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Research Article
Anti-arthritic Effects of Platelets Rich Plasma and Hyaluronic Acid on Adjuvant-induced Arthritis in Rats

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Abstract

Background and Objective: Rheumatoid arthritis (RA) is the commonest form of chronic inflammatory autoimmune disease with unknown etiology that attacks joint tissue for unknown reasons and is influenced by genetic as well as environmental factors. The present study was aimed to investigate the anti-rheumatic effect of platelets rich plasma (PRP) and intra gel hyaluronic acid (HA) on complete freund's adjuvant (CFA) induced RA. **Materials and Methods:** A total of 32 adult male rats were categorized into 4 groups (n = 8): Normal control received vehicles only, RA group received CFA (0.1 mL) injected in the right hind paw, PRP group (50 µL) intra-articularly injected into the right inflamed knee and paw and HA group (50 µL) of HA sodium salt into the inflamed joints. All treatments were administered for 4 weeks. Serum MDA, GSH and GPx levels were investigated using ELISA as oxidative stress biomarkers. Serum CRP, IL-1β, TNF-α, COX-2, ALOX5, MMP-9 and LT-C4 were also determined using ELISA as inflammatory biomarkers. Specific rheumatoid marker COMP and MMP-3 expression levels were detected using qRT-PCR. Histopathological changes in the joint tissues were examined using hematoxylin and eosin (H and E) stain. X-ray was also performed in examining the bone and joints. **Results:** PRP and HA significantly reduced serum MDA and increased GSH and GPx levels. Tested drugs also significantly reduced CRP, IL-1β, TNF-α, COX-2, ALOX5, MMP-9 and LTC4 serum levels. Administration of PRP or HA normalized COMP and MMP-3 expression levels in CFA-induced RA rats. Histopathological analysis and X-ray of PRP or HA groups showed a gradual reduction in joint damage and cartilage erosion. **Conclusion:** In conclusion, PRP and HA possess antirheumatoid effects in CFA-induced RA rats which is mediated through anti-inflammatory, antioxidant and modulation of COMP and MMP-3 expression levels.

Key words: Rheumatoid arthritis, platelets rich plasma, hyaluronic acid, autoimmune, chronic inflammation, joint degeneration, anti-inflammatory

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

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

INTRODUCTION

Rheumatoid arthritis (RA) is a deep-seated systemic progressive autoimmune inflammatory disease that primarily goals synovial joints, resulting in pain and functional limitations. It is the most communal inflammatory arthritis and a noteworthy cause of morbidity and premature mortality[1]. RA affects about 0.5-1% of the population worldwide[2] and leads to permanent disability resulting from progressive and irreversible joint destruction[3].

A pathological symptom of RA is marginal juxta-articular bone erosion, which is positioned in the cartilage-pannus junction. Synovitis-induced inflammatory infiltrates, detected in the trabecular bone close to cartilage-pannus junction, are widely considered to contribute to juxta-articular bone erosion[4].

Drugs, like non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids have been broadly utilized for the handling of RA; however, none of the current drugs can prohibit cartilage degeneration or completely cure this intractable disease. Furthermore, numerous of the drugs are not ideal for long-term curing because of their adverse effects[5].

Several approaches have been encouraged for treating RA. Platelet-rich plasma (PRP) is encouraged as an ideal autologous biological blood derived product that can be exogenously useful to various tissues, where it produces high concentrations of platelet-derived growth factors that improve wound healing, bone healing and tendon healing. When platelets are activated, this causes growth factors to be released and so this initiates the body's natural healing response[6,7].

Autologous platelet-rich plasma has gained publicity as a clinical handling in diverse applications such as soft and hard tissue applications in nearly all fields of surgery, most notably in acute surgical conditions and in the management of chronic non healing wounds[8,9]. Surgeons are utilizing several platelet-rich plasma concoctions to take advantage of an autologous fibrin clot that aid in hemostasis along with providing growth factors in the form of platelet release to potentially boost healing.

The achievement of this curing lies not only in the features of PRP but also in its truthful application. An inappropriate application of PRP, can lead to an unsuccessful biological response and unsatisfactory clinical consequences. Intra-articular infiltrations reach the cartilage and the synovial membrane, boosting a change in the biological milieu of the knee that decelerates the development of arthritis and modulate the clinical features[10].

Regardless of PRP extending medical availability and the euphoria for utilize, their clinical efficiency is scientifically unverified and most patient data are empiric. Nevertheless, intra-articular injection of hyaluronic acid (HA) to cure RA has been also utilized worldwide for pain relief and symptomatic treatment[11].

HA is a naturalistic polysaccharide pertinence to the family of glycosaminoglycans that is accountable for maintaining the viscosity of synovial fluid and the lubricating and damping possessions of articular cartilages like shock absorption and better load distribution[12].

Intra-articular inoculations of HA have been utilized to cure numerous joint disorders including osteoarthritis and significant effectiveness of this procedure was previously recognized[13,14].

In this respect, HA may have mechanical, metabolic or biological actions. The mechanical connotation is that HA preserves lubrication and therefore minimizes wear of articular surfaces, however it plays a vital metabolic role in nutrition of the articular disc and cartilage[15]. Concerning the biological influence, studies *in vitro* and *in vivo* established significant action of HA in the blocking of different inflammatory mediators for example TNF-α, PGE2, IL-1, IL-17 and inducer of nitric oxide synthase (iNOS), in addition to constraining expression of enzymes that degrade the extracellular matrix like matrix metalloproteinases[16] (MMPs) 1, 2, 3, 9 and 13.

HA affects several procedures as well as the induced expression of cytokines, the stimulation of immune and angiogenic processes. A vital function of HA is its contribution in chondrogenesis and providing adequate lubrication of joints, which diminishes the friction between the moving bones, thereby lessening the process of osteoarthritis and RA[17].

Therefore, the purpose of this study was to investigate and compare between the effect of the less costly PRP or the intra gel hyaluronic acid in ameliorating the pain, inflammation and joint destruction caused by induction of complete freund's adjuvant RA rat model for their antioxidant and anti-inflammatory effects.

MATERIALS AND METHODS

Duration and year of study: This study was carried out from the month of December, 2017 to June, 2019 at Biology laboratory of Zoology Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Egypt.

Ethics: The procedures of this study were approved by the Research Ethics Committee of Helwan and Ain Shams

University. The Guidelines for the Care and Use of Laboratory Animals declared by the National Institutes of Health guide and use of Laboratory animals (NIH Publications No. 8023, revised 1978) were followed in all the experimental procedures.

Animals: Adult male albino rats (n = 40) weighing (155±10 g) representing 8-9 weeks of age were obtained from the Animal House Colony of the National Research Centre, Giza, Egypt. Animals were housed in controlled conditions including temperature (25±2°C), humidity (60±10%) and normal photoperiod (12/12 h light-dark cycles) with free access to food and water. The animals were allowed to adapt for 2 weeks to the novel environment before any experiment was performed.

Induction and evaluation of arthritis: Arthritis was induced in rats as previously described by Snekhalatha *et al.*[18]. Rats were injected with 0.1 mL of complete freund's adjuvant (sigma chemical Co. St. Louis, MO, USA) into the right hind paw. To increase the severity of arthritis, a booster injection with 0.1 mL of emulsion was administered in the same manner on day 5. The rats were observed one to four times per week for the evaluation of arthritic signs.

Preparation of platelet-rich plasma (PRP): Blood was collected by cardiac puncture from 8 normal control rats and stored in tubes with sodium citrate anticoagulant[19]. The procedure was conducted in sterile conditions and lysing, or damaging platelets were avoided to prevent the loss of their ability to secrete growth factors. Tubes with collected blood samples were centrifuged at 900 rpm for 10 min to separate red, white and platelet cells[20]. The upper portion of the supernatant, up to the edge of the fog zone that corresponds to plasma and platelets, was collected into new tubes[21]. These tubes were centrifuged at 1800 rpm for 10 min[22]; about 50% of the plasma portion was removed and stored in another tube (portion considered as plasma poor in platelets, PPP). The remaining material containing the platelet pellet was resuspended, originating the PRP portion[21]. PPP presented 733,000 platelets μL^{-1}, PRP presented 2,300,000 platelets μL^{-1} and was considered suitable for the purpose of the study[22].

Injection of PRP: About 50 µL of PRP was intra-articularly injected slowly into the right inflamed knee and ankle joints using a 0.5 mL injection syringe. The injections were performed once[23].

Hyaluronic acid induction: Intra gel hyaluronic acid sodium salt was manufactured by BSA farmaceutici Italia srl, Via Martiri di Cefalonia, 2-26900 Lodi-Italy. The animals were anesthetized and then received an intra-articular injection of 50 µL of intragel of hyaluronic acid sodium salt into the inflamed knee and ankle joint[24].

Experimental settings

Animals and treatment: A total of 32 rats were randomly categorized into 2 groups. 8 rats injected with saline and served as normal control group, while 24 rats were injected with complete Freund's adjuvant for RA: After 2 weeks animals were divided into 3 groups: RA served as positive control rats (n = 8), RA+PRP rats (n = 8) intra-articularly injected with 50 µL of PRP and RA+HA group rats (n = 8) injected with 50 µL of intragel of hyaluronic acid sodium salt into the inflamed joint.

Animals were sacrificed after 4 weeks of the mentioned treatments. They were anesthetized and blood was collected from the retro-orbital venous plexus for preparation of serum by centrifugation at 3000 rpm for 10 min at 4°C using a cooling centrifuge (Sigma 3-30k, USA) and stored at -80°C until analysis. Soon after, the whole knee joints including synovium, bones and ankle joints were separated washed with 10% saline solution. One part was kept in 10% buffered formalin solution for the X-ray and histopathological examination. The other part was used for determination of cartilage oligomeric protein (COMP) and matrix metalloproteinase-3 (MMP-3) expression level using quantitative real time-polymerase chain reaction (qRT-PCR).

Biochemical determinations

Determination of oxidative stress biomarkers: Serum malondialdehyde (MDA) content was determined by a colorimetric method using OxiSelect TBARS assay kit purchased from Cell Biolabs, San Diego, CA, USA. Following the method of Armstrong and Browne[25], the reduced glutathione (GSH) concentration in serum was measured quantitatively using the OxiSelect™ Total Glutathione (GSSG/GSH) Assay kit purchased from Cell Biolabs Co., San Diego, CA, USA according to the method described by Anderson[26], Serum glutathione peroxidase (GPx) level was estimated by enzyme-linked immunosorbent assay (ELISA) using the kit purchased from CUSABIO Co., (USA), according to the manufacturer's instructions provided with the kits.

Determination of auto-immune and serum cytokines levels: Serum c-reactive protein (CRP) was measured by ELISA method using rat c-reactive protein assay kit purchased from

GenWay, Inc. Co., San Diego, CA, USA, according to the method of Eckersall[27]. Serum interleukin-1β (IL-1β) was determined by ELISA technique using rat IL-1β assay kit purchased from IBL Co., Ltd. Aramachi, Takasaki-Shi, Gunma, JAPAN according to manufacturer's instruction provided with the kit. Tumor Necrosis Factor-alpha (TNF-α) was determined by ELISA technique using Rat TNF-α Assay Kit purchased from IBL Co., Ltd. Aramachi, Takasaki-Shi, Gunma, JAPAN according to manufacturer's instruction provided with the kit.

Determination of enzymes activities and rheumatoid biomarkers: Serum cyclooxygenase-2 (COX-2) estimated by ELISA using the kit purchased from IBL Co., Ltd. Aramachi, Takasaki-Shi, Gunma, JAPAN, according to the manufacturer's instructions provided with the kits. Serum arachidonate 5-lipoxygenase (ALOX5) was assayed by ELISA using the kit purchased from CUSABIO Co., (USA), according to the manufacturer's instructions provided with the kit. Serum matrix metalloproteinase 9 (MMP-9) was determined by ELISA technique using kit purchased from Cloud-Clone Corp Co. USA, according to the manufacturer's instructions provided with the kit. Serum leukotriene C4 (LT-C4) was determined by ELISA technique using Rat LT-C4 Kit purchased from Glory Science Co., Ltd, USA, according to manufacturer's instruction.

Molecular study for determination of COMP and MMP-3 expression levels

Total RNA extraction: Total RNA was extracted from joint samples using SV Total RNA Isolation System (Promega, Madison, WI, USA) according to manufacturer's instruction. The RNA concentrations and purity were measured with an ultraviolet spectrophotometer.

Complementary DNA (cDNA) synthesis: The cDNA was synthesized from 1 µg RNA using SuperScript® III First-Strand Synthesis System as described in the manufacturer's protocol (#K1621, Fermentas, Waltham, MA, USA). In brief, 1 µg of total RNA was mixed with 50 µM oligo (dT) 20, 50 ng μL^{-1} random primers and 10 mM dNTP mix in a total volume of 10 µL. The mixture was incubated at 56°C for 5 min and then placed on ice for 3 min. The reverse transcriptase master mix containing 2 µL of 10×RT buffer, 4 µL of 25 mM $MgCl_2$, 2 µL of 0.1 M DTT and 1 µL of SuperScript® III RT (200 U μL^{-1}) was added to the mixture and was incubated at 25°C for 10 min followed by 50 min at 50°C.

Real-time quantitative PCR: Real-time PCR amplification and analysis were performed using an Applied Biosystem with software version 3.1 (StepOne™, USA). The reaction contained SYBR Green Master Mix (Applied Biosystems), gene-specific primer pairs which are shown in Table 1 and designed with Gene Runner Software (Hasting Software, Inc., Hasting, NY) from RNA sequences from the gene bank. All primer sets had a calculated annealing temperature of 60°. Quantitative RT-PCR was performed in a 25 µL reaction volume consisting of 2X SYBR Green PCR Master Mix (Applied Biosystems), 900 nM of each primer and 2 µL of cDNA. Amplification conditions were: 2 min at 50°, 10 min at 95° and 40 cycles of denaturation for 15 sec and annealing/extension at 60° for 10 min. Data from real-time assays were calculated using the v1·7 sequence detection software from PE Biosystems (Foster City, CA). Relative expression of studied gene mRNA was calculated using the comparative Ct method. All values were normalized to GAPDH, which was used as the control housekeeping gene and reported as fold change over background levels detected in the diseased groups.

Bone and joint examination by X-ray: For the determination of radiological evaluation of arthritis, X-ray photographs of the right hind limbs of the control and experimental rats were taken using Toshiba (Model No kx0-15R). This was performed at the National Research Center, Medical Services department, bone density measuring unit, Dokki, Giza, Egypt.

Histopathological examination of joint and bone tissues: The right knee joint was histologically examined in each animal. Joints and bones were fixed in 10% buffered formalin, decalcified in a solution containing 35 mL formic acid and 65 mL sodium citrate for 10 days. Tissue samples were then washed with water and dehydrated through ascending grades of ethyl alcohol and then cleared with xylene. Paraffin blocks were cut at 6 µ thickness using Cambridge rocking microtome and fixed to slides then stained with hematoxylin and eosin (H and E) and Lesions were observed by Nikon microscope[28].

Table 1: List of primers used in qRT-PCR

Genes	Forward primers	Reverse primers
GAPDH	5-GTA TTG GGC GCC TGG TCA CC-3	5-CGC TCC TGG AAG ATG GTG ATG G-3
COMP	5-GGT TCC CTG GCA TAA TCT GA-3	5-GTC ATC GAG ACC CCA AGG TA-3
MMP-3	5-CTG GAA TGG TCT TGG CTC AT-3	5-CTG ACT GCA TCG AAG GAC AA-3

Statistical analysis: The obtained results are represented as the mean±standard errors. Data were analyzed by one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 19 followed by least significant difference (LSD) to compare significance between groups. The level of significance was assorted at $p<0.05$. Percentage the difference representing the percent of variation with respect to the corresponding control group was calculated according to the following equation[29]:

$$\text{Difference (\%)} = \frac{\text{Treated value-Control value}}{\text{Control value}} \times 100$$

RESULTS

Morphological feature: The overall condition of rats was monitored during treatment. The onset time of the paw edema for most rats was 10 days after adjuvant administration. Markers of inflammation were evident in the joints of adjuvant induced arthritis rats. The first signs appeared in metatarsal joints of the paws and progressed to include larger joint area. Morphological alterations were vindicated as shown in Fig. 1a. These were highly represented in adjuvant induced arthritis rats in the form of swollen, inflamed and deformed paws and knees (Fig. 1a). Post-treatment with PRP or intra gel (HA), redness or inflammatory response of the soft tissue around the right hind paw was markedly ameliorated as shown in the Fig. 1a.

Radiological evaluation of RA: According to the radiograph in Fig. 1b marked phalangeal and tarsal changes in the right hind paws of RA rats were observed and appeared as malformed tarsi compared to control (Fig. 1b). PRP and intra gel (HA) treated groups showed remedy incurred changes (Fig.1b).

PRP and HA attenuated oxidative stress and improved antioxidant defense mechanism: To study the role of oxidative stress in CFA-induced RA pathogenesis and the possible antioxidant effects of the tested drugs serum MDA, reduced GSH and GPx levels were determined (Table 2). CFA administration markedly increased serum oxidative stress as proved by significant elevation ($p<0.05$) in serum MDA level and significant reduction ($p<0.05$) in serum GSH and GPx level

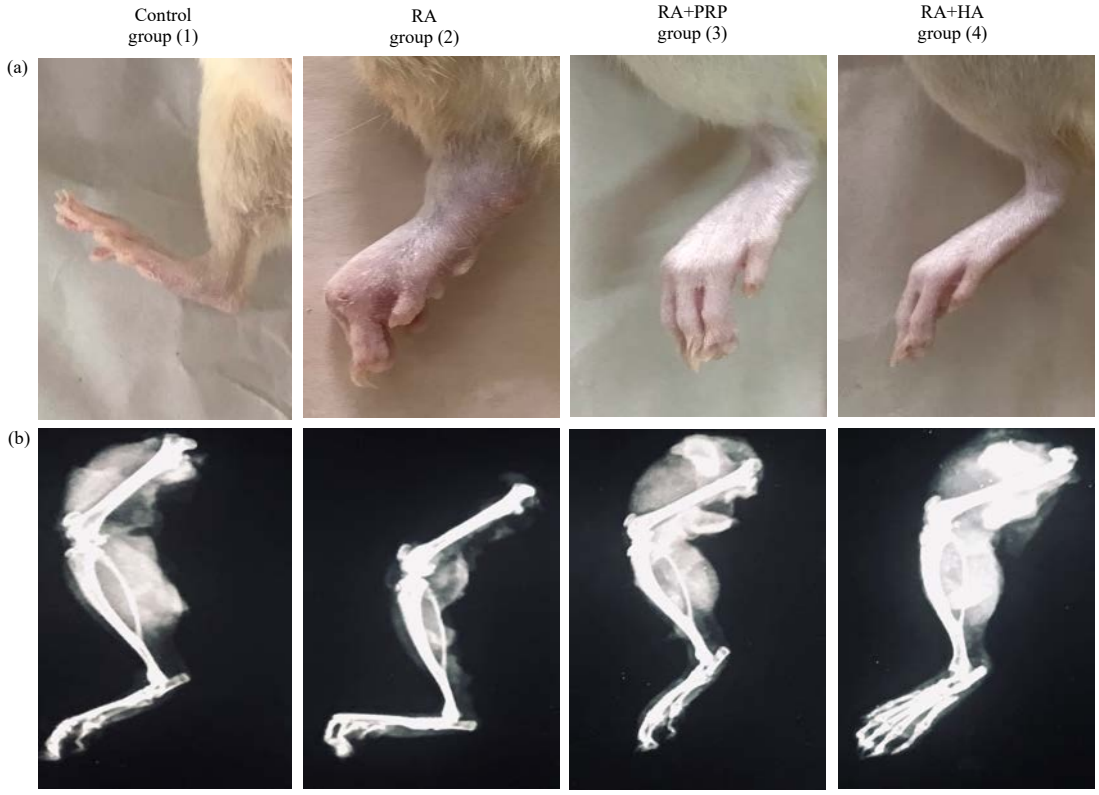

Fig. 1(a-b): (a) Morphology of hind paws of the 4 rat groups and (b) Hind paws of the 4 rat groups imaged by X-ray

Table 2: PRP and HA treatments attenuated oxidative stress and improved serum antioxidant defense mechanism

	Parameters		
Groups	Malondialdehyde (μM mL^{-1})	Reduced glutathione (μM mL^{-1})	Glutathione peroxidase (IU mL^{-1})
Control	0.46±0.06	3.15±0.37	1.65±0.27
RA	1.07±0.13^{a} (130.60%)^{a}	1.67±0.13^{a} (-46.98%)^{a}	0.95±0.10^{a} (-42.42%)^{a}
RA+PRP	0.69±0.05^{bc} (-35.51%)^{b} (13.11%)^{c}	2.43±0.23^{bc} (45.50%)^{b} (-10.00%)^{c}	1.32±0.22^{bc} (38.95%)^{b} (-6.38%)^{c}
RA+HA	0.61±0.03^{b} (-42.99%)^{b}	2.70±0.26^{b} (61.67%)^{b}	1.41±0.17^{b} (48.42%)^{b}

^{a}Significant change at p<0.05 in comparison with the normal control group, ^{b}Significant change at p<0.05 in comparison with the RA group, ^{c}Significant change at p<0.05 in comparison with the RA+HA group

Table 3: PRP and HA attenuated adjuvant-induced inflammatory response

	Parameters		
Groups	C-reactive protein (ng mL^{-1})	IL-1β (Pg mL^{-1})	TNF-α (Pg mL^{-1})
Control	1.60±0.13	2.19±0.35	4.50±0.65
RA	3.58±0.31^{a} (123.75%)^{a}	5.28±0.46^{a} (141.09%)^{a}	9.08±0.89^{a} (101.77%)^{a}
RA+PRP	2.18±0.21^{bc} (-39.11%)^{b} (10.10%)^{c}	3.79±0.57^{bc} (-28.21%)^{b} (19.93%)^{c}	6.88±0.66^{bc} (-24.22%)^{b} (11.50%)^{c}
RA+HA	1.98±0.17^{b} (-44.69%)^{b}	3.16±0.41^{b} (-40.15%)^{b}	6.17±0.45^{b} (-32.05%)^{b}

^{a}Significant change at p<0.05 in comparison with the normal control group, ^{b}Significant change at p<0.05 in comparison with the RA group, ^{c}Significant change at p<0.05 in comparison with the RA+HA group

Table 4: PRP and HA treatments improved enzymes activities disorders and rheumatoid biomarkers in serum induced by adjuvant administration

	Parameters			
Groups	COX-2 (ng mL^{-1})	ALOX5 (ng mL^{-1})	MMP-9 (ng mL^{-1})	LTC4 (pg mL^{-1})
Control	1.12±0.17	0.40±0.02	0.85±0.13	641.50±33.08
RA	2.48±0.36^{a} (121.43%)^{a}	0.78±0.09^{a} (95.00%)^{a}	1.53±0.29^{a} (80.00%)^{a}	1062.71±30.07^{a} (65.66%)^{a}
RA+PRP	1.74±0.23^{bc} (-29.84%)^{b} (-10.82%)^{c}	0.57±0.08^{bc} (-26.92%)^{b} (11.76%)^{c}	1.28±0.25^{bc} (-16.34%)^{b} (7.84%)^{c}	820.50±20.66^{bc} (-22.79%)^{b} (-1.33%)^{c}
RA+HA	1.57±0.27^{b} (-36.69%)^{b}	0.51±0.07^{b} (-34.61%)^{b}	1.02±0.17^{b} (-33.33%)^{b}	831.54±26.32^{b} (-21.75%)^{b}

^{a}Significant change at p<0.05 in comparison with the normal control group, ^{b}Significant change at p<0.05 in comparison with the RA group, ^{c}Significant change at p<0.05 in comparison with the RA+HA group

respectively as compared to normal control group. Treatment with PRP or HA frustrated CFA-induced oxidative stress as established by significant lessening (p<0.05) of CFA-induced elevations in serum MDA levels and elevation of CFA-induced reduction (p<0.05) in serum reduced GSH levels and GPx levels as compared to RA group. The tested treatment with PRP exerted effects nearly close to that of HA which was used as a standard treatment in MDA level and in GSH and GPx levels as compared to HA treated group.

PRP and HA attenuated the inflammatory response: The present data showed on examining inflammatory response that rats with RA presented a significant increase (p<0.05) in serum levels of CRP, IL-1β and TNF-α as compared to the normal control group. Handling with PRP or HA significantly diminished (p<0.05) serum levels of CRP, IL-1β and TNF-α as compared to RA group (Table 3). The tested curing with PRP exerted effects nearly close to that of HA which was used as a standard treatment.

To assess the induction of RA in rats using CFA and the possible effect of the tested drugs in treatment of RA, specific rheumatoid parameters and some enzymes were determined namely: COX-2, ALOX5, MMP-9 and LTC4 in serum. The obtained results showed that CFA significantly increased (p<0.05) serum levels of COX-2, ALOX5, MMP-9 and LTC4 versus to normal control group (Table 4). Treatment with either PRP or HA significantly reduced (p<0.05) serum COX-2, ALOX5, MMP-9 and LTC4 levels as compared to RA group. The effect of the tested PRP treatment was comparable to that of the standard HA. Noteworthy, there are no significant changes (p>0.05) in serum COX-2, ALOX5, MMP-9 and LTC4 levels between RA group treated with PRP and the RA group treated with HA (Table 4).

Effect of PRP and HA on COMP and MMP-3genes expression: In the existing study, COMP and MMP-3 expression values were measured using qRT-PCR in order to investigate the molecular mechanism of the anti-rheumatoid effect exerted by PRP. The present data shows that CFA displayed significant amplification (p<0.05) in the expression of COMP and MMP-3 genes versus to normal control group. Handling of RA group with PRP or HA resulted in a significant suppression (p<0.05) in the expression of COMP and MMP-3 genes for PRP treatment and for HA treatment relative to RA group (Fig. 1, Table 5). The tried curing with PRP exerted similar possessions close to those of HA which was used as a standard

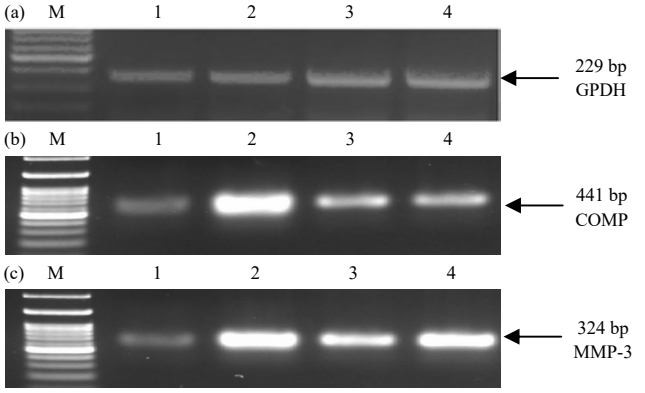

Fig. 2(a-c): Agarose gel electrophoresis showing (a) GPDH, (b) COMP and (c) MMP-3 mRNA expression in the joint tissue by RT-PCR analysis
Lane 1: Control group, Lane 2: RA group, Lane 3: RA+PRP group, Lane 4: RA+HA group, M: DNA ladder (100 bp)

Table 5: Anti-rheumatoid effect of PRP and HA on COMP and MMP-3 genes expression in bone tissue of RA rat model

	Parameters	
Groups	COMP expression	MMP-3 expression
Control	1.23±0.18	1.01±0.07
RA	5.12±0.82[a] (316.26%)[a]	4.71±0.61[a] (366.33%)[a]
RA+PRP	2.41±0.32[bc] (-52.92%)[b] (-14.23%)[c]	1.82±0.23[bc] (-61.35%)[b] (-5.20%)[c]
RA+HA	2.81±0.27[b] (-45.11%)[b]	1.92±0.31[b] (-59.23%)[b]

[a]Significant change at p<0.05 in comparison with the normal control group, [b]Significant change at p<0.05 in comparison with the RA group, [c]Significant change at p<0.05 in comparison with the RA+HA group

treatment versus to the RA+HA group. Nonetheless, there were no noteworthy changes (p>0.05) in the expression of COMP and MMP-3 genes between RA group treated with PRP and the RA group treated with HA (Fig. 2, Table 5).

Histological studies: Histological examination of the knee joints from the right hind limbs from rats in the normal group revealed normal histological architecture as shown in Fig. 3a, b. Conversely, adjuvant induced RA rats showed marked abnormal histological features that appeared as massive reduction of cartilage thickness; increase of surface erosion and significant synovial cell proliferation (Fig. 3c). The synovial stroma showed a considerable number of inflammatory cells as well as congested blood vessels (Fig. 3d).

Various pathological changes including hyperplasia, pannus formation and multiple well-defined aggregates of epithelioid cell (granulomas) were noticed (Fig. 3d). Inflammation extended into the subcutaneous tissue and vascularity increased where inflammation extended into the bony spicules and cartilage (Fig. 3e). Bone erosion was seen. Likewise, loss of hematoxylin-Eosin staining of the cartilage layers, as well as complete disruption of the normal cartilage architecture was noticed (Fig. 3f). Additionally, synovial hyperplasia seemed and the synovial layer presented enlargement of synovial cell lining layer, increased synovial vascularity, giant cells and mononuclear inflammatory infiltration encompassed predominately of lymphocytes and histiocytes (Fig. 3d). The boundary between the fibrous and cartilage layer was unclear. The fibrous layer pervaded into bone marrow (Fig. 3f).

On the contrary, the joints were much less inflamed and showed gradual reduction in joint damage yet cartilage erosion were noticed with no significant increase in blood vessel proliferation, fibrocyte proliferation or granulocyte infiltration observed in either the PRP or the intra gel (HA) group (Fig. 3g, h). Mild synovial proliferation was only detected. Cell morphology was near to ordinary excluding for a small number of inflammatory cells infiltrations. No typical pannus was formed and the cartilage surface was smooth with no obvious damage and no obvious bone erosion (Fig. 3g, h).

DISCUSSION

Rheumatoid arthritis (RA) is considered a form of progressive inflammatory autoimmune disease[30] characterized by bone erosion and destruction, cartilage degradation, synovial inflammation and stiffness of joints[31]. In the present investigation, new treatment PRP was considered and compared with the traditional highly cost HA therapy where it evidenced antirheumatic activity through attenuating oxidative stress, alleviating the inflammatory cascade and modulation of COMP and MMP-3 genes expressions.

Adjuvant-induced arthritis is an ordinarily utilized experimental model for preclinical studies. Due to its short duration of testing, easy measurement and similarities to human RA, this model has been utilized frequently to estimate therapeutic agents[32]. The pannus formation, cartilage erosion, inflammation and hyperplasia exhibited that the adjuvant-induced arthritis model exactly resembles RA[33,34]. Consequently, in the existing study, a rat model of arthritis induced by adjuvant was recognized and used by knee and metatarsal footpad intradermal injection of FCA. X-ray and histological investigations performed in this investigation presented that the RA rats revealed apparent degeneration of joint structure and narrowed joint space in the hind paw, knee and ankle.

In addition, according to histopathological analysis of the knee joint, anomalous hyperplasia of synovial membranes,

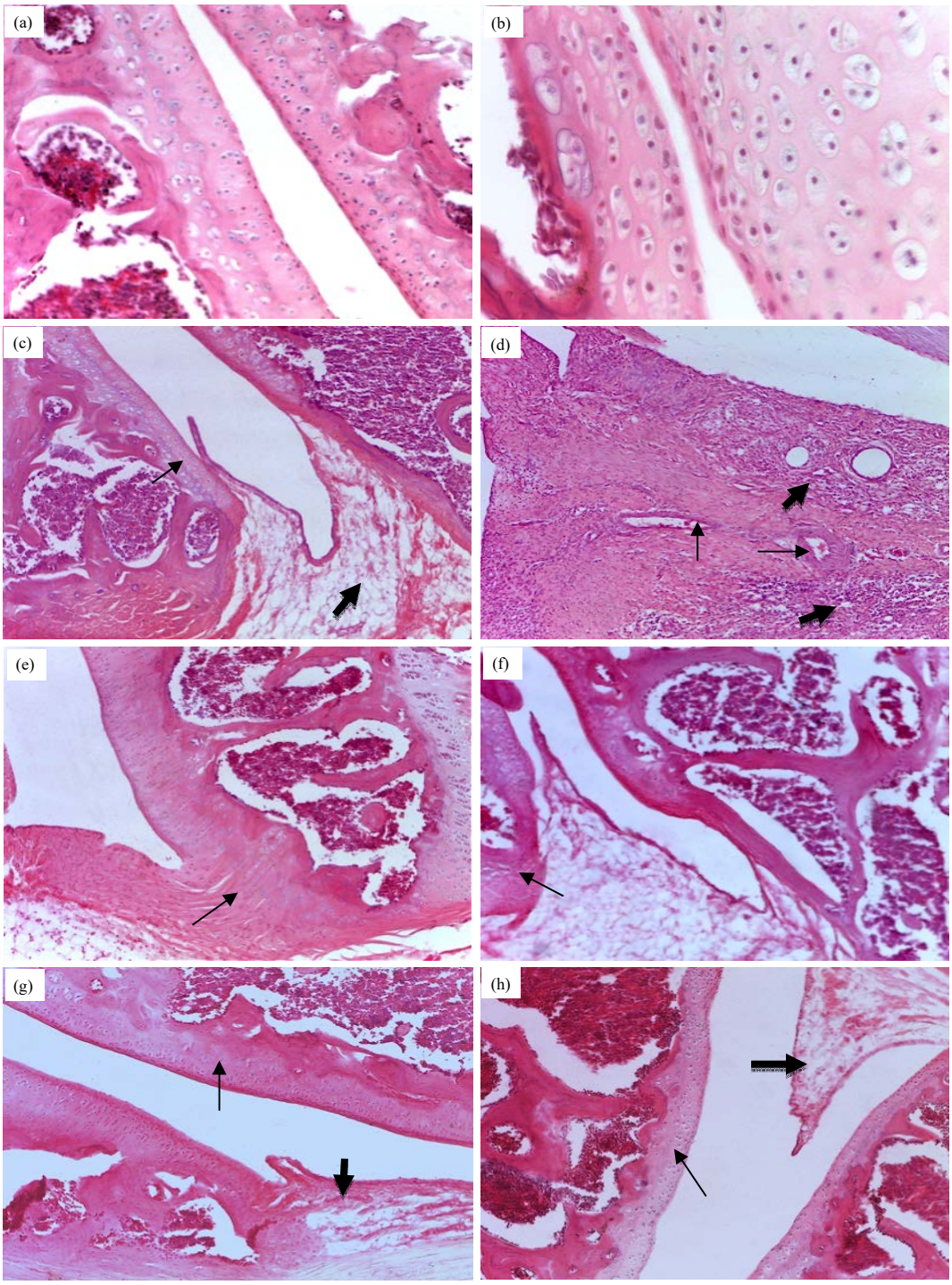

Fig. 3(a-h): Histological examination of knee joints, (a, b) Normal control group showing normal knee joint architecture, (c) RA group showing proliferating synoviocytes (thick arrow) and thinning of articular cartilage plates (thin arrow), (d) RA group showing a considerable number of inflammatory cells (thick arrow) as well as congested blood vessels in the synovial stroma (thin arrow), (e) RA group showing inflammation extended into the bony spicules and cartilage (thin arrow), (f) RA group showing complete disruption of the normal cartilage architecture (thin arrow), (g) RA group treated with PRP and (h) RA group treated with HA intra gel showing mild synovial proliferation (thick arrow) and mild cartilage regeneration (thin arrow)

collagen fiber deposition, large numbers of cartilage, bone erosion and inflammatory cells were noticed in the model group. These phenomena were also described in previous studies[35] and were declined following PRP and HA treatment.

In the current study, dealing with PRP noticeably abridged paw swelling and arthritis index induced by FCA confronted with RA group. This study further confirmed its therapeutic effect by histopathological examination. It was proved effective in reducing the degree of synovial hyperplasia, cartilage and bone destruction, inhibiting inflammatory cell infiltration of the synovium and periarticular inflammation.

In the existing investigation, the RA induction utilizing FCA was mediated through diverse pathways. One of these pathways was initiation of oxidative stress as showed by significant increment in serum MDA with significant diminution in serum reduced GSH and GPx levels. Activated T-cell and macrophage infiltration, chronic inflammation of joints and tissues are primary symptoms of rheumatoid arthritis[36]. Augmented free radical output from inflammatory site results to strengthen rheumatoid arthritis and reduced cellular antioxidant level is a ticklish risk factor for rheumatoid arthritis[37]. Ozturk *et al*.[38] reported that the lipid peroxidation was higher in rheumatoid arthritis compared to normal control. Membrane fatty acid oxidation produces lipid peroxide radicals and causes the cell membrane damage.

The antirheumatic effect of PRP could be attributed to its antioxidant effect. In the present study PRP administration to RA rats significantly reduced the elevations in serum MDA level induced by CFA, while significantly increased the reduction in reduced GSH and GPx levels induced by CFA. These obtained data proved the antioxidant properties of the tested drug. These findings are in accordance with those of Hesami *et al*.[39], who recorded that dealing of rats with PRP for 5 weeks after CCl_4-induced toxicity led to the reduction of hepatotoxicity probably due to lipid peroxidation inhibition and efficacious recovery of the antioxidant protection system. Over and above, Martins *et al*.[40] noticed an enhancement of the enzymatic and non-enzymatic antioxidant values upon curing of skeletal muscle injuries using PRP. Additionally, Bakacak *et al*.[41] stated that intraperitoneal PRP dealing reduced oxidative stress index and total oxidant status in ischemia and ischemia/reperfusion injury in rat ovary. It has been revealed that PRP may prohibit oxidative damage through the instigation of transcription factor nuclear factor erythroid 2-related factor 2(Nrf2) antioxidant response element signaling[42]. Moreover, numerous growth factors emitted from PRP can activate cell activation and activate related signal path as well as the phosphatidylinositol-3 kinase (PI3K)/Akt pathway which can decrease ROS manufacture and increase the level of resistance to oxidation[43].

Furthermore, handling of RA group with HA resulted in significant reduction in serum MDA level and significant augmentation in serum GSH and GPx levels. Parallel results were distinguished when an oxidative insult was induced by either peroxynitrite or xanthine oxidase/hypoxanthine where HA ameliorated the negative effects of reactive oxygen and nitrogen species on mtDNA integrity, mtDNA repair, ATP production and cell viability[44].

Another proposed mechanism for induction of RA via injection of FCA is the stimulation of inflammatory cascade[45,46]. In the present study we measured serum CRP, IL-1β, TNF-α, COX-2, ALOX5 and LTC4 levels as markers for inflammation. Current data demonstrations that FCA recorded a significant increment in CRP, IL-1β and TNF-α levels in serum of rats proving the induction of inflammation in the rat joints. Similar to the present results, previous data showed that FCA significantly elevated serum levels of TNF-α and IL-6 in rats[47].

RA could be activated by a T-cell response to infectious agents. The motivated T-cells activate macrophages, monocytes and synovial fibroblasts and then release a number of pro-inflammatory cytokines and mediators which are responsible for the pain, destruction of bone and cartilage that can lead to severe disability[48] Specifically, pro-inflammatory cytokines TNF-α and IL-1β are two essential pro-inflammatory cytokines that have been revealed to contribute to the clinical manifestations of RA and play a primary role in mediating the pathophysiological processes underlying inflammation and tissue destruction in RA[49]. In addition, TNF-α and IL-1β induce receptor activator of nuclear factor-kB on macrophages, which differentiate into osteoclasts that resorb and destroy bone[50]. IL-1β and TNF-α likewise participate to inflammation by encouraging the releasing of cell- adhesion molecules, other cytokines, chemokines and chemokine receptors, angiogenic agents and minor inflammatory mediators (e.g., PGE2) through the stimulation of cyclo-oxygenase type 2 (COX-2). PGE2 has been exposed to play an vital role in inflammatory arthritis[51]. In addition, COX-2 was considered as one of the vital mediators in the development of RA[52]. Inflammatory activity could be ultimately transferred into a destruction of bone and cartilage[53]. Therefore, it has been proposed that inhibition of these pro-inflammatory cytokines and mediators might be an effective strategy for the treatment of RA.

The present results revealed that PRP has immunomodulatory effect represented by a significant improvement in FCA-induced elevations in serum CRP, IL-1β, TNF-α, COX-2, ALOX5 and LTC4. PRP was notably able to

ameliorate these alterations possibly via its anti-inflammatory activity. Preceding studies backing this theory where PRP was stated to rise the intracellular expression of the anti-inflammatory mediators (IL-4, IL-10 and IL-13) known to play a considerable role in constraining inflammation; the anti-inflammatory effect of PRP had been determined due to the presence of hepatocyte growth factor (HGF)[54].

In this study, the treatment of RA group with HA recorded significant depletion in serum CRP, IL-1β, TNF-α, COX-2, ALOX5 and LTC4. Though, the anti-inflammatory influence of HA was formerly detected but the mechanism was not completely understood; it may be due to a HA and cyclooxygenase-2/prostaglandin (COX/PG) regulatory network, via CD44 expression and further modulated by the presence of inflammatory cytokines and mediators[55]. Furthermore, in osteoarthritis (OA), intra-articular injection of HA is often used to decrease inflammation and promote regeneration[56]. It has been found that the use of HA with molecular weight (MW) between 50 and 120 kDa efficiently suppresses OA pathology-related chemokines and cytokines. HA can treat meniscus tears and cartilage breakdown as well as decrease OA-related immune cells[57]. Furthermore, the use of HA has been revealed to increase the release of growth factors from PRP, which is expected to reduce the time needed for healing[58].

Current results presented that FCA significantly increased serum MMP-9 level and caused significant amplification in the expression of COMP and MMP-3 genes compared to the normal group. In accordance with the current results, previous investigations revealed that FCA induced RA in rats and significantly increased serum MMP-3 and COMP[59]. Studies demonstrated that pro inflammatory cytokines activate synovial fibroblast to emission cartilage-degrading enzyme MMP-3 which is well-thought-out a main marker for cartilage impairment[60]. Cartilage contains a non-collagenous matrix COMP that diffuses into the blood in case of cartilage damage[61]. Moreover, IL-1β stimulation is well known to induce MMPs secretions in RA[62]. MMPs are usually activated by pro-inflammatory cytokines and have been found to be down regulated in response to the anti-TNF therapy[63].

Current study data demonstrated that treatment of RA group with PRP resulted in significant depletion in serum MMP-9 level associated with significant reduction in the expression of COMP and MMP-3 genes. Lately, PRP was stated to have anabolic influences on cartilage and is being used in clinical practice for the treatment of degenerative articular lesions in osteoarthritis, in tendinitis and other sports injuries[64,65]. Moussa *et al.*[66] initiated that PRP amplified the proliferation of chondrocytes, diminished apoptosis and increased autophagy in human osteoarthritic chondrocytes. Moreover, PRP produced a dose-dependent lessening in MMP-3, MMP-13, IL-6 and COX-2 while increased TGF-β, collagen, aggrecan and intracellular anti-inflammatory cytokines IL-4, IL-10 and IL-13.

In the current study, the RA group curing with HA exhibited significant reduction in serum MMP-9 level accompanying with significant lessening in the expression of COMP and MMP-3 genes. This result coincides with that in the study of Kim *et al.*[67], who stated that dealing of osteoarthritis rats with HA resulted a significant decrease in the mRNA expression levels of MMP-3, COX-2, IL-6 and TNF-α. Furthermore, depended on the *in vitro* results of Kim *et al.*[67], who performed *in vivo* animal studies to authorize the anti-inflammatory influences and the preventive effects of cartilage degradation of HA in OA rat models. Chen *et al.*[68], found that HA provided chondrocyte phenotype and enhanced chondrogenesis.

X-ray and histological examinations showed that in PRP and HA-treated RA rats, bone erosion and degradation were scarcely detected and the extent of the narrowing of joint space was detected to be fairly small. High molecular weight HA appears to diminish arthritis at early stage of expansion by blocking the initiators of the inflammatory response, while, by this same way, may not when disease is fully developed. In this context the large use of HA, by joint injection, in human RA and OA may mainly induce pain and swelling reduction[69]. In this situation the mechanism could be various, as the local injection may provide a high HA concentration to the inflamed joints. In addition, as HA binds large amounts of H_2O, the joint amelioration may be due especially to its rheological/fluidizing properties than to a direct interaction with receptor or other protein structures, although other specific unknown interactions couldn't be excluded.

In the current treatise, the dealing of adjuvant Induced RA rats with HA was eligible to diminish inflammation and cartilage erosion. HA is directly accountable for the viscoelastic characteristics of synovial fluid. Consequently, it appears that the purpose of intra-articular therapy with HA is to help replace synovial fluid that has lost its viscoelastic properties. Even though exogenous HA treatment may extend this consequence, the effective protective action of HA is not fully understood[69].

HA likewise has a structural function in cartilage formation in which glycosaminoglycans are bound to HA by link-proteins to form the matured articular proteoglycan. Extra possibility is that HA injection stimulates increased natural HA manufacture by the synovial cells[70].

PRP is well-thought-out as a co-adjunct RA curing when injected into the joint. It produces growth factors for instance epidermal growth factor (EGF) that act on bone proliferation, promoting the formation of periosteal bone and increasing endosteal resorption. The absence of receptors for this growth factor can cause a delay in the primary ossification of cartilage and recruitment of osteoclasts and osteoblasts[71]. In the existing study PRP injected in the adjuvant induced RA rats presented that the joints were much less inflamed and exhibited gradual reduction in joint damage, PRP was efficacious in controlling synovial membrane hyperplasia through constraining the releasing of nuclear factors and blocking the action of pro-inflammatory metabolites that play key roles in the initiation and perpetuation of cellular chronic inflammation in RA[72].

Altogether, these results direct that PRP may be a chief blood derived product that can be useful in initiating the body's natural healing response by reaching the cartilage and the synovial membrane of the knee and decelerating the development of arthritis.

CONCLUSION

Intra-articular injections of PRP and HA provides hope for improvement or treatment of rheumatoid arthritis, so the results clarified that as long as both PRP and HA intra gel are nearly similar in the treatment and reducing RA symptoms due to their anti-inflammatory effects so the more expensive HA may be exchanged by the less costly and equally efficient PRP.

SIGNIFICANCE STATEMENT

This study discover the effective role of the low cost platelets rich plasma (PRP) which when being activated becomes beneficial to various tissues, as the released platelet-derived growth factors initiates the body's natural wound, bone, tendon healing response. This study will help the researcher to uncover the critical areas of treating the degenerative articular lesions of rheumatoid arthritis and other bone injury by intra-articular injection of PRP that many researchers were not able to explore. Thus, a new theory on PRP may be arrived.

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