Original Article

Effects of Intra-Articular Corticosteroid Versus Platelet-Rich Plasma on Induced Knee Osteoarthritis in Adult Albino Rats. Histological and Anatomical Study

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ABSTRACT

Background: Osteoarthritis (OA) is a common degenerative joint disease. Intra-articular injection of microcrystalline corticosteroid and platelet- rich plasma (PRP) are used for pain relief of knee OA.

Aim of the Study: the goal of this study was to assess the possible therapeutic effects of corticosteroid compared to PRP in a rat model of induced OA.

Material and Methods: Intra-articular injection of monosodium iodoacetate (MIA) was used for induction of OA in rats. Blood was collected from12 adult male rats for PRP preparation. 54 adult female albino rats were divided into control groups, OA model group and treated groups which received CS or PRP. All rats were euthanized after 28 days. Left knee joints were processed for histological examination by light microscope after staining by H& E, toluidine blue and masson's trichrome stains. Right knee joints were grossly examined by stereomicroscope and scanning electron microscope (SEM). The obtained data were examined, further statistically analyzed and interpreted.

Results: MIA caused gross anatomical and histological alterations in rat's articular cartilage similar to human knee OA. Articular cartilage of corticosteroid and PRP treated knee joints showed thinning of the articular cartilage, fibrillation and presence of pannus- like tissue. These groups also showed significant increase in histopathological scores compared to the control group and OA model group in addition to gross macroscopic changes that were confirmed by SEM.

Conclusion: This study showed that neither corticosteroid nor PRP could limit the structural changes in osteoarthritis and that different PRP preparation techniques have yielded varying results.

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Key Words: Corticosteroid, gross macroscopy, osteoarthritis, PRP, scanning electron microscope.

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INTRODUCTION

Osteoarthritis (OA) is considered as the most frequent joint disorder, especially in women. It is one of the world's top ten causes of disability[1,2]. Middle-aged and elderly patients are particularly affected with knee OA. Its global prevalence is predicted to be over 250 million patients[1]. By 2030 more than 25% of the adult American people is expected to suffer from knee OA[3].

Knee OA is classified into primary or idiopathic, and secondary. Idiopathic OA is the commonest type of arthritis, it occurs insidiously with advancing age with no obvious predisposing cause[4]. In secondary OA, the predisposing pathology is clearly defined in some cases, like bone infarction, instability of joints (e.g. meniscus lesions), bleeding tendency disorders, joint infections, or overload causing repeated excessive wear (marked obesity, work, sports)[5].

OA is a degenerative joint disease resulting from loss of articular cartilage integrity, alterations in the subchondral bone, and inflammation of the synovial membrane[6,7]. There is a complex association between components of inflammation and many factors (e.g. genetic, biomechanical) that causes the occurrence of OA[1].

No treatment exists for complete cure of knee OA[8]. Due to the restricted life span of joint arthroplasty, the need for disease-modifying therapies or drugs is high. The ideal treatment for OA should address the mechanical strain that generates joint injury as well as the inflammatory reactions which exacerbates the structural damage[9]. Among the non-invasive therapies for OA are weight reduction, exercise, activity adjustment, assistive equipment, oral analgesic medications (paracetamol, non-steroidal anti-inflammatory drugs) Intra-articular injections e.g corticosteroid and platelet-rich plasma (PRP), are preferred in cases of local joint disability and to avoid the complications of systemic administration[10].

Current designed pharmacological OA treatments mainly aim to relieve the joint pain rather than effects on degeneration of the articular cartilage[11]. This may be attributed to the usual late diagnosis of OA at advanced stages. Moreover, these drugs cause many serious

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complications limiting their long term use[7,11]. Thus there is a great need for a new OA therapy that can combine reduction of joint inflammation and restoration of patient's normal activity[12,13].

For 50 years, Intra-articular injection of microcrystalline corticosteroid (CS) has been considered as a secure pain control therapy in non-septic local joint inflammation[12,14]. It is very effectiveness in pain relief in cases of knee OA flare[15,16]. Intra-articular administration of Triamcinolone acetate (TACA) is recommended by Osteoarthritis research society international (OARSI) and American college of Rheumatology (ACR)[17,18].

PRP is a biological therapy that has emerged in recent years for different tissue injuries[19]. It can be obtained in a simple, low and minimally invasive manner to help in tissue regeneration[20]. When compared to whole blood, it has a 3-8 double the concentration of platelets and contains an ultra-physiological amount of autologous growth factors[19]. PRP is frequently prescribed as an intra-articular injection for reduction of pain in knee OA for longer duration[21].

The goal of the current study was to assess the possible therapeutic effects of corticosteroid versus PRP in a rat model of induced OA.

MATERIAL AND METHODS

The present study was carried out, after acceptance of the ethical committee of Alexandria Faculty of medicine, on sixty six adult albino rats[22]. The rats were gotten from the Animal House Center of Physiology Department, Faculty of Medicine, Alexandria University. During the experimental period, the animals were fed following the Egyptian Institute of Nutrition (EIN) recommendations. In order to alleviate any potential irritation, rats were housed two per cage at room temperature.

The rats were divided into:

- 12 healthy adult male rats (weighing about 300 ± 50 g), to obtain blood for PRP preparation. Male rats were used for blood collection for PRP preparation as platelets of male rats have better quality than platelets of female rats[18,23].
- 54 adult female rats (weighing about 200 ± 20 g, and aged 6- 8 weeks) were considered as the experimental group Female rats were used in the experimental groups as OA is known to be more common in females. Female rats have the same hormonal distribution and effects on articular cartilage like human female[24,25].

A) Induction of Osteoarthritis

Following 1 week of acclimatization for 24 female rats, osteoarthritis was induced by a single intra-articular injection of freshly prepared Monosodium Iodoacetate (MIA; Sigma, St. Louis, MO 63103, USA; cat #I2512) after light general anesthesia under sterile conditions[1]. MIA was injected into the both knee joints through the infrapatellar ligaments after shaving then disinfection with povidone-iodine[1,6].1 mg of MIA was dissolved in 25 µl of physiologic saline then was injected using an insulin syringe[11,26].

B) Preparation of PRP

Twelve male albino rats were used for the purpose of PRP preparation. The PRP preparation was conducted at the clinical pathology Lab, Alexandria Faculty of Medicine. The whole blood was drawn from all male rats from the peri-orbital venous plexus under sterile conditions and was pooled in blood tubes containing the anticoagulant citrate phosphate dextrose[4]. Two different protocols of PRP preparation were prepared as follows:

1. **Protocol 1:** The whole blood from 6 male rats was centrifuged at 400 g for 15 minutes, followed by a 5-minute rest period, followed by another cycle of centrifugation at 800 g for 10 minutes[27].
2. **Protocol 2:** The whole blood from the other 6 male rats was centrifuged at 1000 g for 15 minutes, followed by a 5-minute rest period, followed by another cycle of centrifugation at 3000 g for 10 minutes[28,29].

Following the first centrifugation, the plasma was isolated from the concentrated red blood cells, then the supernatant part was extracted during the second centrifugation leaving just about 1 ml of the heavier centrifuged content. This collected fraction is the PRP or platelet concentrate[4]. PRP was isolated using a pipette technique, then the concentration of platelets in the PRP was confirmed using an automated cell counter[30].

C) Experimental design

The 54 female rats were randomly divided into different groups as following:

Group I: (control groups) 30 rats serving as a control group were further subdivided into three subgroups:

- Subgroup Ia: 6 rats received standard diet and free access to water without exposure to any procedure[1].
- Subgroup Ib: 6 rats received single knee intra-articular injection by 25 µl of saline[1].
- Subgroup Ic: 18 rats included 6 rats that received single knee intra-articular injection of 0.1 mg of TACA[31], and The rest of rats; 6 of them received 50 µl of PRP of protocol 1and the other 6 received protocol 2 as single intra-articular injection in the knee joint[30]

Group II: (OA model group) 6 rats received a single knee intra-articular injection of 1 mg of MIA dissolved in 25 µl of saline[1].

Group III: (treated groups) 18 rats received TACA or PRP 7 days after intra-articular injections of MIA:

- Subgroup IIIa: (CS group) 6 rats received a single knee intra-articular injection of 0.1 mg of TACA[31].
- Subgroup IIIb: (PRP1 group) 6 rats received a single knee intra-articular injection of 50 µl of PRP of protocol 1[30].
- Subgroup IIIc: (PRP2 group) 6 rats received a single knee intra-articular injection of 50 µl of PRP of protocol 2[30].

After 28 days, all the 54 female rats were euthanized under anesthesia, left knee joints were collected for histological studies and right knee joints were collected for gross macroscopic and scanning electron microscopic studies[1,32].

D) Histological studies

For each rat, the skin was stripped, soft tissues were gently removed and the left knee joints were separated by being cut at mid-femur and mid- tibia. The intact joints were preserved in neutral buffered formalin (containing 10% formaldehyde) for 48 to 72 hours. The samples were then decalcified using 20% ethylene diamine tetraacetic acid 10% (EDTA) for about 2 weeks. Following the completion of decalcification, the samples were embedded at paraffin blocks. Sagittal tissue sections were prepared and stained with hematoxylin-eosin (H&E) to evaluate architectural degenerative changes, toluidine blue to assess the proteoglycans in cartilage matrix, and Masson's trichrome for detection of fibrosis[6,33].

From each toluidine blue and Masson's trichrome stained subgroups, 5 random images were selected and analyzed by the color threshold tool and the pixels were measured. FIJI/ Image J software (NIH, Bethesda, NJ) was used[34].

The cartilage degeneration of the articular cartilage was blindly evaluated by using modified Mankin grading system on a scale of 14 points with 0 corresponding to a normal joint, and 14 for maximally degenerated joint.

The structure was scored on a scale of 0–5, where 0 = normal; 1= irregular surface, including fissures into the radial layer; 2 = pannus; 3 = absence of superficial cartilage layers; 4 = slight disorganization; 5 = fissure into the calcified cartilage layer. Cellular abnormalities were scored on a scale of 0–3, where 0 = normal; 1 = hypercellularity, including small superficial clusters; 2 = clusters; and 3 = hypocellularity. The matrix staining by toluidine blue was scored on a scale of 0–5, where 0 = normal/ slight reduction in staining; 1 = staining reduced in the radial layer; 2 = staining reduced in the interterritorial matrix; 3 = staining present only in the pericellular matrix; 4 = staining absent and 5= disorganization. The tidemark integrity was scored 0-1, where 0 = normal and 1= Crossed by blood vessels[6,35].

OARSI (osteoarthritis cartilage histopathology assessment system was also used. OARSI grades indicates depth progression into the cartilage: [Grade 0: intact surface and cartilage morphology, grade 1: surface intact, grade 2: surface discontinued, grade 3: vertical fissures, grade 4: erosions, grade 5: denudation, grade 6: deformation]. OARSI stages reveals extent of joint involvement: [Stage 0: no OA activity is seen, stage 1: OA <10% of the articular cartilage, stage 2: OA 10- 25% of the articular cartilage, stage 3: OA 25-50% of the articular cartilage, stage 4: OA > 50% of the articular cartilage]. The recommended calculation of OARSI score is an index of combined grade and stage. The simple formula: score X grade= stage is recommended. This method produces an OA score with a range of 0 to 24 based on the most advanced grade and most extensive stage present[6,35].

E) Gross macroscopic study

The right knee joints of the rats were immediately immersed in 4F1G (4% formaldehyde and 1% gluteraldehyde). They were then carefully dissected by removing ligaments and tendons[36].

The femoral condyle and tibial plateau were obtained, and their articular cartilage surfaces were gross macroscopically examined for detection of surface irregularities at higher magnification. They were then photographed at the experimental embryology lab, Anatomy and Embryology department, Faculty of Medicine, Alexandria University, using Olympus SZ dissecting stereomicroscope (Olympus corporation- Japan through their agent Optoscient company in Egypt)[36].

The gross macroscopic score, set by Yoshimi *et al*. on a scale of 5, was used for blind assessment of the gross appearance of the articular cartilage[36].

F) Scanning Electron Microscopic Study (SEM)

SEM study was selected in the current study to examine the articular surface topography and composition using a focused beam of electrons[37]. Just after gross macroscopic examination, the same samples from the right knee joints were fixed in 4F1G in 0.1M buffer pH 7.2 at 4° C for 2- 24 hours[38]. Specimens were then washed in distilled water. Dehydration was done by ascending grades of alcohol concentrations. The specimens were left to dry in air, then the femur articular cartilages were coated with gold[39]. Morphology of the specimens was examined and photographed in a Jeol scanning electron microscope (JSM-IT200, In Touch Scope Series) at the Electron Microscopy Unit, Faculty of science, Alexandria University.

G) Energy dispersive X-ray spectroscopy (EDX)

Energy dispersive X-ray spectroscopy (EDX) microanalysis is based on emission of distinct pattern of X rays which can reveal the existence of certain elements in the specimen[37,40]. EDX was carried out on the articular cartilage of the right knee joint samples using the EDX microanalysis system (incorporated into the scanning electron microscope, a Jeol JSM- IT200, In Touch Scope Series). This provided a qualitative analysis of the elemental composition at the subchondral bone. The Calcium and

phosphorus peaks were calculated and plotted as graphs by the analysis software[37,40,41].

H) Statistical analysis of the data

Data were inserted to the computer and analyzed using IBM Statistical Package of Social Sciences (SPSS) software package version 20.0. (Armonk, NY: IBM Corp.). In order to check the normal distribution of data, the Kolmogorov-Smirnov test was used. Quantitative data were represented by range (minimum and maximum), mean, standard deviation and median. Significance of the collected results was evaluated at the 5% level[42].

RESULTS

In the present study, one male rat died during blood collection for PRP preparation. No alterations were observed in the appearance or the behavior of the female rats of the control groups.

The female rats in OA model group, CS group and PRP1 group showed normal behavior. However, limping gait was observed in female rats of PRP2 group in comparison with other groups. Also their knee joints were swollen, but without signs of inflammation.

Histological results

The examination of left rat knee joints of control groups stained with H & E showed cartilage with smooth superficial surface and normal thickness. Chondrocytes lied within their lacunae in pale basophilic staining intercellular matrix. They showed light eosinophilic cytoplasm and basophilic nuclei and were surrounded by a clear cartilage capsule (Figures 1a-b).

Different zones of articular cartilage were evident: superficial zone (sz) where collagen fibers were parallel to the surface and chondrocytes were elongated, flattened and parallel to collagen fibers and joint surface; mid zone (mz) subjacent to superficial zone, its collagen fibers were aligned intermediately between superficial and deep zones, chondrocytes were parallel to the collagen fibers; deep zone (dz) where collagen fibers were perpendicular to the joint surface, small rounded chondrocytes lied within chondrons and parallel to the collagen fibers. Tidemark was clearly defined between calcified zone and other non-calcified zones. Subchondral bone appeared with normally acidophilic staining matrix (Figure 1b).

The examination of left rat knee joints of OA model group illustrated diminished articular space between femur and tibia and thinning in articular cartilage thickness (Figure 2a). The articular surfaces showed irregular surface and fibrillation (vertical cracks or articular cartilage discontinuity, confined to the superficial zone). Midzone illustrated abnormal matrix intensity and hypo-cellularity. tide mark was not evident between deep zone and calcified cartilage in some areas (Figure 2b).

Left rat knee joints of CS group showed some areas with smooth cartilage surface. Other areas showed focal structural disorganization with abnormally irregular articular surface and reduced thickness, fibrillation through the superficial zone and denudation (Matrix loss of the whole cartilage zones extending to calcified cartilage interface) (Figure 3a). Pannus- like tissue filled the articular space. The matrix showed variable intensity and absence of definite tidemark. Chondrocytes showed focal areas of hypo-cellularity (Figure 3b).

Left rat knee joints of PRP1 treated group revealed disorganization of the articular cartilage with reduced thickness and irregular surface (Figure 4a). Formation of pannus- like tissue was noted inside the joint cavity or covering the articular surface leaving only a remnant of cartilage layer in some areas (Figures 4a-b). There was slight chondrocyte hypo-cellularity in the different cartilage zones. In addition, there was irregular orientation of tidemark between noncalcified and calcified zones and undefined demarcation of subchondral bone (Figure 4b).

In PRP2 group, the examination of left rat knee joints showed areas of complete loss of cartilage due to the abnormal pannus-like tissue (Figure 5a). However, preservation of the articular cartilage thickness and smooth surface were encountered (Figure 5b). Hyper-cellularity with vacuolated nuclei and clusters of chondrocytes were noticed. Areas with Irregular orientation of tidemarks were noticed (Figure 5b).

Matrix intensity measured by toluidine blue staining showed normal deep staining intensity at the control groups, weak staining intensity at OA model group, CS group and PRP1 group, while PRP2 group revealed moderate staining intensity. (Figure 6) Morphometric analysis illustrated that the means of toluidine blue stain intensity in CS and PRP1 groups were significantly low compared to the control groups, while the means of PRP2 group were significantly less than the means of both control groups and OA model group (Figure 7, Table I).

Masson's trichrome stain used for detection of collagen fiber deposition showed negative staining inside the joint cavities of the control groups and OA model group, while positive staining inside the joint cavities of CS group and PRP groups. (Figure 8) Morphometric analysis of Masson's trichrome staining intensity was significantly high CS and PRP subgroups in comparison to OA model group (Figure 9, Table I).

Scoring results

The Mankin grading score and OARSI score of the different experimental groups are summarized in (Figures 10,11, Table II).

The Mankin grading score was used to assess the structural integrity of the articular surface, intact tidemark, normal cellular distribution, and normal staining with toluidine blue. The mean of control groups was the lowest reflecting good cartilage tissue quality (Figure 10, Table II).

In contrast, the mean scores of OA model group, CS group, and PRP groups were significant in comparison with the control groups, denoting changes in the articular cartilage structure and chondrocytes distribution, decrease in toluidine blue staining and disruption in the tidemark (Figure 10, Table II).

Moreover, the means of Mankin scores of CS group and PRP groups were significantly higher than the mean of the OA model group (Figure 10, Table II).

The mean of total Mnakin score of PRP2 group was significantly less than CS and PRP1 groups. The mean score of PRP1 score was significantly high in comparison with CS group. The mean of articular cartilage structure score of PRP2 group was significantly less than the means of CS group and PRP1group. The mean of chondrocytes score of PRP2 group was not significant with the mean of CS and PRP1 groups. This can suggest that PRP2 gave better results than CS and PRP1 (Figure 10, Table II).

Quantification of histological features by OARSI score showed similar trends to that of Mankin score. OARSI score was significantly higher in OA model group compared to normal control groups. This denotes that both grade and stage are affected. (Figure 11, Table II).

The means of OARSI scores of CS group and PRP groups were also significantly higher than the mean of control group. The mean score of PRP1 group was also significant in comparison with OA model group. However, the means of CS group and PRP2 groups were non-significant (Figure 11, Table II).

The mean of OARSI grade, stage and score of PRP1 group was significantly high in comparison with CS group. The mean of grade was high in PRP1 group due to presence of cartilage loss and denudation that didn't appear at other treated groups. The means of OARSI grade, stage and score of PRP2 group were significantly lower than the means of PRP1 group.

Gross anatomical Results

The cartilage surfaces of the loaded parts of the right femur and tibia were grossly assessed and scored for the extent of degeneration (Figure 12, Table III).

Animals of the control groups showed normal articular cartilage with gross anatomical score of zero. They exhibited a smooth glossy surface with no noticeable evidence of macroscopic degeneration (Figures 12,13a,14a, Table III).

In OA model group, the articular cartilage revealed gross changes including surface irregularities, fibrillations and erosion in some areas. The mean of macroscopic score was significantly high compared to the mean of the control groups (Figures 12,13b,14b, Table III).

The articular cartilages of CS group and PRP1 group have shown ulcerations and cartilage loss. The means of their macroscopic scores were significant in comparison with the control groups and even with OA model group (figures 12,13c-d,13c-d, Table III).

However, the changes in the articular cartilages of PRP2 group varied from just surface irregularities up to cartilage loss in certain focal areas of the articular cartilage, but these changes were less destructive changes than CS group and PRP1 groups. The mean of macroscopic score of PRP2 group was significantly higher than the mean of the control groups, while it was significantly less than the means of CS and PRP1 groups (Figures 12,13e,14e, Table III).

Scanning Electron Microscopic results

Electron microscopic examination of the right articular cartilage of femoral condyles of the control rats depicted normal regular smooth surface (Figures 15a,16a), while right knee joints of OA model group illustrated irregular rough surface and areas with elevations and depressions (Figures 15b,16b).

The articular cartilage of the right femoral condyles of the CS group depicted abnormally destroyed femoral condyles that was rough with cracks and fissures being noticed in some areas. Elevations and depressions or pits were also noticed (Figures 15c,16c).

In PRP1 group, the articular cartilage of right femoral condyles of the showed loss of cartilage integrity, elevations and pits (Figures 15d,16d).

The articular cartilage of right femoral condyles of the PRP2 group revealed focal irregular rough articular surface, elevations and depressions in some areas, while other areas of the articular surface was preserved (Figures 15e,16e).

EDX results

EDX study to detect Calcium and Phosphorus of subchondral bone of the right tibia showed that the mean values of Calcium and Phosphorus peaks in OA model group was similar to the control groups, while in CS group and PRP1 groups, Ca and P peaks were significantly high in comparison with the control groups and OA model group. It was noticed that the mean value of PRP2 group was significant only with the mean of control groups,but nonsignificant with the mean of OA model group (Figures 17- 22, Table IV).

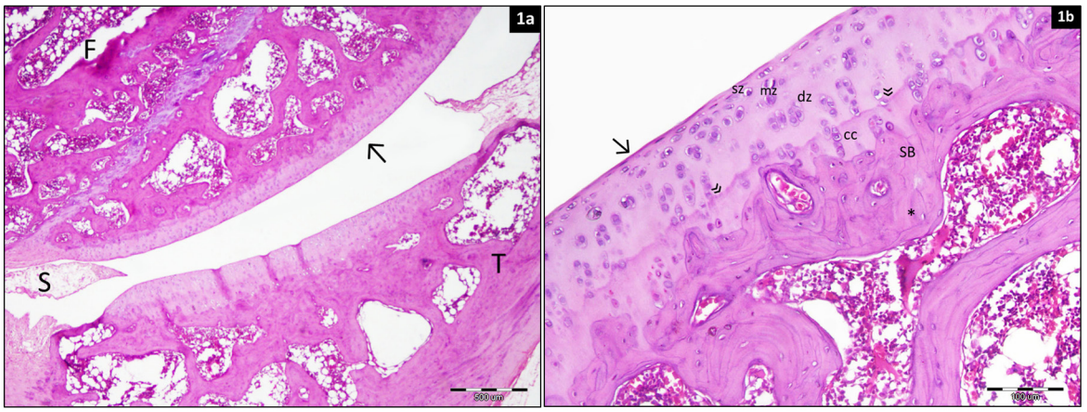

Fig. 1a-b: Light photomicrographs of rat's knee joints of the control groups. (Fig. 1a) shows articular cartilage of normal thickness with smooth non- fibrillated articular surface (↑). (Fig. 1b) reveals different zones including superficial zone (sz), midzone (mz), deep zone (dz), calcified cartilage (cc), and subchondral bone (SB) with its acidophilic staining matrix. Tidemark is clearly defined between non- calcified and calcified zones (»). Femur (F), Tibia (T), Synovium (S). [H&E stain. Mic. Mag. 1a X40, 1b X200]

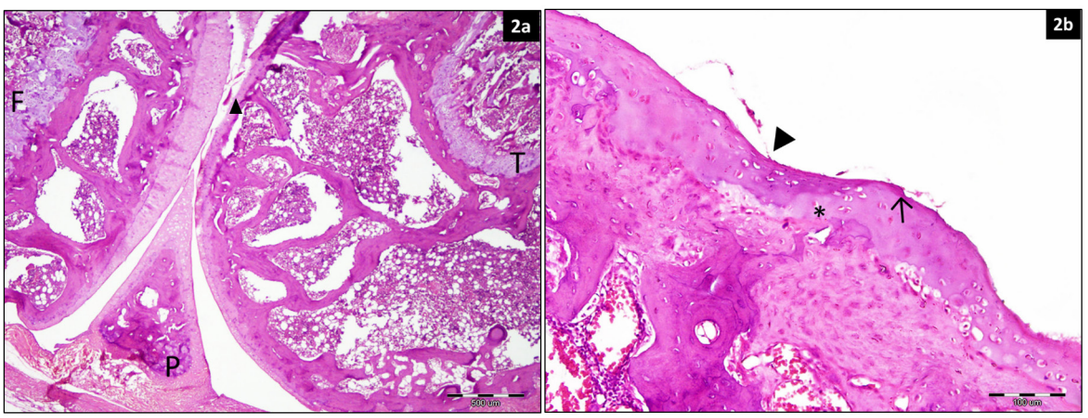

Fig. 2a-b: Light photomicrographs of rat's knee joints of OA model group. (Fig. 2a) reveals diminished articular space and reduced thickness of articular cartilage (►). (Fig. 2b) shows variable thickness of articular cartilage with fibrillation (↑) and areas with low intensity of intercellular matrix (*). Hypo-cellularity and absence of tidemark are noted. Femur (F), Tibia (T) Patella (P). [H&E stain. Mic. Mag. 2a X40, 2b X 200]

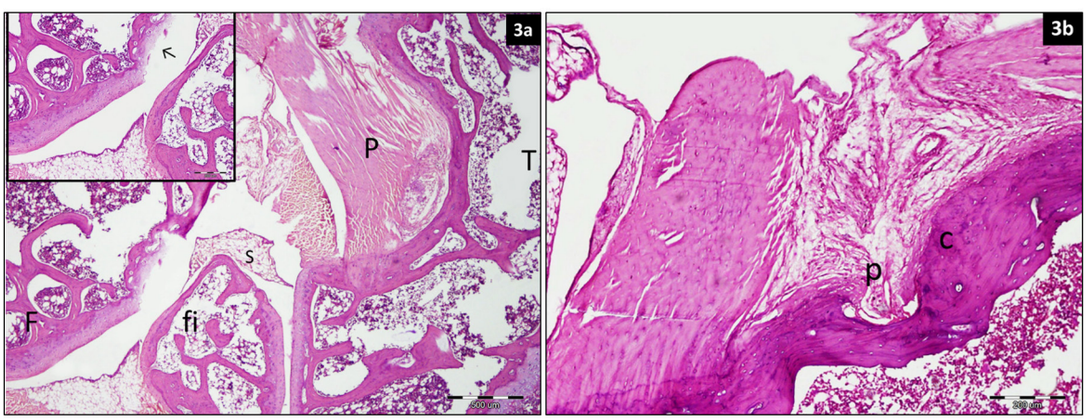

Fig. 3a-b: Light photomicrographs of rat's knee joints of CS group. (Fig. 3a) illustrates structural disorganization with filling of articular space by pannus- like tissue (P). Denudation (↑) is also noted in (inset of Fig. 3a). (Fig. 3b) shows pannus- like tissue (P) pitting the articular cartilage (c) and extending into deeper layers. Tidemark is irregular or absent in focal areas. Femur (F), Tibia (T), Fibula (fi), Synovium (S). [H&E stain. Mic. Mag. 3a X40, 3b X100]

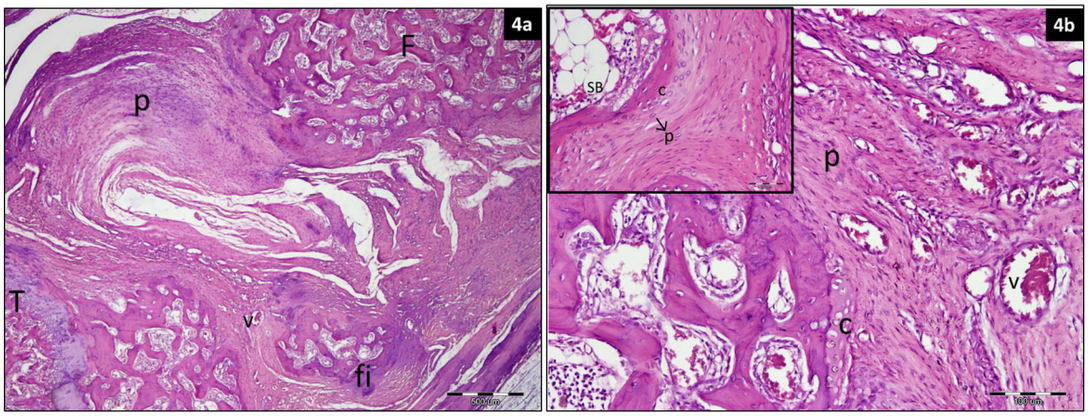

Fig. 4a-b: Light photomicrographs of rat's knee joints of PRP1 group. (Fig.4 a) reveals abnormal pannus-like tissue inside the joint cavity (P) with blood vessels (v). (Fig. 4b) shows a remnant of cartilage layer (c). (Inset of Fig. 4b) shows thinning of subchondral bone trabeculae (SB) and apparent multiple nuclei of fibroblasts (↑). Femur (F), Tibia (T), Fibula (fi). [H&E stain. Mic. Mag. 4a X40, 4b X 200]

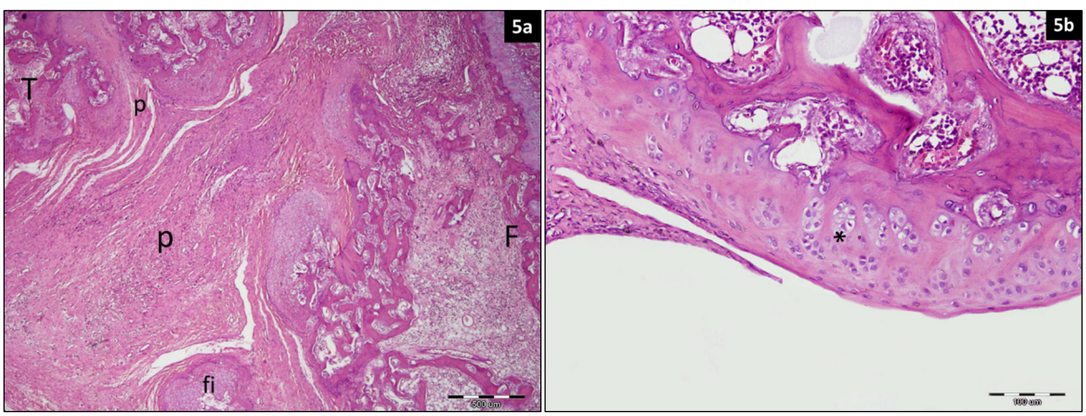

Fig. 5a-b: Light photomicrographs of rat's knee joints of PRP2 group. (Fig. 5a) shows a pannus-like fibrous tissue filling the joint cavity (P) and extending deeply into deep zone of articular cartilage, while in (Fig. 5b), the articular cartilage appears regular and Hypercellularity with clusters of chondrocytes were seen in the matrix (*). Femur (F), Tibia (T), Fibula (fi). [H&E stain. Mic. Mag. 5a X40, 5b X200]

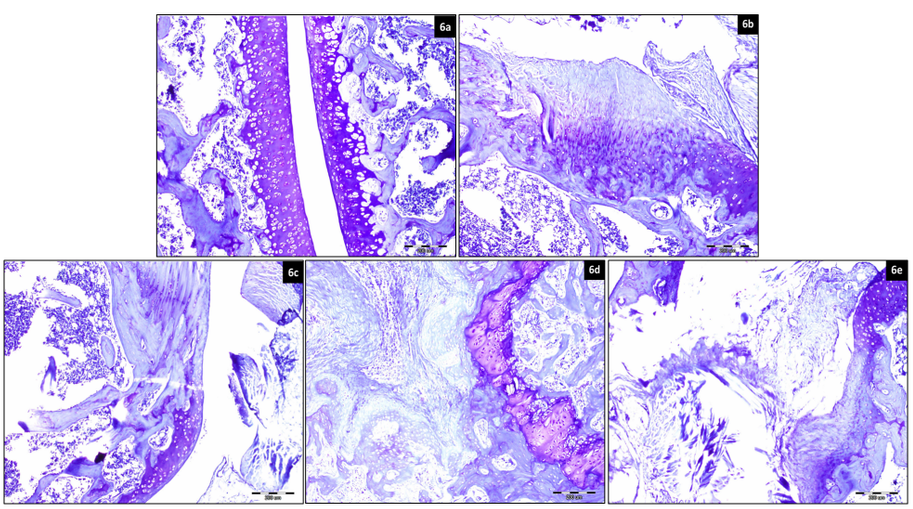

Fig. 6a-e: Light photomicrographs of rat's knee joints showing different toluidine blue staining for detection of intensity of cartilage matrix in different groups. (Fig. 6a: control groups) shows normal deep toluidine blue stain. (Fig. 6b: OA model group), (Fig. 6c: CS group), and (Fig. 6d: PRP1 group) reveal weak toluidine staining, (Fig. 6e: PRP2 group) illustrates areas of moderate toluidine blue stain. [Toluidine Blue stain. Mic. Mag. 6a- e X100]

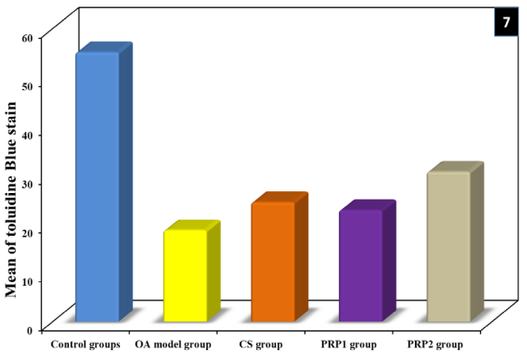

Fig. 7: A bar chart showing comparison between the studied groups according to toluidine blue stain intensity

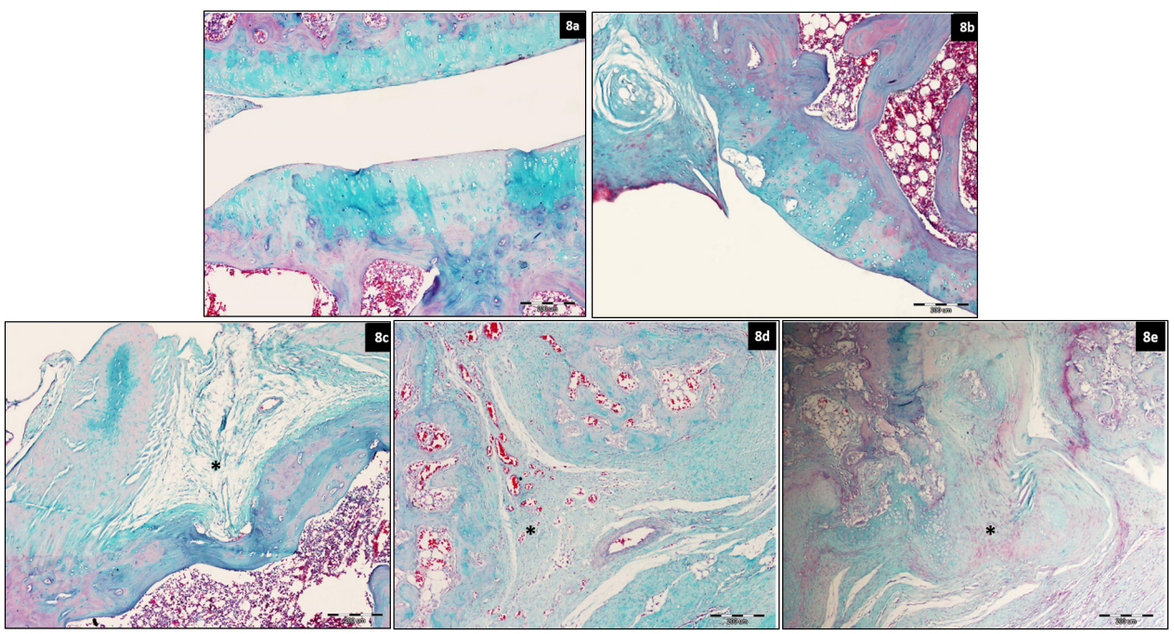

Fig. 8a-e: Light photomicrographs of rat's knee joints of different groups. There was no Masson's Trichrome staining inside the joints cavities (*) in (Fig. 8a: control groups) and (Fig. 8b: OA model group), while it is detected with increased intensity in the joint cavities in (Fig. 8c: CS group), (Fig. 8d: PRP1 group), and (Fig. 8e: PRP2 group). [Masson's trichrome stain. Mic. Mag. 8a- e X100]

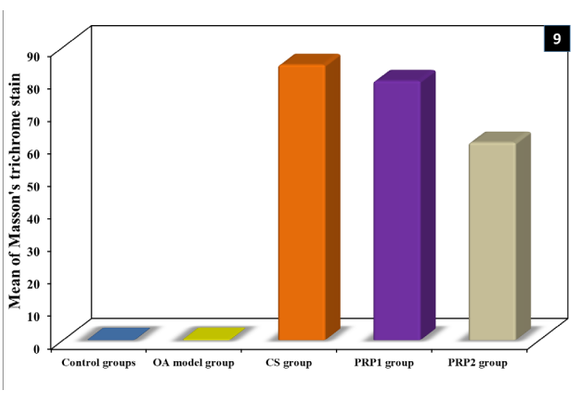

Fig. 9: A bar chart illustrating comparison between the studied groups according to Masson's trichrome stain intensity

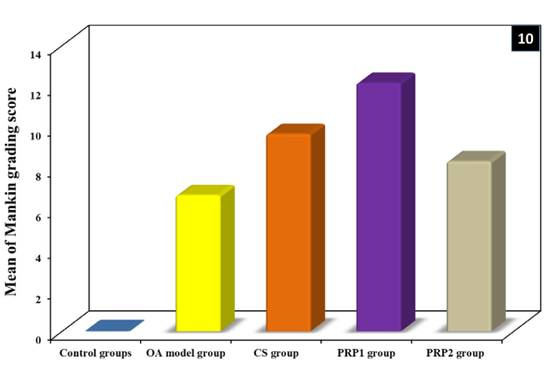

Fig. 10: A bar chart showing comparison between the different studied groups according to different parameters of modified Mankin grading score

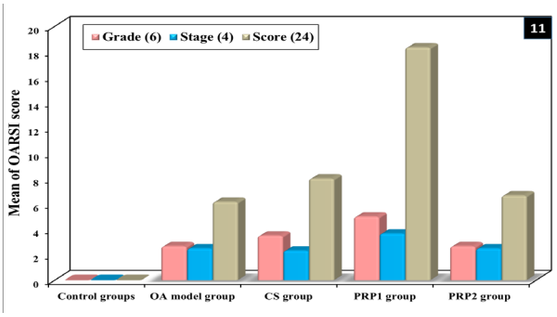

Fig. 11: A bar chart illustrating comparison between the different studied groups according to different parameters of OARSI score

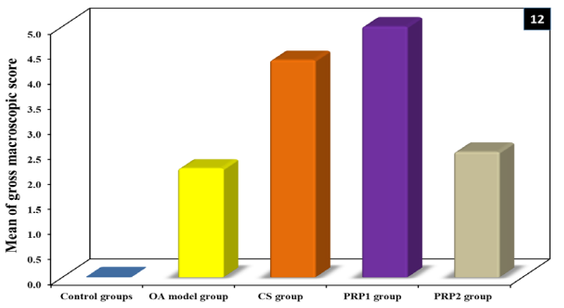

Fig. 12: A bar chart illustrating comparison between the different studied groups according to gross macroscopic score

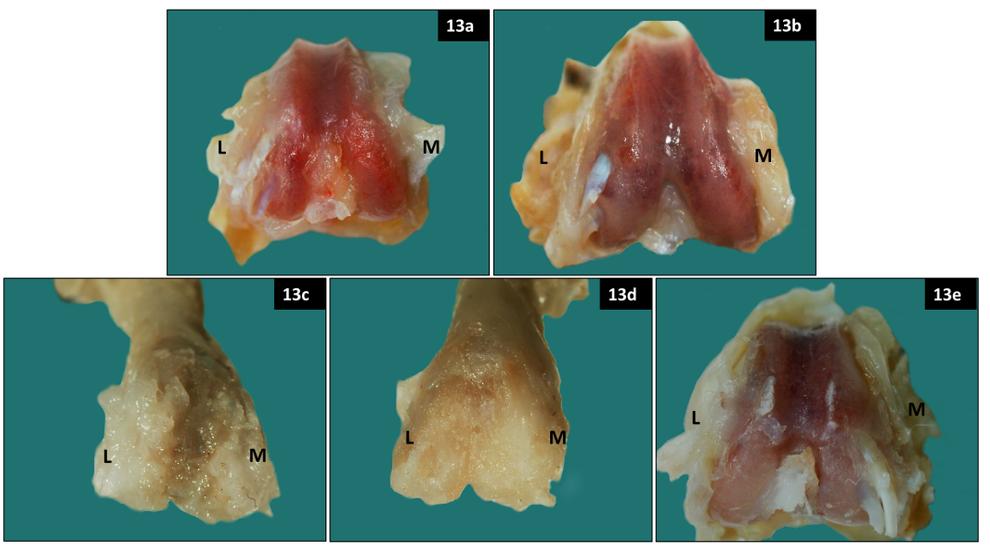

Fig. 13a-e: Macroscopic images of the articular surfaces of femoral condyles in studied groups. (Fig. 13a: control groups) shows regular edges and intact smooth cartilage of both medial (M) and lateral (L) condyles. (Fig. 13b: OA model group) and (Fig. 13e: PRP2 group) reveal surface irregularities and fibrillation, while in (Fig. 13c: CS group) and (Fig. 13d: PRP1 group) there was cartilage loss. [Mic. Mag. 13a- e X4]

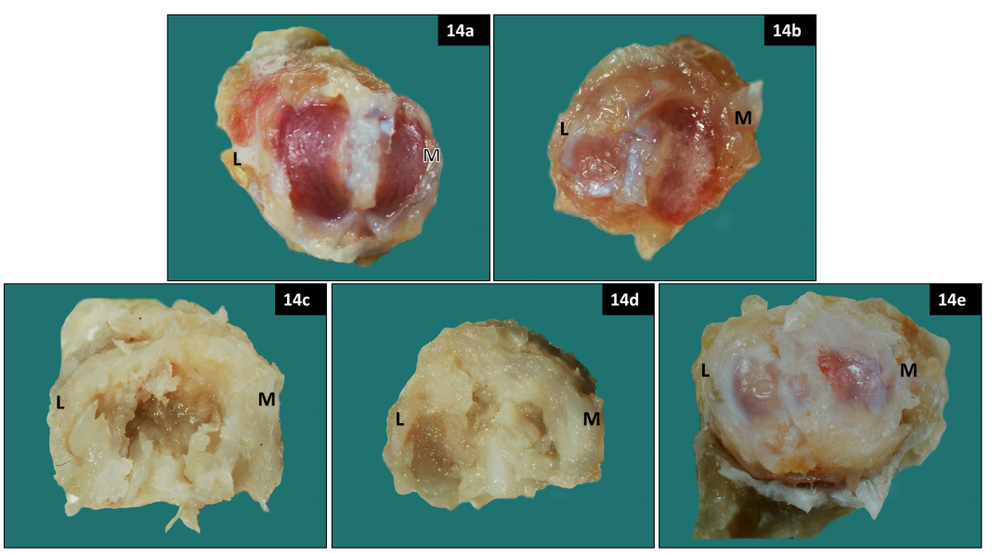

Fig. 14a-e: Macroscopic images of the articular surfaces of tibial condyles in studied groups. (Fig. 14a: control groups) showing regular edges and intact smooth cartilage of both medial (M) and lateral (L) condyles. (Fig. 14b: OA model groups) revealing surface irregularities and fibrillation. (Fig. 14c: CS group) and (Fig. 14d: PRP1 group) there was cartilage loss, while in (Fig. 14e: PRP2 group) there was areas with erosion and other areas there was just surface irregularity. [Mic. Mag. 14a- e X4]

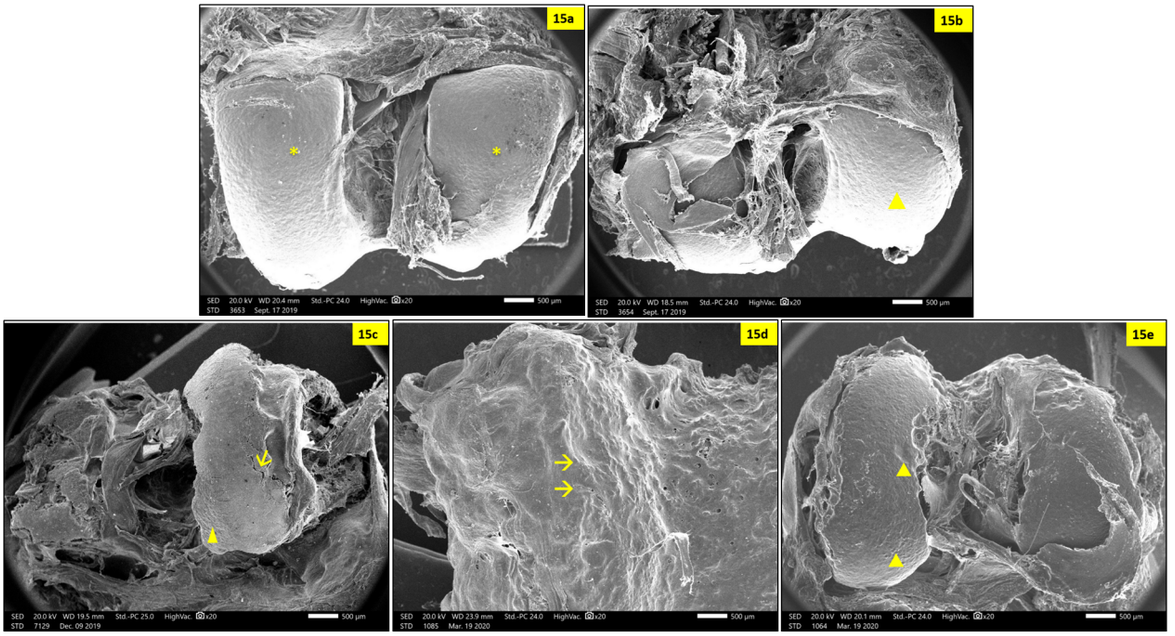

Fig. 15a-e: Scanning electron photomicrographs of articular cartilage of rat's knee joints of different groups at low magnification: (Fig. 15a: control groups) shows normal femoral condyles with regular smooth surface (*). (Fig. 15b: OA model group), (Fig. 15c: CS group), and (Fig. 15e: PRP2 group) illustrate more or less normal articular cartilage and focal irregular rough surfaces (►), cracks and fissures (↑). (Fig. 15d: PRP1 group) reveals loss of femoral articular cartilage integrity (↑↑). [Mic. Mag. 15a- e X20]

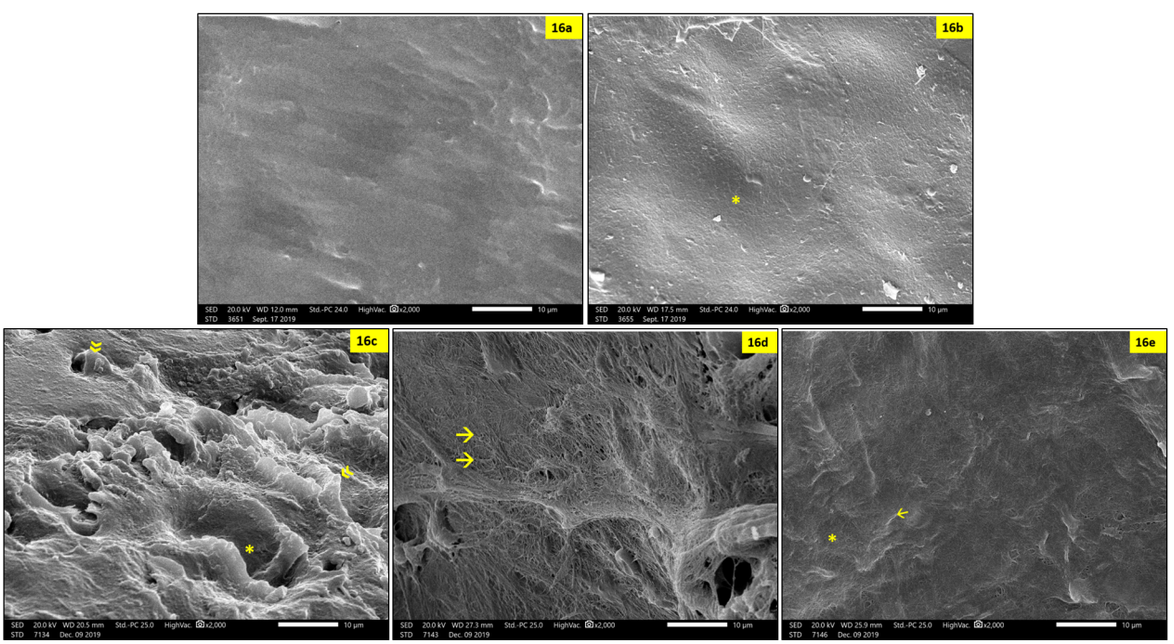

Fig. 16a-e: Higher magnification of scanning electron photomicrographs of articular cartilage of rat's knee joints: (Fig. 16a: control groups) reveals femoral condyles with regular smooth surface. (Fig. 16b: OA model group), (Fig. 16c: CS group), and (Fig. 16e: PRP2 group) show elevations (») and depressions or pits (*). (Fig. 16d: PRP1 group) reveals loss of femoral articular cartilage integrity (↑↑). [Mic. Mag. 16a- e X2000]

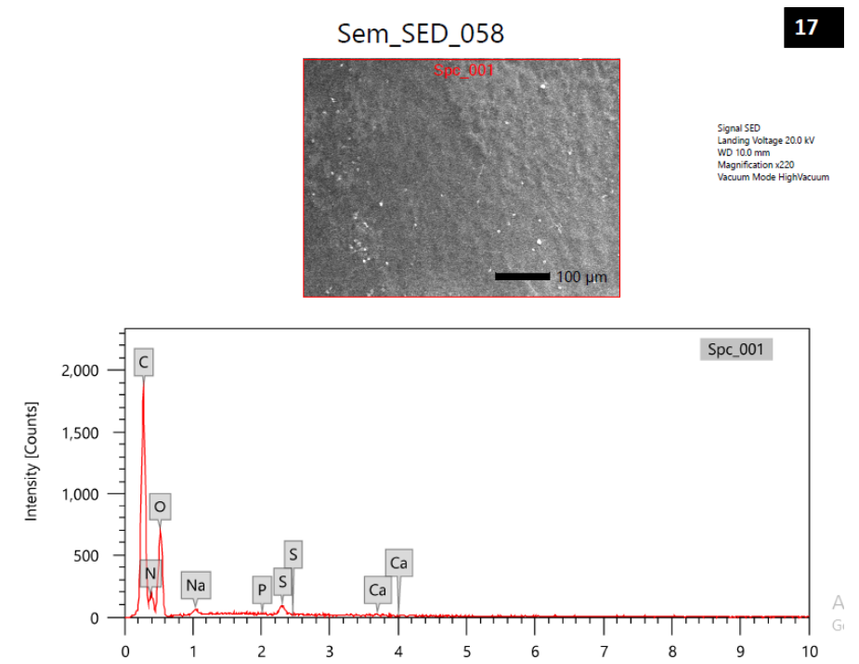

Fig. 17: A graph showing Ca and P analysis of EDX study of the control groups

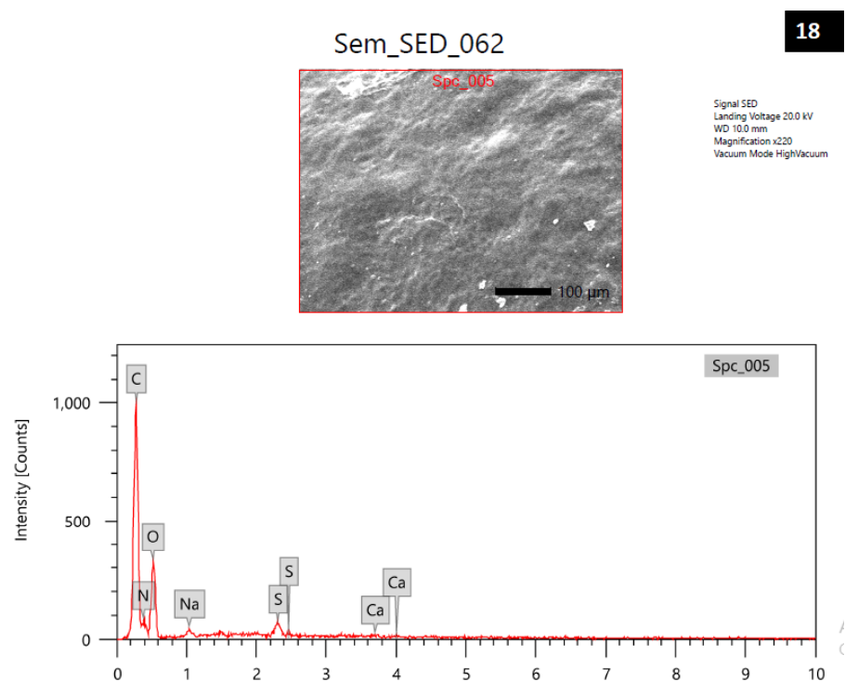

Fig. 18: A graph showing Ca and P analysis of EDX study of OA model group

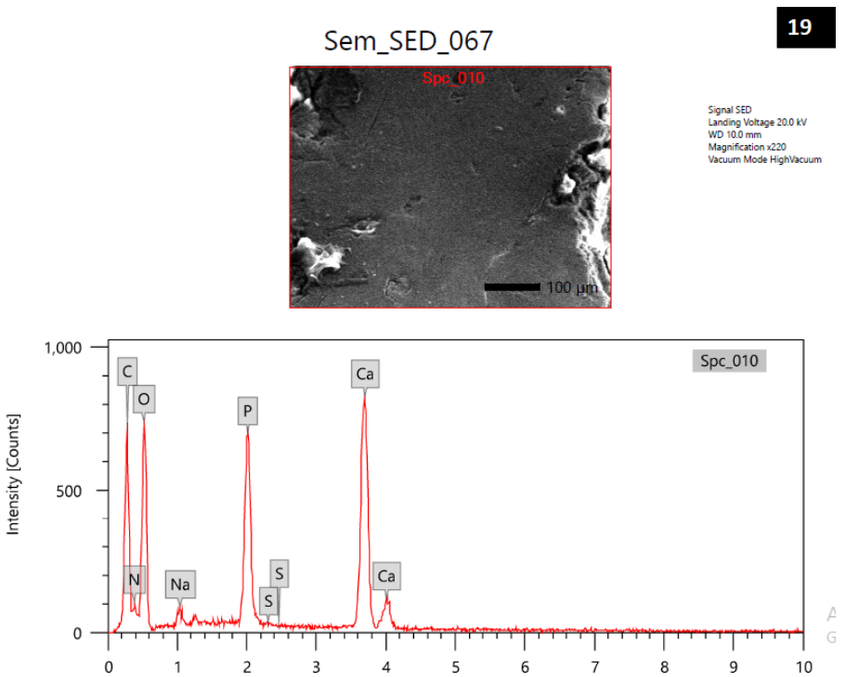

Fig. 19: A graph showing Ca and P analysis of EDX study of CS group

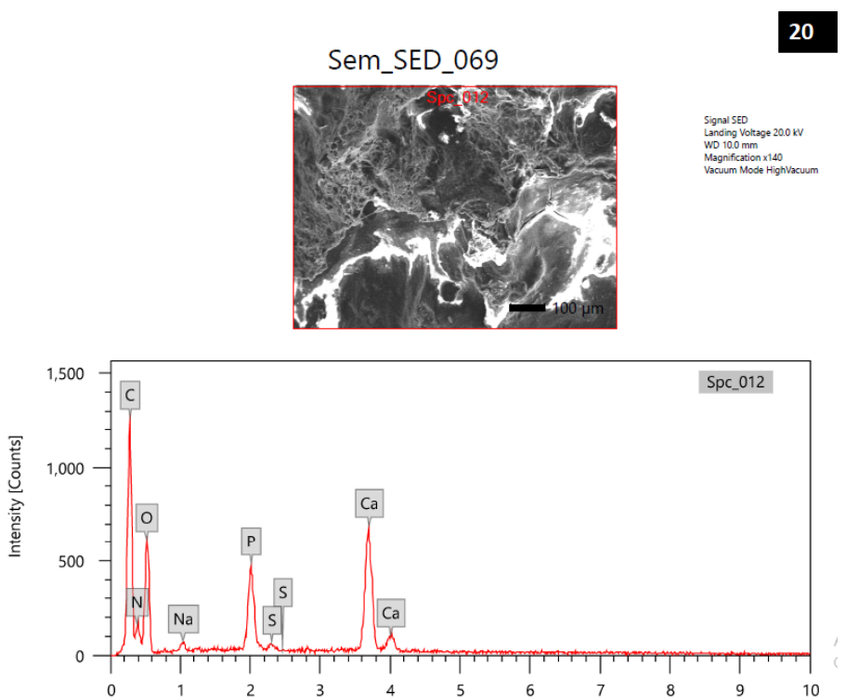

Fig. 20: A graph showing Ca and P analysis of EDX study of PRP1 group

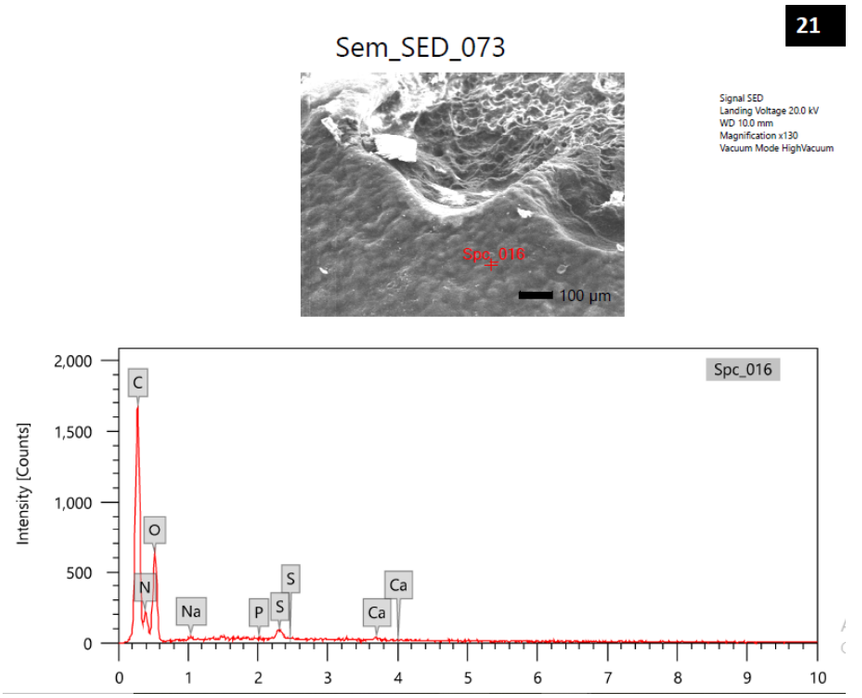

Fig. 21: A graph showing Ca and P analysis of EDX study of PRP2 group

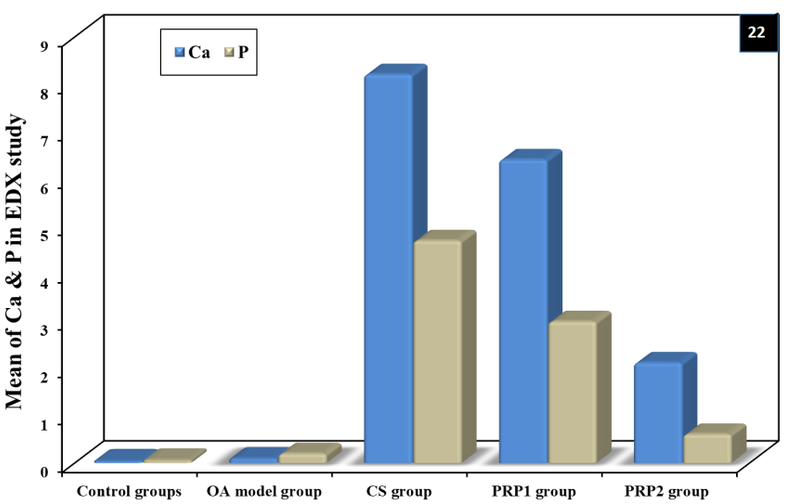

Fig. 22: A bar chart showing comparison between the different studied groups according to Calcium (Ca) and Phosphorus (P) in EDX study

Table I: Comparison between the different studied groups according to Toluidine blue and Masson's trichrome staining intensity

	Control groups (n = 30)	OA model group (n = 6)	CS group (n = 6)	PRP1 group (n = 6)	PRP2 group (n = 6)	Test of Sig.	*p*
Toluidine Blue stain							
Mean ± SD.	55.2 ± 6.6	18.7$^{\#}$ ± 3.4	24.5$^{\#}$ ± 7.2	22.8$^{\#}$ ± 7.2	30.7$^{\#@}$±11.1	F= 65.946*	<0.001*
Median (Min. – Max.)	54.8 (45.8–66.1)	19.3 (13.7–23.4)	26.5 (12.7–32)	23.2 (14 – 31.7)	29 (15.2–46.3)		
Masson's trichrome stain							
Mean ± SD.	0.1 ± 0.1	0.2 ± 0.2	84.6 ± 15.7	79.6 ± 16.9	60.7 ± 14.2	H= 37.012*	<0.001*
Median (Min. – Max.)	0 (0 – 0.3)	0.2 (0 – 0.4)	89.9$^{\#@}$ (61.1–99.9)	78.6$^{\#@}$ (56.6–99.3)	65.2$^{\#@}$ (34.1–72.7)		

H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test)
F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
p: *p value* for comparing between the studied groups
#: Significant with Control groups @: Significant with OA model group
$: Significant with CS group ♦: Significant with PRP1 group
*: Statistically significant at $p \leq 0.05$

Table II: Comparison between the different studied groups according to parameters of modified Mankin grading score and OARSI score

	Control groups (n = 30)	OA model group (n = 6)	CS group (n = 6)	PRP1 group (n = 6)	PRP2 group (n = 6)	F	*p*
Mankin score: Cartilage Structure (5)							
Mean ± SD.	0 ± 0	2.5$^{\#}$ ± 0.6	4$^{\#@}$ ± 1.3	5$^{\#@\$}$ ± 0	3.3$^{\#@\blacklozenge}$ ± 0.5	230.09*	<0.001*
Median (Min. – Max.)	0 (0 – 0)	2.5 (2 – 3)	4.5 (2 – 5)	5 (5 – 5)	3 (3 – 4)		
Chondrocytes (3)							
Mean ± SD.	0 ± 0	1.3$^{\#}$ ± 0.5	2.3$^{\#@}$ ± 0.8	2.7$^{\#@}$ ± 0.5	2.3$^{\#@}$ ± 0.5	114.58*	<0.001*
Median (Min. – Max.)	0 (0 – 0)	1 (1 – 2)	2.5 (1 – 3)	3 (2 – 3)	2.0 (2 – 3)		
Toluidine blue stain (5)							
Mean ± SD.	0 ± 0	2$^{\#}$ ± 0.6	2.3$^{\#}$ ± 0.5	3.5$^{\#@}$ ± 0.8	1.7$^{\#@}$ ± 0.5	130.0*	<0.001*
Median (Min. – Max.)	0 (0 – 0)	2 (1 – 3)	2 (2 – 3)	3 (3 – 5)	2 (1 – 2)		
Tidemark (1)							
Mean ± SD.	0 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	-	-
Median (Min. – Max.)	0 (0 – 0)	1 (1 – 1)	1.0 (1 – 1)	1 (1 – 1)	1 (1 – 1)		
Score (15)							
Mean ± SD.	0 ± 0	6.7$^{\#}$ ± 0.8	9.7$^{\#@}$ ± 0.8	12.2$^{\#@\$}$±0.8	8.3$^{\#@\$\blacklozenge}$±0.8	1171.9*	<0.001*
Median (Min. – Max.)	0 (0 – 0)	6.5 (6 – 8)	9.5 (9 – 11)	12 (11 – 13)	8.5 (7 – 9)		
OARSI score: Grade (6)							
Mean ± SD.	0 ± 0	2.7$^{\#}$ ± 0.5	3.5$^{\#@}$ ± 0.6	5$^{\#@\$}$ ± 0	2.7$^{\#\$\blacklozenge}$ ± 0.5	532.9*	<0.001*
Median (Min. – Max.)	0 (0 – 0)	3 (2 – 3)	3.5 (3 – 4)	5 (5 – 5)	3 (2 – 3)		
Stage (4)							
Mean ± SD.	0 ± 0	2.5$^{\#}$ ± 0.6	2.3$^{\#}$ ± 0.5	3.7$^{\#@\$}$ ± 0.5	2.5$^{\#\blacklozenge}$ ± 0.6	232.75*	<0.001*
Median (Min. – Max.)	0 (0 – 0)	2.5 (2 – 3)	2 (2 – 3)	4 (3 – 4)	2.5 (2 – 3)		
Score (24)							
Mean ± SD.	0 ± 0	6.2$^{\#}$ ± 1.6	8$^{\#}$ ± 1.1	18.3$^{\#@\$}$±2.6	6.7$^{\#\blacklozenge}$ ± 2	320.87*	<0.001*
Median (Min. – Max.)	0 (0 – 0)	6 (4 – 9)	8 (6 – 9)	20 (15 – 20)	6 (4 – 9)		

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
p: *p value* for comparing between the studied groups
#: Significant with Control groups @: Significant with OA model group
$: Significant with CS group ♦: Significant with PRP1 group
*: Statistically significant at $p \leq 0.05$

Table III: Comparison between the different studied groups according to gross macroscopic score

Gross macroscopic score	Control groups (n = 30)	OA model group (n = 6)	CS group (n = 6)	PRP1 group (n = 6)	PRP2 group (n = 6)	F	*p*
Mean ± SD.	0 ± 0	2.2$^{\#}$ ± 0.8	4.3$^{\#@}$ ± 0.8	5$^{\#@}$ ± 0	2.5$^{\#\$\blacklozenge}$ ± 1.4	154.56*	<0.001*
Median (Min. – Max.)	0 (0 – 0)	2 (1 – 3)	4.5 (3 – 5)	5 (5 – 5)	2 (1 – 5)		

H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test)
F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
p: *p value* for comparing between the studied groups
#: Significant with Control groups @: Significant with OA model group
$: Significant with CS group ♦: Significant with PRP1 group
*: Statistically significant at $p \leq 0.05$

Table IV: Comparison between the different studied groups according to Calcium (Ca) and Phosphorus (P) in EDX study

	Control groups (n = 30)	OA model group (n = 6)	CS group (n = 6)	PRP1 group (n = 6)	PRP2 group (n = 6)	H	*p*
Ca							
Mean ± SD.	0.1 ± 0	0.1 ± 0.1	8.2 ± 5.3	6.4 ± 3.3	2.1 ± 4.5		
Median (Min. – Max.)	0.1 (0 – 0.1)	0.1 (0.1 – 0.2)	9.2$^{\#@}$ (0.1 – 13.7)	7.8$^{\#@}$ (0.2 – 9.1)	0.3$^{\#}$ (0 – 11.2)	33.146*	<0.001*
P							
Mean ± SD.	0.1 ± 0	0.2 ± 0.2	4.7 ± 3.9	3 ± 2.1	0.6 ± 0.6		
Median (Min. – Max.)	0.1 (0 – 0.1)	0.1 (0 – 0.5)	5.4$^{\#@}$ (0.1 – 9.5)	3.8$^{\#@}$ (0.4 – 5.2)	0.3$^{\#}$ (0.1 – 1.5)	29.661*	<0.001*

H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test)
F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
p: *p value* for comparing between the studied groups
#: Significant with Control groups @: Significant with OA model group
$: Significant with CS group ♦: Significant with PRP1 group
*: Statistically significant at $p \leq 0.05$

DISCUSSION

Knee OA is a challenging disorder of the entire joint that can cause irreversible structural changes including cartilage loss[4,43]. Those changes lead to many clinical symptoms which can reduce the quality of life, such as joint stiffness, effusion, loss of function and pain[2]. Pain is the key symptom in knee OA, nevertheless the structural pathology underlying this pain is not completely studied[4].

Knee OA has recently become a significant healthcare issue and also a financial burden for the global economy due to high costs of long term therapy in addition to the great effects on patient's activity. Thus, there are noticeable governmental efforts as well as pressing socioeconomic need to enhance both prevention and treatment of OA[4,16,25].

Intra-articular injections e.g. CS and PRP are used extensively as a safe and effective treatment of knee OA when the orally administrated drugs are impotent control symptoms[15]. To the best of our knowledge, very limited studies have compared between the structural changes of intra-articular injection of CS with that of PRP in OA. Triamcinolone hexacetonide was used in the current study being the most insoluble compound that has a longer duration of action and a low rate of systemic absorption[44]. Previous studies have reported that differences in the technique of PRP preparation, platelet count and number of injections can result in different outcomes[19,45]. Two different protocols, proved to be good in control of OA symptoms, were used in the current work[28,29].

The articular cartilage in the OA model group showed irregular surface and fibrillation with hypo-cellularity. Toluidine blue staining was reduced due to reduced matrix proteoglycan. In addition, the Mankin grading score and OARSI score were significantly high when compared to the control groups. This can be attributed to the mechanism of Monosodium iodoacetate (MIA) used for inducing OA model in the current study. MIA leads to death of chondrocytes as it breaks down the glycolytic pathway through inhibiting glyceraldehydes-3 phosphate dehydrogenase in the Krebs cycle of chondrocytes[6,46]. Death of chondrocytes will result in hypo-cellularity and articular surface irregularities, fibrillation in addition to reduced matrix staining due to decreased production of proteoglycans.

Other results of OA model group were in line with the histological studies. The gross macroscopic score was significantly high in comparison with the control groups. The articular surface was irregular and had many depressions when examined by stereomicroscope and scanning electron microscope (SEM).

Histological examination of the CS group and PRP1 groups revealed disorganization of the articular cartilage suggesting that neither CS nor PRP1 had positive effect on the structure of the articular cartilage. Thus, the chondrocytes were still unable to produce sufficient proteoglycans in cartilage matrix resulting in hypo-cellularity, reduced articular cartilage thickness and diminished matrix staining by toluidine blue. Mankin grading score and OARSI score were still higher than the control groups.

Pannus- like tissue (fibroblasts, collagen fibers and blood vessels) has appeared inside the joint cavity of CS group and PRP1 group. Tsai *et al*[47]. have attributed the presence of this pannus- like tissue to vascular endothelial growth factor (VEGF), which is one of the growth factors of PRP. Moreover, Filardo *et al*[48]. have reported that VEGF can endanger homeostasis and regeneration of the articular cartilage. The existence of collagen fibers in this pannus- like tissue was confirmed by positive Masson trichrome staining inside the joint cavity which was highly significant compared to the control groups, indicating presence of fibrosis.

Moreover, this pannus-like tissue in the articular cartilage can elaborate collagenolytic enzymes that can lead to loss of collagen of the articular cartilage[49,50]. This can explain thinning of the articular cartilage, denudation in histological results, as well as ulcerations and cartilage loss in gross macroscopic examination that resulted in significantly high gross macroscopic scores in CS and PRP1 groups. SEM results of these groups also showed loss of articular cartilage integrity with multiple elevations, depressions and gutters.

Loss of areas of the articular cartilage then exposure of the underlying subchondral bone in CS and PRP1 groups may have resulted in high calcium (Ca) and phosphorus (P) elements in EDX study, compared to the control groups and even to OA model group where there was no complete loss of the articular cartilage[51,52].

PRP2 group revealed similar histological results to PRP1 group in some areas, while other areas showed preservation of the thickness and smooth surface of the articular cartilage. Mankin and OARSI mean scores were still significantly higher than the mean of control groups. Hyper-cellularity and chondrocytes cluster formation may indicate some tissue repair by restoration of the activity of chondrocytes[6]. This may emphasize the improvement of the quality of the articular cartilage due to release of proteoglycans and other matrix components resulting in better toluidine blue staining.

Gross macroscopic appearance and SEM studies have shown much less surface irregularities in comparison with PRP1 group. Ca and P mean levels was not significant with the mean of OA model group.In addition, the means of Mankin, OARSI grading score and gross macroscopic score were significantly lower than the means of CS and PRP1 groups suggesting more or less preservation of the articular cartilage. These better results of PRP2 group than PRP1 group could be attributed to the different protocols of preparation that can result in different composition of growth factors[19,45,53].

On the contrary, some previous studies have stated that CS and PRP can provide structural improvement in the osteoarthritic knees[6,30,31]. However, a study by Kumar *et al*[54] showed that CS may not give significant improvement in structural damage of osteoarthritic knee joints. Hermeto *et al*[36] have also found that there was structural disorganization and marked hypo-cellularity in PRP groups. In addition, Huang *et al*[12]. Reported that PRP injection had no improving effect in radiographs of OA patients. Moreover, recent guidelines by osteoarthritis research international society (OARSI) and American college of rheumatology (ACR) don't recommend PRP injection as treatment to Knee OA[17,18].

This can be explained as despite CS and PRP are excellent at alleviation of pain in OA, no previous studied proved a correlation between relief of pain and limitation of the cartilage damage as concomitant results of treatment with CS or PRP[32].

The current work has concluded that neither CS nor PRP could put an end to the progression of the structural damage of the articular cartilage in knee OA. Moreover, different PRP preparation techniques gave different results. Thus, more research is needed for development of a new treatment modality that can prevent, protect the articular cartilage damage, improve the patient's lifestyle, and postpone the need for arthroplasty.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير الكورتيكوستيرويد مقابل البلازما الغنية بالصفائح الدموية لعلاج التهاب المفاصل المستحدث فى مفصل الركبة فى الجرذان البيضاء البالغة. دراسة هيستولوجية و تشريحية

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المقدمه: يعتبر التهاب مفصل الركبة من أكتر الامراض التنكسية شيوعآ. ويتم استخدام الكورتيكوستيرويد و البلازما الغنية بالصفائح الدموية عادة لتخفيف الالام الناتجة عن التهاب مفصل الركبة.

الهدف من البحث: أجريت هذه الدراسة لاستكشاف الدور العلاجي المحتمل للكورتيكوستيرويد و البلازما الغنية بالصفائح الدموية فى علاج التهاب المفاصل المستحدث فى مفصل الركبة فى الجرذان البيضاء البالغة.

مواد وطرق البحث: تم استحداث التهاب المفاصل فى مفصل الركبة عن طريق حقن خلات اليود احادية الصوديوم ثم تم تجميع الدم من اثنى عشرمن ذكور الجرذان البالغة من أجل تحضير البلازما الغنية بالصفائح الدموية. وقد تم تقسيم اربعة وخمسين من اناث الجرذان الى المجموعة المرجعية, مجموعة التهاب المفاصل المستحدث, و المجموعات المعالجة بالكورتيكوستيرويد أو البلازما الغنية بالصفائح الدموية. وتم ذبح الجرذان بعد ٢٨ يوما ثم استخدمت مفاصل الركبة اليسرى من أجل الدراسة الهيستولوجية والفحص بالميكروسكوب الضوئى بعد ان تم صبغها بصبغتى الهيماتوكسيلين والايوسين لفحص التغيرات الهيكلية وبصبغة التولويدين الزرقاء لتقييم البروتيوجليكان داخل الغضروف و ايضا بصبغة ماسون تراي كروم من أجل الكشف عن التليف. تم استخدام مفاصل الركبة اليمنى للفحص التشريحى و الفحص بالميكروسكوب الاليكترونى الماسح. فى النهاية، تم تحليل النتائج النهائية احصائيآ ثم تفسيرها .

النتائج: تسبب حقن خلات اليود احادية الصوديوم داخل مفصل الركبة فى تغيرات غضروفية شبيهة للتغيرات الناتجة عن مرض التهاب مفصل الركبة. الكورتيكوستيرويد او البلازما الغنية بالصفائح الدموية نتج عنه تغيرات هيكلية هيستولوجية مثل انخفاض سمك الغضروف, الرجفان أو ظهور تليف. بالاضافة الى زيادة نقاط مقياسي مانكين و أورسى فى تلك المجموعات المعالجة مقارنة بالمجموعة المرجعية. كما حدثت أيضا تغيرات تشريحية تم تأكيدها بالميكروسكوب الالكترونى الماسح.

الاستنتاج: الكورتيكوستيرويد و البلازما الغنية بالصفائح الدموية لم يمتلكا القدرة على ايقاف تقدم التغيرات الهيكلية الناتجة عن التهاب مفصل الركبة بالاضافة الى ذلك فان اختلاف طرق اعداد البلازما الغنية بالصفائح الدموية قد نتج عنه اختلاف فى النتائج.