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ORIGINAL ARTICLE

Effects of intra-articular platelet-rich plasma on cartilage regeneration and osteoarthritis progression in a chemically induced rat model

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ABSTRACT

Objectives: To evaluate the regenerative effects of intra-articular platelet-rich plasma (PRP) on cartilage regeneration and disease progression in a chemically induced osteoarthritic knee joint rat model.

Methods: This laboratory-based experimental study was conducted at the Department of Anatomy, Army Medical College, Rawalpindi (December 2019–October 2020). Ninety male Sprague-Dawley rats (2-3 months, 250±50g) were randomly divided into three groups (n=30 each). Group A (control) received intra-articular saline (30μl). Groups B and C were induced with osteoarthritis via intra-articular injection of 1 mg monosodium iodoacetate (MIA). Two weeks later, Group C received 0.5 ml PRP, prepared by a double-spin centrifugation method and activated with calcium chloride. After 8 weeks, all animals were sacrificed. Articular cartilage specimens were processed, stained (H&E and toluidine blue), and analyzed for chondrocyte count, matrix loss, and cartilage surface integrity using Image J software. Statistical analysis was performed using ANOVA, Tukey's test, and Chi-square, with $p<0.05$ considered significant.

Results: Group A showed normal articular cartilage (96.9±1.75 chondrocytes). Group B demonstrated significant reduction in chondrocyte count (46.1±1.9, $p=0.000$), matrix loss, and severe surface damage ($p=0.001$). In contrast, PRP-treated group C showed significant improvement in chondrocyte count (86.9±1.4, $p=0.000$), notable matrix preservation, and improved cartilage structure compared to group B ($p=0.001$).

Conclusion: Intra-articular PRP preserved chondrocytes, reduced matrix loss, minimized cartilage damage, and promoted regeneration of articular cartilage, suggesting its therapeutic potential in slowing osteoarthritis progression. Further studies incorporating biochemical and radiological assessments are recommended for validation.

Keywords: Osteoarthritis (MeSH); Osteoarthritis, Knee (MeSH); Platelet-Rich Plasma (MeSH); Monosodium Iodoacetate (MeSH); Iodoacetic Acid (MeSH); Chondrocytes (MeSH); Bone Matrix (MeSH); Cartilage, Articular (MeSH); Injections (MeSH); Injections, Intra-Articular (MeSH)

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INTRODUCTION

Osteoarthritis (OA) is the most prevalent form of arthritis worldwide, characterized by the progressive degeneration of articular cartilage and subchondral bone, most commonly affecting weight-bearing joints such as the knee.[1] Knee osteoarthritis (KOA) substantially limits mobility and quality of life, particularly in older adults, and represents a growing public health concern. The global burden of KOA continues to rise due to increasing life expectancy, obesity, sedentary lifestyles, and population aging.[2] It is among the leading causes of years lived with disability (YLDs) and significantly contributes to the global burden of non-communicable diseases. According to the World Health Organization, approximately 10-15% of individuals over the age of 60 add years are affected by OA, with the knee being the most commonly involved joint.[3] Despite its high prevalence and debilitating impact, effective disease-modifying treatments remain unavailable. Current management strategies, including oral non-steroidal anti-inflammatory drugs (NSAIDs) and intra-articular injections such as corticosteroids and hyaluronic acid, provide symptomatic relief but are associated with potential adverse effects and fail to halt or reverse the underlying degenerative process.[4]

Platelet-rich plasma (PRP) therapy has emerged as a promising regenerative treatment in recent years. Derived from the patient's own blood, PRP concentrates platelets that are rich in cytokines and growth factors, thereby promoting tissue repair and regeneration.[5] Platelets release key mediators such as vascular endothelial growth factor, platelet-derived growth factor, fibroblast growth factor, and epidermal growth factor, all of which play essential roles in angiogenesis and tissue remodeling.[6] PRP is a minimally invasive therapy that enhances the body's natural healing processes without the risks associated with synthetic drugs or surgery.[7] Its favorable effects are largely attributed to an anabolic influence on cartilage growth.[8] While numerous studies have demonstrated the clinical benefits of PRP in reducing pain and improving symptoms, limited research has investigated the underlying histological changes. Platelet concentrates have

transformed the management of a variety of conditions involving bone, cartilage, and skin,[9,10] and PRP injections are increasingly recognized as a safe and effective treatment for KOA.[11] However, limited histological evidence exists regarding its effects on cartilage repair in OA. The present study was designed to determine the effects of intra-articular PRP on cartilage regeneration and osteoarthritis progression, aiming to reverse inflammation and shift the joint environment from a catabolic to an anabolic state.

METHODS

Ninety male Sprague-Dawley rats were procured from the National Institute of Health. Inclusion criteria included male rats aged 80-90 days and weighing 250 ±50 grams, while those with gross joint deformities were excluded. All animals were maintained on a standard diet in the institutional animal facility.[12]

In rats, an intra-articular dose of 1 mg monosodium iodoacetate (MIA) is sufficient to produce an inflammatory response within one day, persisting for approximately five days and resulting in cartilage matrix degeneration and chondrocyte apoptosis. To induce osteoarthritis in Groups B and C, MIA was dissolved at a concentration of 35 mg/mL in 0.9% saline. A 30 μL aliquot (equivalent to 1 mg) of this stock solution was injected into the right knee joint using a 27-gauge, 0.5-inch needle.[13]

Platelet-rich plasma (PRP) was prepared using the double-spin method.[12] Approximately 3 mL of blood was obtained via intracardiac puncture and collected in citrated tubes containing sodium citrate. The first centrifugation was performed at 160 G for 20 minutes at 22°C, yielding a straw-yellow plasma fraction and a red cell fraction. At 1.4 mm below the separation line, a point was marked, and all material above 0.35 mL from the tube's base was transferred to a 5 mL vacuum tube. A second centrifugation at 400 G for 15 minutes separated platelet-poor plasma (PPP) from PRP. To activate, 0.05 mL of 10% calcium chloride was added to each 1 mL of PRP or PPP.[14] After eight weeks, all animals were euthanized, and the lower limbs were disarticulated at the hip joint. The knee joints were exposed by dissecting the skin and ligaments, with the tibia cut 3 mm below the tibial plateau and the femur 5 cm above the condyle. Specimens were fixed in 10% formalin for 24 hours, followed by decalcification in 5% nitric acid for four days. Coronal sections including articular cartilage and subchondral bone were processed, embedded, and cut into 5 μm sections using a rotary microtome. Hematoxylin and eosin staining was performed to evaluate cartilage surface and cellular morphology, while toluidine blue staining was used to assess matrix loss.

Chondrocytes were counted on H&E-stained slides at 40× magnification. Two sites from the periphery and two from the center were selected, and counts were performed using ImageJ's multipoint tool; chondrocytes without nuclei were excluded. The average count per field was calculated.[15] Punctate lesions, defined as localized erosions of the cartilage surface, were assessed for depth using ImageJ's line tool. Lesion depth was graded according to the Mankin system (0–4): 1 =surface degradation, 2=fissures extending to the mid-zone, 3=fissures extending to the deep zone, and 4= complete structural disorganization.[16]

Cartilage matrix degradation was evaluated from the surface to the tidemark. Matrix loss was objectively measured using the ImageJ line tool on recorded images, with values expressed in microns. Areas of complete matrix loss were identified, while artifacts, remnants, or floating debris within the lesion were excluded from analysis.

Surface (0% depth): Matrix destruction was limited to the projected cartilage surface, with intact superficial cartilage on either side. The width of matrix loss was assessed.

Mid-zone (50% depth): Matrix loss extended midway through the cartilage thickness, between the surface and the tidemark, and was measured in width.

Tidemark (100% depth): Complete matrix loss was observed at the tidemark level, and the extent of loss was assessed in breadth.

Data were analyzed using SPSS version 22.0. Frequencies and percentages were calculated for qualitative variables, while quantitative variables were expressed as Mean ± Standard error. Differences in quantitative variables were assessed using one-way analysis of variance (ANOVA) followed by Post Hoc Tukey's test, whereas qualitative variables were analyzed using the Chi-square test. A p-value <0.05 was considered statistically significant.

RESULTS

In control group A, the articular cartilage appeared normal (96.93± 1.75; p=0.000). In contrast, group B demonstrated a significant decline in chondrocyte count (46.16±1.99; p=0.000), marked matrix loss, and severe cartilage damage (p=0.001) [Figures 1-4]. When compared with Group B, Group C showed a significant

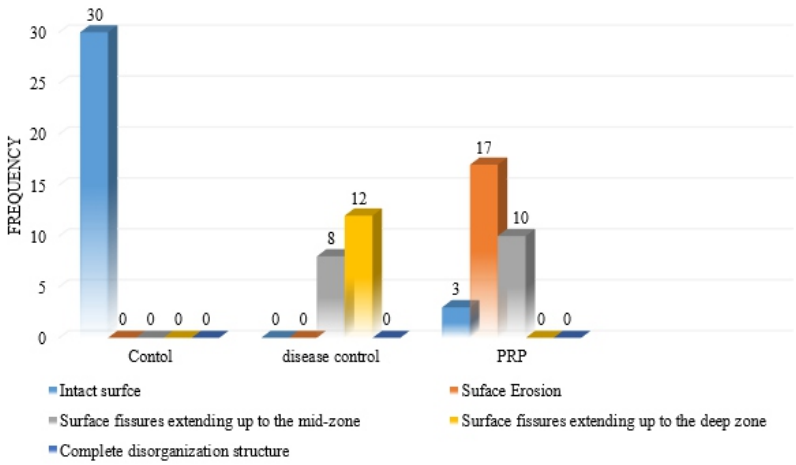

Figure 1: Bar chart showing the comparison of Depth of Punctate depressions among the groups A, B and C

improvement in chondrocyte count (86.9±1.43; p=0.000), along with notable preservation of matrix and articular cartilage structure (p=0.001) [Figures 1-4].

We evaluated the articular cartilage in the control, osteoarthritis, and PRP-treated groups (Figures 1 and 2). Group B exhibited significant surface degradation compared to Group A (p=0.001), whereas Group C demonstrated marked preservation of the articular surface compared to group B (p=0.001). A significant difference was also observed between groups A and C (p=0.000).

We quantified chondrocytes across the three zones of articular cartilage. The osteoarthritis group (Group B) showed a significant reduction in chondrocyte count, averaging 46.1±1.9, with scattered chondrocytes, occasional pyknotic nuclei, and absent lacunae (Figure 3B). In contrast, Group C demonstrated a marked increase in chondrocyte cellularity compared to Group B, with an average of 86.9±1.43 chondrocytes. Chondrocytes in Group C exhibited zonal heterogeneity, maintaining their typical arrangement from the surface to the tidemark, with variation in size and orientation across superficial, mid, and deep zones. A small number of chondrocytes were singly located in lacunae and displayed pyknotic or absent nuclei (Table I).

The frequencies and percentages of matrix loss in toluidine blue-stained sections for Groups A, B, and C are presented in Table I and Figure 4. Group B demonstrated significant matrix deterioration compared to Group A (p =0.001**). In contrast, Group C showed significant preservation of the articular cartilage matrix compared to group B (p=0.00*). A significant difference was also observed between Groups A and C (p = 0.00*).

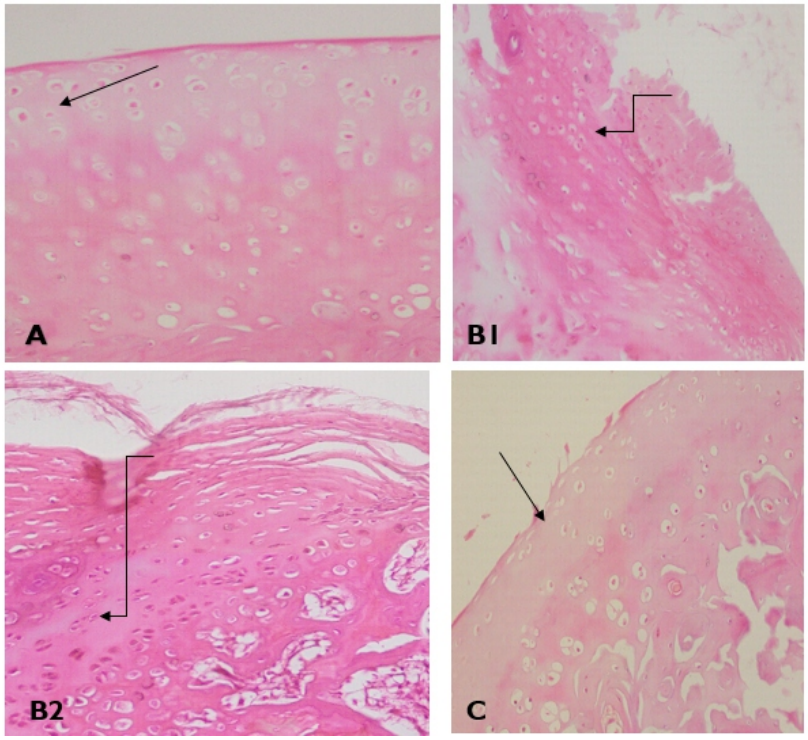

Figure 2: Hematoxyllin and Eosin (H&E) stained sections of the articular cartilage of the experimental groups **A**: Arrow indicates intact surface of articular cartilage (normal Histology), **B1**: Arrow indicating articular cartilage damage from surface erosions till deep zone, **B2**: Arrow indicating complete loss of articular surface-atrix, **C**: PRP treated group articular cartilage (arrow indicating surface erosion)

DISCUSSION

This study evaluated the effect of intra-articular PRP in a monosodium iodoacetate (MIA)-induced OA rat model. Ninety male Sprague-Dawley rats were divided into three groups: Group A (control), Group B (MIA-induced OA), and Group C (OA+ PRP). Group C demonstrated significant improvements in chondrocyte count, matrix preservation, and reduced cartilage degeneration compared to Group B, indicating the protective and regenerative potential of PRP. OA is a progressive joint disorder characterized by cartilage degradation, matrix loss,

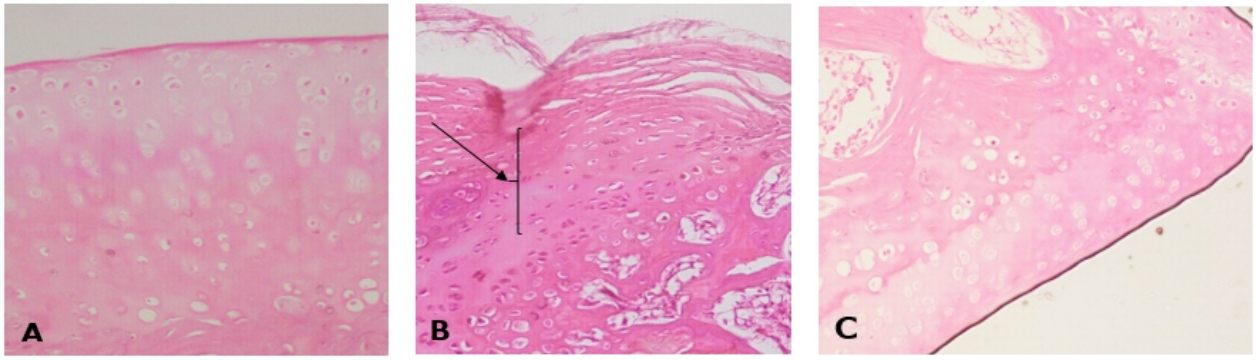

Figure 3: Photomicrograph of normal articular cartilage, group **A**, **B**, **C** showing Number of Chondrocytes [**A**. Chondrocytes are arranged in zones (normal histology), **B**. Osteoarthritis group number arrow indicating number of chondrocytes reduced and cell death noticed, lost zonal arrangement. **C**. Chondrocyte arranged in zones]

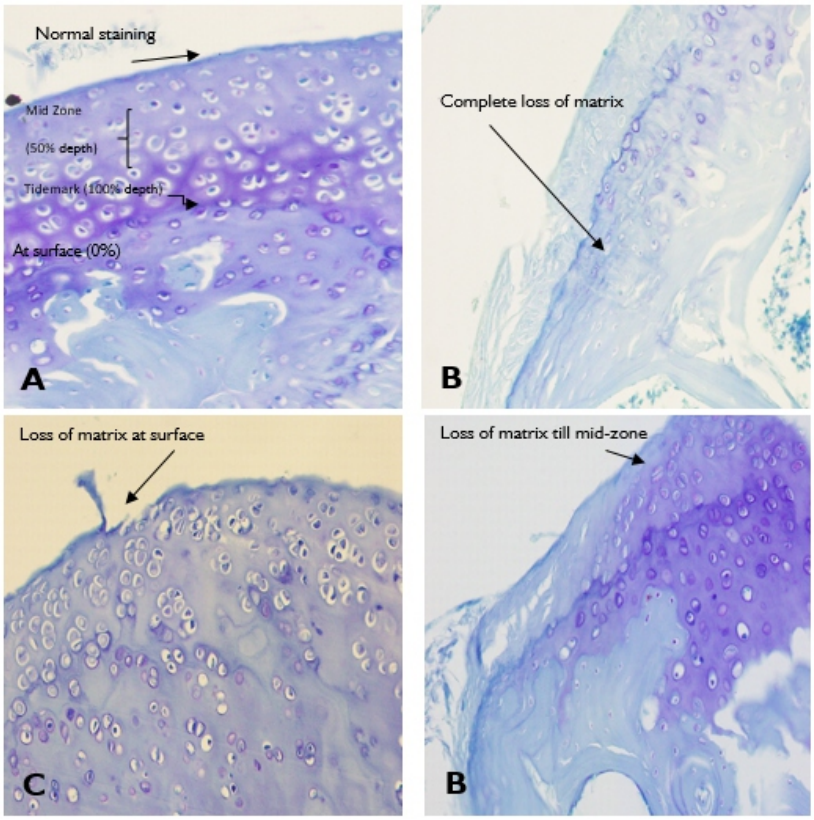

Figure 4: Toludine blue stained section showing **A**. Normal articular cartilage matrix. **B**. Loss of matrix till mid and deep zones, **C**. Slight reduction of matrix.

Table I: Comparison of mean chondrocyte counts among experimental groups

Groups (n=30)	Mean ± SE	Statistical Significance		
		Group A/B	Group A/C	Group B/C
A	96.1±1.9	0.00**	0.00**	0.00**
B	46.9±1.4			
C	86.3±1.4			

SE: Standard error In footnotes; n: Frequency

Table II: Frequency and percentages of matrix loss among experimental groups

Findings	Group A	Group B	Group C	P-Value
Normal	30 (100%)	0 (0.0%)	3 (10%)	0.00**
Slight reduction (surface)	0 (0.0%)	0 (0.0%)	17 (56.6%)	
Moderate reduction (extending down to mid-zone)	0 (0.0%)	18 (60%)	10 (10.0%)	
Severe reduction (entire cartilage thickness from surface to tidemark)	0 (0.0%)	12 (40%)	0 (0.0%)	
No Dye Noted	0 (0.0%)	0 (0.0%)	0 (0.0%)	–

and chondrocyte apoptosis, resulting in pain and disability. Conventional therapies provide only symptomatic relief, whereas PRP, enriched with growth factors such as PDGF, TGF-β1, IGF-1, and VEGF, could promote tissue regeneration, modulate inflammation, and enhance chondrocyte viability.[17]

Histological analysis showed that PRP-treated joints had preserved cartilage architecture, reduced erosion, and improved staining intensity with toluidine blue. Most Group C specimens (63.3%) showed only minor superficial matrix loss, in contrast to the disease group, where 30% demonstrated complete cartilage loss to the tide mark. These findings are consistent with those of Godek P, et al., who reported significant attenuation of cartilage degeneration by PRP.[18] Similarly, Zhou S, et al., observed upregulation of anabolic genes (COL2A1, ACAN) and downregulation of catabolic enzymes (MMP-13) in PRP-treated joints, confirming its dual role in cartilage protection and matrix remodeling.[19] Our results are also in line with clinical studies. Patel S, et al., conducted a randomized controlled trial in India showing improved WOMAC scores and MRI evidence of cartilage preservation in PRP-treated OA knees.[20]

National data further supports our findings. Ghori FS, et al., reported improved cartilage morphology and reduced joint inflammation in osteoarthritic rabbits treated with autologous PRP.[21] Likewise, Yousaf H, et al., demonstrated that PRP significantly preserved chondrocyte morphology and matrix integrity compared to disease controls, closely mirroring the histological outcomes of our study.[22] At the cellular level, the anti-apoptotic and pro-survival effects of PRP on chondrocytes are well recognized. Yousaf H, et al., further showed that PRP suppresses IL-1β-induced apoptosis in vitro and activates the PI3K/AKT signaling pathway in cartilage cells, thereby promoting tissue regeneration.[22] These molecular mechanisms likely account for the higher chondrocyte counts observed in our PRP-treated group. However, the clinical translation of PRP therapy remains challenging due to variability in preparation techniques, platelet concentration, and delivery protocols.

Tey RV, et al., identified this heterogeneity as a major barrier to establishing universal treatment guidelines.[23] While our study employed a single PRP dose, Filardo G, et al., suggested that multiple injections may provide more sustained therapeutic benefits.[24] Furthermore, differences in PRP composition-particularly between leukocyte-rich and leukocyte-poor formulations, may influence inflammatory modulation and clinical efficacy. Therefore, standardized protocols are essential to optimize outcomes and enhance the reproducibility of results.

Our toluidine blue staining demonstrated marked preservation of the cartilage matrix in PRP-treated specimens, with most animals exhibiting only superficial matrix loss. These findings are consistent with those of Park SH, et al., who observed enhanced proteoglycan retention and increased cartilage thickness after PRP administration in animal models.[25] Collectively, national and international evidence supports the role of PRP in fostering a favorable anabolic environment within osteoarthritic cartilage.

This study has certain limitations. Histopathological changes were not assessed alongside biochemical markers or radiological imaging. Only a single PRP dose and short follow-up period were used, and the modest sample size in an animal model may limit generalizability. Future studies should use multiple dosing, longer follow-up, larger cohorts, and incorporate biochemical and radiological assessments to better validate PRP's therapeutic role

CONCLUSION

This study demonstrated that intra-articular administration of PRP effectively preserved articular cartilage structure and reduced the progression of monosodium iodoacetate -induced osteoarthritis in rats. PRP-treated joints showed significantly higher chondrocyte counts, reduced matrix degradation, and shallower cartilage lesions compared to the disease group. These findings highlight the potential of PRP as a disease-modifying therapy for osteoarthritis by promoting cartilage regeneration and inhibiting structural deterioration.

Further research is required to define optimal dosing strategies, long-term outcomes, and clinical applicability. Particular attention should be given to standardizing PRP preparation protocols, especially platelet concentration, as this directly influences the release of growth factors critical for tissue repair and regeneration.

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AUTHORS' CONTRIBUTION

The Following authors have made substantial contributions to the manuscript as under:

MFQ: Conception, acquisition of data, drafting the manuscript, critical review, approval of the final version to be published

KQ: Conception and study design, drafting the manuscript, critical review, approval of the final version to be published

AZ, MMK, SM & KH: Acquisition, analysis and interpretation of data, drafting the manuscript, approval of the final version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest, whether financial or otherwise, that could influence the integrity, objectivity, or validity of their research work.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request