Pharmacogn. Mag.
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ORIGINAL ARTICLE

Ameliorative Efficacy of Oxypaeoniflorin, a Traditional Chinese Medicine Monomer against Adjuvant-Induced Arthritic Inflammation and Pain

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Submitted: 11-Oct-2021 Revised: 04-Nov-2021 Accepted: 17-Dec-2021 Published: 28-Mar-2022

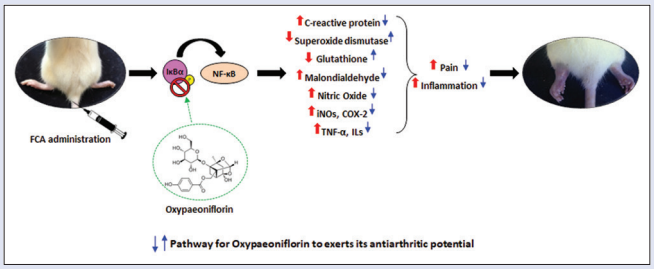
ABSTRACT

Background: Rheumatoid arthritis is a chronic, common autoimmune disorder identified by progressive dysfunction of joints and cartilage damage. Oxypaeoniflorin (OPA), a Traditional Chinese Medicine monomer, has been reported for potential against inflammation and oxidative stress. **Objectives:** The objective of the study is to evaluate the potential of OPA against Freund complete adjuvant (FCA)-induced inflammatory pain in experimental arthritic rats. **Materials and Methods:** FCA was administered in female rats (Wistar strain) to induce polyarthritis, followed by 28 days of oral treatment with either vehicle, leflunomide (10 mg/Kg), or OPA (10, 20, and 40 mg/kg). Various parameters were assessed to determine the effect of OPA on pain and inflammatory pathway. **Results:** Adjuvant-induced arthritis (AIA)-induced elevated paw withdrawal latency and threshold suggested induction of arthritic pain. However, OPA (20 and 40 mg/kg) treatment diminished arthritic pain reflected by amelioration of hyperalgesia and allodynia. In addition, OPA showed an effective inhibition ($P < 0.05$) in FCA-induced alterations in alkaline phosphatase, alanine transaminase, aspartate aminotransferase, serum albumin, and C-reactive protein levels. The AIA-induced elevated oxido-nitrosative stress, protein levels of tumor necrosis factor-α, and interleukins in synovial tissue were effectively reduced ($P < 0.05$) by OPA. Moreover, OPA effectively downregulated ($P < 0.05$) enhanced nuclear factor-kappa beta (NF-κB), Ikβα, iNOs, and COX-2 mRNA expressions in synovial tissue. OPA also reduced histopathology alteration induced in the tibiotarsal joint by FCA. **Conclusion:** OPA exerts its antiarthritic property through inhibition of NF-κB/IκBα pathway to downregulate the activation of pro-inflammatory cytokines and inflammatory mediator thus, ameliorated arthritic inflammation and pain.

Key words: Freund complete adjuvant, nuclear factor-kappa beta, oxypaeoniflorin, pro-inflammatory cytokines, rheumatoid arthritis, traditional Chinese medicine monomer

SUMMARY

- Freund's complete adjuvant (FCA) administration in the subplantar paw region induces arthritis
- Oxypaeoniflorin (OPA) (20 and 40 mg/kg) showed a marked inhibition in FCA-induced decrease in thermal and mechanical hyperalgesia
- OPA also noticeably down-regulated oxido-nitrosative stress, tumor necrosis factor-α, and interleukins levels
- FCA-induced upregulated synovial mRNA expressions of Nuclear factor-kappa beta, Ikβα, iNOs, and COX-2 were significantly inhibited by OPA
- OPA can be measured as one of the essential moieties from plant origin for the management of arthritis.



Abbreviations used: AIA: Adjuvant induced arthritis; ALT: Alanine transaminase; ALP: Alkaline phosphatase; AST: Aspartate Aminotransferase; CRP: C-reactive protein; COX-2: Cyclooxygenase-2; ESR: Erythrocyte Sedimentation Rate; FCA: Freund's Complete Adjuvant; GSH: Reduced Glutathione; Ikβα: Nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor-α; ILs: Interleukins; iNOs: inducible nitric oxide synthase; LF: Leflunomide; MDA: Malondialdehyde; NO: Nitric Oxide; NF-kB: Nuclear factor-kappa beta; OPA: Oxypaeoniflorin; ROS: Reactive Oxygen Species; SOD: Superoxide dismutase; TNF-α: Tumor necrosis factor-alpha.

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DOI: 10.4103/pm.pm_571_21

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INTRODUCTION

Rheumatoid arthritis (RA) is a common, chronic and complex autoimmune disorder characterized by advanced dysfunction of joints and cartilage impacted due to various inflammatory releases. A documented study suggested that women (ages: 40–50 years) are at higher risk of RA development than males.[1] According to the report of Global RA Network, the leading cause of disability is arthritis that affects more than 350 million persons across the globe, and almost 14 million people suffer from RA.[2] The study reported that Chinese RA patients associated with a 43% disability rate considering the disease duration of 5–10 years.[3] This disability is related to a heavy economic burden on the patient and the healthcare system. The cost for the management of RA in Chinese patients is reported as US$12 million per year, with an average economic cost of US$2245 per patient each year considering the Disability Adjusted Life Years in these patients.[4]

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Cite this article as: Lv K, Jiang M. Ameliorative efficacy of oxypaeoniflorin, a traditional chinese medicine monomer against adjuvant-induced arthritic inflammation and pain. Phcog Mag 2022;18:103-11.

Documented reports evidence that releasing pro-nociceptive agents, immune-inflammatory cells, and cytokines into joints induces inflammation into the synovial fluid.[5,6] The influx of prostaglandins and leukotrienes also stimulated primary nociceptive fibers that result in hyperalgesia and nociception at joints.[6,7] In addition, cyclooxygenases (COX) activates immune cells and induces inflammatory cytokines.[8,9] Furthermore, elevated free radical generation, including hydroxyl radicals and reactive nitrogen species, causes a structural perturbation in lipids membrane structure.[6,10,11] Thus, activation of free radicals is thought to be one of the crucial pathways for RA pathogenesis.[6] Therefore, during the last few decades, researchers have done a deep dive to evaluate the potential of new molecules for managing RA.

Disease-modifying antirheumatic drugs and nonsteroidal anti-inflammatory drugs are currently used to treat RA; however, their well-known side effects and high cost limit their routine clinical implications.[6] Thus, several researchers have extensively studied molecules of herbal origin for their effectiveness and safety in the management of RA.[12] Such products from herbal origin specifically targeted defined pain and inflammation mediators.[13] Recently, researchers have shifted their imperative preference to Traditional Chinese medicine (TCM) as a new, safe, and more cost-effective therapeutic option for the management of RA. Several studies reported the potential anti-inflammatory activity of TCM monomers, including sinomenine, paeoniflorin baicalin, artesunate, licochalcone, calycosin, dihydromyricetin, and curcumin.[14] In China, patients with RA are offered TCM monomers for their safety and efficacy over other western medications.[14,15] Oxypaeoniflorin (OPA) is one such TCM monomer, a monoterpene glycoside isolated from *Paeonia lactiflora*. OPA has been reported to inhibit the elevated oxidative stress and inflammation in mesangial cells HBZY-1 through inhibiting monocyte chemoattractant protein-1 and interleukin (IL)-6.[16] In addition, Yoo *et al.*(2018) reported the anti-inflammatory property of OPA through down-regulation of the extracellular signal-related kinase, toll-like receptor, and MAP (p38 mitogen-activated protein) kinases activities *in vitro*.[17] In the experimental model of myocardial ischemia/reperfusion injury, OPA induces cardioprotective efficacy via activation of Sirt1 (silent information regulator factor 2 related enzyme 1)/Foxo1 (forkhead transcription factor FKHR) signaling pathway.[18] Treatment of OPA exerts a protective efficacy in lipopolysaccharide-induced acute lung injury model through regulating PTEN (phosphatase and tensin homolog deleted on chromosome 10)/AKT pathway.[19] However, the potential of OPA in adjuvant-induced arthritis (AIA) remains unclear. Thus, the current study was designed to evaluate the efficacy of OPA against arthritis induced by Freund complete adjuvant (FCA) in experimental rats.

MATERIALS AND METHODS

Study animals

Female Wistar rats weighing from 150 to 180 g were maintained at standard laboratory conditions (temperature: 24°C ± 1°C, relative humidity: 45%–55%, feed: standard pellet and filtered water) in the animal house Affiliated Hospital of the Shaanxi University of Chinese Medicine. The animal ethics committee of Affiliated Hospital of the Shaanxi University of Chinese Medicine approved (Approval no. SUCMDL2021079001) all the protocols related to experimental research.

Induction of adjuvant-induced polyarthritis (adjuvant-induced arthritis)

Previously reported method was used to induce AIA using an intradermal injection of FCA (0.1 mL, Sigma Aldrich, St. Louis, USA) into the tail of the female rat.[20] Post-AIA development, rats were divided into 5 groups (each group contain 18 rats) and received either dimethyl sulfoxide (DMSO, 0.5%, 10 mg/kg) or OPA (OPA, 10, 20 and 40 mg/kg in 0.5% DMSO) or leflunomide (10 mg/kg in 0.5% DMSO) orally for the next 28 days.[21] A separate group of rats (n = 18) was maintained as normal (without administration of FCA). The paw volume, paw withdrawal threshold, and latency were determined according to previously reported methods using plethysmometer, von Frey hair application, and Hargreaves apparatus, respectively.[22-24] At the end of the treatment period, a blood sample was utilized to determine

C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), hemoglobin (Hb), platelets (PLT), red blood cell (RBC), and white blood cell (WBC). In addition, serum was utilized to determine alanine transaminase (ALT), albumin, alkaline phosphatase (ALP), and aspartate transaminase (AST).[22] Isolated synovial tissue was used to evaluate the levels of oxido-nitrosative stress (n = 5-6) markers, including lipid peroxidation (MDA), nitric oxide (NO), reduced glutathione (GSH), and superoxide dismutase (SOD) according to the method reported elsewhere.[25] Another portion of synovial tissue was used to assess the levels of pro-inflammatory cytokine (Tumor necrosis factor-alpha [TNF-α] and ILs) using a commercially available enzyme-linked immunosorbent assay kit (Bethyl Laboratories Inc., Montgomery, TX, USA). The levels of COX-2, Ikβα, iNOs, and Nuclear factor-kappa beta (NF-kβ) mRNA expressions (primer details provided in Table 1) in synovial tissues were determined using Reverse Transcriptase (RT)-polymerase chain reaction approach as per the manufacturer's instructions (MP Biomedicals India Private Limited, India).[25] The histological analysis of the tibiotarsal joint was evaluated using hematoxylin and eosin (H and E) and graded according to the previously described method.[26] GraphPad Prism 5.0 software was used for statistical analysis to compare the difference between AIA control animals and drug-treated groups. Statistically significant was achieved at $P < 0.05$.

RESULTS

Alterations in weight of body, joint diameter, paw volume, paw withdrawal latency, and threshold

FCA-caused induction of arthritis reflected a higher degree of ankle inflammation in AIA control rats [Figure 1b] in contrast to normal rats [Figure 1a]. This ankle inflammation was decreased by leflunomide and OPA treatment [Figure 1c-d].

A noteworthy decrease ($P < 0.05$) in weight of the body and increased ($P < 0.05$) in joint diameter, paw volume, paw withdrawal latency, and threshold were reported in the AIA control group confront to the normal group. However, the administration of leflunomide effectively ($P < 0.05$) lessened AIA-induced alterations in body weight, joint diameter, paw volume, paw withdrawal latency, and threshold than the AIA control group. Treatment with OPA (10 and 20 mg/kg) markedly ($P < 0.05$) increased body weight whereas diminished ($P < 0.05$) joint diameter, paw volume, paw withdrawal latency, and threshold significantly lessened ($P < 0.05$) confront to the AIA control group [Table 2].

Spleen weight, hematological parameters, and erythrocyte sedimentation rate levels

Spleen weight, WBC and platelet count, ESR were amplified effectively ($P < 0.05$), additional levels of RBCs and Hb were markedly

Table 1: Primer sequence for nuclear factor kappa beta, nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor-α, inducible nitric oxide synthase, cyclooxygenase-2, and β-actin

Gene	Sequence		Size (bp)
	Forward primer	Reverse primer	
NF-κB	CCGTTATGTATGTGAAGG	AGAGTCCAGGATTATAGC	338
IκBα	AAGTCGTCGGTGGGAAAC	CCTGAGTGGCTGGAAAGT	405
iNOs	ATCCCGAAACGCTACACTT	TCTGGCGAAGAACAAATCC	314
COX-II	ACAACATTCCTTCCTTC	CCTTATTTCCTTTCACACC	253
β-actin	GTCACCCACACTGTGCCCATCT	ACAGAGTACTTGCGCTCAGGAG	764

NF-kβ: Nuclear factor kappa beta; Ikβα: Nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor-α; iNOs: Inducible nitric oxide synthase; COX-2: Cyclooxygenase-2

Table 2: Effects of oxypaeoniflorin on freund complete adjuva-induced alterations in body weight, change in joint diameter, change in paw volume, paw withdrawal latency, and paw withdrawal threshold (von-frey hair)

Treatment	AUC (body weight [g])	AUC (change in joint diameter [mm])	AUC (change in paw volume [mL])	AUC (paw withdrawal latency [s])	AUC (paw withdrawal threshold [g])
Normal	6403.00±21.28	0.00±0.00	0.00±0.00	230.90±4.78	1735.00±42.26
AIA control	5684.00±20.35$^{\#}$	90.61±1.23$^{\#}$	98.15±1.80$^{\#}$	117.40±3.90$^{\#}$	797.30±39.64$^{\#}$
LF (10)	6187.00±27.89*,$^{\$}$	66.77±1.11*,$^{\$}$	84.55±1.99*,$^{\$}$	140.90±4.06*,$^{\$}$	1034.00±38.92*,$^{\$}$
OPA (10)	5917.00±23.79*	86.61±1.41*	96.44±2.14	125.90±4.28	824.20±46.18
OPA (20)	6011.00±26.58*,$^{\$}$	78.73±1.28*,$^{\$}$	93.23±1.90*,$^{\$}$	132.40±3.75*,$^{\$}$	906.40±41.92*,$^{\$}$
OPA (40)	6092.00±30.65*,$^{\$}$	72.64±1.11*,$^{\$}$	88.14±1.98*,$^{\$}$	136.50±3.98*,$^{\$}$	987.70±44.17*,$^{\$}$

Values in parentheses indicate a dose in mg/kg (n=6). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. For comparison with AIA-control group: *P<0.05, comparison with normal group: $^{\#}P$<0.05 and comparison with one another: $^{\$}P$<0.05. AIA: Adjuvant-induced arthritis; LF: Leflunomide; OPA: Oxypaeoniflorin; AUC: Area under curve

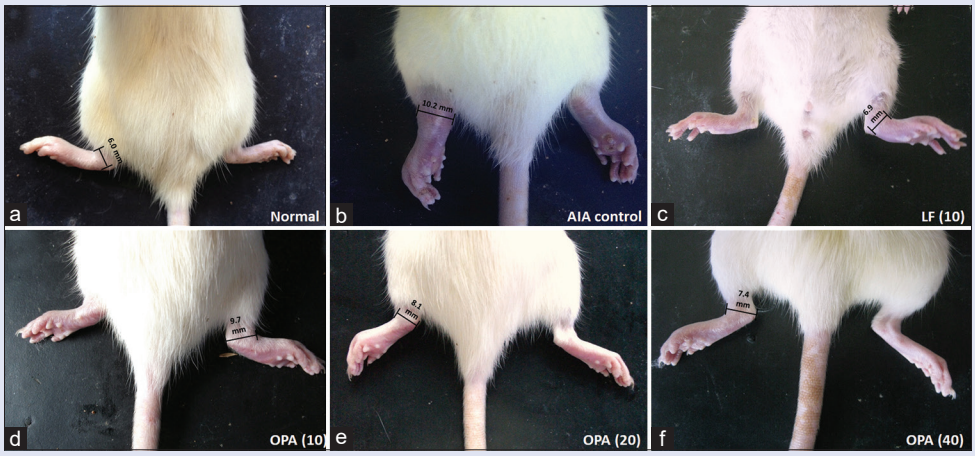

Figure 1: Effects of Oxypaeoniflorin on the severity of ankle inflammation in rats. Representative images of the paw from (a) normal rats, (b) adjuvant-induced arthritis control rats with a higher degree of inflammation, (c) Leflunomide (10 mg/kg) treated rats with reduced ankle inflammation, (d) Oxypaeoniflorin (10 mg/kg) treated rats with severe inflammation, (e) Oxypaeoniflorin (20 mg/kg) and (f) Oxypaeoniflorin (40 mg/kg) treated rats with reduced ankle inflammation

lessened (P < 0.05) in the AIA control group after administration of FCA confront to the normal group. Leflunomide administration meaningfully (P < 0.05) inhibited alteration induced by AIA in spleen weight, ESR, and hematological parameters in contrast to the AIA control group. Treatment with OPA (10 and 20 mg/kg) effectively reduced (P < 0.05) splenic enlargement, WBC and platelet count, ESR levels, whereas RBCs and Hb levels were dramatically elevated (P < 0.05) confront to AIA control group. The alteration in spleen weight, hematological parameters, and ESR were more significantly (P < 0.05) restored by leflunomide treatment compared with OPA [Table 3].

Alanine transaminase, aspartate transaminase, albumin, alkaline phosphatase, C-reactive protein, and rheumatoid factor

The ALT, AST, albumin, ALP, and rheumatoid factor levels were increased (P < 0.05), conversely the level of albumin was effectively declined in the AIA control group in contrast to the normal group. Compared to the AIA control group, leflunomide effectively (P < 0.05) augmented albumin levels and diminished levels of AST, ALP, ALT, CRP, and rheumatoid factor. Administration of OPA (10 and 20 mg/kg) also conspicuously (P < 0.05) attenuated AIA-induced variations in ALT,

Table 3: Effects of oxypaeoniflorin on freund complete adjuva-induced alterations in spleen weight, hematological parameters, and erythrocyte sedimentation rate level

Treatment	Spleen weight (g)	RBC (×106/μL)	WBC (×10³/μL)	Hb (g/dL)	(×10⁹/L)	ESR (mm)
Normal	0.96±0.04	4.17±0.17	5.67±0.33	14.50±0.34	897.00±48.97	3.15±0.40
AIA control	2.27±0.05#	0.50±0.22#	12.00±0.37#	9.67±0.33#	1515.00±53.29#	14.88±0.39#
LF (10)	1.30±0.03*,$	3.17±0.17*,$	6.50±0.34*,$	12.50±0.56*,$	1029.00±40.39*,$	5.50±0.31*,$
OPA (10)	2.06±0.05	1.33±0.33	10.67±0.33	10.83±0.75	1461.00±46.52	12.85±0.28
OPA (20)	1.68±0.05*,$	2.67±0.33*,$	9.33±0.33*,$	12.83±0.54*,$	1234.00±49.20*,$	10.07±0.33*,$
OPA (40)	1.48±0.04*,$	3.67±0.33*,$	8.67±0.21*,$	13.33±0.56*,$	1088.00±60.91*,$	7.90±0.24*,$

Values in parentheses indicate a dose in mg/kg (n=6). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. For comparison with AIA-control group: *P<0.05, comparison with normal group: #P<0.05 and comparison with one another: $$P$<0.05. AIA: Adjuvant-induced arthritis; LF: Leflunomide; OPA: Oxypaeoniflorin; RBC: Red blood cells; WBC: White blood cells; ESR: Erythrocyte sedimentation rate; HB: Hemoglobin; PLTs: Platelets

Table 4: Effects of oxypaeoniflorin on freund complete adjuva-induced alterations in serum aspartate aminotransferase, alanine transaminase, alkaline phosphatase, albumin, C-reactive protein, and rheumatoid factor levels

Treatment	AST (U/mL)	ALT (U/mL)	ALP (U/L)	Albumin (g/dl)	CRP (mg/L)	Rheumatoid factor (IU/mL)
Normal	34.19±3.26	34.41±3.41	63.69±9.94	6.19±0.65	1.98±0.18	0.00±0.00
AIA control	120.60±3.64#	165.60±2.95#	419.80±8.25#	1.96±0.30#	7.24±0.21#	64.02±2.16#
LF (10)	58.56±3.47*,$	69.29±2.52*,$	122.20±11.75*,$	5.85±0.42*,$	2.83±0.18*,$	42.66±2.44*,$
OPA (10)	114.00±4.14	152.40±3.70	380.80±8.84	2.34±0.50	6.75±0.21	61.66±2.42
OPA (20)	79.27±3.70*,$	117.70±3.27*,$	301.00±7.02*,$	3.63±0.33*,$	4.85±0.23*,$	51.99±2.32*,$
OPA (40)	62.53±4.93*,$	74.71±4.43*,$	140.90±9.41*,$	4.51±0.61*,$	3.89±0.25*,$	42.04±3.04*,$

Values in parentheses indicate a dose in mg/kg (n=6). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. For comparison with AIA-control group: *P<0.05, comparison with normal group: #P<0.05 and comparison with one another: $$P$<0.05. AIA: Adjuvant-induced arthritis; LF: Leflunomide; OPA: Oxypaeoniflorin; AST: Aspartate Aminotransferase; ALT: Alanine transaminase; CRP: C-reactive protein; ALP: Alkaline phosphatase

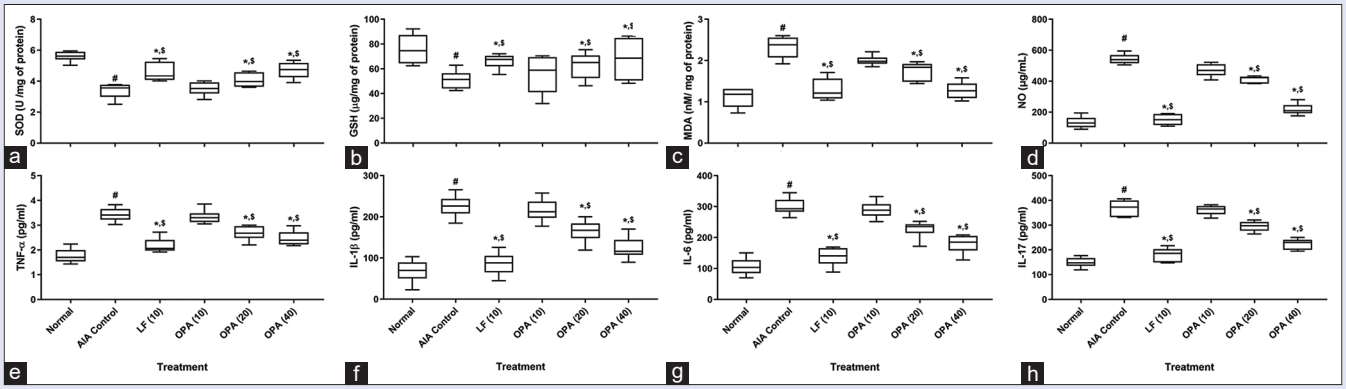

Figure 2: Effects of OPA on FCA-induced alterations in antioxidant parameters, i.e., SOD (a), GSH (b), MDA (c) and NO (d) as well as pro-inflammatory cytokines levels, i.e., TNF-α (e), IL-1 β (f), IL-6 (g), and IL-17 (h) in synovial tissues. Values in parentheses indicate a dose in mg/kg (n = 6). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. For comparison with AIA-control group: *P < 0.05, comparison with normal group: #P < 0.05 and comparison with one another: $$p$ < 0.05. AIA: Adjuvant-induced arthritis, LF: Leflunomide; OPA: Oxypaeoniflorin; SOD: Superoxide dismutase; GSH: Glutathione Peroxidase; MDA: Malondialdehyde; NO: Nitric Oxide; TNF-α: Tumor necrosis factor-alpha; ILs: Interleukins

AST, albumin, ALP, CRP, and rheumatoid factor as confront to the AIA control group. Leflunomide administration exhibited more efficient attenuation (P < 0.05) of ALT, AST, albumin, ALP, CRP, and rheumatoid factor compared to OPA treatment [Table 4].

Oxido-nitrosative stress in synovial fluid

In contrast to the normal group, synovial levels of GSH and SOD were markedly lessened (P < 0.05), inversely levels of NO and MDA in synovial fluid were increased effectively (P < 0.05) in the AIA control group. Leflunomide treatment distinctly (P < 0.05) inhibited oxido-nitrosative stress induced by AIA than the AIA control rats. OPA (10 and 20 mg/kg) administration also noticeably enlarged (P < 0.05) synovial levels of GSH and SOD, whereas levels of NO and MDA were effectively reduced (P < 0.05) in contrast to the control group. Leflunomide has a more pronounced (P < 0.05) potential in inhibiting AIA-induced increased oxidative stress compared to OPA treatment [Figure 2a-d].

Protein levels of tumor necrosis factor-alpha and interleukins in synovial fluid

The protein levels of TNF-α and ILs in synovial fluid were amplified effectively (P < 0.05) in the AIA control group after FCA administration compared to the normal rats. Leflunomide effectively (P < 0.05) condensed synovial levels of TNF-α and ILs protein contrast to the AIA control group. In addition, treatment with OPA (10 and 20 mg/kg) markedly diminished (P < 0.05) levels of TNF-α and ILs in synovial confront to the AIA control group. Increased level of TNF-α and ILs in synovial was more blatantly (P < 0.05) condensed after treatment with leflunomide compared to OPA [Figure 2e-h].

mRNA expressions nuclear factor-kappa beta, Ikβα, cyclooxygenases-2 and iNOs in Synovial Fluid

The NF-kβ, Ikβα, COX-2, and iNOs mRNA expressions in synovial tissue were strikingly upregulated ($P < 0.05$) in the AIA control group confront to normal rats. Compared to the AIA control group, treatment with leflunomide efficiently ($P < 0.05$) down-regulated AIA-induced raised NF-kβ, Ikβα, COX-2, and iNOs mRNA expressions. OPA (10 and 20 mg/kg) administration prominently attenuated ($P < 0.05$) upregulated synovial Ikβα, NF-kβ, COX-2, and iNOs mRNA expressions contrast to the AIA rats. Inhibition of AIA-induced elevated Ikβα, NF-kβ, COX-2, and iNOs mRNA expression in synovial were more prominent ($P < 0.05$) in leflunomide than OPA treatment [Figure 3].

Tibiotarsal joint histopathology

FCA induces apparent obliteration in the tibiotarsal joint reflected by increased synovial proliferation, cellular infiltration, pannus formation, and cartilage erosion which were prominent in the histology of synovial tissue from the AIA control group [Figure 4b]. Histological analysis of the tibiotarsal joint from the normal group portrayed normal architecture with evidence of mild inflammatory infiltration [Figure 4a]. Conversely, leflunomide treatment markedly ($P < 0.05$) attenuated AIA-induced destruction of tibiotarsal joint imitated by reduced synovial proliferation, cellular infiltration, pannus formation, and cartilage erosion in the tibiotarsal joint contrast to the AIA control group [Figure 4c]. OPA (10 and 20 mg/kg) treatment also noticeably lessened ($P < 0.05$) histological

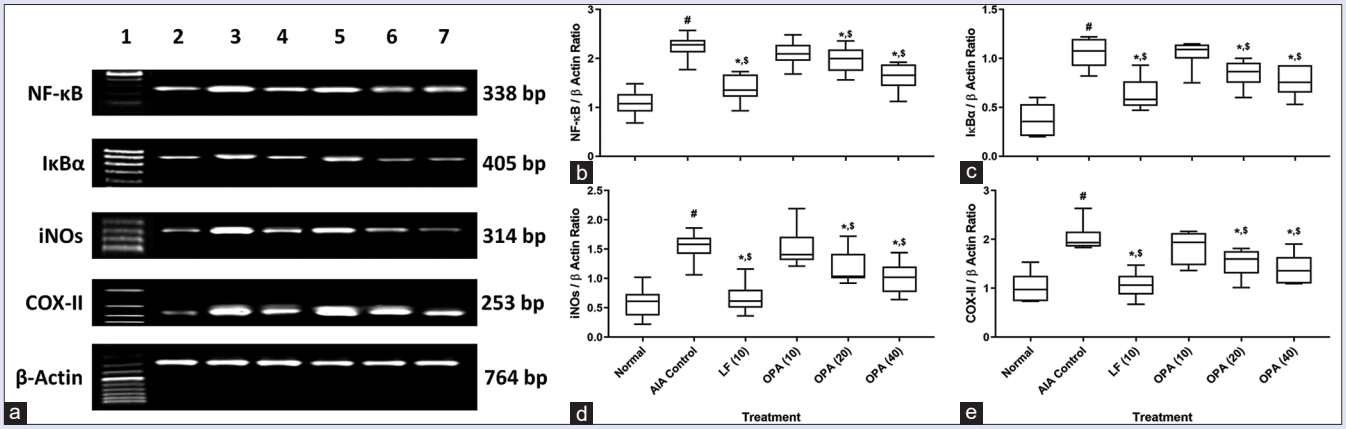

Figure 3: Effects of OPA on FCA-induced alterations in mRNA expression of NF-kβ, Ikβα, iNOs, and COX-2 (a). Quantitative representation of the mRNA expression of NF-kβ (b), Ikβα (c), iNOs (d), and COX-2 (e) in synovial tissues. Data are expressed as mean ± standard error of mean ($n = 6$) and analyzed by one-way ANOVA followed by Tukey's multiple range test. For comparison with AIA-control group: $^{*}P < 0.05$, comparison with normal group: $^{\#}P < 0.05$ and comparison with one another: $^{\$}p < 0.05$. 1000 base pair (bp) ladder (lane 1), Normal group mRNA expression (lane 2); AIA control group mRNA expression (lane 3); Leflunomide group mRNA expression (lane 4) and OPA (10, 20, and 40 mg/kg) group mRNA expression (lane 5-7). AIA: Adjuvant-induced arthritis, LF: Leflunomide; OPA: Oxypaeoniflorin; NF-kβ: Nuclear factor kappa beta; Ikβα: nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor-α; iNOs: inducible nitric oxide synthase; and COX-2: Cyclooxygenase-2

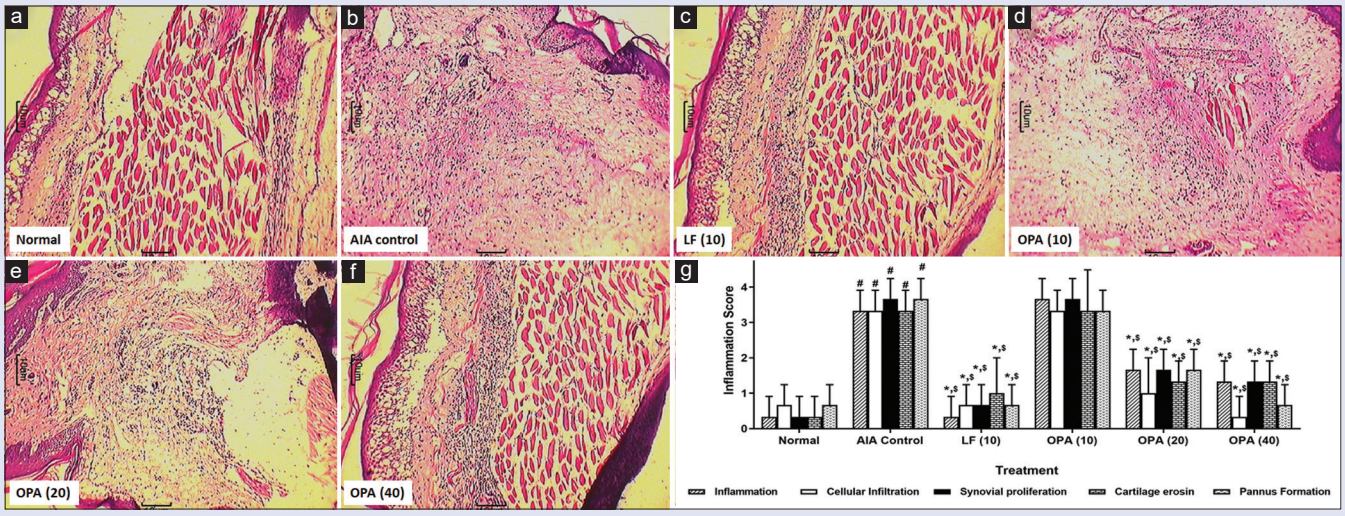

Figure 4: Effects of Oxypaeoniflorin on the histopathology of tibiotarsal joints. Representative histological images from (a) normal, (b) AIA control, (c) Leflunomide (10 mg/kg), (d) Oxypaeoniflorin (10 mg/kg), (e) Oxypaeoniflorin (20 mg/kg) and (d) Oxypaeoniflorin (40 mg/kg) treated rats. Images stained with H and E (×40). The quantitative representation of histological score (g). Data were expressed as mean ± standard error of mean ($n = 3$), and one-way analysis of variance followed by the Kruskal-Wallis test was applied for *post hoc* analysis. For comparison with adjuvant-induced arthritis-control group: $^{*}P < 0.05$, comparison with normal group: $^{\#}P < 0.05$ and comparison with one another: $^{\$}P < 0.05$

aberrations induced by AIA in the tibiotarsal joint confront AIA control group [Figure 4d-g].

DISCUSSION

Rheumatoid arthritis is a complex, chronic disorder of systemic autoimmunity that causes severe inflammation, pain, and damages to the tissue leading to the restriction of limbs movements. The existing pharmacological treatment such as ibuprofen, methotrexate, cyclosporin A, adalimumab, infliximab is effective; however, their unwanted side effect, including gastrointestinal irritation, hepatotoxicity, nephrotoxicity, hematologic toxicity as well as the high cost of treatment, limits their long-term clinical implications.[6] TCM and its monomer in managing various disorders, including RA, have effectively increased in the last several decades.[14,27] In the present investigation, we have also determined the effectiveness of OPA, a TCM monomer, against AIA in FCA-induced arthritic rats. The findings suggested that OPA inhibited the NF-κB/IκBα pathway to downregulate the pro-inflammatory cytokines (TNF-α and ILs) release and inflammatory mediator (COX-II and iNOs) activation thus, ameliorated arthritis-associated inflammation and pain (Graphical abstract).

It has been reported that cachexia, i.e., marked reduction in body weight, is a hallmark of various chronic diseases, including heart or renal failure, arthritis, cancer, diabetes, and Crohn's disease.[28-36] Clinically, it has been shown that RA exhibits hypermetabolism and accelerated protein breakdown, which is a significant reason for increased morbidity and premature mortality in patients.[37,38] In the present investigation, there was a marked reduction in the body weight recorded in AIA control rats even before the manifestation of external signs of the illness, such as destruction of joint integrity and function disability. This result of the current investigation is in accordance with the previous findings.[39] It has been well documented that decreased body weight is affected by immune inflammation and elevated pro-inflammatory cytokines, which play an essential role in leptin regulation.[40,41] In the present investigation, rats administered with FCA showed a marked decrease in body weight, which might be via a reduction of leptin levels. In contrast, administration of OPN showed a significant attenuation in FCA-induced decreased bodyweight, which could be attributed to the inhibition of the release of inflammatory markers.

During inflammatory insult, the pro-inflammatory release (including prostaglandin E2) and pro-inflammatory cytokines are responsible for initiating pain via nociceptor sensitization resulting in threshold decrease.[42,43] Most anti-inflammatory agents possess analgesic, i.e., reduction of allodynia and hyperalgesia, as an essential ancillary property, widely utilized to increase pain threshold in various animal models.[44-46] It has been well established that AIA-induced arthritis is associated with altered allodynia and hyperalgesia,[6,47,48] and in the present study also FCA administration resulted in a marked reduction of allodynia and hyperalgesia evaluated by an array of behavioral assessment using Randall-Selitto, von Frey hair, and Hargreaves test. However, OPN showed effective attenuation of allodynia and hyperalgesia by virtue of its anti-inflammatory property.

AIA is associated with decreased RBC and Hb levels representing an anemic situation in arthritic rats. It is also a common diagnostic marker in chronic arthritis patients.[22,49] This decreased RBC and Hb levels in AIA rats may be due to iron deficiency in the reticuloendothelial system and synovial fluid or failure of bone marrow response in erythropoietin along with the destruction of premature RBCs.[49-51] Studies have shown that the release of inflammatory cytokine plays a vital role in this vicious cycle to bring about decreased Hb and RBC levels during acute phase response.[28,52-55] Furthermore, a moderate rise in WBC count occurred during arthritic conditions due to the release of IL-1 β and mediated increase in the colony-stimulating factors. The spleen plays a major role in RBCs half-life and subsequently anemia in AIA rats, which might cause splenic atrophy.[6,48] ESR serves as a suspension stability index for RBCs in plasma, and it's a measurement of disease activity index during RA.[48,49] In the present investigation, AIA rats exhibited decreased Hb and RBC levels and increased ESR, according to earlier studies.[48,49] Thus, reducing the ESR and improving RBC and Hb count brought about by OPN treatment indicate significant recovery from the anemic condition and further support its anti-arthritic effect.

In the present investigation, a battery of serum chemistry tests was run to assess the functionality of vital organs like the liver after administration of FCA. There were significant alterations in liver functions after chronic oral administration of FCA, reflected by an increase in albumin, ALT, AST, and ALP levels. It has been reported that albumin corresponds to 50% of the total protein.[56] Elevated serum albumin subsequent to FCA administration correlates with elevated synovial protein levels. AST and ALT are abundant enzymes in the synovial tissue,[57] representing liver function, and modulation in their activity suggests liver dysfunction.[58] Administration of FCA caused a marked increase in ALT and AST levels, thus producing hepatotoxicity. A recent study has documented that arthritis patients are associated with primary liver disease.[59] The current study also depicted that FCA-induced arthritis is associated with hepatotoxicity, reflected by elevated AST and ALT levels. However, OPN markedly attenuated these elevated hepatotoxicity markers suggesting its hepatoprotective role, contributing to its anti-arthritic potential.

Considerable evidence suggests that NF-κB (Nuclear factor-kappa B), a critical transcription factor that promotes the accumulation of neutrophils and the adhesion of macrophages that further regulates the release of inflammatory responses.[60] IκBα (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha) is a member of the cellular proteins family responsible for inhibiting the NF-κB transcription factor. Under normal physiological conditions, IκBα masks the nuclear localization signaling of NF-κB via formation of its complex with p65/p50 NF-κB, a heterodimer of NF-κB.[61,62] However, AIA triggers the IκBα phosphorylation, which causes the ubiquitination and degradation of this complex that further subsequently result in NF-κB translocation to nucleus.[63] The activation of NF-κB modulates the expression of the number of inflammatory mediators, including COX-II and iNOs, and pro-inflammatory genes, including iNOs, TNF-α, and ILs.[47,64] In the current investigation, AIA rats showed the upregulated expression of NF-κB and IκBα, further responsible for upregulated levels of pro-inflammatory cytokines and inflammatory mediators. Conversely, administration of OPA inhibited the phosphorylation of IκBα, which could prevent the activation of NF-κB and thus suppress the inflammatory release. Zhang *et al.*(2013) also reported the anti-inflammatory and antioxidative property of OPA via attenuation of NF-κB.[16] The outcomes of the current study were in line with the findings of the earlier investigator.[16]

Cytokines, including ILs and TNF-α, play a central role in RA's pathogenesis.[6] The release of pro-inflammatory cytokines due to antigen-stimulated immune response cause recruitment, activation, and deposition of polymorphonuclear neutrophils (PMNs) into the joint space.[65-67] Further, these PMNs caused the elevated response of ROS, which damages cartilage and joint.[68] It has been reported that differentiation and proliferation of T and B cells and their resorption into bone are inducted by TNF-α and IL-6, whereas IL-1 β causes modulation of immune response via production of NO and

prostaglandin.[9,69,70] Recent evidence demonstrated an elevated response of pro-inflammatory cytokines in RA patients.[71] Thus, measures have been oriented toward the administration of anti-TNF-α antibodies to manage RA.[6] In the current study, FCA caused an increased response of pro-inflammatory cytokines in the synovial fluid. In contrast, treatment with OPN ameliorated this influx of cytokines. The current study results are in line with earlier research findings where OPA efficiently inhibited the elevated response of ILs and TNF-α,[19] thus exerting its anti-inflammatory potential to modulate the pathogenesis of the disease.

CRP has been well documented as a vital marker during various inflammatory diseases.[5,6,49] Rheumatoid arthritis patients also exhibit increased serum CRP levels associated with inflammation and tissue destruction.[48] Moreover, a couple of inducible inflammatory enzymes, including NO and COX-2, play a vital role in activating an inflammatory network of mediators.[52] Numerous findings documented the linkage between elevated NO and pro-inflammatory cytokines in local synovial fluid.[8,52,72] Furthermore, COX-2, an isoenzyme, is abundantly present in activated macrophages responsible for synthesizing prostaglandins that mediate various inflammatory reactions.[73] Thus, dual inhibition of these inducible inflammatory enzymes (NO and COX-2) would be important in terms of symptomatic relief from pain and inflammation. In the present investigation, the activity of NO and COX-2 significantly decreased postadministration of OPN, which, in turn, might have reduced inflammation and modulated the paw withdrawal latency in FCA-induced rats indicating its anti-nociceptive potential.

Nowadays, TCM monomers gained significant attention in managing Chinese patients with RA combined with western medicine to enhance their efficacy. Recently, the National Medical Products Administration of the State Administration for Market Regulation of PRC (formerly China Food and Drug Administration) has approved sinomenine, a TCM monomer for RA treatment.[14,74,75] China's National Health Insurance Directory has accepted sinomenine for managing RA due to its high clinical efficacy and low toxicity.[14] Thus, OPN can serve as an essential moiety for further clinical development during the treatment of RA.

CONCLUSION

The current study demonstrated the efficacy of OPA against AIA in experimental rats. OPA exerts its anti-arthritic property through inhibition of NF-κB/IκBα pathway to downregulate the release of pro-inflammatory cytokines (TNF-α and ILs) and inflammatory mediator (COX-II and iNOs) thus, ameliorated arthritis-associated inflammation and pain.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Linos A, Worthington JW, O'Fallon WM, Kurland LT. The epidemiology of rheumatoid arthritis in Rochester, Minnesota: A study of incidence, prevalence, and mortality. Am J Epidemiol 1980;111:87-98.
2. Cui A, Li H, Wang D, Zhong J, Chen Y, Lu H. Global, regional prevalence, incidence and risk factors of knee osteoarthritis in population-based studies. EClinicalMedicine 2020;29-30:100587.
3. Yun S, Wang X, An Y. A multicenter study of deformity and disability in rheumatoid arthritis patients in China. Chin J Rheumatol 2013;17:526-32.
4. Tian X, Li M, Zeng X. The current status and challenges in the diagnosis and treatment of rheumatoid arthritis in China: an annual report of 2019. Rheumatol Immunol Res 2021;2:49-56.
5. Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis: A review. JAMA 2018;320:1360-72.
6. Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ, Xu J. Rheumatoid arthritis: Pathological mechanisms and modern pharmacologic therapies. Bone Res 2018;6:15.
7. Vohra M, Sharma A, Bagga R, Arora SK. Human umbilical cord-derived mesenchymal stem cells induce tissue repair and regeneration in collagen-induced arthritis in rats. J Clin Transl Res 2020;6:203-16.
8. Jin JH, Kim JS, Kang SS, Son KH, Chang HW, Kim HP. Anti-inflammatory and anti-arthritic activity of total flavonoids of the roots of Sophora flavescens. J Ethnopharmacol 2010;127:589-95.
9. Wong YF, Zhou H, Wang JR, Xie Y, Xu HX, Liu L. Anti-inflammatory and analgesic effects and molecular mechanisms of JCICM-6, a purified extract derived from an anti-arthritic Chinese herbal formula. Phytomedicine 2008;15:416-26.
10. Mei Q, Rao J, Bei HP, Liu Y, Zhao X. 3D bioprinting photo-crosslinkable hydrogels for bone and cartilage repair. Int J Bioprint 2021;7:367.
11. Qian G, Zhang L, Wang G, Zhao Z, Peng S, Shuai C. 3D printed zn-doped mesoporous silica-incorporated Poly-L-lactic acid scaffolds for bone repair. Int J Bioprint 2021;7:346.
12. Ghasemian M, Owlia S, Owlia MB. Review of anti-inflammatory herbal medicines. Adv Pharmacol Sci 2016;2016:9130979.
13. Dudics S, Langan D, Meka RR, Venkatesha SH, Berman BM, Che CT, *et al*. Natural products for the treatment of autoimmune arthritis: Their mechanisms of action, targeted delivery, and interplay with the host microbiome. Int J Mol Sci 2018;19:E2508.
14. Pan HD, Xiao Y, Wang WY, Ren RT, Leung EL, Liu L. Traditional Chinese medicine as a treatment for rheumatoid arthritis: From empirical practice to evidence-based therapy. Engineering 2019;5:895-906.
15. Zhou YY, Xia X, Peng WK, Wang QH, Peng JH, Li YL, *et al*. The effectiveness and safety of tripterygium wilfordii hook. f extracts in rheumatoid arthritis: A systematic review and meta-analysis. Front Pharmacol 2018;9:356.
16. Zhang MH, Feng L, Zhu MM, Gu JF, Wu C, Jia XB. Antioxidative and anti-inflammatory activities of paeoniflorin and oxypaeoniflora on AGEs-induced mesangial cell damage. Planta Med 2013;79:1319-23.
17. Yoo CK, Hwang JH, Lee K, Lee YJ, Kim KJ, Lee BY. Anti-inflammatory effects of moutan cortex radicis extract, paeoniflorin and oxypaeoniflorin through TLR signaling pathway in RAW2647 Cells. J Food Nutr Res 2018;6:26-31.
18. Wang K, Hu W. Oxypaeoniflorin improves myocardial ischemia/reperfusion injury by activating the Sirt1/Foxo1 signaling pathway. Acta Biochim Pol 2020;67:239-45.
19. Guohua F, Tieyuan Z, Rui W, Juan X. Oxypaeoniflorin prevents acute lung injury induced by lipopolysaccharide through the PTEN/AKT Pathway in a sirt1-dependent manner. Oxid Med Cell Longev 2021;2021:6878026.
20. Chou MM, Vergnolle N, McDougall JJ, Wallace JL, Marty S, Teskey V, *et al*. Effects of chondroitin and glucosamine sulfate in a dietary bar formulation on inflammation, interleukin-1beta, matrix metalloprotease-9, and cartilage damage in arthritis. Exp Biol Med (Maywood) 2005;230:255-62.
21. Mukherjee AA, Kandhare AD, Bodhankar SL. Elucidation of protective efficacy of Pentahydroxy flavone isolated from Madhuca indica against arsenite-induced cardiomyopathy: Role of Nrf-2, PPAR-γ, c-fos and c-jun. Environ Toxicol Pharmacol 2017;56:172-85.
22. Patil MV, Kandhare AD, Bhise SD. Anti-arthritic and anti-inflammatory activity of *Xanthium srtumarium* L. ethanolic extract in Freund's complete adjuvant induced arthritis. Biomed Aging Pathol 2012;2:6-15.
23. Kandhare AD, Raygude KS, Ghosh P, Ghule AE, Bodhankar SL. Neuroprotective effect of naringin by modulation of endogenous biomarkers in streptozotocin induced painful diabetic neuropathy. Fitoterapia 2012;83:650-9.
24. Vetal S, Bodhankar SL, Mohan V, Thakurdesai PA. Anti-inflammatory and anti-arthritic activity of type-A procyanidine polyphenols from bark of *Cinnamomum zeylanicum* in rats. Food Sci Hum Wellness 2013;2:59-67.
25. Kandhare AD, Ghosh P, Bodhankar SL. Naringin, a flavanone glycoside, promotes angiogenesis and inhibits endothelial apoptosis through modulation of inflammatory and growth factor expression in diabetic foot ulcer in rats. Chem Biol Interact 2014;219:101-12.
26. Seeuws S, Jacques P, Van Praet J, Drennan M, Coudenys J, Decruy T, *et al*. A multiparameter approach to monitor disease activity in collagen-induced arthritis. Arthritis Res Ther 2010;12:R160.

27. Zhou H, Ma C, Wang C, Gong L, Zhang Y, Li Y. Research progress in use of traditional Chinese medicine monomer for treatment of non-alcoholic fatty liver disease. Eur J Pharmacol 2021;898:173976.
28. Adil M, Kandhare AD, Ghosh P, Venkata S, Raygude KS, Bodhankar SL. Ameliorative effect of naringin in acetaminophen-induced hepatic and renal toxicity in laboratory rats: Role of FXR and KIM-1. Ren Fail 2016;38:1007-20.
29. Adil M, Kandhare AD, Visnagri A, Bodhankar SL. Naringin ameliorates sodium arsenite-induced renal and hepatic toxicity in rats: Decisive role of KIM-1, Caspase-3, TGF-β, and TNF-α. Ren Fail 2015;37:1396-407.
30. Devkar ST, Kandhare AD, Zanwar AA, Jagtap SD, Katyare SS, Bodhankar SL, *et al.* Hepatoprotective effect of withanolide-rich fraction in acetaminophen-intoxicated rat: Decisive role of TNF-α, IL-1β, COX-II and iNOS. Pharm Biol 2016;54:2394-403.
31. Honmore V, Kandhare A, Zanwar AA, Rojatkar S, Bodhankar S, Natu A. Artemisia pallens alleviates acetaminophen induced toxicity via modulation of endogenous biomarkers. Pharm Biol 2015;53:571-81.
32. Kamble H, Kandhare AD, Bodhankar S, Mohan V, Thakurdesai PA. Effect of low molecular weight galactomannans from fenugreek seeds on animal models of diabetes mellitus. Biomed Aging Pathol 2013;3:145-51.
33. Kandhare AD, Ghosh P, Ghule AE, Zambare GN, Bodhankar SL. Protective effect of Phyllanthus amarus by modulation of endogenous biomarkers and DNA damage in acetic acid induced ulcerative colitis: Role of phyllanthin and hypophyllanthin. Apollo Med 2013;10:87-97.
34. Bebe FN, Hu S, Brown TL, Tulp OL. Survival time distribution of advanced stage metastatic melanoma among whites and minority populations in Florida, 1996-2010. Cancer Plus 2019;1:33-42.
35. Ge C, Wang H, Chen H, Yue W, Yan X. The dual characteristics of ten-eleven translocation 1 in Cancer. Cancer Plus 2020;2:13-23.
36. Rengarajan T, Keerthiga S, Duraikannu S, Periyannan V. Exploring the anticancer and anti-inflammatory activities of ferruginol in MCF-7 breast cancer cells. Cancer Plus 2020;1:1-12.
37. Walsmith J, Abad L, Kehayias J, Roubenoff R. Tumor necrosis factor-alpha production is associated with less body cell mass in women with rheumatoid arthritis. J Rheumatol 2004;31:23-9.
38. Carvour ML, Chiu A, Page K. Racial and ethnic disparities in surgical amputations following serious musculoskeletal infections in a diverse New Mexico cohort. J Clin Transl Res 2019;5:25-32.
39. Paval J, Kaitheri SK, Govindan S, Mohammed CA, Kumar RS, Narayanan S, *et al.* Anti-arthritic activity of the plant Tinospora cordifolia Willd. J Herbal Med Toxicol 2011;5:11-6.
40. Blackler R, Syer S, Bolla M, Ongini E, Wallace JL. Gastrointestinal-sparing effects of novel NSAIDs in rats with compromised mucosal defence. PLoS One 2012;7:e35196.
41. Dunn SL, Vera DL, Weaver KF, Garcia JV. Relationships between inflammatory and metabolic markers, exercise, and body composition in young individuals. J Clin Transl Res 2021;7:289-96.
42. Kassuya CA, Ferreira J, Claudino RF, Calixto JB. Intraplantar PGE2 causes nociceptive behaviour and mechanical allodynia: The role of prostanoid E receptors and protein kinases. Br J Pharmacol 2007;150:727-37.
43. Uçeyler N, Schäfers M, Sommer C. Mode of action of cytokines on nociceptive neurons. Exp Brain Res 2009;196:67-78.
44. Kandhare AD, Shivakumar V, Rajmane A, Ghosh P, Bodhankar SL. Evaluation of the neuroprotective effect of chrysin via modulation of endogenous biomarkers in a rat model of spinal cord injury. J Nat Med 2014;68:586-603.
45. Visnagri A, Kandhare AD, Chakravarty S, Ghosh P, Bodhankar SL. Hesperidin, a flavanoglycone attenuates experimental diabetic neuropathy via modulation of cellular and biochemical marker to improve nerve functions. Pharm Biol 2014;52:814-28.
46. Kandhare AD, Raygude KS, Shiva Kumar VS, Rajmane AR, Visnagri A, Ghule AE, *et al.* Ameliorative effects quercetin against impaired motor nerve function, inflammatory mediators and apoptosis in neonatal streptozotocin-induced diabetic neuropathy in rats. Biomed Aging Pathol 2012;2:173-86.
47. Honmore VS, Kandhare AD, Kadam PP, Khedkar VM, Natu AD, Rojatkar SR, *et al.* Diarylheptanoid, a constituent isolated from methanol extract of *Alpinia officinarum* attenuates TNF-α level in Freund's complete adjuvant-induced arthritis in rats. J Ethnopharmacol 2019;229:233-45.
48. Zhang G, Kandhare AD, Mukherjee AA, Bodhankar SL, Yin H. Ameliorative effect of morin, a plant flavonoid against Freund's complete adjuvant-induced polyarthritis in rats. Pharmacogn Mag 2019;15:43.
49. Lin F, Wang X, Liang Y, Liu D, Zhang Y, Zhong R, *et al.* Red blood cell distribution width in rheumatoid arthritis, ankylosing spondylitis and osteoarthritis: True inflammatory index or effect of anemia? Ann Clin Lab Sci 2018;48:301-7.
50. Bozo IY, Deev RV, Smirnov IV, Fedotov AY, Popov VK, Mironov AV, *et al.* 3D printed gene-activated octacalcium phosphate implants for large bone defects engineering. Int J Bioprint 2020;6:275.
51. Zheng C, Attarilar S, Li K, Wang C, Liu J, Wang L, *et al.* 3D-printed HA15-loaded β-tricalcium phosphate/poly (lactic-co-glycolic acid) bone tissue scaffold promotes bone regeneration in rabbit radial defects. Int J Bioprint 2021;7:317.
52. Honmore VS, Kandhare AD, Kadam PP, Khedkar VM, Sarkar D, Bodhankar SL, *et al.* Isolates of *Alpinia officinarum* Hance as COX-2 inhibitors: Evidence from anti-inflammatory, antioxidant and molecular docking studies. Int Immunopharmacol 2016;33:8-17.
53. Bodhankar S, Raygude K, Kandhare A, Ghosh P. The ameliorative effect of fisetin, a bioflavonoid, on ethanol-induced and pylorus ligation-induced gastric ulcer in rats. Int J Green Pharm 2011;5:236-43.
54. Kandhare AD, Alam J, Patil MV, Sinha A, Bodhankar SL. Wound healing potential of naringin ointment formulation via regulating the expression of inflammatory, apoptotic and growth mediators in experimental rats. Pharm Biol 2016;54:419-32.
55. Visnagri A, Kandhare AD, Bodhankar SL. Renoprotective effect of berberine via intonation on apoptosis and mitochondrial-dependent pathway in renal ischemia reperfusion-induced mutilation. Ren Fail 2015;37:482-93.
56. Doweiko JP, Nompleggi DJ. Role of albumin in human physiology and pathophysiology. JPEN J Parenter Enteral Nutr 1991;15:207-11.
57. Tennekoon KH, Jeevathayaparan S, Kurukulasooriya AP, Karunanayake EH. Possible hepatotoxicity of *Nigella sativa* seeds and *Dregea volubilis* leaves. J Ethnopharmacol 1991;31:283-9.
58. Vijayalakshmi T, Muthulakshmi V, Sachdanandam P. Toxic studies on biochemical parameters carried out in rats with Serankottai nei, a siddha drug-milk extract of *Semecarpus anacardium* nut. J Ethnopharmacol 2000;69:9-15.
59. Radovanović-Dinić B, Tešić-Rajković S, Zivkovic V, Grgov S. Clinical connection between rheumatoid arthritis and liver damage. Rheumatol Int 2018;38:715-24.
60. Visnagri A, Adil M, Kandhare AD, Bodhankar SL. Effect of naringin on hemodynamic changes and left ventricular function in renal artery occluded renovascular hypertension in rats. J Pharm Bioallied Sci 2015;7:121-7.
61. Kandhare AD, Liu Z, Mukherjee AA, Bodhankar SL. Therapeutic potential of morin in ovalbumin-induced allergic asthma via modulation of SUMF2/IL-13 and BLT2/NF-kB signaling pathway. Curr Mol Pharmacol 2019;12:122-38.
62. Zhou Z, Kandhare AD, Kandhare AA, Bodhankar SL. Hesperidin ameliorates bleomycin-induced experimental pulmonary fibrosis via inhibition of TGF-beta1/Smad3/AMPK and IkappaBalpha/NF-kappaB pathways. EXCLI J 2019;18:723-45.
63. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther 2017;2:17023.
64. Farrugia M, Baron B. The role of TNF-α in rheumatoid arthritis: A focus on regulatory T cells. J Clin Transl Res 2016;2:84-90.
65. Chen Z, Bozec A, Ramming A, Schett G. Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. Nat Rev Rheumatol 2019;15:9-17.
66. Kaur G, Sultana S. Evaluation of antiarthritic activity of isoeugenol in adjuvant induced arthritis in murine model. Food Chem Toxicol 2012;50:2689-95.
67. Poles J, Karhu E, McGill M, McDaniel HR, Lewis JE. The effects of twenty-four nutrients and phytonutrients on immune system function and inflammation: A narrative review. J Clin Transl Res 2021;7:333-76.
68. Wang X, He X, Zhang CF, Guo CR, Wang CZ, Yuan CS. Anti-arthritic effect of berberine on adjuvant-induced rheumatoid arthritis in rats. Biomed Pharmacother 2017;89:887-93.
69. Zhang RX, Fan AY, Zhou AN, Moudgil KD, Ma ZZ, Lee DY, *et al.* Extract of the Chinese herbal formula Huo Luo Xiao Ling Dan inhibited adjuvant arthritis in rats. J Ethnopharmacol 2009;121:366-71.
70. Daskalakis E, Liu F, Huang B, Acar AA, Cooper G, Weightman A, *et al.* Investigating the influence of architecture and material composition of 3D printed anatomical design scaffolds for large bone defects. Int J Bioprint 2021;7:268.
71. Mateen S, Moin S, Shahzad S, Khan AQ. Level of inflammatory cytokines in rheumatoid

arthritis patients: Correlation with 25-hydroxy vitamin D and reactive oxygen species. PLoS One 2017;12:e0178879.

72. Tambewagh UU, Kandhare AD, Honmore VS, Kadam PP, Khedkar VM, Bodhankar SL, *et al*. Anti-inflammatory and antioxidant potential of Guaianolide isolated from Cyathocline purpurea: Role of COX-2 inhibition. Int Immunopharmacol 2017;52:110-8.
73. Chen X, Zhang C, Wang X, Huo S. Juglanin inhibits IL-1β-induced inflammation in human chondrocytes. Artif Cells Nanomed Biotechnol 2019;47:3614-20.
74. Lin SS, Liu CX, Zhang JH, Wang H, Zhai JB, Mao JY, *et al*. Efficacy and safety of sinomenine preparation for ankylosing spondylitis: A systematic review and meta-analysis of clinical randomized controlled trials. Evid Based Complement Alternat Med 2020;2020:4593412.
75. Liu W, Zhang Y, Zhu W, Ma C, Ruan J, Long H, *et al*. Sinomenine inhibits the progression of rheumatoid arthritis by regulating the secretion of inflammatory cytokines and monocyte/macrophage subsets. Front Immunol 2018;9:2228.