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Comparative efficacy of platelet-rich plasma and dextrose prolotherapy for stifle joint arthritis in a rabbit model

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Abstract

This study aimed to investigate the efficacy of PRP and dextrose PRL in stifle joint arthritis in rabbits. Sixteen healthy adult male rabbits were allocated into groups A (n=8) and B (n=8). After induction of arthritis in all experimental animals, the animals of group A were treated with intra-articular injection of PRP, while the animals of group B were treated with dextrose PRL. Pain scoring was evaluated by the Simple Descriptive Pain scale at 0d, 6d, 13d, and 19d, while radiographic scoring was examined by Kellgren and Lawrence grading system at pre-induction, post-induction, and post-treatment. The hematological parameters, including PLT, HB, WBC, PCV, RBC, monocytes, and lymphocytes, were assessed in blood samples at 0d, 6d, 13d, and 19d. We observed that Pain scoring showed a significantly lower ($P<0.05$) value in group A than in group B at 13d and 19d. Radiographic scoring was significantly lower ($P<0.05$) in group A than the group B during post-treatment. However, during the pre- and post-monoiodoacetate (MIA) induction, there was no significant difference ($P>0.05$) in radiographic scoring between both groups. The PLT concentration was increased, and monocyte was decreased in the group A animals than the group B animals at 13d and 19d ($P<0.05$), while WBC, RBC, PCV, HB, and lymphocyte concentration showed no significant difference ($P>0.05$) between both groups. In conclusion, the application of PRP is an effective method for treating stifle joint arthritis compared to the dextrose PRL in the rabbit model.

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Introduction

Osteoarthritis (OA) is a degenerative joint disease that causes severe pain and functional limitations. It involves not only articular cartilage but also subchondral bone, ligaments, synovium, and periarticular muscles (1). Since cartilage is avascular and has low cellular mitotic activity, once injured, its ability to heal is limited, ultimately leading to irreversible damage. The cause of osteoarthritis is unknown, but several studies suggest joint degeneration results from mechanical stress and biochemical factors (2). Many medications treat arthritis: including NSAIDs, pain relievers, and surgery. The sequence of therapeutic application begins with medical treatment and ends with surgical treatment (3). Intraarticular platelet-rich plasma injection (PRP) is a novel growth factor therapy. PRP is made by centrifuging a blood sample, which boosts platelet concentration by 3 to 5 times over whole blood. Platelets de-granulate and release growth factors when activated by thrombin, calcium, or collagen, triggering a cascade of cell growth and tissue repair/remodeling (4,5). Plasma is a cell-free fraction of a mixture that includes cytokines and thrombin. It also contains other growth factors, including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-beta), transforming growth factor-Ị (TGF-Ị), epidermal growth factor (EGF), insulin-like growth factor, essential fibroblast growth factor, and vascular endothelial growth factor (VEGF). These growth factors trigger a series of molecular events leading to collagen synthesis, angiogenesis, and mesenchymal stem cells undergoing mitosis, as well as chondrocyte growth and differentiation (6,7). Prolotherapy, or hypertonic dextrose injection, is an injection-based treatment for persistent musculoskeletal pain of many forms, including knee osteoarthritis (8). The injection of a small amount of an irritating solution, commonly hypertonic dextrose, into sore ligaments, tendon attachments, and adjacent joint areas is a crucial treatment principle of proliferative therapy. The basic science of the mechanism of action on various types of connective tissue and cells (e.g., fibroblasts/chondrocytes, etc.) is unclear. However, animal studies have reported that peri-tendon injection of glucose consistently results in fibroblast and vascular proliferation, dense collagen deposition, and increases in ligament thickness, energy absorption, and eventual load-bearing capacity (9). Sodium monoiodoacetate (MIA) is the most commonly used compound in osteoarthritis research (10). It prevents glyceraldehyde-3-phosphate dehydrogenase of the Krebs cycle, which leads to chondrocyte death. As a result, osteophytes grow, and articular cartilage deteriorates (11). The result was rapid inflammation and pain lasting 7 days, followed by chronic musculoskeletal pain starting on day 10 post-injection. MIA-induced OA models frequently measure pain behaviors and drug treatments to address pain in animals. This model may be more predictive of drug efficacy than other pain models used to test osteoarthritis drugs (12). It is commonly used in mice and rats (11).

To our knowledge, no studies have compared platelet-rich plasma and dextrose prolotherapy in the Pakistani rabbit model to treat knee arthritis. Therefore, this is the first study to describe the comparative efficacy of platelet-rich plasma and dextrose prolotherapy in treating knee arthritis in a rabbit model. We hypothesized that platelet-rich plasma could be an effective treatment compared to dextrose prolotherapy, which is often responsible for knee arthritis in rabbits.

Materials and methods

Ethical statement

This study and all the procedures were approved and conducted following the rules and regulations of the Ethical Review Committee (Ethical Approval No. DR/996; Dated: 08/10/2019) at the Department of Veterinary Surgery and Pet Sciences, the University of Veterinary and Animal Sciences, Lahore, Pakistan.

Experimental design and grouping

Sixteen local adult male rabbits weighing 1.8±0.2 kg was used in this study. The trial was conducted by inducing arthritis through a chemical method of Monosodium iodoacetate MIA (13). All rabbits were housed with a 12 h light–12 h dark cycle (artificial lighting), and free access to water and food was given. All animals did not get additional exercise and were allowed normal activities in their cages with a ratio of 2 rabbits per cage. Experimental animals were allocated randomly into two equal groups, group A and group B, according to a complete randomized design (CRD). Arthritis was induced by a chemical method by intra-articular injections of MIA into the stifle joint of rabbit after induction of anesthesia (Ketamine 35-40mg/kg with Xylazine 3-5mg/kg) and proper scrubbing and shaving of the injection site. After 28 days of MIA injection, each group received 3 shots of PRP and dextrose PRL on days 1, 7 and 14.

Model induction

Arthritis was induced in rabbits by a single intra-articular injection of monosodium iodoacetate (MIA; Sigma, St. Louis, MO, USA; CAT #I2512) through the infrapatellar ligament of the left knee after proper shaving and scrubbing with surgical chlorhexidine scrub solution of the injection site. MIA was dissolved in physiologic saline and administered in a volume of 50 uL using a 27-gauge, 0.5-inch needle. The Monosodium iodoacetate dose injected into the joint space was calculated based on a study in which the maximal dose resulting in maximum joint discomfort was 3mg/mL (10). Four weeks after MIA injection rabbits, the treatment protocol was started.

Blood sampling and PRP preparation

5mL of blood was collected from the jugular vein of the rabbit. The blood was collected in sodium citrate vacutainer tubes to avoid coagulation, and after that, the tubes were transferred to the Diagnostic Laboratory University of Veterinary and Animal Sciences Lahore for complete blood account (CBC) examination and PRP preparation. PRP preparation was done by using double centrifugation. The first centrifugation was done at 900 rpm for 10 min, and the second centrifugation was done at 2500 rpm for 10 minutes at room temperature. Production of PRP was a labor-intensive process that required two centrifugation spins and multiple transfers and produced roughly 10 percent of PRP by volume of the whole blood drawn (14,15).

Clinical evaluating parameters

The pain was scored using the Simple Descriptive Pain Scale (SDS), described as 0 no pain, 1 mild pain, 2 moderate pain, and 3 severe pain. Radiological scoring was done using the Kellgren and Lawrence grading system (16,17). A composite score of 4 grades osteoarthritic joints. Grade 1 indicates suspected joint space narrowing and possible osteophyte lip, and Grade 2 indicates definite osteophyte and possible joint space narrowing. Grade 3 indicates moderately multiple osteophytes, definite joint space narrowing, and some sclerosis and possible deformity of the bone ends, grade 4 indicates large osteophytes with prominent joint space narrowing, severe sclerosis, and definite deformity of the bone ends. X-ray films were taken in the veterinary surgery X-ray room, dorsal recumbent position, anterior and posterior bit.

Statistical analysis

The data was analyzed statistically by student-paired *t*-test using SPSS version 22. All results were expressed as mean ± SEM; P>0.05 showed no significant difference, and P<0.05 showed a significant difference.

Results

Pain scoring and restoration of function

Pain scoring is well indicative of the restoration of function. Rabbits showed similar signs of pain for both groups A and B on day 0 (before applying treatment) and on day sixth (after the first injection) (P>0.05), while the pain score was lower at day 13 for PRP-treated animals (P<0.05). However, on day 19, rabbits treated with PRP showed minimal pain compared to Dextrose prolotherapy-treated rabbits (P<0.05). In our study, a significant difference of P<0.05 was observed for pain scoring in groups A and B, as shown in table 1.

Table 1: Evaluation of Pain scoring between both groups A and B

Periods	Group A (PRP)	Group B (DPRL)
Day 0	2.875±0.125	3.000±0.000
Day 6	2.500±0.188	2.750±0.163
Day 13	1.750±0.163	2.250±0.163*
Day 19	0.500±0.188	1.625±0.182*

*Indicates the statistical difference (P<0.05).

Radiographic scoring

The radiographic scoring of Rabbits is shown in Table 2. Pre-induction and post-induction radiographs did not differ across both treatments (P>0.05), whereas radiographs showed differences among both treatment groups after applying PRP (treatment A) and dextrose prolotherapy (treatment B) (P<0.05). In our study, a significant difference of P<0.05 was observed for radiographic scoring in groups A and B, as shown in table 2 and figure 1.

Table 2: Radiographic Scoring between both groups A and B

Periods	Group A (PRP)	Group B (DPRL)
Pre-Induction	0.125±0.125	0.250±0.163
Post-Induction	2.375±0.182	2.250±0.163
Post-Treatment	0.375±0.182	1.250±0.250*

*Indicates the statistical difference at P<0.05.

Figure 1: Radiographic Evaluation: A-Post Induction MIA (Group A), B- Post Treatment PRP (Group A). In picture A, Arrow shows slight osteophyte formation. Slight erosions in the joint can also be seen. C-Post Induction MIA (Group B), D-Post Treatment (Prolotherapy). In picture C, the right arrow shows slight osteophyte formation. Slight erosions in the joint can also be seen.

Blood parameters

Complete blood counts of rabbits were taken on days 0, 6, 13, and 19 of applying treatments. The platelet count was similar across both treatments at day 0 (P>0.05), whereas, at day 6, change was observed in the platelet count among both treatments (P>0.05). However, a visible difference in platelet count was observed between both treatments on rabbits at day 13 and day 19 (P<0.05). Similarly, the same trend was observed for monocyte count at day 13 and day 19 among both treatments as for platelet counts, whereas, at day 0 and day 6, there was no difference in monocyte count among both treatments (P>0.05). Blood parameters such as Hb, WBCs, RBCs, Lymphocytes, and PCV did not differ among both treatments at day 0, 6, 13, and 19, respectively (P>0.05). In our study, a non-significant difference of P>0.05 was observed in complete blood count in groups A and B, as shown in table 3.

Discussion

PRP has emerged as an effective treatment method for individuals who have arthritis. A blood sample is obtained from the peripheral blood vessels and then concentrated by centrifugation, and platelet-rich plasma is harvested. The harvested plasma is injected intra-articularly into the patient (18). In the present trial, harvested PRP from rabbits was injected back into the stifle joint of the rabbit to check the effectiveness of treatment compared to that of dextrose prolotherapy.

Table 3: Effects of PRP and DPRL on blood parameters

Parameter	Days	Group A (PRP)	Group B (DPRL)	P Value
Platelets	Day 0	217±6.55	229±3.77	0.117
	Day 6	260±7.79	237±7.99	0.059
	Day 13	312±7.39	270±5.17	<0.001*
	Day 19	401±11.50	334±5.90	<0.001*
Hb	Day 0	8.8±0.11	8.6±0.11	0.238
	Day 6	8.9±0.14	8.8±0.09	0.404
	Day 13	9.6±0.18	9.5±0.16	0.499
	Day 19	10.4±0.10	10.3±0.09	0.399
WBCs	Day 0	12.83±0.17	12.85±0.18	0.955
	Day 6	12.21±0.09	12.40±0.08	0.154
	Day 13	11.82±0.06	11.68±0.07	0.189
	Day 19	11.46±0.09	11.43±0.10	0.876
RBCs	Day 0	4.95±0.16	5.04±0.21	0.765
	Day 6	5.11±0.18	5.33±0.23	0.472
	Day 13	5.48±0.09	5.44±0.11	0.764
	Day 19	5.49±0.10	5.45±0.10	0.818
Lymphocytes	Day 0	86.1±2.46	86.3±2.51	0.961
	Day 6	79.1±1.54	79.2±1.60	0.965
	Day 13	76.5±4.20	76.8±1.22	0.942
	Day 19	67.1±1.55	68.7±4.06	0.719
Monocytes	Day 0	4.6±0.09	4.6±0.07	0.688
	Day 6	4.1±0.07	4.2±0.07	0.383
	Day 13	3.3±0.12	3.8±0.07	0.010*
	Day 19	2.8±0.11	3.5±0.06	<0.001*
PCV	Day 0	37.6±1.66	37.3±2.23	0.912
	Day 6	38.7±1.42	38.8±0.99	0.955
	Day 13	39.0±1.92	38.8±1.24	0.944
	Day 19	39.3±0.85	39.0±1.60	0.888

*Indicates the statistical difference (P<0.05).

Taylor *et al.* (19) performed a trial to assess the pain score by using the SDS pain scoring scale. Lower values for this scale were observed for PRP treatment, indicating the extent of rabbits' relief in PRP treatment compared to dextrose prolotherapy group rabbits (19), who said more cats indicated low pain scores after buprenorphine at 2 and 24 h on a particular time. A recent study has indicated that PRP therapy can reduce pain, joint stiffness, and functional restrictions (20). PRP injection is more effective than PRL for the treatment of knee OA. An additional study also elaborated on the efficacy of PRP in reducing pain in joints extensively to a more extended period when they evaluated the effects of a single PRP dose in knee joint osteoarthritis (21) and said that PRP injection is more efficacious than hyaluronic acid (HA) or placebo for the treatment of osteoarthritis (OA) of the knee. In the present study, the pain score of rabbits treated with PRP was different and low compared to those treated with dextrose prolotherapy. This study suggested that PRP therapy is an effective therapy for pain relief in arthritis which is a good indicator of restoration of function.

Radiography is the least expensive method of imaging joints and is more readily available. It is the tool that helps us access the patient's intra-articular recovery in response to the treatment of osteoarthritis (OA) of the knee (22). In the present study, radiographs of normal rabbits were taken, then arthritis was induced by the same method in all the rabbits, so all rabbits show similar radiographs in the post-induction phase. In the post-treatment period, a specific time is required for treatment to show its effectiveness, so at the end of the trial, post-treatment radiographs were taken, indicating the visibly different effects of the two treatments. PRP treatment resulted in more lowering of the inflammation of synovial membrane in intra-articular spaces as compared to that of dextrose prolotherapy-treated rabbits. This improvement in the condition of rabbits is indicated by the pain score of rabbits in the PRP treatment group. Moreover, the absence of osteophytes in PRP treatment group rabbits indicated better recovery of rabbits.

A CBC (complete blood count) is a blood test that measures the number of blood cells and platelets in the blood. The red blood cell count gives the red blood cells (RBCs) in the blood and hemoglobin. Hemoglobin is vital because it transports oxygen into tissues. Without enough hemoglobin, the tissues cannot get enough oxygen. The WBC count represents the blood's white blood cells (WBC) which are essential for fighting infection. Platelets are small sticky cells. Their job is to stop the bleeding. Without enough platelets, it can be difficult to stop the bleeding. Platelets are released when they are activated or aggregated by collagen. TGF-ß1 and TGF-ß2 have been shown to inhibit bone resorption, osteoclast formation, and osteoclast activity and trigger rapid collagen maturation in early wounds (23). Therefore, a reasonable hypothesis is that increasing platelet concentrations in bone defects may lead to enhanced and faster healing. In the current study, PRP treatment significantly affects the platelet concentration of rabbits compared to dextrose prolotherapy. Platelet count was higher for PRP-treated rabbits on day 13 and day 19, which indicated a higher level of recovery in the rabbits belonging to this group, whereas on day 0, no treatment was conducted, so platelet counts of both the groups were similar. On day 6, platelet count of the PRP group was numerically high, whereas it was statistically non-significant compared to the other treatment group of rabbits. High platelet count in PRP treatment may be due to the exogenous injection of platelets given to the rabbits.

Monocytes are the immature form of macrophages and are the primary inflammation mediators. Other than playing an important role in inflammation, these monocytes are at the origin of bone erosion in arthritis due to their abundant differentiation into osteoclasts. These osteoclasts are isolated cells specialized in bone resorption (24). In our study, at day 0, both treatments had a high monocyte count due to induced arthritis, while the monocytic count decreased after applying both treatments. On days 13 and 19, the monocyte count for PRP treatment was significantly lower than that of the dextrose prolotherapy group, indicating the noticeable recovery in the intra-articular spaces of the rabbits treated with PRP.

Conclusion

In conclusion, the application of PRP is an effective method for treating stifle joint arthritis compared to the dextrose PRL in the rabbit model.

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Conflict of interest

All authors declared no conflict of interest.

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مقارنة فعالية الحقن في المفصل للبلازما الغنية بالصفائح والدكستروز في علاج التهاب المفصل في الارانب

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الخلاصة

هدفت هذه الدراسة إلى تقييم فعالية الحقن بالمفصل للبلازما الغنية بالصفائح والدكستروز على التهاب المفاصل في الأرانب. استخدم ١٦ من الأرانب الذكور البالغين الأصحاء في المجموعتين A (n = 8) وB (n = 8). بعد احداث التهاب المفاصل في جميع حيوانات التجربة، تم علاج حيوانات المجموعة A بالحقن داخل المفصل بالبلازما الغنية بالصفائح، بينما تم علاج حيوانات المجموعة B باستخدام الحقن داخل المفصل بالدكستروز. تم تقييم درجات الألم من خلال مقياس الألم الوصفي البسيط في اليوم ٠ و٦ و ١٣ و ١٩، بينما اعتمد نظام التدريج لكل من Kellgren و Lawrence في فحص درجات التصوير الإشعاعي قبل مرحلة احداث التهاب المفصل و بعد الاحداث وبعد العلاج. تم قياس معايير الدم التي شملت الصفيحات الدموية، الهيموكلوبين، عدد كريات الدم البيض، حجم الخلايا المرصوصة، عدد كريات الدم الحمر، الخلايا وحيدة النواة والخلايا اللمفية في اليوم ٠ و٦ و ١٣ و ١٩. لاحظنا ان درجات الألم أظهرت قيمة أقل بشكل ملحوظ (P < 0.05) في المجموعة A مقارنة بالمجموعة B عند اليوم ١٣ و ١٩. كان تسجيل الأشعة أقل بكثير (P < 0.05) في المجموعة A من المجموعة B أثناء فترة ما بعد العلاج. بينما قبل وبعد استحداث التهاب المفصل بأحادي الأسيتات (MIA) لم يكن هناك فرق معنوي في درجات الأشعة بين كلتا المجموعتين. وجد زيادة في تركيز الصفيحات الدموية، وانخفضت نسبة الخلايا وحيدة النواة في المجموعة A بالمقارنة مع مع حيوانات المجموعة B عند اليوم ١٣ و ١٩ (P < 0.05)، بينما لم يظهر الهيموكلوبين، عدد كريات الدم البيض، حجم الخلايا المرصوصة، عدد كريات الدم الحمر، وتركيز الخلايا الليمفاوية أي فرق معنوي بين كلا المجموعتين. يستنتج ان تطبيق استخدام البلازما الغنية بالصفائح طريقة فعالة لعلاج التهاب المفاصل مقارنة بالدكستروز عند الحقن في المفصل في نموذج الأرانب.