Original Article

Pro-inflammatory, Anti-inflammatory and Antioxidant Activity of Platelet Rich Plasma (PRP) on Arthritic Rats

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Abstract

Arthritis is an acute or chronic inflammation of one or more joints which results in marked physiological changes. Platelet rich plasma (PRP) is a concentrated source of autologous platelets and has many therapeutic effects in infections and injuries. In addition, it also accelerates tissue healing due to presence of abundant source of growth factors. In Iraq, this appears to be the first in vivo physiological study which investigated deleterious impact of arthritis on inflammatory and antioxidant markers. Moreover, this study also evaluated the role of PRP in restoration of the normal physiological status. The results revealed significantly higher levels of inflammatory markers FABP and IL-1 and lower levels of IL-10, TNF-a and TGF in arthritis group (AG) when compared to negative control group (NCG). Regarding antioxidants in AG, significant reduction in catalase, glutathione peroxidase and superoxide peroxidase, and elevation in malondialdehyde was observed. However, levels of inflammatory and antioxidant markers in arthritis-PRP group (APRPG) were found to be significantly restored to normal. Moreover, significant variation in paw volume was observed. While comparing the values between AG and APRPG, no significant differences were detected at 3rd, 6th and 9th day of experiment; however, significant progressive increase was detected from the 12th day to the last day of experiment. Among rats of AG, the highest increase in paw volume was found at 24th and 27th day of experiment; while in rats of APRPG, the highest value was seen at 9th day of experiment. With respect to the scores of arthritis, no clinical signs were detected among rats of NCG and PRPG; whereas, significant increase was reported in rats of AG (score: 2) and in rats of APRPG (score: 1). In conclusion, PRP can be used as a safe and easy therapeutic product for reversion of physiological changes due to arthritis. Further studies might help in elucidating the activity of PRP on other physiological parameters or diseases.

Keywords: Catalase, Autologous platelets, Glutathione peroxidase, Malondialdehyde, Iraq

Introduction

Arthritis is the inflammation and loss of function of one or more joints due to infectious, immunological, metabolic and toxic agents, which mainly affects the adult population worldwide.[1] The worldwide prevalence of this disease differs widely depending on the geographical location, age of the people surveyed and study protocol.[2,3] Arthritis is often diagnosed as acute or chronic with pain, swelling and stiffness in joints however, specific symptoms vary depending on the type of illness.[4] The greater understanding for the etiology of arthritis and the role of environmental chemicals can open the way to new approaches in disease prevention and treatment. Various types of diseases show similar sumptoms which can confuse clinical detection leading to the dilemma of choosing the appropriate therapy.[5]

Rheumatoid arthritis (RA) is a disease in which joints are inflamed locally and systemically. In last few decades, interleukins (ILs) and tumor necrosis factors (TNFs) have been described as markers which play important role in arthritis through regulation or dysregulation of cartilage and remodeling of bone characteristics.[6] Transforming growth factor (TGF) has a different influence on hematopoiesis and immunity.[7] Fatty acid binding proteins (FABPs) are abundant proteins which

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Fatty acid binding proteins (FABPs) are abundant proteins which have a specificpattern of expression. Although FABP appears to have various roles which are still incompletely known, yet, recent studies found that it has the ability to enhance the production of pro-inflammatory markers.[8,9] Oxygen derived species of biological system have received much attention due to their implicated role as a mediator for damaging of tissues in arthritis and other pathological conditions.[10] In recent years, many studies refer to the ability of the body to heal itself through utilization of Platelet Rich Plasma (PRP) which is a concentrated source of autologous platelets.[11] This type of therapy represents a form of regenerative medicine that can amplify the natural growth factors used by the body to heal the injured tissues.[12] In addition to acceleration of tissue healing, PRP has many therapeutic effects in infections and injuries.[13] In Iraq, this appears to be the first *in vivo* study aimed to detect deleterious effects of arthritis on anti-inflammatory and antioxidant markers and the role of PRP in reversion of these effects.

Materials and Methods

Ethical approval

This study received a license from the Scientific Committee of the College of Education for Pure Sciences, Wasit University, Wasit Iraq.

Animals

In total, 60 adult *Wistar* albino rats were purchased from a private farm for laboratory animals in Baghdad province (Iraq). These included 40 males of ± 2 months old age and 90-120 grams of weight. In addition, 20 female rats were included having an age of ± 2 months and 70-100 grams of weight. All study rats were subjected to a preparation period for 2 weeks, supplemented with balanced diet and kept in plastic cages under standard conditions [temperature (20-24ºC), humidity (40-60%) and daily lighting cycle].

PRP preparation

As described by other studies[14,15] blood samples from heart of 20 female rats were collected in sodium citrate-anticoagulant tubes. The samples were centrifuged at 1000 rpm for 10 min and the supernatant was transferred to new tubes and subjected for additional centrifugation. Finally, the clear supernatant was removed and residual material was considered as PRP. Prior to injection, 50ml of calcium chloride (10%) was added to each 3 ml PRP tube to activate the PRP.

Experiment

After the preparation of PRP, 40 males were allocated equally and randomly to the following 4 groups:

1. Normal control group (NCG): Rats did not receive formaldehyde solution or PRP.
2. Arthritis group (AG): Rats received 0.1 ml of formaldehyde (2% v/v) in sub-planter region to induce arthritis.[16]
3. PRP group (PRPG): Rats received 100ml of PRP into the ankle joint of right hind paw at 0, 8, 16 and 24 days of experiment.
4. Arthritis-PRP group (APRPG): Rats received 0.1 ml of formaldehyde (2% v/v) in sub-planter region to induce arthritis. Then, they received 100ml of PRP at 0, 8, 16 and 24 days of experiment.

The paw thickness of study animals was measured at 3 days' intervals during the period of 3 to 27 days of experiment (1 month).

Assessment of arthritis in injected hind paws

The signs of arthritis were assessed as described in a study.[17] Swelling was measured using the digital caliper by assessing the ankle joint width, while erythema was evaluated by observation. Based on severity of symptoms, following score scale was established:

1. Score (0): No signs of disease.
2. Score (1): Swelling of paw and fingers.
3. Score (2): Swelling of paw and joint.
4. Score (3): Severe swelling of paw and joint with erythema.
5. Score (4): Severe swelling of paw with deformities or ankylosis.

The percentage of increased swelling and total score of arthritis severity in study subjects were calculated using the established protocols.[17,18]

Specimen Collection

On the last day of experiment, study rats were given diethyl ether anesthesia and blood was drawn directly from their heart (cardiac puncture) using disposable syringes with a 23G1 needle.[19] The blood samples were collected in a labeled free-anticoagulant glass tube and centrifuged. The serum samples obtained were saved in 1.5 ml eppendorf tubes and later preserved at -20ºC.

Laboratory testing of sera

Levels of markers (FABP, IL-1, IL-10 and TNF-a), protein (TGF), malondialdehyde (MDA) and antioxidants [superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT)] were assessed through specific ELISA kits (Sunlong Biotech, China).

Statistical analysis

The data was analyzed statistically by the GraphPad Prism Software (version 6.0.1). One-way *ANOVA* and Chi-square (x^2) tests were used to detect significant differences between means (Mean ± Standard Error) of anti-inflammatory and antioxidant markers of the study groups as well as between scores of arthritis. The $p<0.05$ (*) and $p<0.01$ (**) was considered as statistically significant while $p>0.05$ was considered non-significant.

Results

In this study, significant differences in levels of anti-inflammatory markers AG, PRPG and APRPG were observed when compared to NCG (P£0.0001) (Figure 1). The highest level of FABP was detected in AG (16.57 ± 0.85) while the lowest was observed in PRPG (12.45 ± 0.7). However, of its level in APRPG and NCG was found to be 13.7 ± 0.72 and 12.52 ± 0.71 respectively. Likewise, IL-1 was found to be significantly increased in AG (347.49 ± 12.07), whereas, its significant decrease was observed in PRPG (250.42 ± 21.59) and APRPG (272.91 ± 16.33). Regarding IL-10, of its level in PRPG (63.86 ± 3.02) varied insignificantly (P>0.05) in comparison with NCG (60.55 ± 3.32). However, in both these groups, its level was significantly higher than in AG (20.22 ± 2.16) and APRPG (34.82 ± 1.87). The TNF-a showed a significant elevation in AG (289.52 ± 11.78) and significant decrease in PRPG (148.78 ± 6.26) and APRPG (203.52 ± 7.05). However, the variation was insignificant among the groups PRPG, APRPG and NCG (152.23 ± 6.02). Similarly, TGF showed insignificant variation between PRPG (164.45 ± 6.38) and NCG (160.55 ± 9.11), while in AG (75.16 ± 2.85), it was reduced significantly than in APRPG (102.29 ± 3.92).

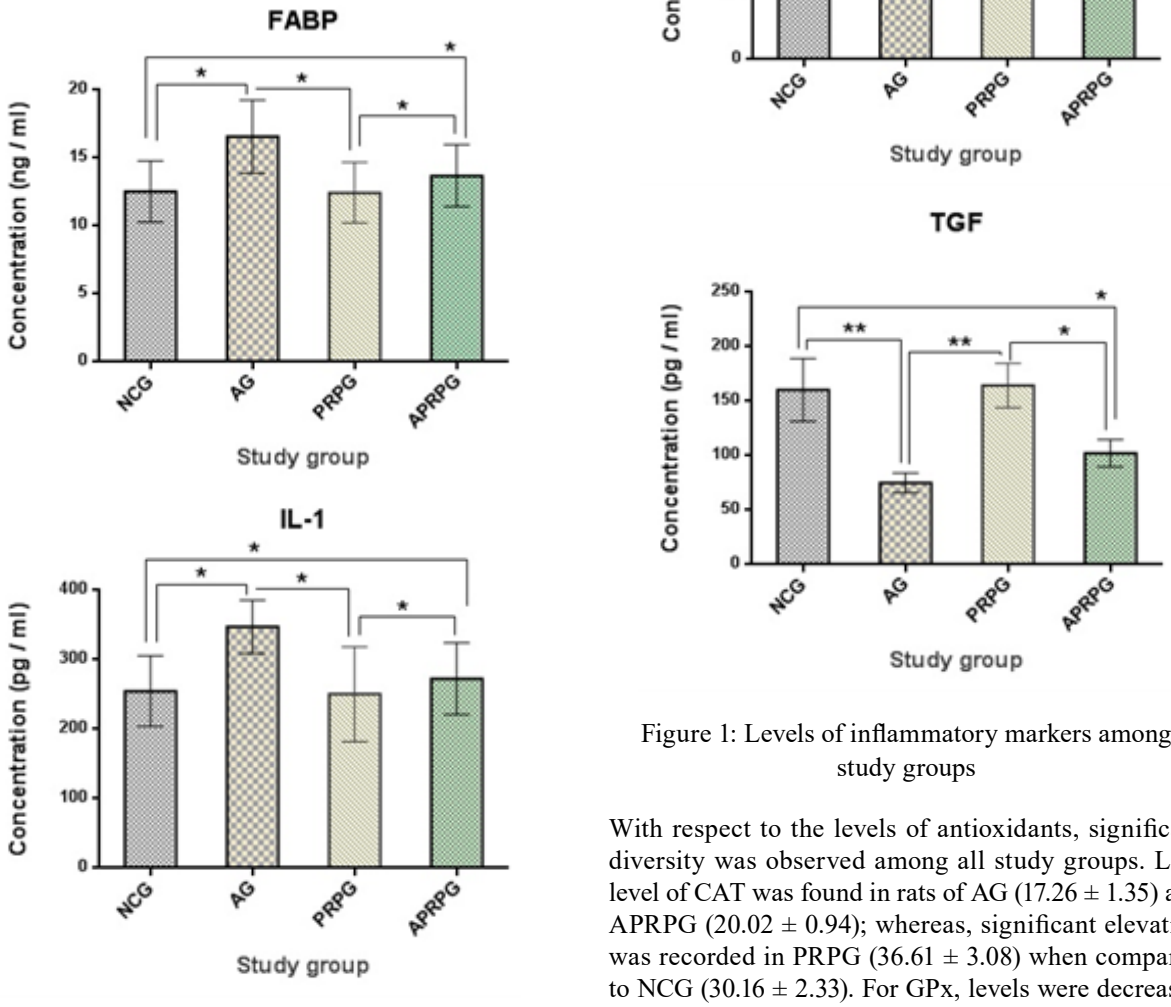
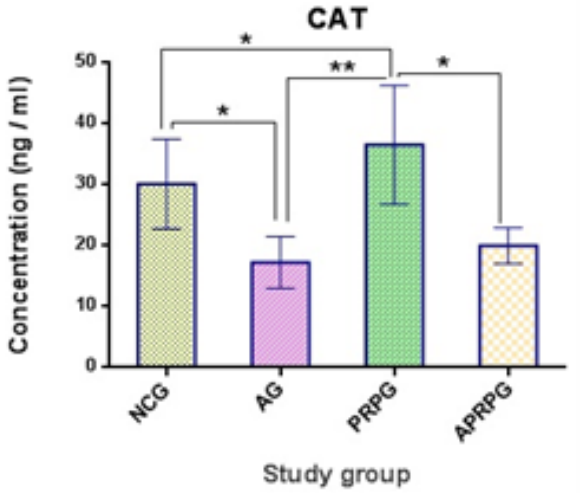
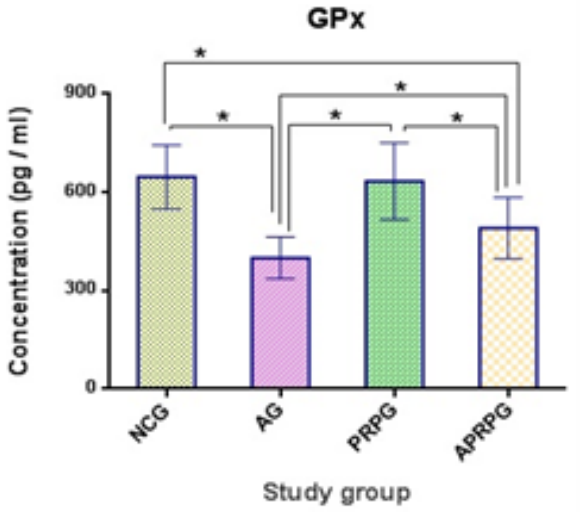


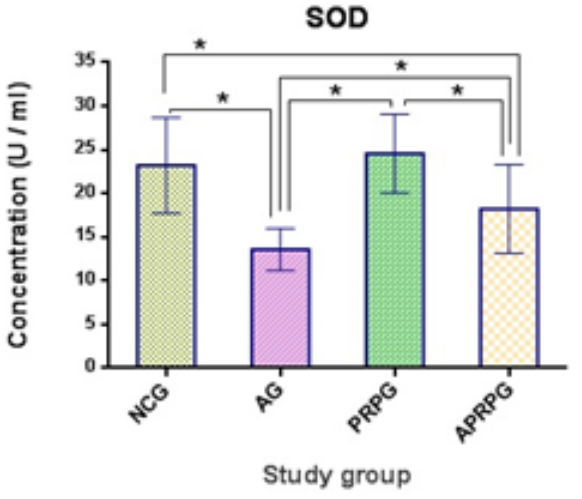
Figure 1: Levels of inflammatory markers among study groups

With respect to the levels of antioxidants, significant diversity was observed among all study groups. Low level of CAT was found in rats of AG (17.26 ± 1.35) and APRPG (20.02 ± 0.94); whereas, significant elevation was recorded in PRPG (36.61 ± 3.08) when compared to NCG (30.16 ± 2.33). For GPx, levels were decreased

significantly in APRPG (491 ± 29.43) when compared to the levels in PRPG (633.81 ± 37.1) and NCG (646.77 ± 30.81). Similarly, SOD level was reduced significantly in AG (13.57 ± 0.76). Conversely, level of MDA was significantly elevated in AG (182.77 ± 10.47).







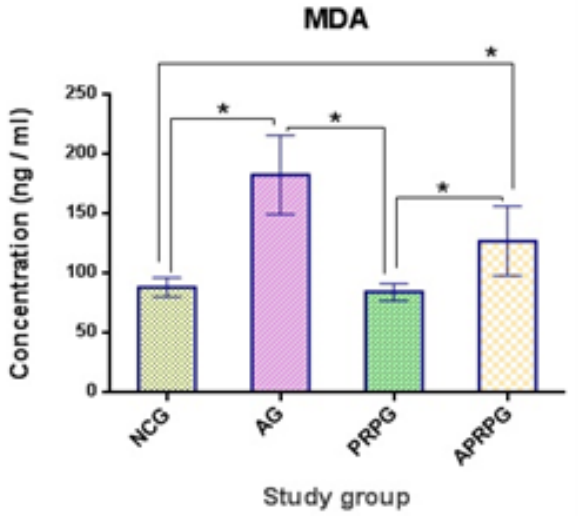


Figure 2: Levels of antioxidants among study groups

In the present study, significant variation in the paw volume was observed among different study groups (Table 1). The AG and APRPG showed no significant variation at 3rd, 6th and 9th day of experiment; however, significant progressive increase was detected from the 12th day to the last day of the experiment. Among rats of AG, the highest increase in paw volume was showed at 24th (79.72 ± 6.25) and 27th (84.05 ± 7.11) day of experiment; while in rats of APRPG, the highest change was seen at 9th day (45.27 ± 3.92) of experiment (Table 1). Regarding the scores of arthritis, no clinical signs were detected among rats of NCG and PRPG; whereas, significant elevation was found among rats of AG (score: 2) and of APRPG (score: 1) (Table 2).

Table 1: Values of percentage of increasing paw volume in rats of AG and APRPG

Day of experiment	AG (%)	APRPG (%)	x^2
3	19.04 ± 3.29	20.18 ± 3.52	1.671 NS
6	36.67 ± 4.81	38.02 ± 3.62	2.029 NS
9	47.06 ± 4.28	45.27 ± 3.92 *	2.306 NS
12	56.43 ± 5.7	37.79 ± 3.58	4.842 S
15	63.83 ± 5.41	29.26 ± 3.04	5.227 S
18	71.24 ± 5.16	24.48 ± 2.41	7.005 S
21	75.86 ± 5.57	17.91 ± 2.16	8.486 S
24	79.72 ± 6.25 *	13.74 ± 1.82	10.319 S
27	84.05 ± 7.11 *	11.17 ± 1.78	13.623 S
p-value	£0.0001	£0.01	-

(S): Significant (P<0.05); (NS): Non-significant (P>0.05)

Table 2: Scores of arthritis in all study groups

Score	NCG	AG	PRPG	APRPG
0	10 (100%) *	0 (0%)	10 (100%) *	0 (0%)
1	0 (0%)	2 (20%)	0 (0%)	7 (70%) *
2	0 (0%)	5 (50%) *	0 (0%)	3 (30%)
3	0 (0%)	3 (30%)	0 (0%)	0 (0%)
4	0 (0%)	0 (0%)	0 (0%)	0 (0%)
p-value	£0.0001	£0.012	£0.0001	£0.0057

Each value represents the number (percentage), Significance * (P<0.05)

Discussion

In different forms of arthritis, early detection and therapeutic intervention is paramount to control and prevent the additional damages and disabilities to the affected joint. However, inappropriate diagnostic criteria might lead to the delay in choosing the suitable therapy.[5] The results of this study showed that the concentrations of anti-inflammatory markers; FABP, IL-1 and TNF-a were elevated and that of IL-10 and TGF markers were reduced significantly. FABP is an adipokine that is expressed predominantly in mature adipocytes and macrophages to influence both metabolic and inflammatory pathways.[20] Several studies have confirmed an association of FABP to different types of arthritis such as rheumatoid, osteoarthritis, ankylosing spondylitis and gout.[14,21-23] The increased levels of FABP in AG suggests that this marker is part of pathogenesis in arthritis. According to a study, the level of FABP is elevated in RA individuals, in particular those having high levels of adipokine and cholesterol, but not in osteoarthritis patients which suggests that FABP has a role in increasing the risk of atherosclerotic alterations.[15] Similarly, another study reported higher concentration of FABP in plasma of osteoarthritis patients than in controls. In addition, its concentration is found even higher in synovial fluid than in plasma.[8] recorded study showed that while FABP does not always correlate with arthritis, the serum levels increase among the infected persons when compare to negative control and decrease with suitable therapeutic scheme.[24] The increased concentration of IL-1 in acute and chronic arthritis is demonstrated by different studies.[25-27] IL-1 is cloned firstly in 1980, and found to be implicated in mediation of tissue damage in RA joints and inducing intracellular responses.[28] In arthritis, no adequate IL-1Rs are effectively blocked by IL-1 due to significant decrease in IL-1Ra and increase in IL1Rb.[29] Moreover, this marker can stimulate the process of synthesizing and activating the matrix metalloproteinases and other enzymes which participate in destruction of cartilage in osteoarthritis and RA. This increases the bone resorption through stimulation of the process of differentiation and activation of osteoclast.[30] Many IL-1 inhibitors have been examined initially in RA to regulate IL-1 activity, thereafter, in treating certain pathogenic conditions such as arthritis,[31] cardiovascular diseases,[32] cancer,[33] and epilepsy.[34] Inflammation is a very important part of innate immunity, and is regulated in many steps such as marker network in particular TNF-a, a cytotoxic marker that causes tumor necrosis. In arthritis, inflammation and joint destruction are found related with the existence of TNF-a at high levels in synovial fluid.[35] Additionally, TNF-a induces the expression of RANKL, stimulates bone loss reduces bone formation and induces clustering of pro-inflammatory leukocytes such as T-cells, monocytes as well as neutrophils. Hence, the slowing down of bone erosion and prevention of progression of systemic bone loss are related with inhibition of TNF-a.[36,37] Several studies have reported IL-10 to suppress joint swelling and deformation in arthritis animal model.[38-40] Additionally, systemic administration of IL-10 is found to interrupt the sequence of events and ameliorates the disease process.[41] These results show that IL-10 decreases the expression of inflammatory mediators, protein production and stimulation of thymocytes and mast cells.[42] Low levels of IL-10 production are suggested to have an association with each type of RA.[43] Studies on anti-TGFb shows that its administration increases the susceptibility and disease severity.[44] In contrast, other studies report that administration of TGF can participate in ameliorating of arthritis and repairing of articular cartilage in arthritic mice.[45,46]

The present study showed significant decrease in concentration of antioxidants (CAT, GPx and SOD) in rats of AG and significant increase in concentration of lipid peroxidase (MDA). These findings are in agreement with the results of other studies.[47-49] In PRPG, the concentration of antioxidants increased significantly while the levels of MDA reduced insignificantly when compared to animals of healthy group. Many studies indicate that alteration in antioxidant status of arthritis patients may occur due to increase in inflammatory cell infiltration, vascularity and hyperplasia[50,51] Defects in system of the antioxidants can cause an imbalance in concentration of inflammatory markers, which play great role in arthritis pathogenesis.[52] Recent studies provide evidence for the involvement of reactive oxygen species in pathogenesis of RA.[53,54] In RA patients, synovial fluid of affected joints could swarm by the activated neutrophils which produce huge quantities of ROS, causing an oxidative stress that results in severe metabolic malfunction and damage for macromolecules.[55-57] Catalase activity effects expression of anti-inflammatory genes and their low levels are responsible for high inflammation in arthritic patients.[58] In a study, role of SOD has been demonstrated in many health problems in humans. According to this study, SOD can help in cure process of different illnesses due to its capability of reducing damages by healing of injuries.[59] The diminution of effectiveness of plasma GPx as detected in current study might be associated with decreasing activity of glucose-6-phosphate dehydrogenase which is required in regeneration of GPx and may be active towards oxidative damage in RA.[60-62] Many studies have shown that both behavioral and cellular responses can be induced by injecting a low-dose of formaldehyde.[63-65] The current study revealed that the percentage of increase in paw volume was elevated progressively in rats of AG; whereas, the administration of PRP participated greatly in lowering this percentage. Moreover, no obvious clinical signs of arthritis were recorded during the period of experiment in rats of both AG and PRPG; however, scores 2 and 1 were significantly prevalent among rats of AG and APRPG, respectively. The effects of PRP in reversing the destructive effects of arthritis, observed in this study, are in agreement with that detected worldwide.[66-68] The

effect of PRP on chondrogenesis and its regenerative role to restore disc degeneration and osteoporosis have been indicated by a study.[69] Many studies have reported that PRP products play a role in the initial wound healing and releasing multiple growth factors as it contains the dense granules that have many bioactive factors and non-growth factors such as adenosine, calcium, dopamine, histamine and serotonin.[70,71]

Conclusion

The findings of this study revealed significant correlation between different novel biomarkers and arthritis. Moreover, the effective role of PRP in restoring and supporting health status of study animals has also been demonstrated. Results suggest that PRP can be used as a safe and easy to prepare product for the treatment of arthritis and for bone deficiency healing as well. Additional studies are necessary to elucidate its role in treatment of different types of arthritis and other diseases in humans.

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Conflict of Interest

The author declares no conflict of interest.

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