Bäcklund R, Drake I, Bergström U, Compagno M, Sonestedt E, Turesson C (2023) Diet and the risk of rheumatoid arthritis – a systematic literature review Semin Arthritis Rheum 58: 152118 doi: 10.1016/j.semarthrit.2022.152118
6. Liu H, Kong L, Cao D, Zhan X, Gao X, Sun H, et al. (2024) Efficacy and mechanism of the Ermiao San series of formulas for rheumatoid arthritis based on Chinmedomics strategy Phytomedicine 132:155903 doi: 10.1016/j.phymed.2024.155903
7. Boissier M-C, Assier E, Falgarone G, Bessis N (2008) Shifting the imbalance from Th1/Th2 to Th17/Treg: the changing rheumatoid arthritis paradigm Joint Bone Spine 75: 373–375 doi: 10.1016/j.jbspin.2008.04.005
8. Zhu X, Du L, Zhang L, Ding L, Xu W, Lin X (2024) The critical role of toll-like receptor 4 in bone remodeling of osteoporosis: from inflammation recognition to immunity Front Immunol 15: 1333086 doi: 10.3389/fimmu.2024.1333086
9. Singh VS, Dhawale SC, Shakeel F, Faiyazuddin M, Alshehri S (2021) Antiarthritic potential of *Calotropis procera* leaf fractions in FCA-induced arthritic rats: involvement of cellular inflammatory mediators and other biomarkers *Agriculture* 11: 68 doi: 10.3390/agriculture11010068
10. Bell PG, Walshe IH, Davison GW, Stevenson E, Howatson G (2014) Montmorency cherries reduce the oxidative stress and inflammatory responses to repeated days of high-intensity stochastic cycling Nutrients 6: 829–843
11. Casas R, Sacanella E (2014) The immune protective effect of the Mediterranean diet against chronic low-grade inflammatory diseases Endocr Metab Immune Disord Drug Targets 14: 245–254 doi: 10.2174/1871530314666140922153350
12. Sharma R, Sharma S, Dar BN, Singh B (2021) Millets as potential nutri-cereals: a review of nutrient composition, phytochemical profile and techno-functionality Int J Food Sci Technol 56: 3703–3718 doi: 10.1111/ijfs.15044
13. Mishra A, Dutta T, Baitharu I (2022) Nutritional values and potential health benefits of millets J Nutrients 8: 9–26 doi: 10.18488/87.v8i1.3176
14. Manna T (2023) Role of millets in food security: in the context of the International Year of Millets Krishi Parasar School of Agriculture and Allied Sciences 3(1): April 2023
15. Chen H, Pan T, Liu P, Wang P, Xu S (2019) Baihu Jia Guizhi decoction improves rheumatoid arthritis inflammation by regulating succinate/SUCNR1 metabolic signaling pathway Evid Based Complement Alternat Med 2019: 3258572 doi: 10.1155/2019/3258572
16. Gao Q, Qin H, Zhu L, Li D, Hao X (2020) Celastrol attenuates collagen-induced arthritis via inhibiting oxidative stress in rats Int Immunopharmacol 84: 106527 doi: 10.1016/j.intimp.2020.106527
17. Seth P, Srinivas RV, Wang J, Zhang Q, He JS, Zhao D, et al. (1981) Circulating immune-complexes in cervical cancer – simple method for detection and characterization Indian J Med Res 73: 405–412
18. Wang J, Zhang Q, Jin S, He D, Zhao S, Liu S (2008) Genistein modulates immune responses in collagen-induced rheumatoid arthritis model Maturitas 59: 405–412 doi: 10.1016/j.maturitas.2008.04.003
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin-phenol reagent J Biol Chem 193: 265–275
20. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction Anal Biochem 95: 351–358 doi: 10.1016/0003-2697(79)90738-3
21. Kakkar P, Das B, Viswanathan PN (1984) A modified spectrophotometric assay of superoxide dismutase Indian J Biochem Biophys 21: 130–132
22. Benke GM, Cheever KL, Mirer FE, Murphy SD (1974) Comparative toxicity, anticholinesterase action and metabolism of methyl parathion and parathion in sunfish and mice Toxicol Appl Pharmacol 28: 97–109 doi: 10.1016/0041-008X(74)90135-5
23. Bradley PP, Priebat DA, Christensen RD, Rothstein G (1982) Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker J Invest Dermatol 78: 206–209 doi: 10.1111/1523-1747.ep12506462
24. Habashy NH, Abu-Serie MM (2024) Attenuation of carbon tetrachloride-induced nephrotoxicity by gum Arabic extract via modulating cellular redox state, NF-κB pathway, and KIM-1 Biomed Pharmacother 173: 116340 doi: 10.1016/j.biopha.2024.116340
25. Simizu S, Imoto M, Masuda N, Takada M, Umezawa K (1996) Involvement of hydrogen peroxide production in erbstatin-induced apoptosis in human small cell lung carcinoma cells Cancer Res 56: 4978–4982
26. Patil MVK, Kandhare AD, Bhise SD (2012) Anti-arthritic and anti-inflammatory activity of Xanthium strumarium L. ethanolic extract in Freund's complete adjuvant induced arthritis Biomed Aging Pathol 2: 6–15 doi: 10.1016/j.biomag.2012.01.002
27. Laborde CM, Castro-Santos P, Díaz-Peña R (2020) Contribution of multiplex immunoassays to rheumatoid arthritis management: from biomarker discovery to personalized medicine J Pers Med 10: 202 doi: 10.3390/jpm10040202
28. Magyari L, Varszegi D, Kovesdi E, Sarlos P, Farago B, Javorhazy A, Sumegi K, Banfai Z, Melegh B (2014) Interleukins and interleukin receptors in rheumatoid arthritis: research, diagnostics and clinical implications World J Orthop 5: 516–536 doi: 10.5312/wjo.v5.i4.516
29. Refaat R, Salama M, Abdel Meguid E, El Sarha A, Gowayed M (2013) Evaluation of the effect of losartan and methotrexate combined therapy in adjuvant-induced arthritis in rats Eur J Pharmacol 698: 421–428 doi: 10.1016/j.ejphar.2012.10.024
30. Li W, Mao X, Wang X, Liu Y, Wang K, Li C, et al. (2021) Disease-modifying anti-rheumatic drug prescription Baihu-Guizhi decoction attenuates rheumatoid arthritis via suppressing toll-like receptor 4-mediated NLRP3 inflammasome activation Front

Pharmacol 12: 743086 doi: 10.3389/fphar.2021.743086
31. Xiong R-B, Li Q, Wan W-R, Guo J-Q, Luo B-D, Gan L (2014) Effects and mechanisms of vitamin A and vitamin E on the levels of serum leptin and other related cytokines in rats with rheumatoid arthritis Exp Ther Med 8: 499–504 doi: 10.3892/etm.2014.1777
32. Bisset S, Sobhi W, Attoui A, Lamaoui T, Jardan YAB, Das S, Alam M, Kanouni KE, Rezgui A, Ferdjioui S, Derradji Y, Khenchouche A, Benguerba Y (2023) Targeting oxidative stress markers, xanthine oxidase, TNFRSF11A and cathepsin L in curcumin-treated collagen-induced arthritis: a physiological and COSMO-RS study Inflammation 46(1): 432–452 doi: 10.1007/s10753-022-01745-7
33. Mathew LE, Rajagopal V, Helen A (2017) Betulinic acid and fluvastatin exhibits synergistic effect on toll-like receptor-4 mediated anti-atherogenic mechanism in type II collagen induced arthritis Biomed Pharmacother 93: 681–694 doi: 10.1016/j.biopha.2017.06.053
34. Xu R, Liu Z, Hou J, Huang T, Yang M (2018) Osthole improves collagen-induced arthritis in a rat model through inhibiting inflammation and cellular stress Cell Mol Biol Lett 23: Article 30 doi: 10.1186/s11658-018-0086-0
35. Vincent FB, Morand EF, Schneider P, Mackay F (2014) The BAFF/APRIL system in SLE pathogenesis Nat Rev Rheumatol 10:365–373 doi: 10.1038/nrrheum.2014.33
36. Nurmurodovna B, Mekhriniso BD (2025) Morphological changes of the spleen in autoimmune diseases: a literature review Web Med J Med Pract and Nurs 3:36–43.
37. Talkov RH, Bauer W, Short CL (1942) Rheumatoid arthritis associated with splenomegaly and leukopenia N Engl J Med 227:395–399 doi: 10.1056/NEJM194209102271101
38. Chang Y, Jia X, Wei F, Wang C, Sun X, Xu S, Yang X, Zhao Y, Chen J, Wu H, Zhang L (2016) CP-25, a novel compound, protects against autoimmune arthritis by modulating immune mediators of inflammation and bone damage Sci Rep 6:26239 doi:10.1038/srep26239
39. Peter J, Sabu V, Aswathy IS, Krishnan S, Lal Preethi SS, Simon M, Helen A (2020) Dietary amaranths modulate the immune response via balancing Th1/Th2 and Th17/Treg response in collagen-induced arthritis Mol Cell Biochem 472:57–66 doi:10.1007/s11010-020-03783-x
40. Fang Q, Zhou C, Nandakumar KS (2020) Molecular and cellular pathways contributing to joint damage in rheumatoid arthritis Mediators Inflamm 2020:3830212 doi:10.1155/2020/3830212
41. Pu J, Fang FF, Li XQ, Shu ZH, Jiang YP, Han T, Peng PW, Zheng CJ (2016) Matrine exerts a strong anti-arthritic effect on type II collagen-induced arthritis in rats by inhibiting inflammatory responses Int J Mol Sci 17:1410. doi:10.3390/ijms17091410
42. Sundaram K, Nishimura R, Senn J, Youssef RF, London SD, Reddy SV (2007) RANK ligand signaling modulates the matrix metalloproteinase-9 gene expression during osteoclast differentiation. Exp Cell Res 313:168–178. doi:10.1016/j.yexcr.2006.10.001
43. Pant T, Uche N, Juric M, Zielonka J, Bai X (2024) Regulation of immunomodulatory networks by Nrf2-activation in immune cells: Redox control and therapeutic potential in inflammatory diseases Redox Biol 70:103077 doi:10.1016/j.redox.2024.103077
44. Niu Y, Feng Q, Cui M, Fan C, Wang T, Yuan R, Tsering D, Huang S, Li B (2024) Siweixizangmaoru Decoction ameliorated type II collagen-induced arthritis in rats via regulating JAK2–STAT3 and NF-κB signaling pathway. Biol Pharm Bull 47:1511–1524. doi:10.1248/bpb.b24-00362
45. Adefegha SA, Okeke BM, Oboh G, Ijomone OM, Oyeleye SI (2018) Modulatory effect of eugenol on arginase, nucleotidase, and adenosine deaminase activities of platelets in a carrageenan-induced arthritis rat model: A possible anti-arthritic mechanism of eugenol Biomed Pharmacother 106:1616–1623. doi:10.1016/j.biopha.2018.07.143
46. Sindhu G, Ratheesh M, Shyni GL, Nambisan B, Helen A (2012) Anti-inflammatory and antioxidative effects of mucilage of Trigonella foenum-graecum (Fenugreek) on adjuvant induced arthritic rats Int Immunopharmacol 12:205–211 doi:10.1016/j.intimp.2011.11.012
47. Dey S, Saxena A, Kumar Y, Maity T, Tarafdar A (2022) Understanding the antinutritional factors and bioactive compounds of kodo millet (*Paspalum scrobiculatum*) and little millet (*Panicum sumatrense*) J Food Qual 2022:1–19 doi:10.1155/2022/1578448