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Original Article

Is Platelet Rich Plasma Effective in the Treatment of Formaldehyde Induced Arthritis in Rats? Application to Osteoarthritic Patients

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ABSTRACT

Introduction: In this study, the efficacy of platelet rich plasma (PRP) in the treatment of formaldehyde (FA) induced arthritis was evaluated.

Material and Methods: Forty two adult male albino rats were divided into three groups. Group I (control group): eighteen rats were divided into I-a, I-b, and I-c subgroups. Group II (arthritic group): twelve rats were subjected to intra-articular injection with single dose of 0.02 ml of 5% FA in the right knee and were further subdivided into II-a and II-b subgroups. Group III (arthritic group treated with PRP): twelve rats were subjected to induced arthritis as in group II. Then they were subjected to intra-articular injection with single dose of 0.3 ml of PRP in the same right knee and were further subdivided into III-a and III-b subgroups.

Results: After sacrifice, the knee joints were fixed, decalcified, and processed for paraffin sections. Different stains were applied and immunohistochemical staining for Caspase-3 enzyme was done. Sections were examined by light microscope. The arthritic groups revealed irregular articular surface and bone eburnation. There was apparent hypocellularity and disorganization of the chondrocytes. Osteoclasts and osteoblasts invaded the osteochondral junction. The synovial membrane showed deposition of thick collagen bundles with inflammatory cell infiltrate and numerous blood vessels. Caspase-3 immunoreactivity was apparent in many chondrocytes. All these changes were ameliorated in group III-a. Many of these changes were seen in Group III-b. The morphometric results and statistical analysis confirmed the histological findings.

Conclusion: It was concluded that intra-articular injection of PRP demonstrated transient effective role on articular cartilage healing.

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Key Words: Articular cartilage, efficacy of platelet rich plasma, formaldehyde, induced arthritis, platelet rich plasma.

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INTRODUCTION

Osteoarthritis (OA) is the most common form of joint diseases. It is one of the top ten causes of disability worldwide that leads to major public health problem especially with aging and increasing obesity. OA is a progressive disorder based on degeneration of the articular cartilage[1].

Osteoarthritis was commonly mistaken as degenerative joint disease including series of wear and tear. However, it was proved to be more complex process driven by inflammatory mediators within the affected joint[2].

Although cartilage destruction is the hallmark of OA, yet all other components of the joint could be affected. Synovitis, subchondral bone remodeling, degeneration of ligaments, and hypertrophy of the joint capsule can take parts in the pathogenesis of OA[3].

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Conservative therapies of OA include oral administration of nonsteroidal anti-inflammatory drugs. Although these drugs are effective in controlling the symptoms, they don't suppress the degeneration of the articular cartilage[4].

With the progression of OA, oral drugs become ineffective. Intra-articular injection of corticosteroids or hyaluronic acid is used as the last non-operative therapeutic modality. However, these agents need to be injected repeatedly as they are effective only in short term therapy[5]. In addition, some researchers stated that the beneficial role of intra-articular injection of these agents was inconclusive for their efficacy in functional improvement of the joint after injection[6].

Total joint replacement is the surgical treatment for end-stage OA. Although clinical results confirm usefulness of this procedure, however, the surgical treatment has the problems of invasiveness and high financial burden[7].

Animal models of osteoarthritis are used to evaluate potential anti-arthritic drugs for clinical use. In animal models, OA often occurs in the weight-bearing joints, which are the knee and hip joints[8]. Osteoarthritis can be induced by intra-articular injection of chemical agents such as collagenase[9], monoiodoacetate[10], or formaldehyde (FA)[11]. Osteoarthritis may be also induced surgically in the knee joint by different methods such as meniscal tear or transection of the anterior cruciate ligament[12].

Numerous researchers have studied the regeneration of the injured articular cartilage by the use of various growth factors. These growth factors accelerate cartilage metabolism and/or inhibit cartilage degeneration[13].

Recently, platelet-rich plasma (PRP) has gathered attention as an autologous source of growth factors. It is prepared from autologous blood by centrifugation, and the obtained sample contains a platelet concentration of four to five times higher than that of normal blood[14].

Being a blood product, PRP is considered as a simple and low cost source of many growth factors and biologically active molecules. It contains high concentrations of autologous growth factors such as PDGF, TGF-β1 and IGF-1[15].

Platelet rich plasma promotes wound healing and tissue regeneration. It has been used clinically in many fields like sport injuries, oral surgeries, and plastic surgeries[16]. It is also used in dermatological field to treat hair loss and for cosmetic purposes.

Nevertheless, the use of PRP in OA was found to be associated with reduced inflammation, pain relief, improved function, and possible cartilage regeneration[17].

By reviewing the literature, few experimental studies were focusing on the histological effect of PRP usage on the articular cartilage in induced osteoarthritis. So, this study aimed to evaluate the potential therapeutic effect of PRP on the articular cartilage using a rat model of FA-induced osteoarthritis in the knee joint.

MATERIAL AND METHODS

Animals:

Forty two adult male albino rats, weighing 180-200 gm, obtained from the animal house of the Medical Research Centre were used. The rats were fed the standard rat chow, supplied water ad libitum, and kept under the same circumstances throughout the experiment.

Experimental design:

The animals were divided randomly into three groups:

Group I (control group): Eighteen rats were divided into three subgroups:

Group I-a: Six rats were left without any intervention. Three of them were sacrificed after three weeks while the other three were sacrificed after six weeks.

Group I-b: Six rats were subjected to a single intra-articular injection with 0.3 ml saline in the right knee and sacrificed after three weeks.

Group I-c: Six rats were subjected to a single intra-articular injection with 0.3 ml saline in the right knee and sacrificed after six weeks.

Group II (arthritic group): Twelve rats were subjected to intra-articular injection of a single dose of 0.02 ml of 5% FA[18] in the right knee and further subdivided into two subgroups:

Group II-a (arthritis for three weeks): Six rats were sacrificed after three weeks.

Group II-b (arthritis for six weeks): Six rats were sacrificed after six weeks.

Group III (arthritic group treated with PRP): Twelve rats were subjected to intra-articular injection of a single dose of 0.02 ml of 5% FA in the right knee. Then, one week later, they were subjected to intra-articular injection with single dose of 0.3 ml of PRP[9] in the same right knee and were further subdivided into two subgroups:

Group III-a: Six rats were sacrificed three weeks after FA injection.

Group III-b: Six rats were sacrificed six weeks after FA injection[9].

All intra-articular injections were performed after light anesthesia with ether inhalation. The hair over the knees was shaved and the knees were sterilized with Betadine antiseptic solution. Each intra-articular injection was performed through the infrapatellar ligament using insulin syringe while the knee joint was maintained in the full flexion position[19].

Preparation of Platelet rich plasma (PRP):

The rats of group III (arthritic group treated with PRP) were anesthetized with intraperitoneal injection of 60 mg pentobarbital/Kg body weight, then whole blood were extracted from the retro-orbital plexus of vessels with a capillary tube[20]. Blood was immediately deposited in sodium citrate test tube and placed in a centrifuge to obtain the PRP[21].

Histological study:

At the end of the experiment, rats were anesthetized by ether inhalation then sacrificed by decapitation. The knee joints were extracted, fixed and decalcified in the chelating agent disodium EDTA solution containing 10% formalin. Decalcification lasted for about 5 weeks, during which the solution was renewed every 2 days until the tissues became softened. The decalcified knees were cleaved longitudinally in a coronal plane along the central portion and specimens were processed for paraffin blocks. Serial sections of 5 µm thickness were obtained and stained with H&E, toluidine blue, and Masson's trichrome (MTC) then examined by light microscope[22].

Immunohistochemistry:

In some paraffin sections, immunohistochemical staining for Caspase-3 was performed for detection of any apoptotic chondrocytes. The reaction appeared as brownish cytoplasmic granules with some amount of nuclear staining[23].

Image analysis and statistics:

Six different non-overlapping fields from six different stained sections of six different rats were examined in each group for measuring the thickness of the non-calcified cartilage, and counting the chondrocytes, and inflammatory cells in the synovial membrane. Also scoring of articular cartilage damage in OA groups was done according to Mankin's grading system using 0-14 points scale (Table 1)[24]. All measurements were taken using the image analyzer Leica (Q 500 MC program, Wetzlar, Germany). The mean values and standard error of mean (SEM) were calculated by SPSS statistical program version 17 (IBM Corporation, New York, USA). Analysis of variance (one way ANOVA) followed by post hoc test was performed to compare between the studied groups. With regard to probability, a *P value* less than 0.05 was considered significant and those < 0.001 were considered highly significant. Image analysis was performed by an examiner who does not know the coding of the study groups to avoid bias.

Table 1: Mankin's grading system for scoring of articular cartilage damage in OA (Mankin *et al*., 1971):

Category	Grade
1) Structure	
• Normal	0
• Surface irregularity	1
• Pannus and surface irregularity	2
• Clefts to transitional zone	3
• Clefts to radial zone	4
• Clefts to calcified zone	5
• Complete disorganization	6
2) Cells	
• Normal	0
• Diffuse hypercellularity	1
• Cloning	2
• Hypocellularity	3
3) Toluidine blue staining	
• Normal	0

• Slight reduction	1
• Moderate reduction	2
• Severe reduction	3
• No dye noted	4
4) Tide mark integrity	
• Intact	0
• Crossed by blood vessels	1

RESULTS

1. Histological and immunohistochemical results:

Group I (control group):

Examination of different stained sections of the knee joint of the positive control subgroups (group I-b and group I-c) revealed similar findings as compared to the control subgroup (group I-a). H&E stained sections of the knee joint showed the articulated lower end of femur and upper end of tibia with both menisci in between. The articular cartilage appeared as a typical hyaline cartilage with regular surface, lacking the perichondrium and the joint space appeared clear. The cartilage thickness in the lower end of femur appeared less than that in the upper end of tibia (Fig. 1).

The articular cartilage consisted of an area of non-calcified cartilage and another one of calcified cartilage. The tidemark line was seen as a well-defined boundary separating the non-calcified from the calcified cartilage. The subchondral bone with bone trabeculae separating the bone marrow spaces was seen deep to the calcified cartilage (Fig. 2).

The non-calcified cartilage consisted of three ill demarcated zones: superficial tangential, middle transitional, and deep radial zones. The superficial tangential zone comprised flattened chondrocytes parallel to the surface of the cartilage. The middle transitional zone consisted of more rounded and larger chondrocytes in a scattered manner. The deep radial zone consisted of rows of chondrocytes perpendicular to the surface. The calcified cartilage had scattered chondrocytes inside their lacunae (Fig. 3).

The synovial membrane was formed of bundles of thin and widely spaced collagen fibers. The cells of the synovial membrane (synoviocytes) appeared as spindle shaped cells with flattened nuclei. They were arranged into two to three layers at the outer layer of the synovial membrane, and were sporadically dispersed at the inner layer. Moreover, small sized blood vessels were also observed in the inner layer of the synovial membrane (Fig. 4).

The synovial membrane appeared as fine, loosely arranged bundles of collagen fibers which were stained green in MTC stained sections (Fig. 5).

The matrix of the articular cartilage appeared to have homogenous affinity to toluidine blue staining in both the non-calcified and calcified cartilages (Fig. 6).

Immunohistochemical staining for Caspase-3 showed minimal positive immunoreaction in few chondrocytes which appeared as brownish cytoplasmic granules with some amount of nuclear staining (Fig. 7).

Group II (arthritic group):

Group II-a (arthritic for three weeks):

Hematoxylin and eosin stained sections of the knee joint showed areas of disorganized articular cartilage with irregular surface, hypocellularity, dispersed rough collagen fibers in the matrix and loss of the tidemark. The synovial membrane showed encroachment over the affected area with pannus formation (Figs. 8 & 9). Moreover, the calcified cartilage was invaded by aggregations of different shaped cells. Some of these invading cells were multinucleated and surrounded by absorption bay, resembling osteoclasts, and the other cells were cuboidal in shape with eccentric nuclei and cytoplasmic processes, resembling osteoblasts (Fig. 9).

Examination of the synovial membrane of this group showed apparent increase in the number of the synoviocytes with the presence of dark rounded inflammatory cells. Macrophages with large nuclei and prominent nucleoli were also detected (Fig. 10).

Masson's trichrome stained sections showed the synovial membrane with deposition of dense thick collagen bundles in some areas with numerous dilated and congested blood vessels. Heavy infiltration with inflammatory cells was also noticed (Fig. 11).

Toluidine blue stained sections showed apparent decrease in the affinity to the stain in all layers of the non-calcified cartilage (Fig. 12).

Immunohistochemical staining showed positive caspase-3 immunoreaction in most of the chondrocytes (Fig. 13).

Group II-b (arthritic for six weeks):

Examination of the H&E stained sections of this group showed that the thickness of the non-calcified cartilage apparently decreased as compared to the control group. The calcified cartilage was infiltrated by spindle shaped fibroblasts together with osteoclasts (Fig. 14).

Some sections showed areas of loss of the articular cartilage especially at the periphery with exposure (eburnation) of the subchondral bone with the presence of osteoblasts and osteocytes. The synovial membrane was seen encroaching over the affected areas (Fig. 15).

Examination of the synovial membrane revealed congested blood vessels with minimal mononuclear inflammatory cell infiltrate through dense bundles of collagen fibers (Fig. 16).

Masson's trichrome stained sections showed condensation of dense bundles of collagen fibers in most areas of the synovial membrane together with presence of numerous blood vessels (Fig. 17).

Toluidine blue stained sections showed apparent decrease in the affinity to the stain in the majority of the non-calcified cartilage as compared to the control group with the appearance of minimal areas with higher affinity to the stain (Fig. 18).

Immunohistochemical staining showed positive Caspase-3 immunoreaction in few chondrocytes which was apparently lesser than that in the previous group (group II-a), but more than that in the control group (Fig. 19).

Group III (arthritic group treated with PRP):

Group III-a (three weeks arthritic group treated with PRP):

Hematoxylin and eosin stained sections showed that the articular cartilage of this group had a regular surface similar to that of the control group. Moreover, the extracellular matrix showed homogeneity with regular arrangement of the collagen fibers. The thickness of the non-calcified cartilage was nearly the same as the control group and the tidemark line was intact and single in most of the sections (Fig. 20).

However, in some areas the chondrocytes showed diffuse hypercellularity and irregular arrangement, while others appeared having shrunken and darkly stained nuclei (Fig. 21).

Examination of the synovial membrane revealed thin bundles of collagen fibers with minimal inflammatory cell infiltrate. Synoviocytes were seen as spindle shaped cells with flattened nuclei (Fig. 22).

Masson's trichrome stained sections showed the synovial membrane composed of thin bundles of collagen fibers together with small sized blood vessels (Fig. 23).

Examination of the toluidine blue stained sections showed homogenous affinity to the stain in both calcified and non-calcified cartilage (Fig. 24).

The Caspase-3 stained sections showed minimal positive immunoreaction in the chondrocytes which was nearly the same as the control groups (Fig. 25).

Group III-b (six weeks arthritic group treated with PRP):

Hematoxylin and eosin stained sections showed the articular cartilage with a continuous regular surface and intact tide mark; however, there were minimal surface irregularities or fibrillation in some areas. The thickness of the articular cartilage was apparently variant; thick in some areas and thin in others. The bone trabeculae of the subchondral bone appeared normally separated (Fig. 26).

Aggregations of osteoclasts, osteoblasts and fibroblasts with flattened nuclei were seen invading the calcified cartilage (Fig. 27).

Examination of the synovial membrane showed that it was formed of dense bundles of collagen fibers with apparent increase in the synoviocytes as compared to group III-a (three weeks arthritic group treated with PRP). Few

inflammatory cells were also seen (Figs. 28 & 29).

Toluidine blue stained sections showed apparent decrease in the affinity to the stain in the non-calcified cartilage (Fig. 30).

Immunohistochemical staining showed apparent increase in the positive Caspase-3 immunoreaction in the chondrocytes as compared to group III-a (three weeks arthritic group treated by PRP) (Fig. 31).

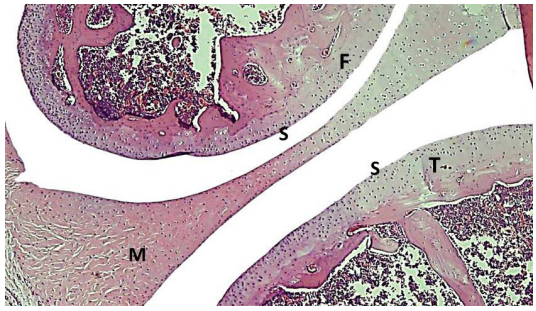


Fig. 1: A photomicrograph of a coronal section in the knee joint of a rat (control group, I-a) showing the articulating lower end of femur (F) and upper end of tibia (T). Notice the regular surface of the articular cartilage (S), the meniscus (M), and the clear joint space. (H&E, x40).

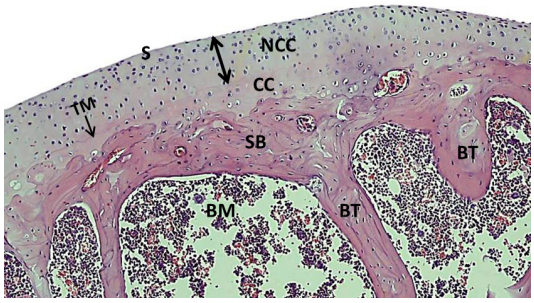


Fig. 2: A photomicrograph of a coronal section in the articular cartilage of lower end of femur of a rat (control group, I-a) showing the articular cartilage with regular surface (S). The non-calcified cartilage (NCC) and calcified cartilage (CC) are separated by the tidemark line (TM). The subchondral bone (SB) shows bone trabeculae (BT) between the bone marrow (BM) spaces. Notice the thickness (double headed arrow) of the non-calcified cartilage. (H&E, x100).

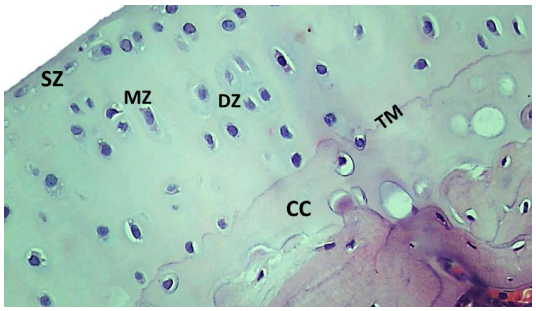


Fig. 3: A photomicrograph of a coronal section in the articular cartilage of upper end of tibia of a rat (control group, I-a) showing different zones of the articular cartilage; superficial tangential zone (SZ), middle transitional zone (MZ), and deep radial zone (DZ). Notice the tidemark line (TM) and the calcified cartilage (CC). (H&E, x400).

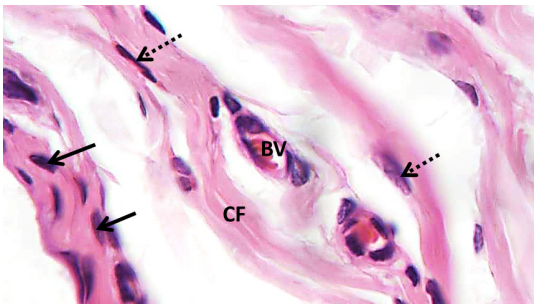


Fig. 4: A photomicrograph of a coronal section in the knee joint of a rat (control group, I-a) showing the synovial membrane with fine bundles of collagen fibers (CF) trapping two to three layers of spindle shaped synoviocytes with flattened nuclei (arrows) in its outer layers together with sporadic ones (dotted arrows) within its inner layers. Notice the presence of small blood vessels (BV). (H&E, x1000).

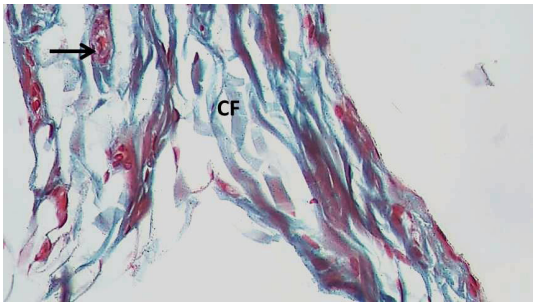


Fig. 5: A photomicrograph of a coronal section in the rat's knee joint of (control group, I-a), showing the arrangement of the collagen fibers (CF) of the synovial membrane. Notice the small blood vessels (arrow). (MTC, x400).

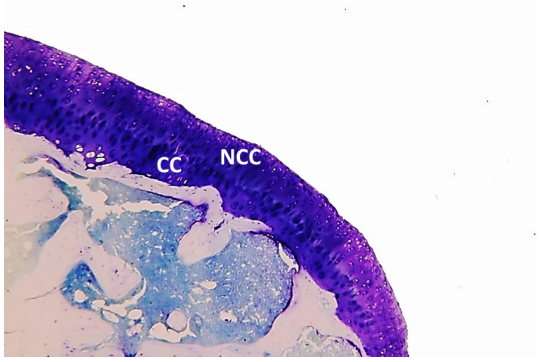


Fig. 6: A photomicrograph of a coronal section in the articular cartilage of lower end of femur of a rat (control group, I-a) showing the homogenous affinity to toluidine blue stain in both non-calcified cartilage (NCC) and calcified cartilage (CC). (Toluidine blue, X100).

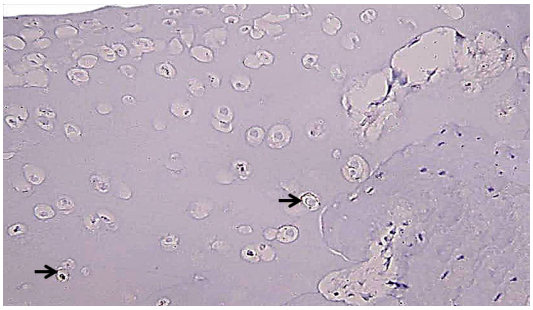


Fig. 7: A photomicrograph of a coronal section in the articular cartilage of upper end of tibia of a rat (control group, I-a) showing minimal positive Caspase-3 immunoreaction (arrows) in the chondrocytes. (Caspase-3, x400).

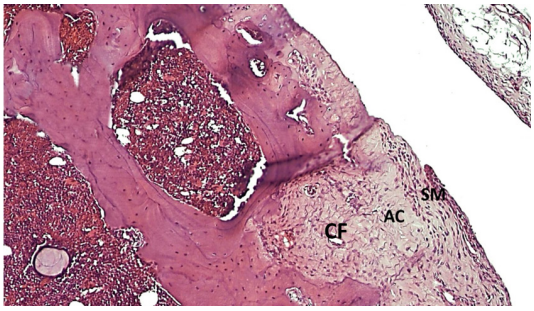


Fig. 8: A photomicrograph of a coronal section in the articular cartilage of lower end of femur of a rat (arthritic group for three weeks, II-a) showing the appearance of disorganized area of articular cartilage (AC) with dispersed rough collagen fibers (CF) in the matrix. Notice the encroachment of the synovial membrane (SM) over the affected area in the form of a pannus. (H&E, x100).

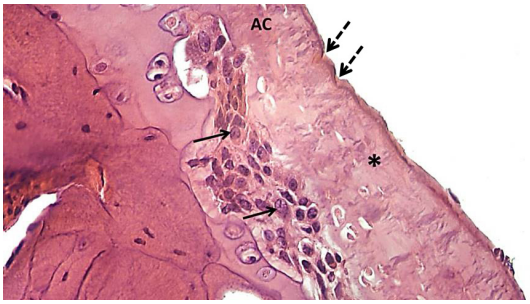


Fig. 9: A photomicrograph of a coronal section in the articular cartilage of upper end of tibia of a rat (arthritic group for three weeks, II-a) showing the surface irregularities (dotted arrows) of the articular cartilage (AC) with apparent hypocellularity (*) and loss of the tidemark line. Notice the aggregation of osteoblasts (black arrows). (H&E, x400).

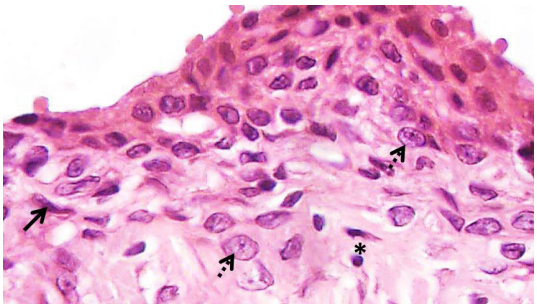


Fig. 10: A photomicrograph of a coronal section in the articular cartilage of upper end of tibia of a rat (arthritic group for three weeks, II-a) showing spindle shaped synoviocytes (arrow) together with macrophages having large nuclei and prominent nucleoli (dotted arrows). Notice the dark rounded inflammatory cells (*). (H&E, x1000).

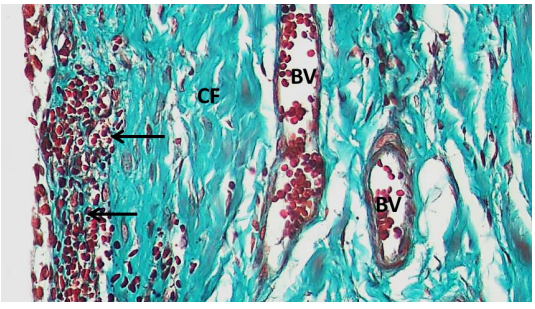


Fig. 11: A photomicrograph of a coronal section in the knee joint of a rat (arthritic group for three weeks, II-a) showing the synovial membrane having thick bundles of collagen fibers (CF) with numerous blood vessels (BV). Note the heavy infiltration with inflammatory cells (arrows). (MTC, x400).

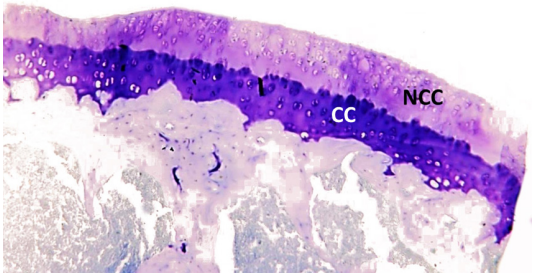


Fig. 12: A photomicrograph of a coronal section in the articular cartilage of upper end of tibia of a rat (arthritic group for three weeks, II-a) showing an apparent decrease in the affinity to the stain in all layers of the non-calcified cartilage (NCC). CC= calcified cartilage. (Toluidine blue, x100).

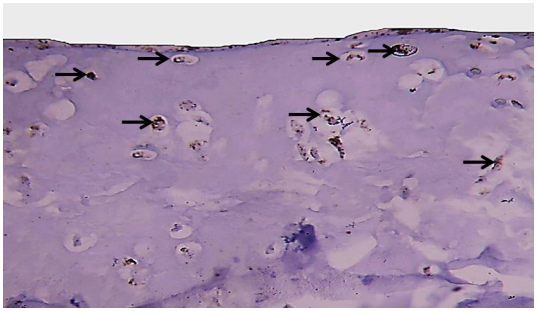


Fig. 13: A photomicrograph of a coronal section in the articular cartilage of lower end of femur of a rat (arthritic group for three weeks, II-a) showing positive Caspase-3 immunoreaction (arrows) in most of the chondrocytes. (Caspase-3, x400).

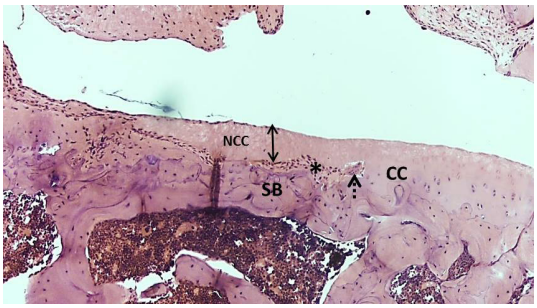


Fig. 14: A photomicrograph of a coronal section in the articular cartilage of upper end of tibia of a rat (arthritic group for six weeks, II-b) showing apparent decrease in the thickness (double headed arrow) of the non-calcified cartilage (NCC). Notice the invasion of the calcified cartilage (CC) by aggregation of spindle shaped fibroblasts (*) and osteoclast (dotted arrow). SB = subchondral bone. (H&E, x100).

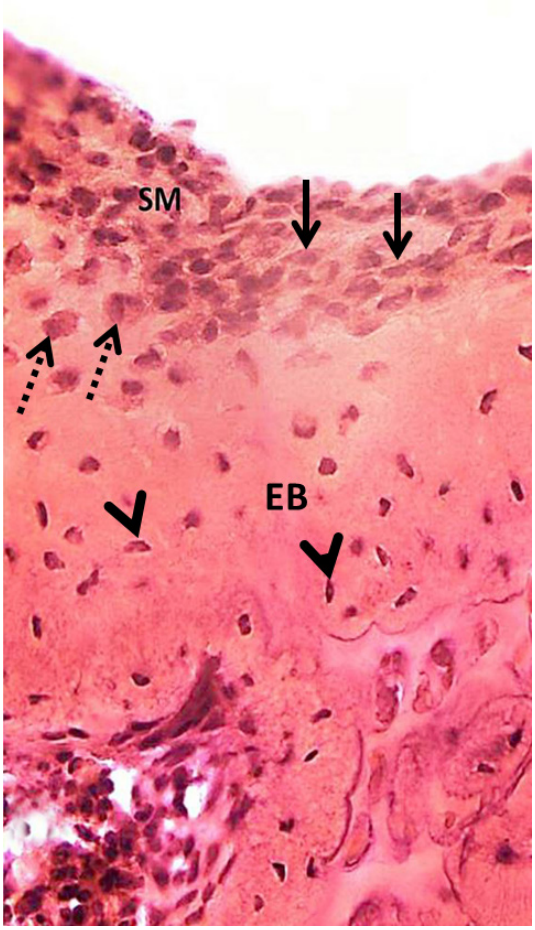


Fig. 15: A photomicrograph of a coronal section in the knee joint of a rat (arthritic group for six weeks, II-b) showing the encroaching synovial membrane (SM) with its spindle shaped synoviocytes (arrows). Notice the eburnated bone (EB) with osteoblasts (dotted arrows) and osteocytes (arrow heads). (H&E, x400).

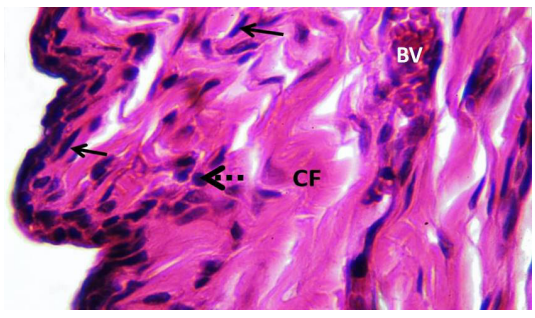


Fig. 16: A photomicrograph of a coronal section in the knee joint of a rat (arthritic group for six weeks, II-b) showing spindle shaped synoviocytes (arrows) and minimal mononuclear inflammatory cells (dotted arrow). Notice the congested blood vessel (BV). CF = bundles of collagen fibers. (H&E, x1000).

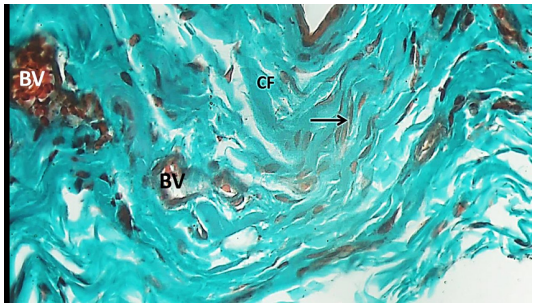


Fig. 17: A photomicrograph of a coronal section in the knee joint of a rat (arthritic group for six weeks, II-b) showing the synovial membrane composed of dense bundles of collagen fibers (CF) with numerous blood vessels (BV). Notice the spindle shaped synoviocytes (arrow). (MTC, x400).

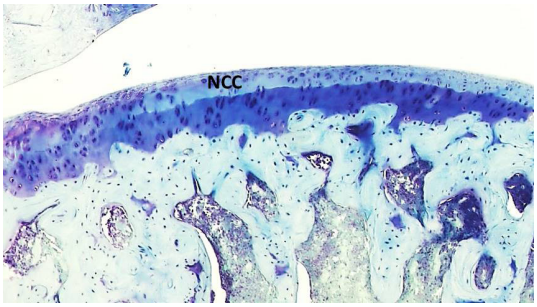


Fig. 18: A photomicrograph of a coronal section in the articular cartilage of lower end of femur of a rat (arthritic group for six weeks, II-b) showing an apparent decrease in the affinity to the stain in the majority of the non-calcified cartilage (NCC). (Toluidine blue, x100).

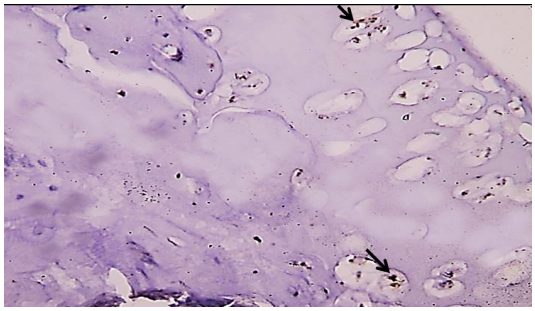


Fig. 19: A photomicrograph of a coronal section in the articular cartilage of lower end of femur of a rat (arthritic group for six weeks, II-b) showing positive Caspase-3 immunoreaction (arrows) in few chondrocytes. (Caspase-3, x400).

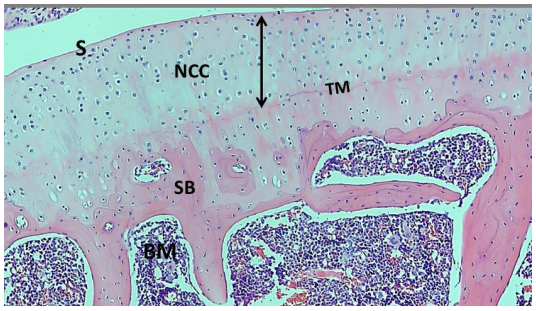


Fig. 20: A photomicrograph of a coronal section in the articular cartilage of upper end of tibia of a rat (three weeks arthritic group treated with PRP, III-a) showing the articular cartilage with regular surface (S) together with regular arrangement of the chondrocytes. Notice the thickness of the non-calcified cartilage (NCC) (double headed arrow) and the intact tidemark line (TM). SB = subchondral bone, and BM = bone marrow spaces. (H&E, x100).

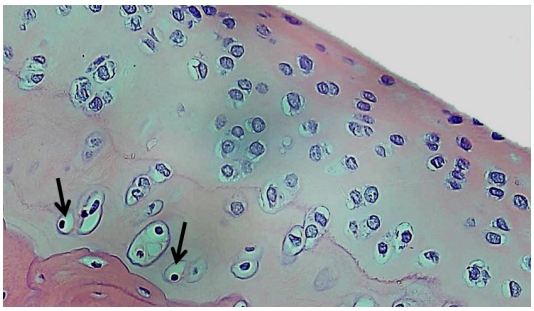


Fig. 21: A photomicrograph of a coronal section in the articular cartilage of upper end of tibia of a rat (three weeks arthritic group treated with PRP, III-a) showing apparent increase in the number of chondrocytes which are irregularly arranged. Notice the presence of some chondrocytes with shrunken and deeply stained nuclei (arrows). (H&E, x400).

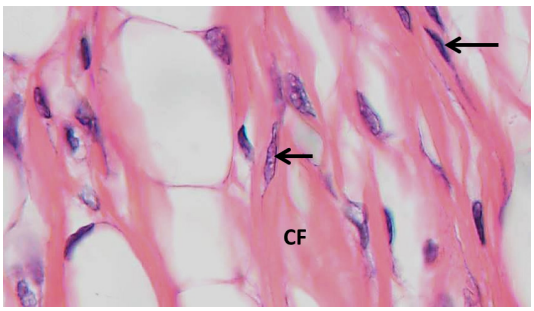


Fig. 22: A photomicrograph of a coronal section in the knee joint of a rat (three weeks arthritic group treated with PRP, III-a) showing the synovial membrane with its spindle shaped synoviocytes (arrows) lying within fine bundles of collagen fibers (CF). (H&E, x1000).

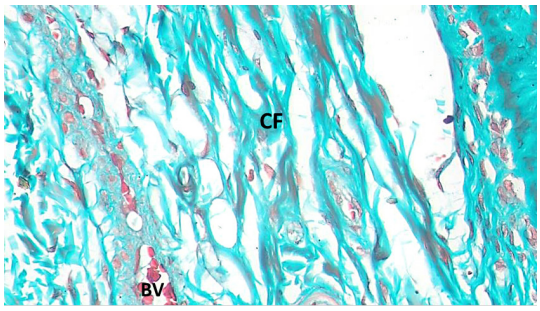


Fig. 23: A photomicrograph of a coronal section in the knee joint of a rat (three weeks arthritic group treated with PRP, III-a) showing the synovial membrane with thin bundles of collagen fibers (CF). BV = blood vessel. (MTC, x400).

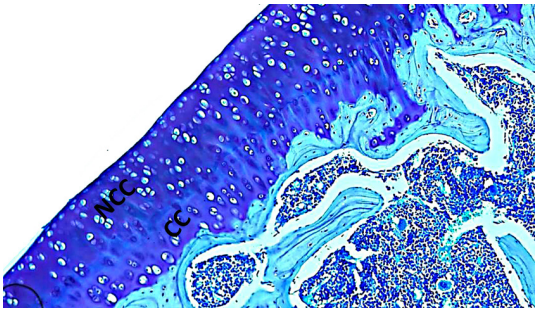


Fig. 24: A photomicrograph of a coronal section in the articular cartilage of upper end of tibia of a rat (three weeks arthritic group treated with PRP, III-a) showing the articular cartilage having almost the same affinity to toluidine blue stain in both non-calcified cartilage (NCC) and calcified cartilage (CC). (Toluidine blue, x100).

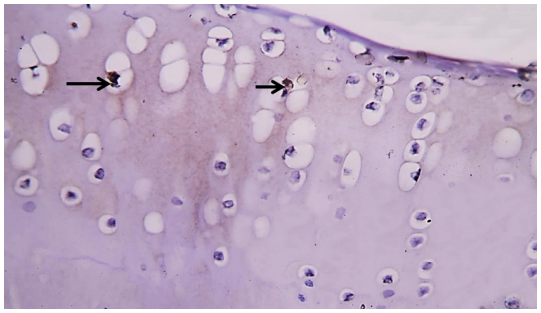


Fig. 25: A photomicrograph of a coronal section in the articular cartilage of upper end of tibia of a rat (three weeks arthritic group treated with PRP, III-a) showing minimal positive caspase-3 immunoreaction (arrows) in the chondrocytes. (Caspase-3, x400).

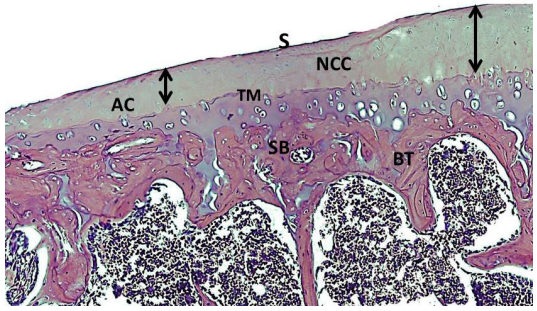


Fig. 26: A photomicrograph of a coronal section in the articular cartilage of lower end of femur of a rat (six weeks arthritic group treated with PRP, III-b) showing continuous regular surface (S) of the articular cartilage (AC) with intact tidemark line (TM) and normally separated bone trabeculae (BT) of the subchondral bone (SB). The thickness of the non-calcified cartilage (NCC) appears variable (double headed arrows). (H&E, x100).

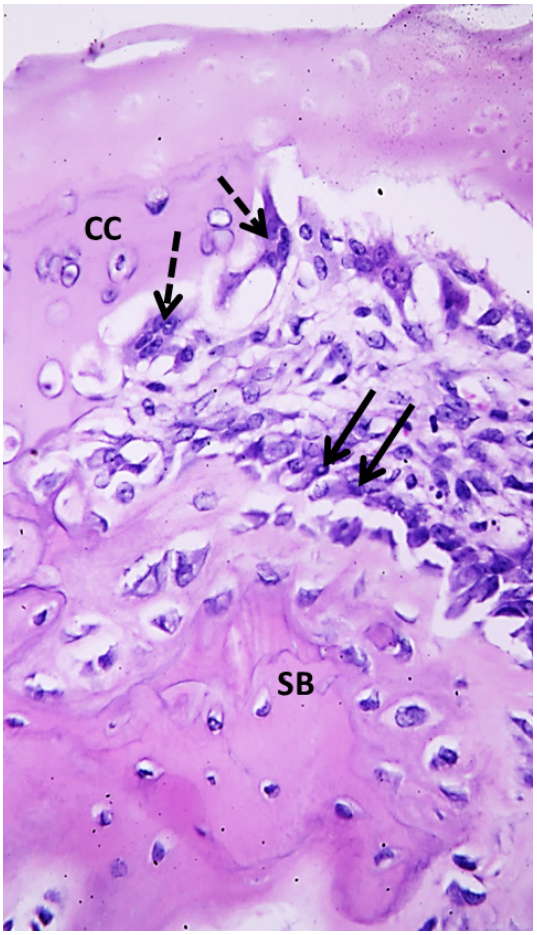


Fig. 27: A photomicrograph of a coronal section in the articular cartilage of lower end of femur of a rat (six weeks arthritic group treated with PRP, III-b) showing invasion of the calcified cartilage (CC) by cuboidal shaped osteoblasts (arrows). Notice the presence of multinucleated osteoclasts (dotted arrows) surrounded with an area of bone resorption. SB = subchondral bone. (H&E, x400).

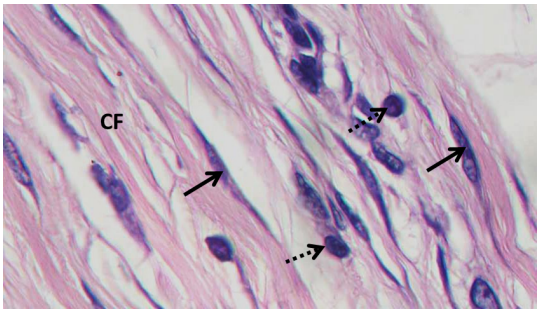


Fig. 28: A photomicrograph of a coronal section in the knee joint of a rat (six weeks arthritic group treated with PRP, III-b) showing the synovial membrane with its spindle shaped synoviocytes (arrows) lying within bundles of collagen fibers (CF). Notice the inflammatory cellular infiltration (dotted arrows). (H&E, x1000).

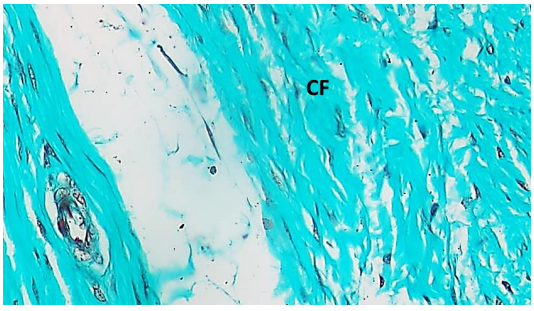


Fig. 29: A photomicrograph of a coronal section in the knee joint of a rat (six weeks arthritic group treated with PRP, III-b) showing the synovial membrane with dense bundles of collagen fibers (CF). (MTC, x400).

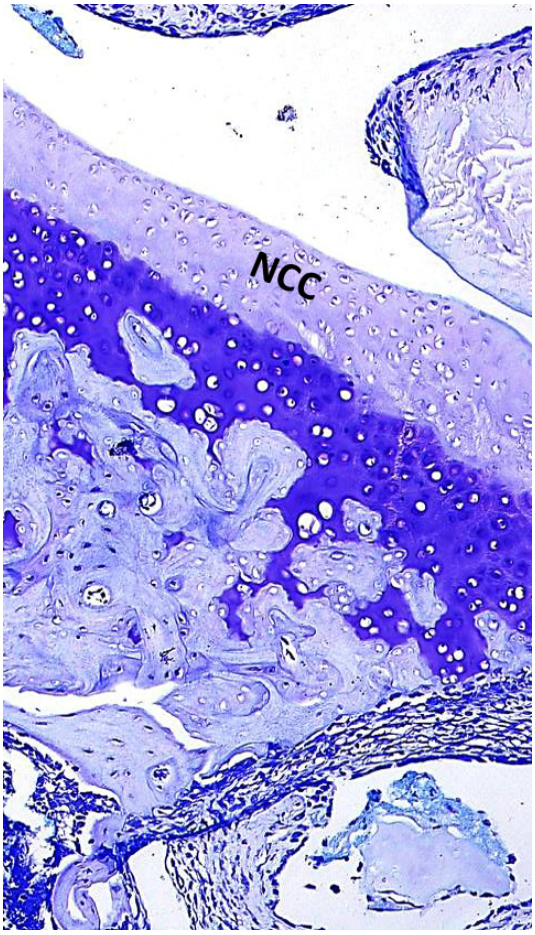


Fig. 30: A photomicrograph of a coronal section in the articular cartilage of upper end of tibia of a rat (six weeks arthritic group treated with PRP, III-b) showing an apparent decrease in the affinity to the stain in the non-calcified cartilage (NCC). (Toluidine blue, x100).

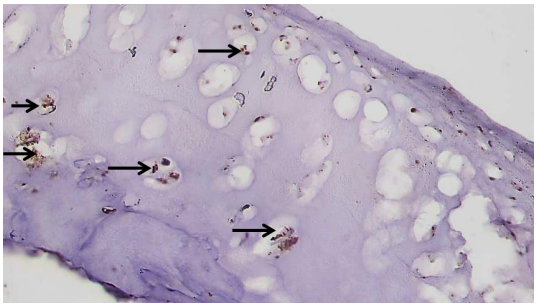


Fig. 31: A photomicrograph of a coronal section in the articular cartilage of upper end of tibia of a rat (six weeks arthritic group treated with PRP, III-b) showing positive Caspase-3 immunoreaction (arrows) in many chondrocytes. (Caspase-3, x400).

2. Morphometric results and statistics:

A morphometric study was conducted and statistically analyzed. No significant differences were noted in the control groups (I-b, and I-c) as compared with the control group (I-a).

Thickness of the non-calcified cartilage: (Tables 2 & 3) and (Histograms 1 & 2)

The mean thickness of the non-calcified cartilage in both arthritic groups (II-a, and II-b) revealed a significant decrease for the lower end of femur and highly significant decrease for the upper end of tibia as compared with the control groups.

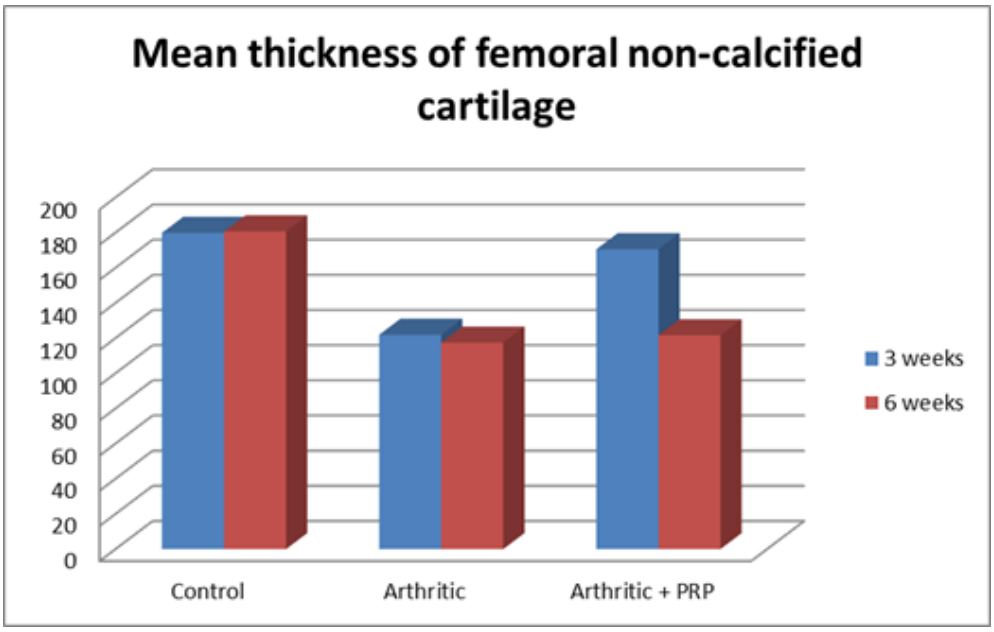
In the three weeks arthritic group treated with PRP (III-a), the mean thickness of the non-calcified cartilage showed a significant increase in femur and a highly significant increase in tibia as compared with arthritic group (II-a), and non-significant decrease as compared with control group (I-b).

In the six weeks arthritic group treated with PRP (III-b), the mean thickness of the femoral and tibial non-calcified cartilage showed non-significant increase as compared with arthritic group (II-b), and highly significant decrease as compared with control group (I-c)

Table 2: Comparison of the mean thickness of the femoral and tibial non-calcified cartilage (µm) ± SEM between the three weeks experimental groups:

	Control (I-b)	Arthritic (II-a)	Arthritic + PRP (III-a)
Femur	179.88 ± 7.08	121.93 ± 5.83	170.55 ± 7.14
		(P=0.00197)[a]	*(P= 0.0072)[b]*
Tibia	203.698 ± 8.69	113.11 ± 6.23	190.91 ± 7.21
		(P=0.00001)[c]	*(P=0.0000099)[d]*

a) Significant decrease as compared with control group.
b) Significant increase as compared with arthritic group.
c) Highly significant decrease as compared with control group.
d) Highly significant increase as compared with arthritic group.

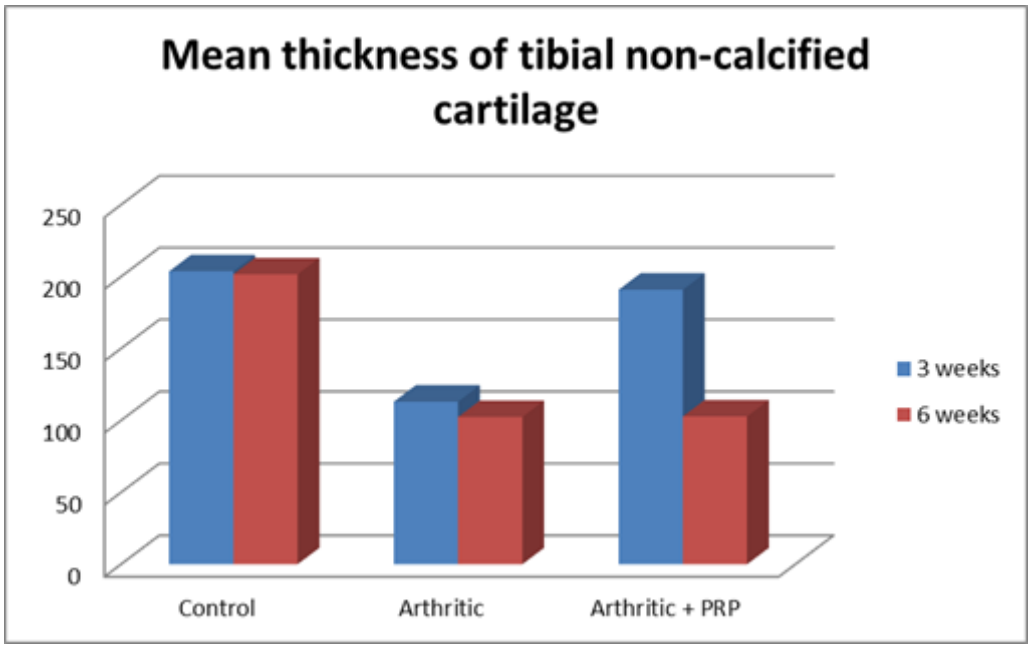


Histogram 1: Mean thickness of femoral non-calcified cartilage.

Table 3: Comparison of the mean thickness of the femoral and tibial non-calcified cartilage (µm) ± SEM between the six weeks experimental groups:

	Control (I-c)	Arthritic (II-b)	Arthritic + PRP (III-b)
Femur	180.65 ± 6.52	117.41 ± 4.61	121.64 ± 6.65
		(P=0.0013)[a]	*(P= 0.000143)*[b]
Tibia	201.65 ± 8.71	102.42 ± 3.35	102.83 ± 2.54
		(P=0.0000012)[b]	*(P< 0.000001)*[b]

a) Significant decrease as compared with control group
b) Highly significant decrease as compared with control group



Histogram 2: Mean thickness of tibial non-calcified cartilage.

Chondrocytes count: (Tables 4 & 5) and (Histograms 3 & 4)

The mean number of femoral and tibial chondrocytes in both arthritic groups (II-a and II-b) showed highly significant decrease as compared with the control groups (I-b and I-c).

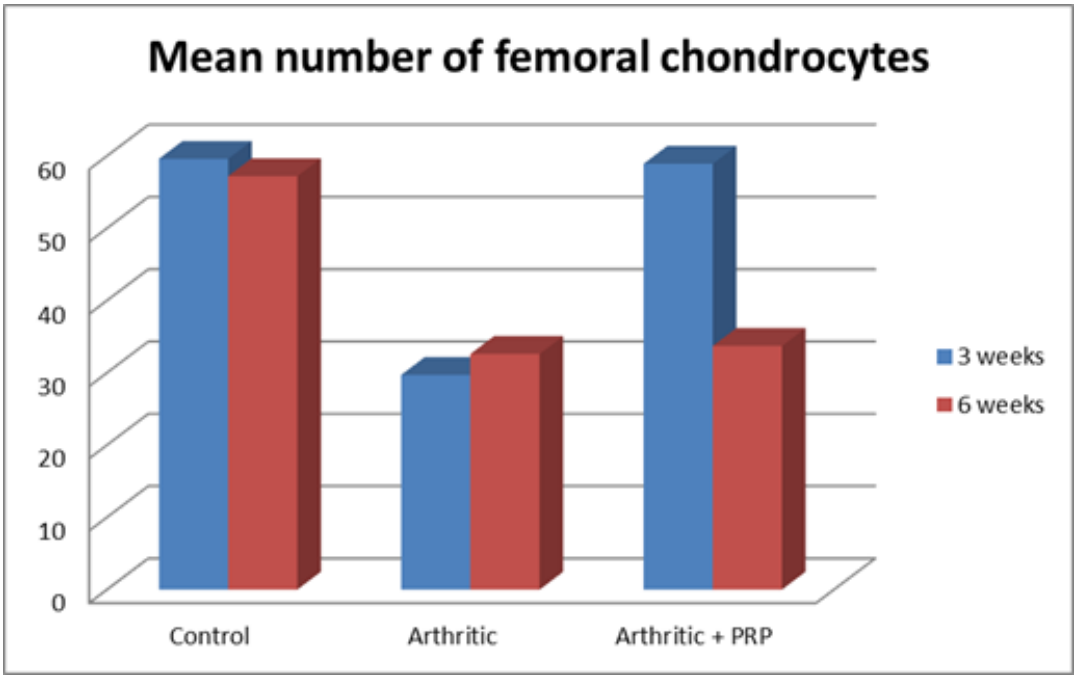
In the three weeks arthritic group treated with PRP (III-a), the mean number of the femoral and tibial chondrocytes showed highly significant increase as compared with arthritic group (II-a). Their number showed a non-significant decrease in femur and an increase in tibia as compared with control group (I-b).

In the six weeks arthritic group treated with PRP (III-b), the mean number of the femoral and tibial chondrocytes showed non-significant increase as compared with arthritic group (II-b), but highly significant decrease as compared with control group (I-c).

Table 4: Comparison of the mean number of the femoral and tibial chondrocytes ± SEM between the three weeks experimental groups:

	Control (I-b)	Arthritic (II-a)	Arthritic + PRP (III-a)
Femur	59.6 ± 4.09	29.7 ± 4.7	58.9 ± 3.84
		(P= 0.00043)[a]	*(P= 0.00016)[b]*
Tibia	68.1 ± 5.33	31.2 ± 3.81	68.9 ± 3.55
		(P= 0.000017)[a]	*(P= 0.00000096)[b]*

a) Highly significant decrease as compared with control group
b) Highly significant increase as compared with arthritic group

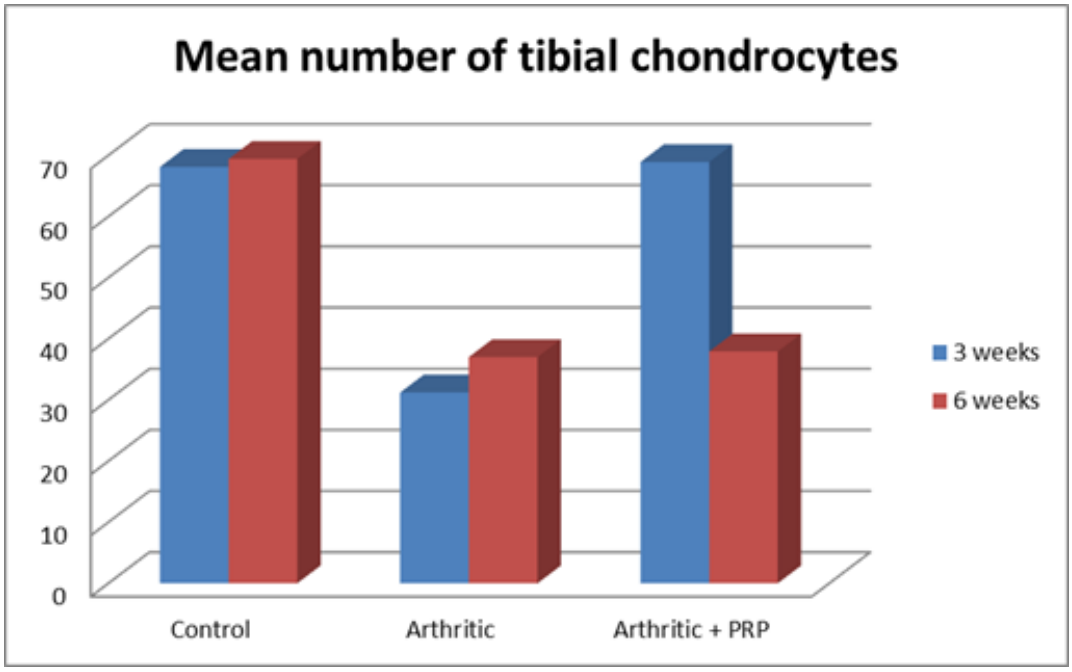


Histogram 3: Mean number of femoral chondrocytes.

Table 5: Comparison of the mean number of the femoral and tibial chondrocytes ± SEM between the six weeks experimental groups:

	Control (I-c)	Arthritic (II-b)	Arthritic + PRP (III-b)
Femur	57.2 ± 5.01	32.6 ± 3.57	33.7 ± 4.09
		(P=0.0004)[a]	*(P= 0.00093)[a]*
Tibia	69.4 ± 5.21	37 ± 4	37.9 ± 4.99
		(P=0.00013)[a]	*(P= 0.00043)[a]*

a) Highly significant decrease as compared with control group



Histogram 4: Mean number of tibial chondrocytes.

Inflammatory cells count in the synovial membrane: (Tables 6 & 7) and (Histogram 5)

In the arthritic groups (II-a, and II-b), counting the number of the inflammatory cells in the synovial membrane showed highly significant increase in group (II-a), and significant increase in group (II-b) as compared with control groups.

In the three weeks arthritic group treated with PRP (III-a), the inflammatory cells count showed highly significant decrease as compared with arthritic group (II-a), and non-significant increase as compared with control group (I-b).

On the other hand, in the six weeks arthritic group treated with PRP (III-b), the inflammatory cells count showed non-significant decrease as compared with arthritic group (II-b), and significant increase as compared with control group (I-c).

Table 6: Comparison of the mean number of the inflammatory cells in the synovial membrane between the three weeks experimental groups:

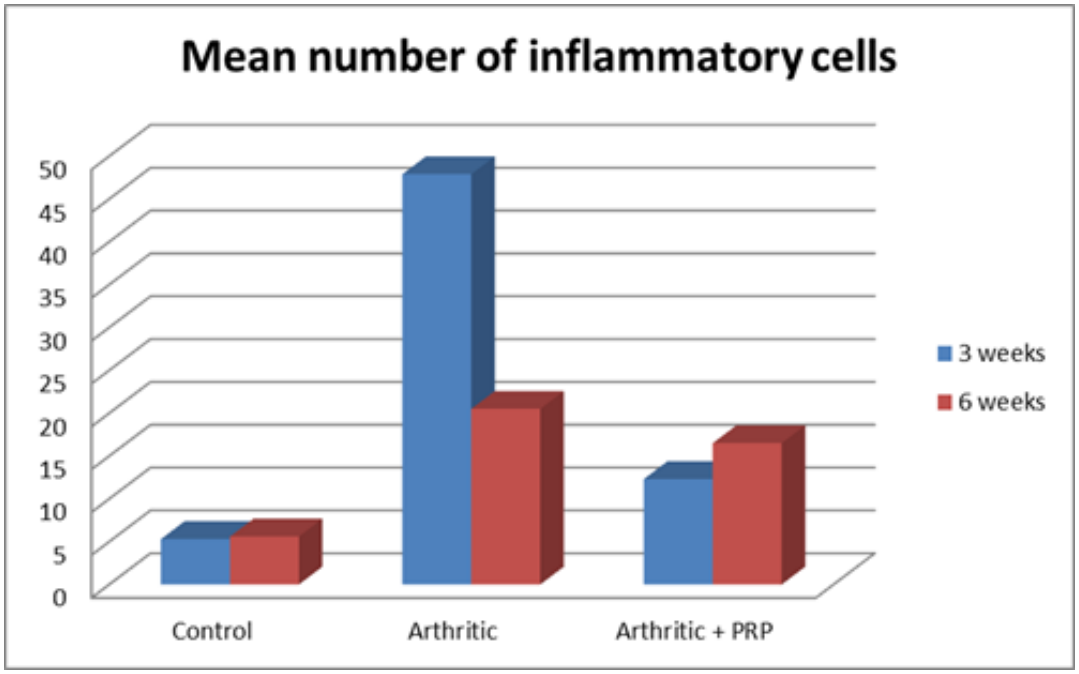
	Control (I-b)	Arthritic (II-a)	Arthritic + PRP (III-a)
Mean number of inflammatory cells ± SEM	5.3 ± 1.98	47.9 ± 4.08	12.3 ± 2.19
		(P<0.0000001)[a]	*(P<0.0000001)*[b]

a) Highly significant increase as compared with control group
b) Highly significant decrease as compared to arthritic group

Table 7: Comparison of the mean number of the inflammatory cells in the synovial membrane between the six weeks experimental groups:

	Control (I-c)	Arthritic (II-b)	Arthritic + PRP (III-b)
Mean number of inflammatory cells ± SEM	5.6 ± 1.01	20.5 ± 3.67	16.5 ± 3.83
		(P=0.00309)[a]	*(P= 0.0258)*[a]

a) Significant increase as compared with control group



Histogram 5: Mean number of inflammatory cells in the synovial membrane.

Mankin's score: (Tables 8 & 9) and (Histogram 6)

The mean Mankin's score showed highly significant increase in both arthritic groups (II-a, and II-b) as compared with control groups (I-b and I-c).

In the three weeks arthritic group treated with PRP (III-a), mean Mankin's score recorded highly significant decrease as compared with arthritic group (II-a), and non-significant increase as compared with control group (I-b).

Moreover, in the six weeks arthritic group treated with PRP (III-b), the mean Mankin's score showed non-significant decrease as compared with arthritic group (II-b), and highly significant increase as compared with control group (I-c)

Table 8: The mean Mankin's score of the articular cartilage damage between the three weeks experimental groups:

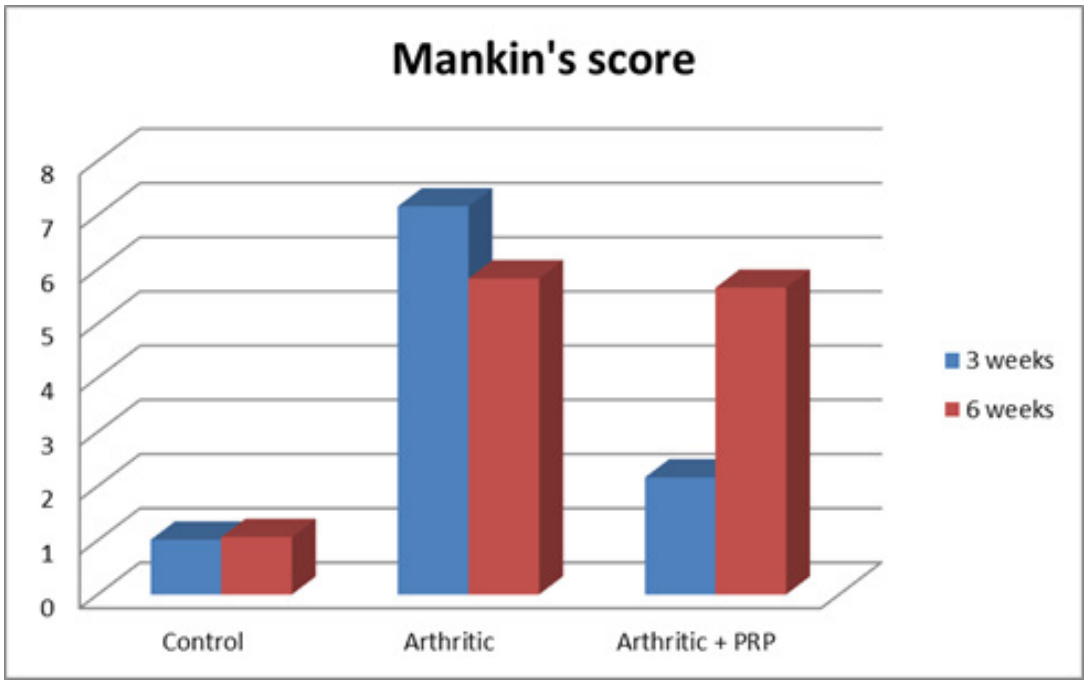
	Control (I-b)	Arthritic (II-a)	Arthritic + PRP (III-a)
Mean Mankin's score of the articular cartilage damage	1.01 ± 0.34	7.16 ± 0.945	2.16 ± 0.472
		(P=0.0004)[a]	*(P=0.00081)[b]*

a) Highly significant increase as compared with control group
b) Highly significant decrease as compared with arthritic group

Table 9: The mean Mankin's score of the articular cartilage damage between the six weeks experimental groups:

	Control (I-c)	Arthritic (II-b)	Arthritic + PRP (III-b)
Mean Mankin's score of the articular cartilage damage	1.06 ± 0.215	5.83 ± 0.654	5.66 ± 0.557
		(P= 0.0000727)[a]	*(P =0.000034)[a]*

a) Highly significant increase as compared with control group



Histogram 6: Mean Mankin's score.

DISCUSSION

Osteoarthritis is a progressive disease with no complete cure till now. The different treatments of OA are aiming to minimize the joint damage to reduce the pain and improve the quality of life. Although numerous researches are exploring new modalities for cartilage regeneration, yet there is little success[25].

In human, OA often occurs in hand, cervical and lumbar vertebrae, knee, and hip joints. On the other hand, spontaneous OA in animal models is localized to the weight-bearing joints; typically the knee and/or hip joint[8]. So, the knee joint was chosen for experimental osteoarthritis in the present study. Moreover, rats models of OA were chosen because their reliability and structural similarity to human OA. Also, adult male rats were chosen not only because males tend to grow faster than females, thus reaching greater body weight and therefore tend to have more consistent pathological alterations, but also because estrogen has a protective effect against OA. This was confirmed by the work of Ma *et al*.[26] who revealed that ovariectomized female rats developed more severe OA than the control females, while orchiectomized male rats developed less severe OA than the control males.

In the present study, OA was induced by single intra-articular injection as the approach of Mendoza *et al*.[11] who concluded that local injection of FA was enough to induce joint arthritis that resembles the characteristics of human OA.

The present work showed the progressive effect of the FA on the different components of the knee joint as Wheeler-Aceto and Cowan[27] stated that FA has biphasic inflammatory effect on the joints and an early neurogenic component followed by a delayed tissue mediated response.

In the present study, the control groups showed the regular surface of the articular cartilage and the particular arrangement of the chondrocytes in its different zones. However, the arthritic groups showed cellular disorganization with the presence of apoptotic chondrocytes. These findings were in agreement with those of Cimen *et al*.[28] who revealed that FA injection in the knee joint of rats caused several pathological changes including surface irregularities with chondrocyte degeneration and disorganization.

The delayed tissue response to FA is mediated by prostaglandin, serotonin, histamine, and cytokines, such as interleukin-1 beta, interleukin-6, tumor necrosis factor-alpha,

eicosanoids, and nitric oxide[29]. Meanwhile, nitric oxide has a toxic effect on the chondrocytes' mitochondria resulting in dysfunction of the cell respiration and low production of ATP, hence the occurrence of apoptosis[30].

OA causes variant response in the number of chondrocytes. This response was explained by Adams[31] who stated that degeneration of cartilage in OA is characterized by two phases: a biosynthetic phase, during which the chondrocytes attempt to repair the damaged extracellular matrix by forming clusters shortly after induction of OA, and a degradation phase, in which the activity of enzymes produced by the chondrocytes digests the matrix with inhibition of matrix synthesis, and the consequent erosion of the cartilage is accelerated.

The clusters formed during the biosynthetic phase are usually evident in the superficial zones of the articular cartilage. Many studies have demonstrated that the cells isolated from the superficial layer of articular cartilage have progenitor characteristics with high colony formation capacity[32].

The degradation phase of OA was evident in the three and six weeks post arthritic groups of the present study. The chondrocytes showed hypocellularity in both groups.

In the present study, the concentration of the proteoglycan in the matrix of the articular cartilage among different groups was demonstrated by the affinity to the toluidine blue stain. Being a cationic dye, toluidine blue staining visualizes proteoglycans in a tissue because of its high affinity for the negative charges in the sulfate groups in proteoglycans. Therefore, it is widely accepted that the degree of positive staining corresponds to the amount of proteoglycans[33]. Both arthritic groups of the present work showed decreased affinity to toluidine blue stain. Similar finding was observed by Ucuncu *et al*.[34] after one month of intra-articular injection of FA. Moreover, the decrease in the affinity to stain was apparent in the upper zones of the non-calcified cartilage. This can be explained by the fact that upper zones contain lesser proteoglycan concentration than the other zones of the articular cartilage[35].

Decreased proteoglycans and collagen content in OA is caused by malfunctioning chondrocytes that produce decreased concentrations of these components. This results in weakening of the articular cartilage with fibrillations and cracks formation[36].

Additionally, the disruption in the organization of the extracellular matrix macromolecules results in increase in permeability and water content of the articular cartilage leading to rarefaction of the extracellular matrix and the presence of empty lacunae[35].

In three weeks arthritic group treated with PRP of the present work, the articular cartilage showed regular surface and nearly normal chondrocytes count. The extracellular matrix showed high affinity to the toluidine blue stain. The Mankin's score was nearly equal to that of the control group. Mifune *et al*.[10] mentioned that PRP increases mRNA expression of proteoglycan core protein in the articular cartilage and decreases chondrocyte apoptosis, hence; suppresses the progression of OA. Moreover, PRP has anti-inflammatory activity which can diminish the matrix degradation by IL-1, thus PRP interrupts the progressive loss of the extracellular matrix[37]. In addition, PRP has chondroprotective effect caused by its high content of IL-4 that inhibits the degradation of proteoglycans and stimulates the synthesis of type-II collagen and aggrecan. PRP also reduces the elevated levels of nitric oxide in osteoarthritic joints[38].

On the contrary, Guner and Buyukbebeci[18] have observed that in FA-induced arthritis, there was no difference in the articular cartilage healing process between the PRP treated group and the untreated group. Moreover, Saito *et al*.[39] noticed that the rabbits which treated by PRP, after surgical induction of OA, had surface irregularities, chondrocyte clusters with decreased cartilage thickness.

In the six weeks arthritic group treated with PRP of the present study, irregular surface of the articular cartilage and a decrease in the number of chondrocytes were noticed. Moreover, the Mankin's score was nearly equal to that of the arthritic group. This may be due to the difficulty in maintaining the effect of growth factors in PRP throughout the widespread degenerative cartilage of an osteoarthritic joint. This was in accordance with Saito *et al*.[39] who stated that maintaining therapeutic concentrations of growth factors typically requires the administration of large

amounts of these factors with frequent injections of PRP.

In the present study, both arthritic groups (II-a and II-b), and six weeks arthritic group treated with PRP (III-b) showed decrease in the thickness of the non-calcified cartilage. Goldring[40] has cited that duplication of the tidemark in OA increases the mechanical stresses in the deep zones of the cartilage matrix leading to articular cartilage thinning and deterioration.

In all groups of the current study, especially arthritic groups, there was invasion of the calcified cartilage by different types of cells like fibroblasts, osteoblasts and osteoclasts. These cells were present since the third week after induction of arthritis. They were invading through the cartilage-subchondral bone junction. This was in agreement with Guzman *et al*.[41] who noticed the presence of osteoclasts together with single to several rows of large reactive osteoblast lining the trabeculae of the subchondral bone after one week of induction of arthritis in experimental animals.

The researchers have used different explanations for the source of these invading cells. Some researchers stated that synovial membrane is the source of these cells as they migrate to cover any chondral defect[42], while Banaszkiewicz and Kader[43] mentioned that they are undifferentiated cells from the marrow and the underling bone in a trial to restore the damaged tissue with new one. However, the new tissue is different in the matrix composition and mechanical properties from the original tissue as it was shown in the three weeks post-arthritic group of the present study, in accordance with the conclusion of Buckwalter *et al*.[44].

Moreover, Fransès *et al*.[45] have explained that chondrocytes were found to produce inhibitors of angiogenesis, such as tissue inhibitor of metalloproteinase. These angiogenesis inhibitors prevent blood vessels from penetrating the articular cartilage. In OA there is a reduction in the production of angiogenesis inhibitors due to deterioration of the chondrocytes metabolism, thus resulting in the penetration of the articular cartilage by vessels from the subchondral bone.

In the present study, invasion of the articular cartilage by blood vessels was noticed among both arthritic groups (II-a and II-b). On the other hand, PRP treated groups showed minimal vascular invasion of the articular cartilage. This may be due to low levels of metalloproteinase expression in these groups as it was concluded by Arican *et al*.[46] who conducted their study on experimental dogs. They cited that arthritic dogs treated by PRP showed low levels of metalloproteinase-2 and metalloproteinase-9 as compared to untreated dogs.

Furthermore, increased osteoclastic activity in OA may contribute to neuronal excitation and pain. So, the drugs that inhibit the osteoclasts would be analgesic. Additionally, osteoclasts release chemical mediators that cause chondrocyte death with subsequent mechanical erosion of the articular surface and cracking of the calcified cartilage with focal loss of the subchondral bone[47].

In the present work, the three weeks arthritic group showed the peak of the inflammatory process in the synovial membrane. This was similar to observation of Cimen *et al*.[28]. Synovial membrane is a source of proinflammatory and catabolic products, including metalloproteinases and aggrecanases, which contribute to articular matrix degradation. Furthermore, different cytokines and chemokines are released in cases of synovitis which have catabolic effect on the chondrocytes. Therefore, any alteration in the synovial membrane can lead to decreased concentrations of cartilage-protecting factors with increased production of factors contributing to the degradation of the articular matrix[48].

In the present study, the three weeks arthritic group treated with PRP (III-a) showed marked reduction in the number of synovial membrane inflammatory cells which was statistically significant when compared to the three weeks post-arthritic group. PRP has anti-inflammatory effect carried out by stimulation of production of different protective cytokines like intracellular interleukins 4, 10, and 13 which counterbalance the toxic effects of interleukin 1[49].

Moreover, our study demonstrated spontaneous regression of the inflammatory process in the synovial membrane after six weeks of induction of arthritis. Poulet *et al*.[50] have mentioned that synovitis in experimental models of OA appeared to be transient. This may be the same condition explained by Hoshino *et al*.[51] who concluded

that structural changes in synovial tissues were reversible after induction of OA by low doses of monoiodoacetate (MIA), while high doses of MIA led to irreversible synovial tissue damage.

In the present study, Masson's trichrome stain showed progressive deposition of dense bundles of collagen fibers and fibrosis of the synovial membrane in the both untreated arthritic groups (II-a and II-b) and six weeks arthritic group treated with PRP (III-b). This was in agreement with Oehler *et al*.[52] who noticed that in case of early OA, there was more inflammation, while more fibrosis was noticed in late-stage OA suggesting that the factors inducing fibrosis are upregulated in the inflammatory phase. Moreover, Remst *et al*.[53] have concluded that high levels of transforming growth factor beta (TGF-β) in OA plays a key role in many pro-fibrotic processes as it enhances fibroblast proliferation and matrix deposition.

Limitations of the study:

Although the results of this study have been informative about the efficacy of PRP as a therapeutic tool for FA-induced OA, it has certain limitations. The study included single intra-articular injection of PRP. The study also lacks the comparison between the therapeutic effect of PRP and other traditional therapeutic modalities. So, it is recommended to conduct further studies including different therapeutic agents and to put into consideration the use of multiple injections of the PRP.

CONCLUSIONS

It was concluded that intra-articular injection of PRP in case of OA demonstrated advantageous role on articular cartilage healing, however, these effects appeared to be transient. So the need of multiple injections of PRP has to be considered.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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هل البلازما الغنية بالصفائح الدموية فعالة في علاج التهاب المفاصل المستحث باستخدام الفورمالدهايد في الفئران؟ تطبيق لمرضى التهاب المفاصل

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ملخص البحث

في هذه الدراسة ، تم تقييم فعالية البلازما الغنية بالصفائح الدموية في علاج التهاب المفاصل الناجم عن الفورمالدهايد. تم تقسيم اثنين وأربعين من ذكور الفئران البيضاء إلى ثلاث مجموعات. المجموعة الأولى (المجموعة الضابطة): تم تقسيم ثمانية عشر فأرًا إلى مجموعات فرعية ١- أ و ١- ب و ١- ج. المجموعة الثانية (مجموعة التهاب المفاصل): تم إخضاع اثني عشر فأرًا للحقن داخل المفصل بجرعة واحدة قدرها ٠,٠٢ مل من الفورمالدهايد تركيز ٥٪ في الركبة اليمنى وتم تقسيم الفئران إلى مجموعات فرعية ٢- أ و ٢- ب. المجموعة الثالثة (مجموعة التهاب المفاصل المعالجة بواسطة البلازما الغنية بالصفائح الدمرية): تم تعريض اثني عشر فأرًا لالتهاب المفاصل المستحث كما هو الحال في المجموعة الثانية، ثم تعرضوا للحقن داخل المفصل بجرعة واحدة قدرها ٠,٣ مل من البلازما الغنية بالصفائح الدموية في نفس الركبة اليمنى وتم تقسيمهم إلى مجموعات فرعية ٣- أ و ٣- ب. بعد التضحية بالفئران ، تم تشريح مفاصل الركبة ، ونزعها ، ثم معالجتها للفحص بالمجهر الضوئي. تم صبغ قطاعات البارافين بالصبغات المختلفة وتم إجراء صبغ مناعي للإنزيم كاسبيس-٣. كشف الفحص عن وجود سطح مفصلي غير منتظم وتآكل للعظام في مجموعة التهاب المفاصل. كان هناك أيضًا عدم انتظام ونقص في الخلايا الغضروفية وكان هناك انتشار للخلايا العظمية المختلفة التي غزت منطقة الاتصال العظمي الغضروفي للمفصل. أظهر الغشاء الزلالي ترسب حزم الكولاجين الكثيفة مع تسلل الخلايا الالتهابية والعديد من الأوعية الدموية. كان نشاط المناعة لإنزيم كاسبيس-٣ واضحًا في العديد من الخلايا الغضروفية. تم اكتشاف تحسن كل هذه التغييرات في المجموعة الثالثة أ. بيد أنه شوهد الكثير من هذه التغييرات في المجموعة الثالثة ب. أكدت النتائج القياسية والتحليل الإحصائي نتائج الفحص النسيجية. ونتج أن حقن البلازما الغنية بالصفائح الدموية داخل المفصل له دور فعال مؤقت في التئام الغضروف المفصلي.