Effect of Autologous Bone Marrow Derived Mesenchymal Stem Cells in Treatment of Rheumatoid Arthritis.

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Research Article

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Abstract

Background

Chronic inflammation causes articular bone and cartilage degeneration in people with rheumatoid arthritis (RA). Despite recent advances, management of RA is frequently accompanied with negative consequences, and ineffectiveness. Access to treatment is still hampered by many factors including financial restrictions. As a result, medications that reduce inflammation and bone resorption that are both safer and less expensive are required. Mesenchymal stem cells have recently been identified as a potential therapy for rheumatoid arthritis.

Aim of Study:

The aim of this study was to examine the anti-arthritic effect of rat bone marrow-MSCs, oligosaccharides and human placental extract, individually and combined on RA model, using Complete Freund’s adjuvant (CFA) induced arthritis in rats.

Materials and Methods

In female rats, rheumatoid arthritis was induced by injecting Complete Freund's adjuvant in the paw of hind limbs. Treatments with rat bone marrow-mesenchymal stem cells via intravenous route, oligosaccharides, and human placental extract were given individually and in combination via intra-peritoneal route. Measurement of complete blood picture (CBC), ESR, cortisol, urea, uric acid, liver and renal biochemical parameters were carried out to determine the safety and efficacy of the different combinations. Inflammatory cytokines TNF-α, IL-6 and IL-10 were measured to assess anti-inflammatory effects. Histopathological analysis of bone sections was carried out too.

Results

Our findings showed that adding innovative treatments involving oligosaccharides and human placental extract to rat-bone marrow mesenchymal stem cells in a CFA-induced arthritic rat model resulted in a favorable anti-arthritic inflammatory effect as shown in reduction of inflammatory cytokines, with complete safety on blood picture, renal and liver functions, cortisol, and ESR. Histopathological analysis went concurrent with these results showing healing and remodeling of osteoporotic lesions induced in arthritic rat model.

Conclusions

The combination of rBM-MSCs via intravenous route, with oligosaccharides, and human placental extract could be employed as a powerful therapeutic medication to treat rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA) is a systemic chronic inflammatory disease characterized by joint discomfort and swelling. Rheumatoid arthritis develops when the proinflammatory and anti-inflammatory states are out of balance, resulting in synovial membrane inflammation and joint destruction. One of the methods through which RA promotes
joint injury is increased expression of cytokines such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), IL-1b, and IL-1α.

Four classes of medications are used for treatment of RA: steroidal hormones, biological agents, immunosuppressants and anti-artrhetic drugs. Use of anti-arthritic drugs is limited due their high cost, side effects and low specificity. Antibodies like anti-TNF, anti-IL-1, and anti-IL-6 target and neutralize inflammatory cytokines. However, they are not always effective and have short-term effects; 50 percent of patients who are prescribed these treatments stop using them within two years due to a decrease in symptomatic relief or the absence of symptoms alleviation. Treatment of RA with mesenchymal stem cells (MSCs) is a multifaceted therapy. Administration of MSCs results in reduced levels of TNF-α in the circulation and can reduce the degree of arthritic inflammation up to 30% in most RA experimental models.

Other novel treatments for RA include human placental extract (HPE) and oligosaccharides (Os). HPE has an anti-inflammatory effect and may act by increasing growth of chondrocytes and repressing cartilage damage. Experiments performed on arthritic animal model showed that local injection of HPE reduced the symptoms of arthritis and the expression of inflammatory cytokines. Oligosaccharide (Os) therapy for RA proved to disrupt the glycosylation on the Fc domain of IgG, which is elevated in the serum and synovial fluid of rheumatoid patients.

The aim of this study was to examine the anti-arthritic effect of rat bone marrow-MSCs (rBM-MSCs), Os and HPE, individually and combined on RA model, using Complete Freund's adjuvant (CFA) induced arthritis in rats.

**Materials And Methods**

**Drugs and chemicals**

Sprague Dawley (SD) rats aged 8–12 weeks were used to isolate mesenchymal stem cells. Cells were extracted from the tibias and femurs and separated at room temperature using an 80 percent Percoll gradient centrifuge at 1300 rpm for 10 minutes. Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 g/mL streptomycin sulphate was used to wash cells in the low-density fraction (HyClone, USA). The cells were then seeded onto 10 cm culture dishes (SPL, South Korea) containing a control medium and grown as adherent cells for 3–4 weeks in a humidified atmosphere at 5% CO2 and 37 °C. Every three days, the media was replaced. Cells were subcultured at a ratio of 1: 4 for up to four passes after achieving a confluence of 70–80%. For the characterization of rat bone marrow derived mesenchymal stem cells (rBM-MSCs) from SD rats, anti-CD34 monoclonal antibody was employed as a negative control and anti-CD29 and anti-CD44 as positive markers. Only positive markers stained rBM-MSCs were included in the study.

Oligosaccharides (3′-Sialyllactose, Product no: A8681), CFA (product no F5881) were purchased from Sigma Aldrich Chemical Co USA, and HPE (Laennec) from Japan Bio Products. ELISA kits for estimating inflammatory cytokines and diagnostic kits for measuring liver enzymes and kidney functions were purchased from Abcam Company, United Kingdom.

**Animals and experimental design**

Eighty female Sprague Dawley (SD) rats weighing 150–200 g was housed in standard animal house conditions under relative humidity 30-70% and temperature 20-26°C according to animal research committee, 2021(ARC) temperature and humidity standards. Rats were allowed free access to water and standard pellet diet. An adaptation
period of one week was allowed before beginning the experiment. Experimental animals were kept and used in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

All experimental protocols were approved by the Ethics Committee of the Faculty of Pharmacy, Suez Canal University (Ismailia, Egypt) (code #201911PHDA3).

Rats were divided into eight groups of ten rats each as follows:

Group I: Negative control group.

Group II: Positive control group, rats were injected intradermally with a single dose of 0.1 mL CFA into right hind paw for induction of arthritis.

Group III: CFA induced arthritic rats treated with rBM-MSCs at the first day of the experiment intravenously (IV) (2 million / rat/ once /month).

Group IV: CFA induced arthritic rats treated with Os I.P(intraperitoneal) (0.2 mL / rat/) twice weekly for one month.

Group V: CFA induced arthritic rats treated with HPE I.P (0.4 mL / rat) once weekly for one month.

Group VI: CFA induced arthritic rats treated with rBM-MSCs (2 million/ rat/) once +Os (0.2 mL / rat) twice weekly for one month

Group VII: CFA induced arthritic rats treated with rBM-MSCs (2million/ rat) once/ +HPE (0.4 mL / rat) once weekly for one month

Group VIII: CFA induced arthritic rats treated with rBM-MSCs (2 million/once) +Os (0.2 mL / rat) twice weekly + HPE (0.4 mL / rat) once weekly for one month.

Measurement of biochemical parameters and inflammatory cytokines

At the appropriate time after 4 weeks and after 24 hours of the last dose, rats of all studied groups were anesthetized with light halothane inhalation. Blood samples were collected to do laboratory analysis for CBC, serum cortisol, erythrocyte sedimentation rate (ESR), serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), urea, uric acid, inflammatory cytokines TNF-α, IL-6 and IL-10 according to manufacturer instructions. Then, all animals were sacrificed by decapitation under anesthesia according to the Ethics Committee of the Faculty of Pharmacy, Suez Canal University (Ismailia, Egypt) (code #201911PHDA3).

Histopathologic analysis

Specimen preparation: the head of each femur of all animals was carefully dissected out and processed for histological studies. Each femur head was cleaned from attached muscle, trimmed or dissected as appropriate, and immediately fixed in 10% formal saline solution for 7 days. The specimens were washed by tap water for half an hour then they were decalcified in the chelating agent disodium EDTA. The head of femur of all animals was taken and immediately fixed in glutaraldehyde solution then prepared for scanning electron microscopy, the decalcified head of femur was cut longitudinally in a coronal plane along the central portion and specimens were processed to form paraffin blocks, Serial sections of 5 micrometer thickness were obtained and subjected to histological stained
sections Hematoxylin and Eosin (H & E): was used to demonstrate the general histological structures of rat’s head of femur among various experimental groups.

**Statistical analysis**

Data were processed using the Statistical Package for Social Science (SPSS), version 20. Results are expressed as mean ± SD. One-way analysis of variance, ANOVA, followed by least significant difference (LSD) post hoc test for multiple comparisons were employed for statistical analysis. A p value less than 0.05 was considered statistically significant.

**Results**

**Biochemical parameters in the studied groups**

Induction of arthritis with CFA resulted in reduction in hemoglobin concentration, mean corpuscular hemoglobin concentration (MCHC) and WBCs count and in an elevation in mean corpuscular volume and platelet count showing the best improvement in group treated with RBM-MSCs, Os and HPE (table 1).

Serum levels of liver enzymes (ALT and AST), urea and uric acids were significantly elevated in CFA induced arthritic group compared to negative control group. Combination therapy of RBM-MSCs, OS and HPE have proven the best effect in treatment of arthritic groups (table 2).

Erythrocyte sedimentation rate and cortisol markers were significantly higher in CFA induced arthritic group compared to negative control group. Combination therapy of rBM-MSCs, Os and HPE have proven the best effect in treatment of arthritic groups compared to other groups (table 3).

There was the best significant reduction in the serum levels of these inflammatory cytokines in the group receiving combination therapy of rBM-MSCs, Os, and HPE compared with other treated groups (table 4).

**Histopathological analysis of the studied groups**

**a: Photomicrograph of bone tissue of negative control group (fig1a)**

Photomicrograph of bone tissue showed normal cortical structures (blue arrow), normal osteocytes (black arrow) and normal haversian canals (yellow arrow). 400 X H&E stain

**b: Photomicrograph of bone tissue of CFA induced arthritic group (fig1b)**

Photomicrograph of bony tissue showed early osteodystrophic changes with characteristic endo-osteoblastic proliferation (orange arrow) and osteoporotic reactions (yellow arrow) in the form of osteocytes apoptosis (green arrows) and osteodegenerative areas with increase activity of the osteoclasts (red arrows) especially around the degenerated areas (green arrows). Bone matrix showed hypocellularity with deep bluish coloration denoting demineralization (blue arrow). 400 X H&E stain

**c: Photomicrograph of bone tissue of group treated with combination therapy of rBM-MSCs, Os and HPE (fig1c)**

Photomicrograph of bone tissue showed healing and remodeling processes of previous osteoporotic lesions with presence of a few reactive osteoclastic change (black arrow) and focal osteo-fibroblastic dysplasia and endosteoblastic hyperplasia (blue and brown arrows). A few bone cells still suffered osteo-degenerative changes
although the chondroplasty compensatory process is remarkable (red arrows). The haversian canals appear moderately dilated with hyperemic sinusoids (green arrows). 200, 400 H&E X stain

**Discussion**

Disease-modifying antirheumatic medications (DMARDs) and non-steroidal anti-inflammatory drugs (NSAIDs) are two of the few therapeutic options for RA, and they both focus on symptom control rather than preventing disease progression. Anti-TNFα, anti-IL-1, and anti-IL-6 biological antibodies are another RA therapeutic option. These drugs target and neutralize inflammatory cytokines. They don't, however, function in every circumstance. As a result, identifying effective treatments for patients who do not respond to currently available drugs is critical.

Our findings showed that adding innovative treatments involving Os and HPE to rBM-MSCs in a CFA-induced arthritic rat model resulted in a favorable anti-arthritic inflammatory effect, with complete safety on blood picture, renal functions, cortisol, ESR, and liver enzymes levels. In CFA-induced arthritic rat group II, increased levels of TNFα, IL-6, and IL-10 were observed. Group III (treated with rBM-MSCs) had lowered levels of inflammatory cytokines than the CFA arthritic group II.

Our findings backed up Zhang et al findings which showed that intravenous injection of MSCs into the body reduced systemic and joint inflammation. Group IV (treated with Os) demonstrated a significant anti-inflammatory effect. Our findings matched those of Rademacher et al who invented a way to treat immune complexes in a body fluid such as serum or synovial fluid by exposing them to an oligosaccharide with a structural component that inhibits or disrupts the degree of occupancy of the Fc carbohydrate binding site on IgG.

This beneficial antiarthritic impact was superior to therapy in group (III), with the better results in groups VI (treated with rBM-MSCs + Os) and the highest in group VIII (treated with rBM-MSCs + OS + HPE). Arthritic rats, group V (treated with HPE) showed an improvement in lowering inflammatory cytokines when compared to groups IV, III. Our findings were unlike those of Park et al who discovered that HPE had no anti-inflammatory effect in RA. The effect of this group was doubled in our study when rBM-MCs and Os were added to HPE which had shown the best potency against inflammatory cytokines generated in RA.

To demonstrate the safety of these treatments, we investigated several biochemical indicators, complete blood count, kidney functions (urea, uric acid), cortisol, ESR and liver enzymes (AST, ALT).

Groups III, IV, V, VI, VII, and VIII showed approximately identical improvements in exacerbated blood count levels, ESR, renal and liver function tests, occurred in group II with the most improvement in group VIII. We may deduce from these biochemical measurements that rBM-MCs, Os, and HPE had the best anti-inflammatory effect with a good biochemical result in general.

Furthermore, histopathological analysis of bone tissue sections from rats treated with BM-MSCs, OS, and HPE in groups III, IV, and V revealed less improvement in osteodystrophy changes than multiple combinations in groups VI, VII, and VIII, which showed the best healing and remodeling processes.

**Conclusions**

In conclusion, rBM-MSCs, OS, and HPE combination had the most powerful healing effect on CFA-induced arthritis in animal models, better than individually or in other combinations, with no effect on liver enzymes or renal
functions.

Future clinical trials will be needed to better understand the mechanism of action of this combination in arthritis, with the goal of producing medications with higher therapeutic efficacy and fewer side effects than current therapies.

Declarations

Ethics approval: All experimental protocols were approved by the Ethics Committee of the Faculty of Pharmacy, Suez Canal University (Ismailia, Egypt) (code #201911PHDA3).

Experimental animals were kept and used in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

Consent to Participate: Not applicable, because it is an animal study

Consent for publication: Not applicable, because it is an animal study

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Declaration of conflicting interests: The authors declare that there is no conflict of interest.

Data availability statement: Data generated and analyzed during this study are included in the published article.

Authors’ contributions: All authors contributed in the design of the study. All authors participated in data collection and analysis, data interpretation and manuscript writing.


- AE. SB. , and AM. suggested the protocol and developed the codes, supervised the research.
- YE., SB., NM. and AM. performed the experiments.
- AE., DA., AR., and YE. wrote and revised the manuscript.
- AE. is the corresponding author

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I confirm hereby that the manuscript has not been submitted or is not simultaneously being submitted elsewhere, is not at the time of submission under consideration by another journal or other publication, and that no portion of the data has been or will be published elsewhere while the manuscript is under review by the journal, unless rejected or withdrawn by the author.

References


### Tables

**Table 1: Complete blood picture of the studied groups**

<table>
<thead>
<tr>
<th></th>
<th>RBC (x 10⁶/µL)</th>
<th>Hb (g/dL)</th>
<th>Hct (%)</th>
<th>MCV (fL)</th>
<th>MCHC (g%)</th>
<th>PLT (x 10³/µL)</th>
<th>WBC (x 10³/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td>6.02±0.09</td>
<td>13.39±0.19</td>
<td>34.0±0.65</td>
<td>55.2±0.61</td>
<td>39.5±1.36</td>
<td>561.8±30.22</td>
<td>10.21±0.89</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td>5.77±0.13</td>
<td>11.99±0.32</td>
<td>33.45±1.12</td>
<td>58.04±1.61</td>
<td>20.8±1.69</td>
<td>999.3±55.13</td>
<td>7.78±0.64</td>
</tr>
<tr>
<td><strong>Group 3</strong></td>
<td>6.74±0.13</td>
<td>13.66±0.06</td>
<td>34.98±0.11</td>
<td>51.88±1.03</td>
<td>39.11±1.09</td>
<td>518.2±44.08</td>
<td>9.8±0.98</td>
</tr>
<tr>
<td><strong>Group 4</strong></td>
<td>6.22±0.12</td>
<td>12.59±0.3</td>
<td>33.02±0.57</td>
<td>53.08±0.38</td>
<td>38.11±0.65</td>
<td>801.8±12.04</td>
<td>14.94±.89</td>
</tr>
<tr>
<td><strong>Group 5</strong></td>
<td>6.54±0.12</td>
<td>14.24±0.21</td>
<td>36.12±0.49</td>
<td>54.38±0.59</td>
<td>39.31±0.77</td>
<td>751±14.63</td>
<td>13.08±0.45</td>
</tr>
<tr>
<td><strong>Group 6</strong></td>
<td>5.99±0.15</td>
<td>13.08±0.16</td>
<td>33.17±0.58</td>
<td>55.91±1.12</td>
<td>39.3±0.99</td>
<td>665.1±37.12</td>
<td>9.66±0.41</td>
</tr>
<tr>
<td><strong>Group 7</strong></td>
<td>6.33±0.09</td>
<td>13.36±0.1</td>
<td>34.32±0.38</td>
<td>53.78±0.81</td>
<td>39.13±0.87</td>
<td>661.8±20.35</td>
<td>9.84±0.42</td>
</tr>
<tr>
<td><strong>Group 8</strong></td>
<td>6.5±0.06</td>
<td>14.11±0.09</td>
<td>35.64±0.27</td>
<td>55.38±0.21</td>
<td>39.67±0.98</td>
<td>651±23.77</td>
<td>15.26±0.99</td>
</tr>
</tbody>
</table>

Data are represented as mean ± SD and analyzed using ANOVA followed by LSD post-hoc test at p value <0.05, n=10 for each group. RBC, red blood cells count, Hb, hemoglobin, Hct, hematocrit value, MCV, mean corpuscular volume, MCHC, mean corpuscular hemoglobin concentration, PLT, platelet count, WBC, white blood cells count.

Group 1, negative control group, group 2, CFA induced arthritic group, group 3, treated with MSCs, group 4, treated
with Os, group 5, treated with HPE, group 6, treated with MSCs and Os, group 7, treated with MSCs and HPE, group 8, treated with MSCs, Os and HPE. Different letters in each column indicate a significant difference. a, statistical significant difference from negative control, b, statistical significant difference from positive control, c, statistical significant difference from the group treated with MSCs, d, statistical significant difference from the group treated with Os, e, statistical significant difference from the group treated with HPE, f, statistical significant difference from the group treated with MSCs and Os, g, statistical significant difference from the group treated with MSCs and HPE.

Table 2: Serum levels of liver enzymes (ALT and AST), urea and uric acid in the studied groups

<table>
<thead>
<tr>
<th></th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>Urea (mmol/L)</th>
<th>Uric acid (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>66.71±5.79</td>
<td>55.56±3.52</td>
<td>28.37±2.1</td>
<td>3.68±0.62</td>
</tr>
<tr>
<td>Group 2</td>
<td>143±20</td>
<td>455±17</td>
<td>38.48±3</td>
<td>5.04±0.38</td>
</tr>
<tr>
<td>Group 3</td>
<td>76±12.76</td>
<td>92.94±17.54</td>
<td>21.48±4.62</td>
<td>3.29±0.41</td>
</tr>
<tr>
<td>Group 4</td>
<td>92.02±5.95</td>
<td>134.34±16.67</td>
<td>19.48±1.45</td>
<td>4.09±0.5</td>
</tr>
<tr>
<td>Group 5</td>
<td>88.64±4.4</td>
<td>106.1±9.66</td>
<td>26.12±3.14</td>
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<tr>
<td>Group 6</td>
<td>107.25±17</td>
<td>134.5±22.86</td>
<td>29.99±1.57</td>
<td>3.82±0.35</td>
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<tr>
<td>Group 7</td>
<td>94.88±18.8</td>
<td>125.18±15.71</td>
<td>35.92±3</td>
<td>3.76±0.44</td>
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<tr>
<td>Group 8</td>
<td>96.91±10.9</td>
<td>129.34±21.44</td>
<td>25.28±5.91</td>
<td>3.86±0.14</td>
</tr>
</tbody>
</table>

Data are represented as mean ± SD and analyzed using ANOVA followed by LSD post-hoc test at \( p \) value <0.05, \( n=10 \) for each group. ALT, alanine aminotransferase, AST, aspartate aminotransferase. Group 1, negative control group, group 2, CFA induced arthritic group, group 3, treated with MSCs, group 4, treated with Os, group 5, treated with HPE, group 6, treated with MSCs and Os, group 7, treated with MSCs and HPE, group 8, treated with MSCs, Os and HPE. Different letters in each column indicate a significant difference. a, statistical significant difference from negative control, b, statistical significant difference from positive control, c, statistical significant difference from the group treated with MSCs, d, statistical significant difference from the group treated with Os, e, statistical significant difference from the group treated with HPE, f, statistical significant difference from the group treated with MSCs and Os, g, statistical significant difference from the group treated with MSCs and HPE.
Table 3: Serum ESR1, ESR2 and cortisol in the studied groups

<table>
<thead>
<tr>
<th></th>
<th>ESR 1 (mm/h)</th>
<th>ESR 2 (mm/h)</th>
<th>Cortisol (nmol/L)</th>
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</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td>6±0.82</td>
<td>10.8±0.92</td>
<td>5.25±0.36</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td>8.9±0.9</td>
<td>14.5±0.96</td>
<td>20.5±0.89</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td><strong>Group 3</strong></td>
<td>12±1</td>
<td>20.9±1.52</td>
<td>5.92±0.88</td>
</tr>
<tr>
<td></td>
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<td>b</td>
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<tr>
<td><strong>Group 4</strong></td>
<td>12.4±1.05</td>
<td>22.3±1.07</td>
<td>8.65±0.82</td>
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<tr>
<td><strong>Group 5</strong></td>
<td>10.3±1.16</td>
<td>16.7±1.06</td>
<td>6.45±0.68</td>
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<tr>
<td><strong>Group 6</strong></td>
<td>10.5±1.08</td>
<td>18.3±1.25</td>
<td>8.3±0.82</td>
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<td>abce</td>
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<tr>
<td><strong>Group 7</strong></td>
<td>11.7±0.82</td>
<td>20.1±1.4</td>
<td>3.13±0.36</td>
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<tr>
<td><strong>Group 8</strong></td>
<td>10.4±1.17</td>
<td>19.5±1.08</td>
<td>3.77±0.99</td>
</tr>
<tr>
<td></td>
<td>abcdg</td>
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<td>abcdeg</td>
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</tbody>
</table>

Data are represented as mean ± SD and analyzed using ANOVA followed by LSD post-hoc test at p value <0.05, n=10 for each group. ESR, erythrocyte sedimentation rate. Group 1, negative control group, group 2, CFA induced arthritic group, group 3, treated with MSCs, group 4, treated with Os, group 5, treated with HPE, group 6, treated with MSCs and Os, group 7, treated with MSCs and HPE, group 8, treated with MSCs, Os and HPE. Different letters in each column indicate a significant difference. a, statistical significant difference from negative control, b, statistical significant difference from positive control, c, statistical significant difference from the group treated with MSCs, d, statistical significant difference from the group treated with Os, e, statistical significant difference from the group treated with HPE, f, statistical significant difference from the group treated with MSCs and Os, g, statistical significant difference from the group treated with MSCs and HPE.

Table 4: Inflammatory cytokines IL-10, TNF-α and IL-6 in the studied groups
<table>
<thead>
<tr>
<th>Group</th>
<th>IL-10 (pg/mL)</th>
<th>TNF-α (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>27.18±1.75</td>
<td>33.44±1.13</td>
<td>30.67±0.81</td>
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<tr>
<td>Group 2</td>
<td>122.08±1.84 a</td>
<td>148.88±3.18 a</td>
<td>130.04±5.42 a</td>
</tr>
<tr>
<td>Group 3</td>
<td>82.17±5.51 ab</td>
<td>102.17±3.56 ab</td>
<td>100.17±1.64 ab</td>
</tr>
<tr>
<td>Group 4</td>
<td>62.24±2.67 abc</td>
<td>74.2±4.96 abc</td>
<td>81.69±3.75 abc</td>
</tr>
<tr>
<td>Group 5</td>
<td>49.55±2.17 abcd</td>
<td>59.18±1.69 abcd</td>
<td>68.63±1.26 abcd</td>
</tr>
<tr>
<td>Group 6</td>
<td>40.06±1.18 abcde</td>
<td>50.13±1.63 abcde</td>
<td>55.3±3.06 abcde</td>
</tr>
<tr>
<td>Group 7</td>
<td>33.75±0.41 abcdef</td>
<td>42.31±2.69 abcdef</td>
<td>46.64±1.46 abcdef</td>
</tr>
<tr>
<td>Group 8</td>
<td>28.03±0.49 bcdefg</td>
<td>35.44±0.97 bcdefg</td>
<td>39.82±0.29 bcdefg</td>
</tr>
</tbody>
</table>

Data are represented as mean ± SD and analyzed using ANOVA followed by LSD post-hoc test at p value <0.05, n=10 for each group. IL-10, interleukin 10, TNF-α, tumor necrosis factor α, IL-6, interleukin 6. Group 1, negative control group, group 2, CFA induced arthritic group, group 3, treated with MSCs, group 4, treated with Os, group 5, treated with HPE, group 6, treated with MSCs and Os, group 7, treated with MSCs and HPE, group 8, treated with MSCs, Os and HPE. Different letters in each column indicate a significant difference. a, statistical significant difference from negative control, b, statistical significant difference from positive control, c, statistical significant difference from the group treated with MSCs, d, statistical significant difference from the group treated with Os, e, statistical significant difference from the group treated with HPE, f, statistical significant difference from the group treated with MSCs and Os, g, statistical significant difference from the group treated with MSCs and HPE.

**Figures**
Figure 1

a: Photomicrograph of bone tissue of negative control group

Photomicrograph of bone tissue showed normal cortical structures (blue arrow), normal osteocytes (black arrow) and normal haversian canals (yellow arrow). 400 X H&E stain

Photomicrograph of bone tissue of CFA induced arthritic group

Photomicrograph of bony tissue showed early osteodystrophic changes with characteristic endo-osteoblastic proliferation (orange arrow) and osteoporotic reactions (yellow arrow) in the form of osteocytes apoptosis (green arrows) and osteodegenerative areas with increase activity of the osteoclasts (red arrows) especially around the
degenerated areas (green arrows). Bone matrix showed hypocellularity with deep bluish coloration denoting demineralization (blue arrow). 400 X H&E stain

**Photomicrograph of bone tissue of group treated with combination therapy of rBM-MSCs, Os and HPE**

Photomicrograph of bone tissue showed healing and remodeling processes of previous osteoporotic lesions with presence of a few reactive osteoclastic change (black arrow) and focal osteo-fibroblastic dysplasia and endosteoblastic hyperplasia (blue and brown arrows). A few bone cells still suffered osteo-degenerative changes although the chondroplasty compensatory process is remarkable (red arrows). The haversian canals appear moderately dilated with hyperemic sinusoids (green arrows). 200, 400 H&E X stain

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementary tables.docx](#)