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Swimming Exercise Improves Rheumatoid Arthritis in Rat
(*An Animal Study***)**

Abstract

Background: Elevated free radical generation in inflamed joints and impaired antioxidant system have been implicated in rheumatoid arthritis (RA). It is suggested that exercise improves the symptoms of rheumatoid arthritis, although the mechanism is not well understood. The aim of this study was to investigate the effect of swimming on the glutathione antioxidant system and oxidative changes induced by rheumatoid arthritis in rats.

Methods: 8-week-old female Wistar rats were distributed within three groups (10 in each): A normal control group (C), a rheumatoid arthritis control group (RA+C), and a rheumatoid arthritis swimming exercise group (RA+EX). Rats at nine weeks of age were induced for rheumatoid arthritis by injection of complete Freund's adjuvant (20mg/kg) into the tail of Wistar rats. The arthritis was assessed grossly by walking ability, skin redness, and swelling in the joints. The RA+EX rats were conditioned to swim for the 4-week period (~20-60 min/day), whereas the other two groups remained sedentary in their cages. 48 hours after the last exercise session, a venous sample was collected to determine metabolic parameters, including glutathione peroxidase, glutathione reductase and lipid peroxidation products (malondialdehyde). The variance analysis test and the Tukey post-hoc test were applied to analyze the data ($P<0.05$).

Results: Rheumatoid arthritis significantly increased levels of malondialdehyde, and swimming training prevented this response ($P<0.05$). Glutathione peroxidase was significantly decreased in the RA+C group compared to the RA+EX and C groups ($P<0.05$). In addition, the severity of rheumatoid arthritis clinical signs with RA+EX group was significantly lower than RA+C group ($P<0.05$).

Conclusion: Our findings suggest that swimming may be useful in preventing the negative changes in glutathione antioxidant system and oxidative stress parameters of rheumatoid arthritis in rats.

Key words: : Antioxidants, Glutathione Peroxidase, Rats, Rheumatoid Arthritis, Swimming

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Introduction

Card Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease characterized by stiffness, swelling, and gradual destruction of the joints. The annual prevalence of RA is reported to be about one percent of the total population in developed and developing countries [(1)]. Rheumatoid arthritis affects 1 in 50 people and is more common in women than men. The disease is common after the age of 40, but can occur at any age [(2)]. In addition, patients with RA have an increased risk of cardiovascular disease (CVD). High levels of inflammation in RA patients (indicated by interleukins and C-reactive protein) may cause oxidative stress, a condition characterized by an imbalance between the production of free radicals and antioxidants. On the other hand, there is evidence that oxidative stress leads to joint destruction and increases the risk of CVD in RA patients [(3)]. It has been suggested that oxidative stress and inflammation can mutually regulate each other, and this is especially evident in RA patients. This interaction may exacerbate joint destruction and the risk of CVD in RA patients. Therefore, some studies have shown that free radicals and oxidative stress play a prominent role in the pathogenesis of RA. The pathogenic effects of reactive oxygen species (ROS) are limited by the antioxidant system to suppress free radical oxidation processes [(4)]. Antioxidants are compounds that prevent the formation of free radicals or scavenge free radicals that are produced. The glutathione system consists of glutathione peroxidase, glutathione reductase,

and glutathione tripeptide, which act as a reducing factor in the glutathione peroxidase-mediated reaction. In fact, the enzyme glutathione peroxidase catalyzes a powerful antioxidant reaction to scavenge free radicals. Glutathione reductase regenerates oxidized glutathione during a glutathione peroxidase-mediated reaction [5]. Evidence shows that in RA patients, the antioxidant defense of glutathione is weaker than normal individuals, and on the other hand, the improvement of this antioxidant system is associated with a reduction in RA symptoms [6]. Therefore, strengthening antioxidant defenses, including the state of the glutathione system, may be considered a therapeutic procedure for RA patients.

On the other hand, regular exercise is associated with a reduction in multiple cardiovascular risk factors and also improves joint function. In RA patients, regular exercise improves patients' health, increases functional capacity and reduces disease activity [7]. In addition, exercise has recently been shown to improve CVD risk factors and endothelial function in RA patients [8]. There is evidence in healthy individuals that repetitive stimuli induced by exercise increase mitochondrial biogenesis and protein expression of antioxidant enzymes, which is ultimately associated with a reduction in oxidative stress levels [9]. However, there are limited studies on the effect of exercise on oxidative stress in RA . For example, Rall et al. found that after a period of resistance training, there was no significant change in urinary DNA oxidation index in RA patients [10]. In another study, Wadley et al reported that aerobic exercise did not increase oxidative stress markers (3-nitrotyrosine) in RA patients and the disease activity decreased after exercise [11]. To the best of our knowledge, no study has examined the effect of exercise on oxidative stress markers and the antioxidant status in RA patients. Therefore, the aim of this study was to evaluate the markers of oxidative stress and antioxidant defense of glutathione in response to a period of swimming exercises in RA-induced rats .

Methods

The research method was experimental. In determining the sample size, according to the sample size formula for continuous scores, if the expected differences are equal to 1.5, with a test power of 80% at a significance level of $\alpha = 0.05$, the number of subjects in each group was set at 10. Initially, the procedures and protocols used in the present study, which were in accordance with the guidelines for the care and use of laboratory animals, were approved by the Animal Ethics Committee of the University of Medical Sciences (IR.IAU.ARAK.REC.1397.007). In this study, 20 female Wistar rats (220-270 g), prepared from the Animal Care Center of Arak University of Medical Sciences, were selected as a sample and transferred to the University Research Center. The animals were kept at 22 ±2° C, humidity 55 ±5% and dark cycle at 12:12 hours in polycarbonate cages (5 rats per cage). The animals received enough water and food (in the form of pellets) throughout the study. After transferring the rats to the laboratory and adapting to the environment, induction of rheumatoid arthritis was performed and the rats were randomly divided into two groups of rheumatoid arthritis swimming exercise (RA + EX) (n=10) and rheumatoid arthritis control (RA + C) (n=10). The rats in the exercise group performed a 4-week (5 days a week) aerobic exercise program, while the other rats did not participate in any exercise program. In addition, another group of rats that did not have rheumatoid arthritis was considered as a healthy control group (C) (n=10).

Induction of rheumatoid arthritis

At first, rats were anesthetized by intraperitoneal injection of ketamine-xialzin (70 mg / kg body weight of ketamine and 6 mg / kg xylezine). Rheumatoid arthritis was then performed with Freund's adjuvant inoculation. Arthritis was induced by a single intradermal injection of 0.1 ml of Complete Freund's adjuvant containing 10 mg/ml of heat killed Mycobacterium tuberculosis into hind paw in Wistar rats. The development of the disease during the two weeks after injection was assessed by macroscopic assessments of walking ability, skin redness, and swelling in the ankle, wrist, and little finger joints [12].

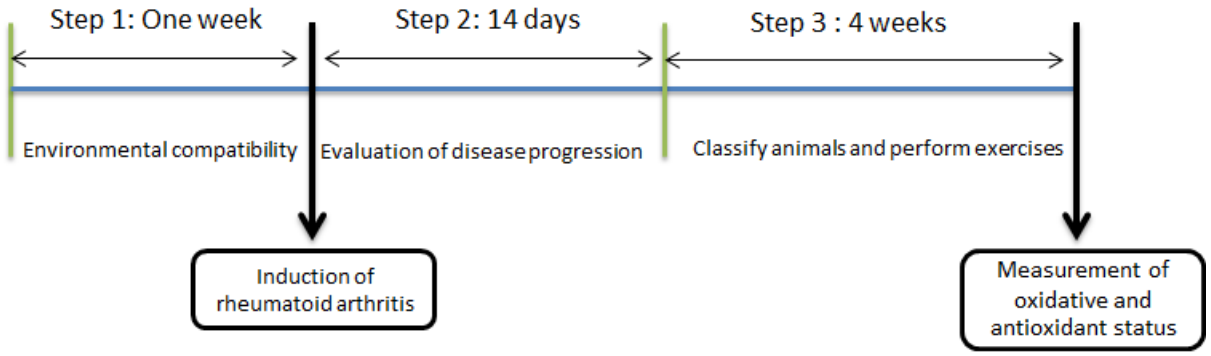


Figure1. The process of conducting the study

Swimming exercise program

Two weeks after the induction of rheumatoid arthritis, the rats were ready to participate in an exercise program. First, the rats were taught how to practice swimming in the pool for a week. At this stage, the rats swam in the water for 15 minutes daily. The next step was the main exercise. At this stage, the rats of the exercise group swam in the pool (150 cm and 50 cm deep) 5 days a week at a temperature of 32°C (temperature was controlled by thermometer and thermostat). The duration of the first session was 20 minutes, which was gradually increased (10 minutes per week) during the training period until it reached 60 minutes in the last session. This exercise program is a model of moderate intensity endurance training [13]. During the program, rats were encouraged to swim with light manual stimuli. Control animals were placed in a pool at least three times a week to provide a similar environment.

Laboratory methods and measurement of variables

The course of the disease from 14 to 42 days after injection was evaluated according to the standard between the toes. The lesions on all four legs of each rat were independently graded from zero to four according to the size of the edema and erythema in the joints, so the maximum degree of arthritis in each rat would be 16 [14]. The rats were anesthetized 48 hours after the last exercise by intraperitoneal injection of a combination of ketamine and xylazine. Then, 10 ml of blood was drawn from the heart with a syringe by incision in the abdomen and chest and poured into tubes containing EDTA. The collected samples were rapidly centrifuged for 10 minutes at a speed of 2800 rpm. The obtained serum was poured into a numbered microtube. The samples were then transferred to a freezer at -70 ° C for further research.

Laboratory kits of the German company Zellbio were used to determine the activity of glutathione peroxidase and glutathione reductase enzymes. The activity of antioxidant enzymes was expressed in conventional units per unit in mg and the amount of serum MDA in nanomoles per gram. For this purpose, according to the instructions of the kit, hemolyzes were prepared at the beginning and then prepared with the reagents of the kit. Then the samples were read by spectrophotometry at 330 and 510 nm and the enzymatic activity of each was determined.

The serum provided in the previous step was used to determine the level of malondialdehyde or TBRS index according to the method of Yoshika et al. For this purpose, 0.1 cc of serum was first combined with 0.3 cc of 20% trichloroacetic acid and centrifuged for 15 minutes. Then 2 cc of thiobarbituric 0.8% was added and placed in a hot water bath for 20 minutes and cooled immediately. In addition, 0.5cc N-butanol was added and the amount of MDA on the surface was determined at 540 and 530 nmol against butanol. Tetratoxypropane 5, 10 and 20 nmol / ml were used as standard to determine the standard curve [15].

Statistical analysis

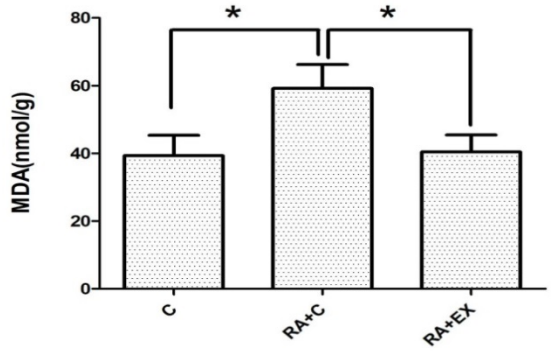
After confirming the normal distribution of data using Shapiro-Wilk test, one-way analysis of variance and Tukey post hoc test were used for statistical analysis. All data were

presented as mean ± standard deviation. Calculations were performed using SPSS software version 18 and the significance level of the tests was considered p≤0.05.

Results

At the beginning of the study, the body weight of rats in the study groups was the same (about 260 g). At the end of the study, the weight of rats in RA+EX group (243±10 g) was significantly lower than RA+C (271±12 g) and C (284±11 g) groups (P=0.02). Apparently, after the induction of rheumatoid arthritis, arthritic and inflammatory disorders in the ankle appeared from about days 12 to 14. In rheumatoid arthritis cases, movements such as walking were slower and weaker. At the end of the research protocol, the severity of arthritis in rats of RA+EX group (5.8±1.3) was significantly lower than RA+C group (7.6 ±1.6) (P=0.04)(figure 2).

Serum malondialdehyde levels and glutathione peroxidase and glutathione reductase activity in the study groups are shown in Figure 2. As can be seen, the serum level of malondialdehyde in the RA+C group is significantly higher than the RA+EX (P=0.04) and C (P=0.02) groups. In addition, we found that glutathione peroxidase activity in RA+C group was significantly lower than RA+EX (P=0.03) and C (P=0.01) groups. On the other hand, no significant difference was observed in the level of glutathione reductase activity between the studied groups (P>0.05).



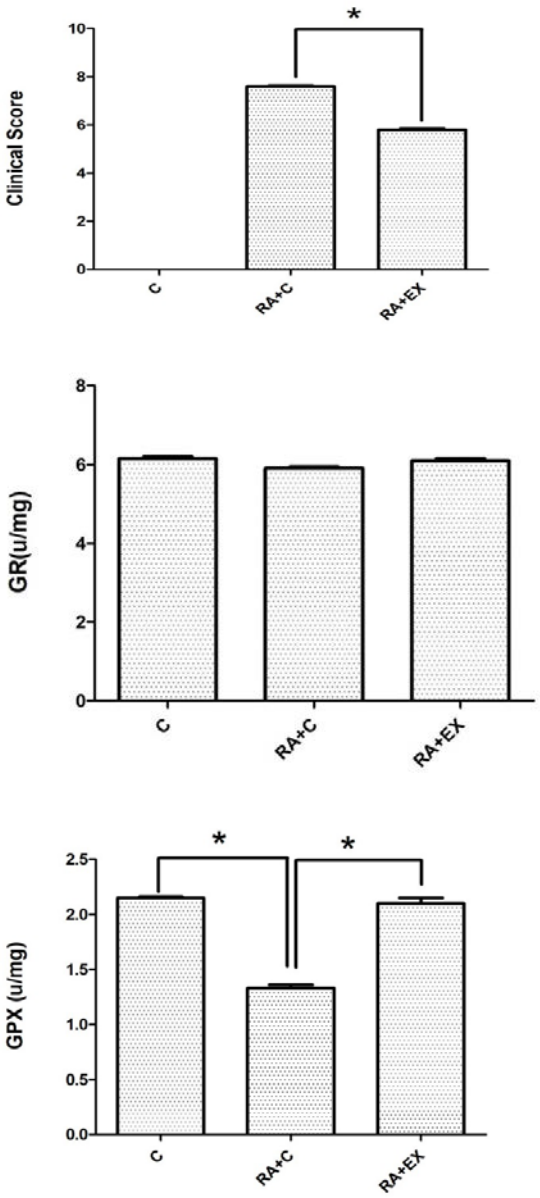


Figure2. Comparison of groups in terms of indicators: a: MDA (malondialdehyde), b: clinical symptoms, c: GPx (glutathione peroxidase) and d: GR (glutathione reductase). *Indicates a significant difference (P<0.05) between RA+C group and C and RA+EX groups.

Discussion

Rheumatoid arthritis is a systemic autoimmune disorder characterized by chronic inflammation that primarily affects the joints [14]. In the present study, RA symptoms such as the ability to walk, redness of the skin and swelling at the ankle and wrist joints and small finger joints peaked from the 15th day after induction and this is consistent

with some evidence in this regard [15]. In the conventional treatment of RA patients, more emphasis is placed on the use of drugs that relieve inflammatory and immune disorders. The limitations of these treatments are known toxicity and diversity in clinical effect. Therefore, in recent years, the effect of a variety of natural products (antioxidant compounds) and non-pharmacological strategies such as exercise training against inflammation and osteoarthritis has been emphasized [16]. In RA, unregulated oxidative metabolism plays an important role in the pathophysiology of the disease. It has been suggested that ROS produced within the joints may contribute significantly to the pathogenesis of osteoarthritis, as these inorganic oxidants are able to destroy matrix components by direct action or by indirect activation of latent collagenases [17]. The aim of this study was to investigate the behavioral activity of systemic oxidants and antioxidants over a period of swimming training using a model of adjuvant-induced rheumatoid arthritis. Lipid peroxidation has been suggested as a key mechanism of degradation and damage during RA. In this regard, Mousavian et al. showed that in patients with RA with a significant increase in plasma MDA content, the level of antioxidant defense is weak [18]. Other similar studies have reported high levels of oxidative stress in patients with RA [5]. Consistent with this evidence, the present study also found that serum MDA levels in rats with RA were significantly higher than healthy rats. It has been suggested that defects in the antioxidant defense system of glutathione play a key role in oxidative stress and pathogenesis of RA [6]. According to these evidences, in the present study, it was also observed that RA rats had lower levels of glutathione peroxidase activity compared to healthy rats. Similarly, previous studies have reported that in the RA state, plasma lipid peroxidation levels increase but the activity of antioxidant enzymes decreases [19]. Glutathione plays an important role in protecting cells and tissue structures. Its role includes detoxifying xenobiotics, free radicals, peroxides and regulating the function of the immune system. The enzyme glutathione peroxidase plays a key role in glutathione metabolism and a large part of the increase in oxidative stress in RA patients has been attributed to dysfunction of this enzyme [20]. Glutathione peroxidase in the aqueous phase of cell membranes destroys highly reactive lipid hydroperoxide. Decreased activity of these enzymatic antioxidants may be due to the accumulation of H2O2, which in turn inhibits these enzymes [21]. In the present study, it was also observed that there was no significant difference in glutathione reductase activity between the healthy control group and the rheumatoid arthritis groups. In fact, our finding is consistent with some studies that have reported that glutathione reductase levels are less affected by RA [6]. Therefore, in the present study, it seems that at least part of the increase in MDA in RA rats is related to the decrease in glutathione peroxidase activity. Taken together, the above data suggest antioxidant therapy strategies for the prevention and treatment of RA.

Studies have also shown that acute intense physical exercise can increase the production of reactive oxygen species, which in turn causes muscle damage and increased levels of oxidative stress in several systems. In contrast, much evidence has shown that regular exercise is associated with decreased ROS production and increased activity of antioxidant enzymes. However, the effect of acute and long-term exercise is still not well understood in terms of its oxidative effects and mechanisms in rheumatoid arthritis [22]. According to a study by Garrado et al., swimming exercise can significantly reduce the formation of TBARS (oxidative stress index) in rats [23]. Nonato et al. also reported that 8 weeks of swimming training reduced lipid peroxidation and increased the activity of glutathione peroxidase and superoxide dismutase enzymes in rats [24]. In fact, these findings are conclusive. In another study [25], moderate physical activity was found to be beneficial and safe for patients with rheumatoid arthritis and to increase antioxidant activity for at least 24 hours. In the present study, we found that a 4-week period of swimming exercise was able to improve antioxidant defense, even in an

experimental model of adjuvant-induced rheumatoid arthritis rats. Our results suggest that swimming training is associated with a reduction in lipid peroxidation as well as an increase in GPx activity. This finding is consistent with other studies that have reported decreased lipid peroxidation and increased antioxidant activity following aerobic exercise [24, 25, 26]. In addition, in the present study, it was observed that after 4 weeks of swimming training, no significant change in glutathione reductase activity occurs, and this is in agreement with the results of some studies that have suggested a longer training period to affect the activity of this enzyme [25]. Therefore, if the training period was longer in the present study, there was a possibility of an increase in glutathione reductase activity. However, in our study, the animals had a high lipid peroxidation index before exercise due to the induced rheumatoid arthritis process. This improvement supports the idea that moderate-intensity aerobic exercise promotes adaptive responses by enhancing the antioxidant defense system and combating excess oxygen species that improve tissue resistance to oxidative stress [26].

However, this study was not without limitations: Among other things, the present study, in addition to being cross-sectional with small number of samples, subgroup analyzes was not performed. Recording and controlling the food intake of subjects was not done. Because RA disease has long-term effects, the short period of this research cannot accurately investigate the effects of exercise on the disease. Therefore, it is recommended to use longer courses in future studies. Of course, the present study also has several strengths, including the fact that all biochemical tests were performed twice and that all subjects in the study were identical in terms of possible interfering factors such as age, sex and weight.

Conclusion

Overall, our study suggests that swimming exercise, , may reduce clinical symptoms and oxidative stress and improve glutathione antioxidant defense status in rats with RA. However, more evidence is needed to support the antioxidant effects of swimming at the molecular level in RA patients.

Acknowledgments

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Authors' Contribution

All authors met the writing standards based on the recommendations of the International Committee of Medical Journal Publishers.

Conflicts of Interest

None declared.

References

1- Wang L, Gao C, Zhu D, Chen LH. Effect of functional exercises on patients with rheumatoid arthritis: a meta-analysis. Beijing Da Xue Xue Bao Yi Xue Ban. 2018; 50(6):991-7.

2- Narváez J. Treatment of rheumatoid arthritis. Med Clin (Barc). 2016; 147(4):176-80.

3- da Fonseca LJS, Nunes-Souza V, Goulart MOF, Rabelo LA. Oxidative stress in rheumatoid arthritis: what the future might hold regarding novel biomarkers and add-on therapies. Oxid Med Cell Longev. 2019; 1:7536805-11.

4- Mateen S, Moin S, Khan AQ, Zafar A, Fatima N. Increased reactive oxygen species formation and oxidative stress in rheumatoid arthritis. PLoS One. 2016; 11(4):0152925-32.

5- Attia AM, Ibrahim FA, Abd El-Latif NA, Aziz SW. Therapeutic antioxidant and anti-inflammatory effects of laser acupuncture on patients with rheumatoid arthritis. Lasers Surg Med. 2016; 48(5):490-7.

6- Kadry MO. Liposomal glutathione as a promising candidate for immunological rheumatoid arthritis therapy. Heliyon. 2019; 5(7):e02162-9.

7- Metsios GS, Kitas GD. Physical activity, exercise and rheumatoid arthritis: Effectiveness, mechanisms and implementation. Best Pract Res Clin Rheumatol. 2018; 32(5):669-82.

8- Lange E, Kucharski D, Svedlund S, Svensson K, Bertholds G. Effects of aerobic and resistance exercise in older adults with rheumatoid arthritis: a randomized controlled trial. Arthritis Care Res (Hoboken). 2019; 71(1):61-70.

9- Powers SK, Deminice R, Ozdemir M, Yoshihara T, Bomkamp MP, Hyatt H. Exercise-induced oxidative stress: friend or foe? J Sport Health Sci. 2020; 9(5):415-25.

10- Rall LC, Roubenoff R, Meydani SN, Han SN, Meydani M. Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) as a marker of oxidative stress in rheumatoid arthritis and aging: effect of progressive resistance training. J Nutr Biochem. 2000; 11(11-12):581-4.
11- Wadley AJ, Veldhuijzen van Zanten JJ, Stavropoulos-Kalinoglou A. Three months of moderate-intensity exercise reduced plasma 3-nitrotyrosine in rheumatoid arthritis patients. Eur J Appl Physiol. 2014; 114(7):1483-92.
12- Asquith DL, Miller AM, McInnes IB, Liew FY. Animal models of rheumatoid arthritis. Eur J Immunol. 2009; 39(8):2040-4.
13- Kalantari A, Saremi A, Shavandi N, Foroutan Nia A. Impact of four week swimming exercise with alpha-tocopherol supplementation on fertility potential in healthy rats. Urol J. 2017; 14(5):5023-6.
14- Fischer BD, Adeyemo A, O'Leary ME, Bottaro A. Animal models of rheumatoid pain: experimental systems and insights. Arthritis Res Ther. 2017; 19(1):146-52.
15- González-Chávez SA, Quiñonez-Flores CM, Espino-Solís GP. Exercise exacerbates the transcriptional profile of hypoxia, oxidative stress and inflammation in rats with adjuvant-induced arthritis. Cells. 2019; 8(12):1493-9.
16- Burmester GR, Pope JE. Novel treatment strategies in rheumatoid arthritis. Lancet. 2017; 389(10086):2338-48.
17- Phull AR, Nasir B, Haq IU, Kim SJ. Oxidative stress, consequences and ROS mediated cellular signaling in rheumatoid arthritis. Chem Biol Interact. 2018; 281:121-36.
18- Moosavian SP, Paknahad Z, Habibagahi Z. A randomized, double-blind, placebo-controlled clinical trial, evaluating the garlic supplement effects on some serum biomarkers of oxidative stress, and quality of life in women with rheumatoid arthritis. Int J Clin Pract. 2020; 74(7):13498-503.
19- Shan L, Tong L, Hang L, Fan H. Fangchinoline supplementation attenuates inflammatory markers in experimental rheumatoid arthritis-induced rats. Biomed Pharmacother. 2019; 111:142-50..
20- Hassan MQ, Hadi RA, Al-Rawi ZS, Padron VA, Stohs SJ. The glutathione defense system in the pathogenesis of rheumatoid arthritis. J Appl Toxicol. 2001; 21(1):69-73.
21- Sindhu G, Ratheesh M, Shyni GL, Nambisan B, Helen A. Anti-inflammatory and antioxidative effects of mucilage of Trigonella foenum graecum (Fenugreek) on adjuvant induced arthritic rats. Int Immunopharmacol. 2012; 12(1):205-11.
22- Powers SK, Radak Z, Ji LL. Exercise-induced oxidative stress: past, present and future. J Physiol. 2016; 594(18):5081-92.
23- Guaraldo SA, Serra AJ, Amadio EM, Antônio EL, Silva F. Lasers Med Sci. 2016; 31(5):833-40. Lasers Med Sci. 2016; 31(5):833-40.
24- Nonato LF, Rocha-Vieira E, Tossige-Gomes R, Soares AA, Soares BA, Freitas DA, Oliveira MX, Mendonca VA, Lacerda AC, Massensini AR, Leite HR. Swimming training attenuates oxidative damage and increases enzymatic but not non-enzymatic antioxidant defenses in the rat brain. Braz J Med Biol Res. 2016; 49:5310-16.
25- Tuna Z, Duger T, Atalay-Guzel N, Aral A, Basturk B, Haznedaroglu S, Goker B. Aerobic exercise improves oxidant-antioxidant balance in patients with rheumatoid arthritis. J Phys Ther Sci. 2015; 27:1239–42.
26- Dos Santos SA, Dos Santos Vieira MA, Simões MCB. Photobiomodulation therapy associated with treadmill training in the oxidative stress in a collagen-induced arthritis model. Lasers Med Sci. 2017; 32(5):1071-79.