OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.XX.XX

Research Article
Effect of Aloe Vera Polypeptide Fraction for Bone Repair in Adjuvant-Induced Arthritic Rats

[1]Yang Chenhui, [2]Qingling Liu, [3]Zhiheng Guo and [4]Zemin Jiang

[1]Department of Rehabilitation and Reconstruction, Tianshui Hand and Foot Surgery Hospital, Tianshui, 741000, China
[2]Department of Nursing, Tianshui Hand and Foot Surgery Hospital, Tianshui, 741000, China
[3]Department of Traumatology and Orthopedics, Tianshui Hand and Foot Surgery Hospital, Tianshui, 741000, China
[4]Department of Hand Surgery, Tianshui Hand and Foot Surgery Hospital, Tianshui, 741000, China

Abstract

Background and Objective: Osteoarthritis (OA) is a degenerative, debilitative, multifactorial illness affecting the knee joints of millions of people globally. The peptide/polypeptide fraction from Aloe Vera (AV-PP) has shown anti-inflammatory potential by inhibiting inflammatory cytokines. To evaluate the efficacy and potential mechanism of action of AV-PP against bone healing during surgically induced bone damage in a rat model of Adjuvant-Induced Arthritis (AIA). **Materials and Methods:** A bone fracture was created in adjuvant-induced arthritis rats using a surgical drill in the anterolateral wall of the lateral condyle of the femur. Then rats were treated with either distilled water or alendronate (3.0 mg kg^{-1}) or AV-PP (0.25, 0.5 and 1.0 mg kg^{-1}) for the next 28 days. **Results:** AIA-induced increased paw volume and spleen weight was effectively ($p<0.05$) reduced by AV-PP (0.5 and 1.0 mg kg^{-1}) treatment. The elevated levels of synovial TNF-α, IL-1β, IL-6 and IL-17 and diminished levels of IL-4 and IL-10 were distinctly ($p<0.05$) restored by AV-PP treatment. Bone injury-induced up-regulated synovial RANKL and osteocalcin, whereas down-regulated OPG and Runx2 mRNA expressions were prominently ($p<0.05$) inhibited by AV-PP treatment. Histopathology findings of the tibiotarsal joint showed administration of AV-PP effectively ($p<0.05$) reduced bone inflammation and improved bone healing. **Conclusion:** AV-PP showed efficacy against surgically induced bone damage in a rat model of adjuvant-induced arthritis by inhibiting RANKL/OPG signalling pathway, activating osteocalcin and balancing inflammatory cytokine levels in AIA rats.

Key words: Aloe vera, bone healing, osteoarthritis, osteocalcin, osteoprotegerin, polypeptide, RANKL, runx2

Citation: Chenhui, Y., Q. Liu, Z. Guo and Z. Jiang, 2022. Effect of Aloe vera polypeptide fraction for bone repair in adjuvant-induced arthritic rats. Int. J. Pharmacol., 18: XX-XX.

Corresponding Author: Yang Chenhui, Department of Rehabilitation and Reconstruction, Tianshui Hand and Foot Surgery Hospital, Tianshui, 741000, China

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Osteoarthritis (OA) is a chronic progressive multifactorial illness that commonly impacts joints, leading to chronic pain, inflammation and damage to tissue which causes limb functional difficulty[1-4]. The epidemiological study documented that incidence and prevalence of knee OA are directly correlated with the age and highest at the age of 70-79 years[1]. In the elderly patient, knee OA is a significant cause of disability and in 2020, approximately 654.1 (95% CI, 565.6-745.6) million people age 40 years and older suffering from knee OA worldwide[1,5]. It has been well documented that the prevalence and incidence of OA are higher in females than males, with a ratio of 1.69 (95% CI, 1.59-1.80) and 1.39 (95% CI, 1.24-1.56), respectively[1]. A Cost-utility analysis conducted by Gui *et al.*[3] reported that the total cost for hospitalization for Chinese OA was about ¥57,940.55 ($8987) which significantly (81.59%) contributed to out-of-pocket expenses. Thus, OA is associated with a higher economic and humanistic burden.

Cartilage degradation and bone remodelling are the main pathophysiological features of OA[4]. Researchers have well documented the role of pro-inflammatory and anti-inflammatory cytokines in bone remodeling[4,6]. The balance between pro-inflammatory cytokines (ILs (interleukins)-1β, IL-6, IL-17 and Tumour Necrosis Factor (TNF)-α) and anti-inflammatory (IL-4, IL-10, IL-13) has been suggested as an important approach for the management of OA[7-10]. The altered levels of these cytokines have been reported in synovial fluids of OA patients[11]. The new emerging treatment targeted the regulators mainly involved in the joint bone erosion and thus maintained osteoclast homeostasis[12,13]. These regulators include RANKL (receptor activator of nuclear factor kappa-B ligand), OPG (osteoprotegerin), RUNX2 (Runt-related transcription factor-2), Col I (collagen I), BSP (Bone Sialoprotein), Osteocalcin (OCN) and Osteopontin (OPN)[14-16]. RANKL has been reported as an essential apoptosis regulatory gene for OPG[17]. Thus, inhibition of these new targets may provide safe and effective treatment options for OA management.

The current first-line treatment medications for the management of OA include acetaminophen, NSAIDs (Nonsteroidal anti-inflammatory drugs including ibuprofen and naproxen), duloxetine which aid in relieving OA pain[18,19]. However, these agents are associated with adverse events, including cardiovascular and digestive difficulties, bleeding problems and damage to the kidney and liver[18]. Recently, investigators have established the relationship between the efficacy of bisphosphonate (such as alendronate) and pain modulation[20]. Alendronate has been reported to protect the cartilage against its degeneration and prevent subchondral bone remodelling, thus helping to protect against OA and decrease knee pain[21]. However, little evidence reported that alendronate is unable to inhibit long-term joint degeneration[22]. The available surgical and other optional procedures, including corticosteroid or lubrication (hyaluronic acid) injections or joint replacement are effective with a minimal side effect. However, these therapeutic regimens are associated with significant cost. Thus, OA patients are preferring therapeutic regimens from complementary and alternative medicinal system. Thus, researcher have taken efforts to evaluate emerging safe and effective therapy from natural origin that can be implemented for long-term management of OA.

The increasing popularity of complementary and alternative medicines, including herbal remedies, has raised significant awareness in treating various disorders. Aloe vera (A. vera, Aloe barbadensis Mill., Family: Liliaceae) have been reported to possess many pharmacological activities[23]. Among various active phytoconstituents of A. vera gel, researchers have documented sterols, saccharides, polyphenols, flavonoids and peptide/polypeptides as important bioactive for its therapeutic potential[23]. In the randomized placebo-controlled trials, polypeptides have been demonstrated their therapeutic potential in the management of osteoarthritis[2,24,25]. Peptide/polypeptides from A. vera gel have been documented for their anti-inflammatory and anti-diabetic potential[26,27]. The peptide/polypeptide fraction from Aloe Vera (AV-PP) has shown anti-inflammatory potential by inhibiting pro-inflammatory cytokines (TNF-α and IL-6)[26]. However, the potential of Aloe vera peptide/polypeptide is still unknown against OA. Hence, the purpose of the present investigation was to evaluate the efficacy and potential mechanism of action of AV-PP against bone healing during surgically induced bone damage in rat models of adjuvant-induced arthritis.

MATERIAL AND METHODS

Study area: The experiment was performed in the Institute's Tianshui Hand and Foot Surgery Hospital, Tianshui, China, from 17th June-27th August, 2021. All the experiments were carried out between 08:00 and 17:00 hrs in a quiet laboratory environment.

Animals: Wistar rats (Adult female, 150-180 g, n = 140) were procured from the animal house of Tianshui Hand and Foot Surgery Hospital, Tianshui, China. The rats were maintained in-house at 24±1°C, 12:12 hrs dark-light cycle, with standard

pellet feed and filtered water during the experimental time of 08:00 and 17:00 hrs in a quiet laboratory environment. The animal ethics committee of Tianshui Hand and Foot Surgery Hospital approved all the experimental research protocols (No. 2021-071201).

Chemicals and reagents: To determine the levels of TNF-α (Tumour Necrosis Factor-α) and ILs (Interleukin-4, IL-1β, IL-6, IL-10 and IL-17) a rat specific ELISA (Enzyme-Linked Immunosorbent Assay) kits were procured from Bethyl Laboratories Inc., Montgomery, TX, USA. To induce AIA, FCA was purchased from Sigma Aldrich, St. Louis, USA. Microliter syringe was purchased from Hamilton, Bonaduz, Switzerland. Laboratory grade chemicals including methanol, ethyl acetate, toluene and formic acid were purchased from Merck Life Science Pvt Ltd, India. The One-step Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) kit and Total RNA Extraction kit were purchased from MP Biomedicals India Private Limited, Mumbai, India.

Aloe vera extract preparation and enrichment of peptide/polypeptide fraction: Aloe vera was obtained from Muzi Agricultural Ltd., Shaanxi Province, China. The Aloe vera extract was prepared according to the previously reported method[26]. The peptide/ polypeptide fractions from the extract were enriched through 30% Trichloroacetic Acid (TCA) precipitation. In brief, the Aloe vera extract was dissolved in water and kept for shaking for 24 hrs. TCA solution was added to the extract and centrifuged at 5000 rpm. The pellet was added with acetone and washed 3 times under the same condition. The resulting peptide/polypeptide fraction obtained was stored at -80°C for further use. Bradford assay was carried out to estimate the amount of protein in the PPF[28].

Induction of adjuvant-induced arthritis (AIA): FCA (0.1 mL, intradermal) was injected into the paw of the rats to induce AIA in female rats (150-180 g, 5 groups, i.e., group III to VII, n = 18 each group)[29]. Following the injection, 12 days were allowed for the development of arthritis. A separate group of rats (group I and II, n = 18) was maintained as normal and sham and did not receive FCA.

AIA rat model combined with bone injury model: After developing AIA (13 days) in groups III to VII, a critical size femur defect was performed in AIA rats as previously described[30,31]. Briefly, knees were shaved and disinfected and then an incision was made in the skin and muscles surrounding the right femur were retracted. After lateral knee arthrotomy, a cylindrical defect (FD, 3×4 mm, diameter×depth) was created using a surgical drill in the anterolateral wall of the lateral condyle of the femur. Sham (group II) animals underwent surgery but did not receive any injury to the femur. Surgeries were performed under general anaesthesia with volatile isoflurane. After surgery, the joint was irrigated with sterile saline solution and both capsule and skin were closed with 4-0 nylon sutures. For the sham-operated group, the wounds were sutured after exposing the knee joint cartilage surface. The animals were injected intramuscularly with antibiotics (1.0-1.3 mg/cefotiam hydrochloride) for 3 days after surgery and allowed to recover.

Drug treatment: After 24 hrs of surgery, animals received either Distilled Water (DW) (1.0 mg kg^{-1}, p.o. in groups I, II and III, i.e., normal, sham and Arthritic Control [AC]) or alendronate (Ale, 3.0 mg kg^{-1} as a standard in group IV) or aloe vera peptide/polypeptide fraction (AV-PP, 0.25, 0.5 and 1.0 mg kg^{-1} in group V to VII) for the next 28 days. The dosage of AV-PP was selected based on previously reported study[26].

During the experimental period, the paw volume was determined at different days using Plethysmometer (UGO Basile Italy)[32]. On the last day of the study (day 43), rats were sacrificed by cervical dislocation. Immediately synovial tissues and spleen were isolated and stored at -70°C.

Determination of synovial inflammatory cytokines (TNF-α and ILs) levels: The synovial level (n = 6) of inflammatory cytokine including ILs and TNF-α were determined using ELISA and presented as pg Ml^{-1}.

Determination of synovial mRNA expressions of RANKL, OPG, Osteocalcin and Runx2: The levels of mRNA were analyzed in synovial tissues (n = 6) using RT-PCR as described previously[33]. Single-stranded cDNA was synthesized from 5 µg of total cellular RNA using a reverse transcriptase kit (MP Biomedicals India Private Limited, India) as described previously. The primer sequence for receptor activator of nuclear factor-kappa-B ligand (RANKL), Osteoprotegerin (OPG), Osteocalcin (OCN), Runt-related transcription factor 2 (Runx2) and β-actin are presented in Table 1. Amplification of β-actin served as a control for sample loading and integrity. The intensity of mRNAs was standardized against that of the β-actin mRNA from each sample and the results were expressed as the PCR-product/β-actin mRNA ratio.

Histopathology of tibiotarsal joint: To assess the potential of AV-PP on tibiotarsal histology, ankle joints (n = 3/group) were

Table 1: Primer sequence for RANKL, OPG, OCN, runx2 and β-actin

Gene	Sequence forward primer	Reverse primer	Size (bp)
RANKL	GGGAATTACAAAGTGCACCAG	GCCATCCTTCTCAAAGTTGT	69
OPG	TCAAGTGCTTGAGGGCATAC	TGGAGATCGAATTCTGCTTG	119
OCN	AAGCAGGAGGGCAATAAGGT	CAAGCAGGGTTAAGCTCACA	240
Runx2	TGTTCTCTGATCGCCTCAGTG	CCTGGGATCTGTAATCTGACTCT	146
β-actin	GTCACCCACACTGTGCCCATCT	ACAGAGTACTTGCGCTCAGGAG	764

OCN: Osteocalcin, OPG: Osteoprotegerin, RANKL: Receptor activator of nuclear-factor kappa-β ligand and Runx2: Runt-related transcription factor 2

isolated and stored in formaldehyde solution (10%). Then, microtomes were used to cut these sections into the thickness of 5 μm followed by deparaffinated and H and E (hematoxylin and eosin) staining. An impression of histopathological features was evaluated using a light microscope. A previously reported method was used to determine histological aberrations intensity in the tibiotarsal joint[34].

Statistical analysis: GraphPad Prism 5.0 software (GraphPad, San Diego, CA) was used to perform data analysis. Data are expressed as Mean±Standard Error mean (SEM) and analyzed using Two-Way ANOVA followed by Tukey's multiple range post hoc analysis (for body weight and paw volume) and One-Way ANOVA followed by Tukey's multiple range post hoc analysis (for spleen weight, cytokines levels and mRNA expressions (RANKL, OPG, Osteocalcin and Runx2)) and Kruskal-Wallis test for post hoc analysis (non-parametric tests, i.e., histopathology score). A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Bodyweight and hind paw volume: There was a significant decrease ($p<0.05$) in body weight (Fig. 1a), whereas hind paw volume decidedly increased ($p<0.05$, Fig. 1b) in the AC group as compared to the normal and sham groups. However, the administration of alendronate effectively ($p<0.05$) increased body weight and lessened paw volume compared to the AC group. Treatment with AV-PP (0.5 and 1.0 mg kg^{-1}) markedly amplified ($p<0.05$) body weight and hind paw volume was suggestively decreased ($p<0.05$) when compared to the AC group. However, the administration of alendronate showed more momentous inhibition in AIA-induced alteration in body weight and hind paw volume compared to the AV-PP treated group (Fig. 1a, b).

Spleen weight and spleen index: Spleen weight (Fig. 1c) and spleen index (Fig. 1d) amplified markedly ($p<0.05$) in the AC group after FCA administration compared to the normal and sham groups. Administration of alendronate effectively ($p<0.05$) reduce spleen weight and spleen index compared to the AC group. Additionally, AV-PP (0.5 and 1.0 mg kg^{-1}) treatment meaningfully decreased ($p<0.05$) spleen weight and spleen index compared with the AC group. The elevated spleen weight and spleen index were more effectively ($p<0.05$) decreased after treatment with alendronate than AV-PP (Fig. 1c-d).

Synovial ILs and TNF-α protein levels: When compared with the normal and sham group, synovial TNF-α, IL-1β, IL-6 and IL-17 levels were distinctly increased ($p<0.05$), whereas IL-4 and IL-10 levels were markedly lessened ($p<0.05$) in the AC group. Alendronate treatment blatantly ($p<0.05$) inhibited AIA-induced alterations in TNF-α and ILs in synovial tissue compared to the AC group. Administration of AV-PP (0.5 and 1.0 mg kg^{-1}) also noticeably enhanced ($p<0.05$) synovial IL-4 and IL-10 levels, whereas synovial TNF-α, IL-1β, IL-6 and IL-17 levels were markedly reduced ($p<0.05$) when compared with the AC group. Alendronate treatment was more effective in inhibiting AIA-induced alterations in TNF-α and ILs in synovial tissue than in the AV-PP treatment (Table 2).

Synovial RANKL, OPG, Osteocalcin and Runx2 mRNA expressions: Induction of bone injury in AIA-induced arthritic rats caused significant alterations ($p<0.05$) in synovial RANKL, OPG, osteocalcin and Runx2 mRNA expressions (Fig. 2a). The mRNA expressions of synovial RANKL (Fig.2b) were strikingly up-regulated ($p<0.05$), synovial OPG (Fig. 2c) effectively ($p<0.05$) down-regulated, osteocalcin (Fig. 2d) were strikingly up-regulated ($p<0.05$) and Runx2 (Fig. 2e) mRNA expressions were effectively ($p<0.05$) down-regulated in the AC group compared with the normal and sham groups. Treatment with alendronate effectively ($p<0.05$) down-regulated AIA-induced raised RANKL and osteocalcin mRNA expressions in synovial tissue whereas up-regulated synovial OPG and Runx2 mRNA expressions as compared to AC group. AV-PP (0.5 and 1.0 mg kg^{-1}) administration flagrantly attenuated ($p<0.05$) RANKL and osteocalcin mRNA expressions whereas

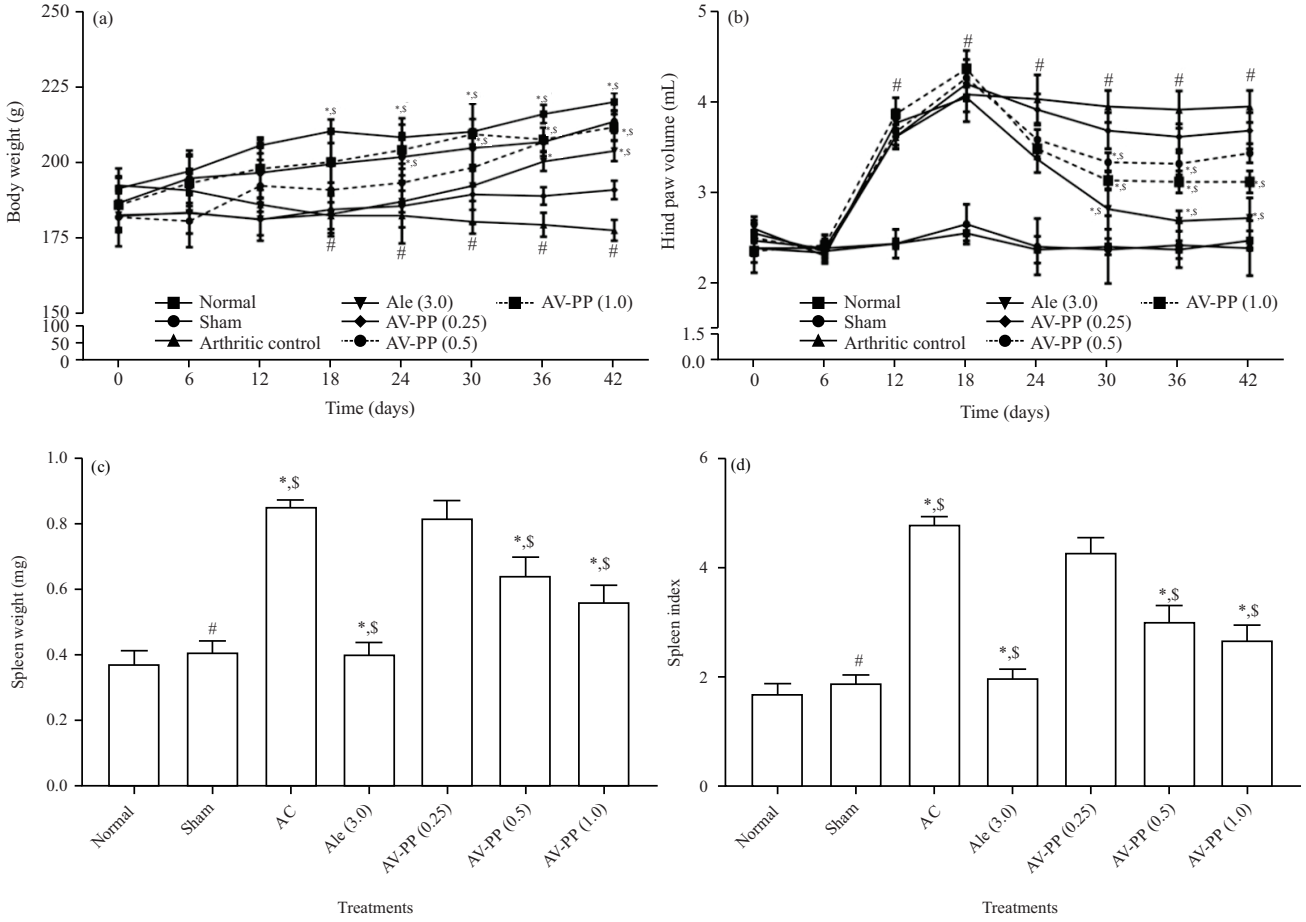

Fig. 1(a-d): Effects of AV-PP on alteration in (a) Body weight, (b) Paw volume, (c) Spleen weight and (d) Spleen index in AIA rats

Values in parentheses indicate a dose in mg kg-1 (n = 6). Two-way ANOVA analyzed data for body weight and paw volume, whereas data for spleen weight and spleen index was analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. For comparison with AIA-control (AC) group: *p<0.05, comparison with normal group: #p<0.05 and comparison with one another: $p<0.05. AC: Adjuvant-induced arthritis control, Ale: Alendronate and AV-PP: Aloe vera peptide/polypeptide fraction

Table 2: Effects of AV-PP on FCA-induced alterations in inflammatory cytokine levels in synovial tissues

Treatments	TNF-α (pg mL^{-1})	IL-1β (pg mL^{-1})	IL-4 (pg mL^{-1})	IL-6 (pg mL^{-1})	IL-10 (pg mL^{-1})	IL-17 (pg mL^{-1})
Normal	1.68±0.07	60.31±4.04	349.60±12.59	99.27±4.12	75.18±2.75	100.20±13.15
Sham	1.92±0.05	65.41±4.39	342.20±13.38	122.00±3.97	67.03±2.64	126.20±14.41
AC	3.33±0.06$^{\#}$	231.40±3.37$^{\#}$	42.51±13.86$^{\#}$	298.40±3.67$^{\#}$	7.72±1.75$^{\#}$	335.10±13.52$^{\#}$
Ale (3.0)	2.15±0.06$^{*,\$}$	87.93±3.35$^{*,\$}$	302.30±13.86$^{*,\$}$	141.60±6.15$^{*,\$}$	60.68±2.88$^{*,\$}$	157.10±11.88$^{*,\$}$
AV-PP (0.25)	3.20±0.07	206.10±4.15	67.95±12.16	286.00±3.95	9.27±1.53	335.30±11.55
AV-PP (0.5)	2.68±0.05$^{*,\$}$	157.70±3.43$^{*,\$}$	169.10±13.32$^{*,\$}$	235.80±2.31$^{*,\$}$	25.36±2.74$^{*,\$}$	276.60±11.85$^{*,\$}$
AV-PP (1.0)	2.50±0.04$^{*,\$}$	115.70±3.69$^{*,\$}$	222.60±12.38$^{*,\$}$	186.00±5.50$^{*,\$}$	43.94±2.87$^{*,\$}$	205.30±11.38$^{*,\$}$

Values in parentheses indicate a dose in mg kg^{-1} (n = 6). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. For comparison with AIA-control (AC) group: *p<0.05, comparison with normal group: #p<0.05 and comparison with one another: $p<0.05. AC: Adjuvant-induced arthritis control, Ale: Alendronate, AV-PP: Aloe vera Peptide/Polypeptide fraction, ILs: Interleukins, and TNF-α: Tumour Necrosis Factor-α

prominently (p<0.05) up-regulated synovial OPG and Runx2 mRNA expressions as compared to the AC group.

Histopathology of the tibiotarsal joint: Histological analysis of tibiotarsal joints from normal and sham groups portrayed normal architecture of tibiotarsal joints with evidence of mild synovial proliferation, cartilage erosion and inflammation (Fig. 3a, b). AIA followed by bone injury cases marked destruction in the tibiotarsal joints reflected by elevated cellular infiltration, synovial proliferation, cartilage

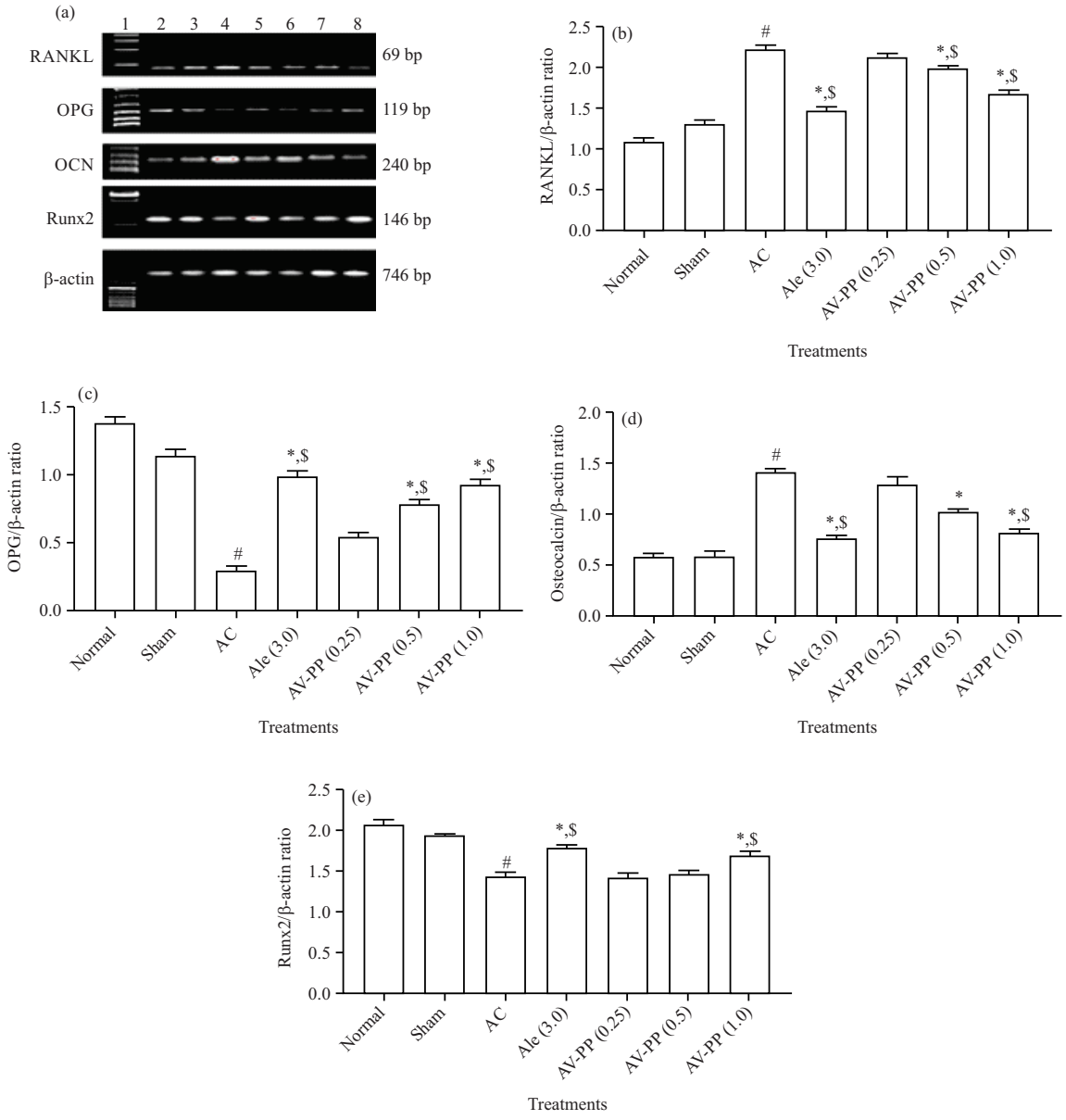

Fig. 2(a-e): Effects of AV-PP on FCA-induced alterations in mRNA expressions of RANKL, OPG, Osteocalcin and Runx2 in synovial tissues, (a) Qualitative, (b) quantitative representation of the mRNA expression of RANKL, (c) OPG, (d) Osteocalcin and (e) Runx2

Data are expressed as Mean ± SEM (n = 6) and analyzed by one-way ANOVA followed by Tukey's multiple range test. For comparison with AIA-control (AC) 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); sham group mRNA expression (lane 3); AC control group mRNA expression (lane 4); Alendronate group mRNA expression (lane 5) and AV-PP (0.25, 0.5 and 1.0 mg kg^{-1}) group mRNA expression (lane 6-8). AC: Adjuvant-induced arthritis control, Ale: Alendronate, AV-PP: Aloe vera peptide/polypeptide fraction, OCN: Osteocalcin, OPG: Osteoprotegerin, RANKL: Receptor activator of nuclear factor kappa-B ligand and Runx2: Runt-related transcription factor 2

erosion and inflammation, which were evident in the synovial tissue from the AC group (Fig. 3c). Conversely, alendronate treatment effectively (p<0.05) inhibited AIA-induced destruction of tibiotarsal joints imitated by reduced cellular infiltration, synovial proliferation, cartilage erosion and inflammation as compared to the AC group (Fig. 3d). Administration of AV-PP (0.5 and 1.0 mg kg^{-1}) also noticeably decreased (p<0.05) AIA-induced histological modifications in tibiotarsal joints when compared with the AC group (Fig. 3e-g).

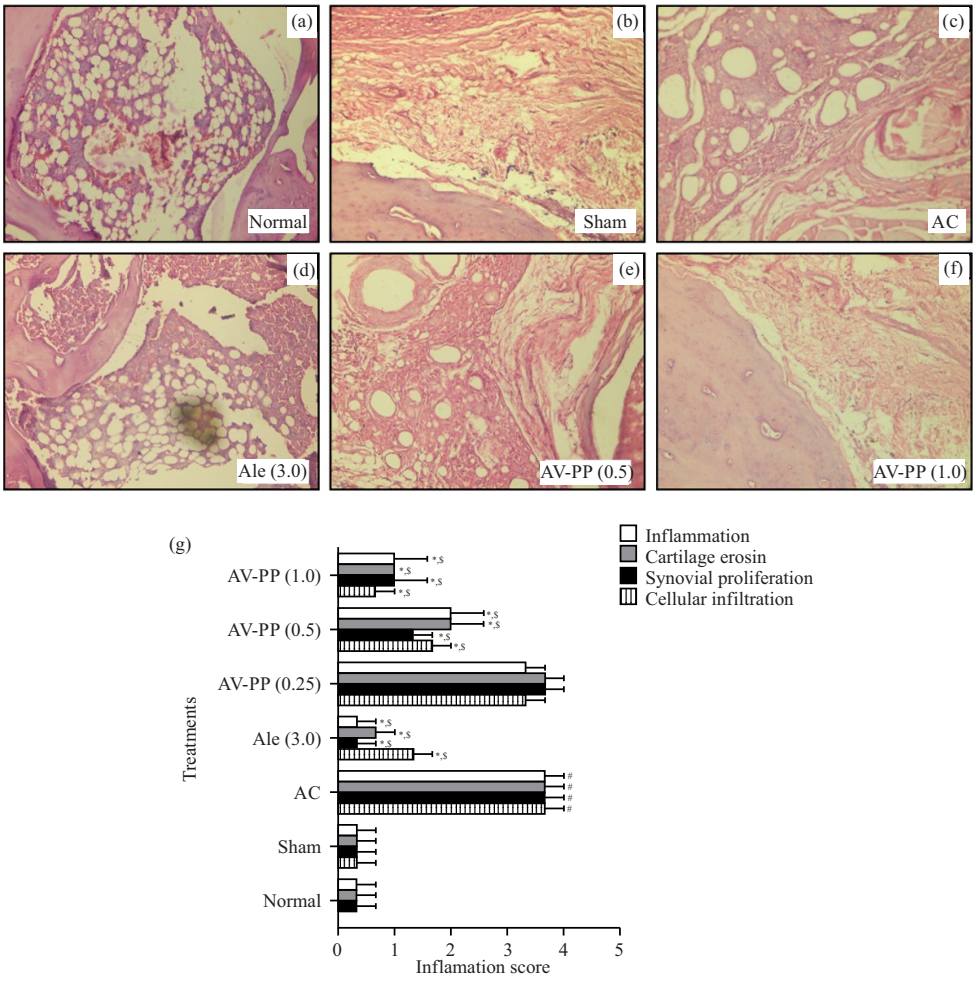

Fig. 3(a-g): Effects of AV-PP on the histopathology of tibiotarsal joints, representative histological images from (a) Normal, (b) Sham, (c) AIA control, (d) Alendronate (3.0 mg kg^{-1}), (e) AV-PP (0.5 mg kg^{-1}), (f) AV-PP (1.0 mg kg^{-1}) treated rats. Images stained with H and E (X 40). The quantitative representation of (g) Histological score

Data were expressed as Mean±SEM (n = 3) and one-way ANOVA followed by the Kruskal-Wallis test was applied for post hoc analysis. For comparison with AIA-control group: *p<0.05, comparison with normal group: #p<0.05 and comparison with one another and $p<0.05

DISCUSSION

Osteoarthritis (OA) is a degenerative, debilitative, painful and the most common type of arthritis, affecting knee joints of almost 100 million people globally[1]. Surgically induced open fracture in the femur of Adjuvant-Induced Arthritic (AIA) rats is an experimental model which determined its bone healing characteristics during arthritis. This experimental model resemblance with the clinical characteristic of OA reflected by systemic bone loss, focal bone erosion at the joint and osteoporosis in a patient with OA[35]. The present investigation evaluated the potential of peptide/polypeptide fraction from Aloe vera (AV-PP) against surgically induced bone damage in a rat model of adjuvant-induced arthritis. Findings suggest that AV-PP improve bone repair and attenuate cartilage degeneration via inhibition of the RANKL/OPG signalling pathway, activating osteocalcin and balancing inflammatory cytokines levels in AIA rats.

A decline in the body weight and halting of growth has been observed in the AIA control rats indicated induction of disease, which is following earlier report[36,37]. Body weight is considered an indirect index of health status, thus, it can evaluate the recovery from illness and maintain a healthy weight[37]. Effects of body weight on the development and restoration of health were during immune-inflammatory conditions, including arthritis, have been well studied by the previous investigator[36-38]. In addition, the severity of arthritis is characterized by various macroscopic signs, including swelling of the hind paw and ankle joints. In the present study, AIA control rats showed the swelling of hind paw suggested by

increased paw volume during Plethysmometer analysis. Furthermore, splenic enlargement is a hallmark for the association of various inflammatory conditions during immune response[30,39]. AIA-induced arthritis has been well linked with increased spleen weight which was observed with increased spleen index in AIA-control rats. However, administration of AA-PP showed inhibition in AIA-induced decreased bodyweight, suggesting the restoration of health and halting of disease progression. Moreover, attenuation of increased hind paw swelling and spleen weight by AA-PP reflects its anti-inflammatory, which might be exerted via inhibition of inflammatory release.

In the current study, diminished IL-4 and IL-10, whereas elevated levels of TNF-α, IL-1β, IL-6 and IL-17 have been observed in the synovial fluid of AIA-control rats. Noteworthy, AA-PP treatment inhibited AIA-induced alteration in pro-inflammatory and anti-inflammatory cytokine levels. Previous investigators also documented polypeptide's anti-inflammatory potential from aloe vera via down-regulation of TNF-α and IL-6 levels[26]. The results of the present investigation were following the findings of the previous researcher[26]. Although the exact mechanism of pathogenesis of OA remains unclear, however, it has been reported that pro-inflammatory cytokines produced via activated synovial cells play a crucial role in the pathogenesis of this illness[8]. Thus, researchers have considered these cytokines as a potential target for treating OA[39-41]. The activation of the inflammatory response by polynuclear neutrophils in the joint space is initiated by the production of inflammatory cytokines and their effects on the joint surface. This process can trigger the onset of joint damage and decrease the functionality of the cartilage membrane. The presence of cytokines, such as ILs and TNF-α in the synovial membrane contributes to the development of joint destruction and the proliferation of the joint. Furthermore, a researcher reported that elevated expressions of TNF-α induce IL-1β production[8,26,40]. IL-6 is another important pro-inflammatory cytokine that served as a hallmark for the systemic activation of pro-inflammatory cytokines[37]. Participation of IL-17 has been documented during the establishment of several inflammatory diseases such as inflammatory OA[41,42]. Moreover, patients with OA reported elevated levels of IL-6 and Il-17 in synovial fluid[41,43]. Conversely, IL-4 and IL-10 are vital anti-inflammatory cytokines reported to down-regulate the expressions of pro-inflammatory cytokines[44,45]. Recently, IL-10 has been reported to inhibit the production of TNF-α and its mediated events during the development of OA[44].

In the present investigation, elevated synovial RANKL/OPG ratio suggested the bone loss in AIA control rats, however, administration of AA-PP showed bone protection via inhibition of the RANKL/OPG pathway and thus halted the disease progression and improved the bone healing process. RANKL is a member of the TNF superfamily mainly involved in regulating osteoclast formation and bone resorption[46]. Studies in knock-out mice suggested that up-regulated expression of RANKL and TNF expedited the disease onset thus, increasing the severity of OA[47]. Additionally, elevated expression of RANKL has been reported in the cartilage samples from OA patients[48]. Therefore, the crucial role of RANKL has been well reported during bone loss and inflammation. On the other hand, OPG is another member of the TNF receptor family that acts as a decoy receptor to oppose the binding of RANKL[49]. A decreased expression of OPG has been reported in the serum of patients with knee osteoarthritis[49]. OPG is responsible for the inhibition of the physiological function of RANKL, thus preventing the process of osteoclastogenesis. The researcher documented the importance of the RANKL: OPG ratio during the pathogenesis of OA[48,49]. Evidence suggested that processes such as postmenopausal osteoporosis, bone fracture and bone loss are associated with the imbalance in the ratio of RANKL: OPG[46,50]. The abnormal RANKL: OPG ratio is known to cause osteoclast-induced bone destruction in OA. This process is triggered by the interactions between RANKL and OPG, limiting the formation of osteoclasts. The increased expression of the RANKL and the low levels of the antagonist OPG increase the risk of joint osteoblastosis. Moreover, osteoblasts and osteoclasts are the important regulators of bone metabolism and osteoclast homeostasis[51]. The increased activity of these two regulators leads to dynamic changes in bone turnover and loss.

Elevated osteocalcin and diminished RUNX2 expressions in AIA control rats suggested their importance during the bone healing process, which is in line with the previous investigators[52-54]. Conversely, AA-PP treatment attenuated bone fracture-induced alterations in osteocalcin and RUNX2 mRNA expressions depicting its bone healing property. Numerous pieces of evidence documented the association of bone GLA protein, osteocalcin with bone development and cartilage regeneration[53,55]. Osteocalcin is a non-collagenous protein of the bone matrix thus, it is a sensitive marker of bone metabolism and plays a role in the formation of bone. A recent study by Bihlet *et al.*[54] documented a significant association of osteocalcin levels with the severity of female patients with knee OA. Furthermore, RUNX2 (also known as Core-binding factor-alpha-[CBFA1]) protein is a crucial component of chondrocyte and osteoblast differentiation[52]. In chondrocyte-specific Runx2 knock-out mice, elevated expression of RUNX2 prevented DMM-induced cartilage degradation suggesting its

importance during the cross-talk between the subchondral bone and cartilage[52]. It is an important transcription factor that plays a crucial role in the induction of proliferative chondrocyte hypertrophy and the development of osteoblast differentiation. Thus, Runx2 is well known for its role in the development and maintenance of OA. A previous study showed that when cartilage tissue was damaged after knee joint instability, global Runx2 expression levels decreased[56]. Studies have shown the postnatal role of RUNX2, evidenced by its minimal expression in articular cartilage[52,53]. The decrease in RUNX2 expression was also evidenced by the accelerated rate of cartilage degeneration and delayed bone healing[57].

Recently it has been well understood that drug development for managing complex and relapsing disorders, including OA, has more relevance by using a multitarget approach for better clinical benefits. Single molecule-target-based management of chronic diseases may result in drug resistance or disease progression may occur quickly. Thus, in the present investigation, we have observed that administration of peptide/polypeptide fraction from Aloe vera improved bone repair in AIA rats via inhibition of RANKL/OPG signalling pathway, activating osteocalcin and balancing inflammatory cytokines levels.

CONCLUSION

The present study demonstrated that a peptide/polypeptide fraction from Aloe vera showed the potential efficacy against surgically induced bone damage in a rat model of adjuvant-induced arthritis. AV-PP improves bone repair and attenuates cartilage degeneration by inhibiting the RANKL/OPG signalling pathway, activating osteocalcin and balancing inflammatory cytokines levels in experimental arthritic rats.

SIGNIFICANCE STATEMENT

This study discovered that the peptide/polypeptide fraction from Aloe vera has repair potential against osteoporotic fractures among osteoarthritis patients. Additionally, findings revealed putative mechanisms of bone repair by inhibiting the RANKL/OPG signalling pathway, activating osteocalcin and balancing inflammatory cytokines. This study will help the researcher to find new complementary and alternative medicine for managing bone repair during osteoarthritis.

REFERENCES

1. Michael, C.R., 1978. Color-sensitive complex cells in monkey striate cortex. J. Neurophysiol., 41: 1250-1266.
2. García-Coronado, J.M., L. Martínez-Olvera, R.E. Elizondo-Omaña, C.A. Acosta-Olivo, F. Vilchez-Cavazos, L.E. Simental-Mendía and M. Simental-Mendía, 2019. Effect of collagen supplementation on osteoarthritis symptoms: A meta-analysis of randomized placebo-controlled trials. Int. Orthop., 43: 531-538.
3. Gui, Q., X. Zhang, L. Liu, F. Zhao, W. Cheng and Y. Zhang, 2019. Cost-utility analysis of total knee arthroplasty for osteoarthritis in a regional medical center in China. Health Econ. Rev., 10.1186/s13561-019-0231-0.
4. Man, G.S. and G. Mologhianu, 2014. Osteoarthritis pathogenesis-a complex process that involves the entire joint. J. Med. Life, 7: 37-41.
5. Carvour, M.L., A. Chiu and K. Page, 2019. Racial and ethnic disparities in surgical amputations following serious musculoskeletal infections in a diverse new Mexico cohort. J. Clin. Transl. Res., 5: 25-32.
6. Bozo, I.I., R.V. Deev, I.V. Smirnov, A.Y. Fedotov and V.K. Popov *et al*., 2020. 3D printed gene-activated octacalcium phosphate implants for large bone defects engineering. Int. J. Bioprinting, Vol. 6. 10.18063/ijb.v6i3.275.
7. Farrugia, M. and B. Baron, 2016. The role of TNF-α in rheumatoid arthritis: A focus on regulatory T cells. J. Clin. Transl. Res., 2: 84-90.
8. Molnar, V., V. Matišić, I. Kodvanj, R. Bjelica and Ž. Jeleč *et al*., 2021. Cytokines and chemokines involved in osteoarthritis pathogenesis. Int. J. Mol. Sci., Vol. 22. 10.3390/ijms22179208.
9. Dunn, S.L., D.L. Vera, K.F. Weaver and J.V. Garcia, 2021. Relationships between inflammatory and metabolic markers, exercise and body composition in young individuals. J. Clin. Transl. Res., 7: 289-296.
10. Rengarajan, T., S. Keerthiga, S. Duraikannu and V. Periyannan, 2019. Exploring the anticancer and anti-inflammatory activities of ferruginol in MCF-7 breast cancer cells. Cancer Plus, Vol. 1. 10.18063/c+.v1i4.255
11. Hoff, P., F. Buttgereit, G.R. Burmester, M. Jakstadt and T. Gaber *et al*., 2013. Osteoarthritis synovial fluid activates pro-inflammatory cytokines in primary human chondrocytes. Int. Orthop., 37: 145-151.
12. Zheng, C., S. Attarilar, K. Li, C. Wang and J. Liu *et al*., 2021. 3D-printed HA15-loaded β-tricalcium phosphate/poly (Lactic-co-glycolic acid) bone tissue scaffold promotes bone regeneration in rabbit radial defects. Int. J. Bioprinting, Vol. 7. 10.18063/ijb.v7i1.317.
13. Daskalakis, E., F. Liu, B. Huang, A.A. Acar and G. Cooper *et al*., 2021. Investigating the influence of architecture and material composition of 3D printed anatomical design scaffolds for large bone defects. Int. J. Bioprinting, Vol. 7. 10.18063/ijb.v7i2.268.

14. Liu, Y., Y. Wang, X. Sun, X. Zhang, X. Wang, C. Zhang and S. Zheng, 2018. RUNX2 mutation reduces osteogenic differentiation of dental follicle cells in cleidocranial dysplasia. Mutagenesis, 33: 203-214.
15. Vohra, M., A. Sharma, R. Bagga and S.K. Arora, 2020. Human umbilical cord-derived mesenchymal stem cells induce tissue repair and regeneration in collagen-induced arthritis in rats. J. Clin. Transl. Res., 6: 203-216.
16. Ge, C., H. Wang, H. Chen, W. Yue and X. Yan, 2020. The dual characteristics of ten-eleven translocation 1 in cancer. Cancer Plus, 2: 13-23.
17. Ono, T., M. Hayashi, F. Sasaki and T. Nakashima, 2020. Rankl biology: Bone metabolism, the immune system and beyond. Inflammation Regener., Vol. 40. 10.1186/s41232-019-0111-3.
18. Grässel, S. and D. Muschter, 2020. Recent advances in the treatment of osteoarthritis. F1000Research, Vol. 9. 10.12688/f1000research.22115.1.
19. Ghosh, P., A.D. Kandhare, V.S. Kumar, A.R. Rajmane, M. Adil and S.L. Bodhankar, 2012. Determination of clinical outcome and pharmacoeconomics of anti-rheumatoid arthritis therapy using CDAI, EQ-5D-3L and EQ-VAS as indices of disease amelioration. Asian Pac. J. Trop. Dis., 2012: S671-S678.
20. Xing, R.L., L.R. Zhao and P.M. Wang, 2016. Bisphosphonates therapy for osteoarthritis: A meta-analysis of randomized controlled trials. SpringerPlus, Vol. 5. 10.1186/s40064-016-3359-y.
21. Strassle, B.W., L. Mark, L. Leventhal, M.J. Piesla and X.J. Li *et al*., 2010. Inhibition of osteoclasts prevents cartilage loss and pain in a rat model of degenerative joint disease. Osteoarthritis Cartilage, 18: 1319-1328.
22. Khorasani, M.S., S. Diko, A.W. Hsia, M.J. Anderson, D.C. Genetos, D.R. Haudenschild and B.A. Christiansen, 2015. Effect of alendronate on post-traumatic osteoarthritis induced by anterior cruciate ligament rupture in mice. Arthritis Res. Ther., 17: 1-30.
23. Radha, M.H. and N.P. Laxmipriya, 2015. Evaluation of biological properties and clinical effectiveness of *Aloe vera*: A systematic review. J. Traditional Complementary Med., 5: 21-26.
24. Kumar, S., F. Sugihara, K. Suzuki, N. Inoue and S. Venkateswarathirukumara, 2015. A double-blind, placebo-controlled, randomised, clinical study on the effectiveness of collagen peptide on osteoarthritis. J. Sci. Food Agric., 95: 702-707.
25. Oesser, S., C.H. Schulze, D. Zdzieblik and D. König, 2016. Efficacy of specific bioactive collagen peptides in the treatment of joint pain. Osteoarthritis Cartilage, Vol. 24. 10.1016/j.joca.2016.01.370.
26. Babu, S.N. and A. Noor 2019. *Aloe barbadensis* miller peptide/polypeptide fraction alleviates inflammation through inhibition of proinflammatory cytokines and mediators *in vitro* and in rats with freund's adjuvant-induced hind paw edema. Asian Pac. J. Trop. Biomed., 9: 524-530.
27. Babu, S.N., S. Govindarajan, M.A. Vijayalakshmi and A. Noor, 2021. Role of zonulin and GLP-1/DPP-IV in alleviation of diabetes mellitus by peptide/polypeptide fraction of *Aloe vera* in streptozotocin-induced diabetic wistar rats. J. Ethnopharmacol., Vol. 272. 10.1016/j.jep.2021.113949.
28. Rekowski, A., G. Langenkämper, M. Dier, M.A. Wimmer, K.A. Scherf and C. Zörb, 2021. Determination of soluble wheat protein fractions using the bradford assay. Cereal Chem., 98: 1059-1065.
29. Chou, M.M., N. Vergnolle, J.J. McDougall, J.L. Wallace, S. Marty, V. Teskey and A.G. Buret, 2005. Effects of chondroitin and glucosamine sulfate in a dietary bar formulation on inflammation, interleukin-1β, matrix metalloprotease-9 and cartilage damage in arthritis. Exp. Biol. Med., 230: 255-262.
30. Teixeira, J.H., A.M. Silva, M.I. Almeida, M. Bessa-Gonçalves, C. Cunha, M.A. Barbosa and S.G. Santos, 2019. The systemic immune response to collagen-induced arthritis and the impact of bone injury in inflammatory conditions. Int. J. Mol. Sci., Vol. 20. 10.3390/ijms20215436.
31. Honmore, V.S., A.D. Kandhare, P.P. Kadam, V.M. Khedkar, A.D. Natu, S.R. Rojatkar and S.L. Bodhankar, 2019. 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., 229: 233-245.
32. Patil, M.V.K., A.D. Kandhare and S.D. Bhise, 2012. Anti-arthritic and Anti-inflammatory activity of *Xanthium srtumarium* L. ethanolic extract in freund's complete adjuvant induced arthritis. Biomed. Aging Pathol., 2: 6-15.
33. Kandhare, A.D., P. Ghosh and S.L. Bodhankar, 2014. Naringin, a flavanone glycoside, promotes angiogenesis and inhibits endothelial apoptosis through modulation of inflammatory and growth factor expression in diabetic foot ulcer in rats. Chemico-Biol. Interact., 219: 101-112.
34. Seeuws, S., P. Jacques, J. Van Praet, M. Drennan and J. Coudenys *et al*., 2010. A multiparameter approach to monitor disease activity in collagen-induced arthritis. Arthritis Res. Ther., Vol. 12. 10.1186/ar3119.
35. Hu, Y., T. Zhang, H. Huang, W. Cheng and Y. Lai *et al*., 2018. Fracture healing in a collagen-induced arthritis rat model: Radiology and histology evidence. J. Orthop. Res., 36: 2876-2885.
36. Honmore, V., A. Kandhare, A.A. Zanwar, S. Rojatkar, S. Bodhankar and A. Natu, 2015. *Artemisia pallens* alleviates acetaminophen induced toxicity via modulation of endogenous biomarkers. Pharmaceut. Biol., 53: 571-581.
37. Wang, X., X. He, C.F. Zhang, C.R. Guo, C.Z. Wang and C.S. Yuan, 2017. Anti-arthritic effect of berberine on adjuvant-induced rheumatoid arthritis in rats. Biomed. Pharmacother., 89: 887-893.
38. Poles, J., E. Karhu, M. McGill, H.R. McDaniel and J.E. Lewis, 2021. The effects of twenty-four nutrients and phytonutrients on immune system function and inflammation: A narrative review. J. Clin. Transl. Res., 7: 333-376.

39. De Marchi, E., E. Orioli, A. Pegoraro, S. Sangaletti and P. Portararo *et al*., 2019. The P2X7 receptor modulates immune cells infiltration, ectonucleotidases expression and extracellular ATP levels in the tumor microenvironment. Oncogene, 38: 3636-3650.
40. Haleagrahara, N., S. Miranda-Hernandez, M.A. Alim, L. Hayes, G. Bird and N. Ketheesan, 2017. Therapeutic effect of quercetin in collagen-induced arthritis. Biomed. Pharmacother., 90: 38-46.
41. Snelling, S.J.B., S. Bas, G.J. Puskas, S.G. Dakin and D. Suva *et al*., 2017. Presence of IL-17 in synovial fluid identifies a potential inflammatory osteoarthritic phenotype. PLOS ONE, Vol. 12. 10.1371/journal.pone.0175109.
42. Mateen, S., S. Moin, S. Shahzad and A.Q. Khan, 2017. Level of inflammatory cytokines in rheumatoid arthritis patients: Correlation with 25-hydroxy vitamin D and reactive oxygen species. PLOS ONE, Vol. 12. 10.1371/journal.pone.0178879.
43. Bebe, F.N., S. Hu, T.L. Brown and O.L. Tulp, 2019. Survival time distribution of advanced stage metastatic melanoma among whites and minority populations in florida, 1996-2010. Cancer Plus, 1: 33-42.
44. Barker, T., V.E. Rogers, V.T. Henriksen, R.H. Trawick, N.G. Momberger and G.L. Rasmussen, 2021. Circulating IL-10 is compromised in patients predisposed to developing and in patients with severe knee osteoarthritis. Sci. Rep., Vol. 11. 10.1038/s41598-021-81382-6.
45. Chen, Z., A. Bozec, A. Ramming and G. Schett, 2019. Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. Nat. Rev. Rheumatol., 15: 9-17.
46. Papadaki, M., V. Rinotas, F. Violitzi, T. Thireou, G. Panayotou, M. Samiotaki and E. Douni, 2019. New insights for RANKL as a proinflammatory modulator in modeled inflammatory arthritis. Front. Immunol., Vol. 10. 10.3389/fimmu.2019.00097.
47. Rinotas, V., A. Niti, R. Dacquin, N. Bonnet and M. Stolina *et al*., 2014. Novel genetic models of osteoporosis by over expression of human RANKL in transgenic mice. J. Bone Min. Res., 29: 1158-1169.
48. Upton, A.R., C.A. Holding, A.A.S.S.K. Dharmapatni and D.R. Haynes, 2012. The expression of RANKL and OPG in the various grades of osteoarthritic cartilage. Rheumatol. Int., 32: 535-540.
49. Mirzaii-Dizgah, M.R., M.H. Mirzaii-Dizgah, I. Mirzaii-Dizgah, M. Karami and B. Forogh, 2021. Osteoprotegerin changes in saliva and serum of patients with knee osteoarthritis. Rev. Española Cirugía Ortopédica Traumatología, 10.1016/j.recot. 2021.02.003.
50. Takeshita, A., K. Nishida, A. Yoshida, Y. Nasu and R. Nakahara *et al*., 2021. RANKL expression in chondrocytes and its promotion by lymphotoxin-α in the course of cartilage destruction during rheumatoid arthritis. PLOS ONE, Vol. 16. 10.1371/journal.pone.0254268.
51. Chen, X., Z. Wang, N. Duan, G. Zhu, E.M. Schwarz and C. Xie, 2018. Osteoblast-osteoclast interactions. Connective Tissue Res., 59: 99-107.
52. Catheline, S.E., D. Hoak, M. Chang, J.P. Ketz, M.J. Hilton, M.J. Zuscik and J.H. Jonason, 2019. Chondrocyte specific RUNX2 overexpression accelerates post traumatic osteoarthritis progression in adult mice. J. Bone Min. Res., 34: 1676-1689.
53. Liao, L., S. Zhang, J. Gu, T. Takarada and Y. Yoneda *et al*., 2017. Deletion of Runx2 in articular chondrocytes decelerates the progression of dmm-induced osteoarthritis in adult mice. Sci. Rep., Vol. 7. 10.1038/s41598-017-02490-w.
54. Bihlet, A.R., I. Byrjalsen, A.C. Bay-Jensen, J.R. Andersen, C. Christiansen, B.J. Riis and M.A. Karsdal, 2019. Associations between biomarkers of bone and cartilage turnover, gender, pain categories and radiographic severity in knee osteoarthritis. Arthritis Res. Ther., Vol. 21. 10.1186/s13075-019-1987-7.
55. Maruotti, N., A. Corrado and F.P. Cantatore, 2017. Osteoblast role in osteoarthritis pathogenesis. J. Cell. Physiol., 232: 2957-2963.
56. Roseti, L., G. Desando, C. Cavallo, M. Petretta and B. Grigolo, 2019. Articular cartilage regeneration in osteoarthritis. Cells, Vol. 8. 10.3390/cells8111305.
57. Chen, D., D.J. Kim, J. Shen, Z. Zou and R.J. O'Keefe, 2020. Runx2 plays a central role in osteoarthritis development. J. Orthop. Transl., 23: 132-139.