The effect of aerobic exercise combined with bone marrow mesenchymal stem cells on inflammatory biomarkers levels of the brain in a model of osteoarthritic rat

Mohamad Fatahi[1], Naser Behpoor[2*], Sedighe Hosseinpourdelavar[1], Parvin Farzaneghi[3]

1. Department of Exercise Physiology, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran
2. Department of Exercise Physiology, Razi University, Kermanshah, Iran
3. Department of Exercise Physiology, Sari Branch, Islamic Azad University, Sari, Iran

***Corresponding author:**Tel: +98 9123271873 Fax:+98 -
Address: Department of Exercise Physiology, Razi University, Kermanshah, Iran
E-mail:n_behpoor@yahoo.com
Received; 20/05/2020 Revised; 22/06/2020 Accepted; 16/07/2020

Abstract

Introduction: Osteoarthritis is a degenerative disease that has many public health problems. Interleukin 10 (IL-10) and tumor necrosis factor alfa (TNF-α) are considered as the main factors regulating inflammation and pathology of knee osteoarthritis. This study was done to investigate the effects of mesenchymal stem cells (MSCs) and aerobic training on inflammatory biomarkers (IL-10 and TNF- α) in the brain tissue of osteoarthritic rats.

Materials and Methods: In this experimental study, 48 male Wistar rats were randomly divided into six groups (n=8 in each group) as follows: 1. Naive (healthy control), 2. Osteoarthritis (Ost), 3. Osteoarthritis + saline (Sal), 4. Osteoarthritis + mesenchymal cells (Mes), 5. Osteoarthritis + training (Tra), and 6. Osteoarthritis + training + mesenchymal cells (Tra + Mes). An essential aerobic exercise program was performed for eight weeks as follows: Speed: 15-22 meters per minute; Slope: 0^{o} for 25-64 minutes. Inflammatory biomarkers, including TNF-α and IL-10 cytokines, in the brain tissue of rats, were measured using enzyme-linked immunosorbent assay.

Results: The combination of training and treatment with MSCs in the brain of osteoarthritic rats significantly increased the level of IL-10 in comparison with the use of MSCs or aerobic exercise alone. Moreover, the combined use of exercise and MSCs caused a significant decrease in TNF-α concentration.

Conclusion: It seems that the combined use of MSCs and eight weeks of aerobic exercise improves the concentration of inflammatory biomarkers in the brain of a rat model of osteoarthritis.

Keywords: Exercise, Osteoarthritis, Inflammation, Stem cells

Introduction

Osteoarthritis is the most prevalent chronic joint disease and a frequent cause of joint pain, function loss, and disability (1). Osteoarthritis represents the second leading cause of work disability in men aged beyond 50. Furthermore, osteoarthritis is responsible for approximately 2% of all public health expenses, and large indirect costs due to decreased productivity. Many treatments have been proposed for osteoarthritis; however, poor clinical results without cartilage repair have been reported (2).

Exercise remains an extremely popular leisure-time activity in many countries throughout the Western world and has become a big part of modern lifestyle. Exercise is widely suggested because of various health benefits such as weight

control, disease management in cardiovascular diseases and diabetes, as well as for improving psychological well-being (3). In addition to exercise, new investigations have shown that using multipotent cells can open new ways to treat incurable diseases (4).

Mesenchymal stem cells (MSCs) are multipotent cells that can differentiate into osteoblasts, adipocytes, and chondrocytes. MSCs can be obtained from different sources, such as umbilical cord blood, bone marrow, adipose tissue, and lung (5). Exercise can affect MSCs. It may facilitate MSC migration by increasing interleukins and recruiting stem cells in the site of injury. Additionally, the secretum of MSCs is responsible for hematopoietic stem and progenitor cell (HSPC) mobilization and proliferation, and exercise induces homing of HSPCs to extramedullary sites (6). Moreover, MSCs are considered as a novel strategy for treating a wide variety of diseases, including autoimmune, bone and cartilage diseases, inflammatory airway disorders, muscle diseases, neurodegenerative diseases, spinal cord injuries, etc. (7). MSCs have been demonstrated to secrete a broad range of bioactive or trophic factors such as TNF-α and interleukin-10 (IL-10) which are essential in the cellular microenvironment for survival, protection, immune modulation, and differentiation (8-10).

Regardless of circulating cytokines sources, TNF-α and IL-6 are the most frequent factors among many markers of systemic inflammation, (11). High levels of these cytokines are strong predictors of mortality (in middle-aged people and the elderly) (12), and are also the risk factor for other diseases such as diabetes and certain cancers (13, 14). Although it has been reported that MSCs and exercise separately could have beneficial effects on the inflammatory indices in the brain tissues (15-17), simultaneously effects of aerobic exercise and MSCs on the levels of inflammatory biomarkers such as IL-10 and TNF-α in the brain tissue have not yet been investigated . This study examined whether bone marrow MSC transplantation combined with eight weeks of aerobic exercise could affect IL-10 and TNF-α levels in brain tissue of a rat model of osteoarthritis.

Materials and Methods

In this experimental study, male Wistar rats (250-300 g) were obtained from the " Laboratory of the Animal Research Center from Islamic Azad University, Sari Branch, Iran." One week before the start of the experiment, the animals were familiarized with laboratory environment. They were maintained at the standard temperature (21 ± 2°C) under a 12/12 h light/dark cycle, with food and water available *ad libitum*. The study protocol was approved by the Ethics Committee for Animal Research of Islamic Azad University, Sari Branch, Iran (ethical code: NO.19.33.2018), and also was in accordance with the last update of the Helsinki Declaration. The rats were randomly divided into six groups (n=8) as follows: 1. Naive (healthy control), 2. osteoarthritis (Ost), 3. Osteoarthritis + saline (Sal), 4. Osteoarthritis + mesenchymal cells (Mes), 5. Osteoarthritis + training (Tra), and 6. Osteoarthritis + training + mesenchymal (Tra + Mes).

Bone marrow MSCs were isolated and cultured by the whole bone marrow adherence method (18). Based on the rats' body weight, 2% pentobarbital was intraperitoneally injected at 35 mg/kg into the rats, and they were anesthetized. The rats' skin was disinfected with 75% ethanol and the femur and tibia were removed aseptically. Then, the femur and tibia were soaked in 75% ethanol for 10 min. The sample was transferred to a clean bench and dried in a sterile petri dish. The ends of the two bones were cut off, and the two bone marrow

cavities were exposed. The marrow cavity was washed by serum-free Dulbecco's modified Eagle's medium (repeat 2-3 times), and the solution was centrifuged at 1,000 rpm for 5 minutes. After centrifugation, the supernatant was discarded and the cells were suspended in a DMEM medium containing 10% fetal bovine serum. The cells were counted on the counting plate, and the cell density was adjusted to 5,000 cells/mL in a culture flask. The culture was incubated at 37°C (5% CO2, wet saturation). After 24 hours, the medium was changed. Then, the medium was changed every 3 days. When 80% to 90% of the cells covered the wall of the culture medium, cells were digested with 0.25% trypsin.

The animals were anesthetized through intraperitoneal injection of a cocktail containing both xylazine (5 mg/kg) and ketamine (60 mg/kg). After shaving with razors, a para-patellar skin incision (1 cm) was made on the medial side of the right knee joint and on the medial side of the patellar tendon. The patella was then dislocated laterally to provide access to the joint space and the anterior cruciate ligament was transected in the flexed knee. A positive anterior drawer test confirmed the complete transection of the ligament. The joint was then irrigated with sterile saline to avoid ancillary inflammation, and a purposefully made suture was inserted (19).

Before performing the aerobic exercise program, in order to acquaint the subjects with the research environment and treadmill, a light exercise program for 1 week, including 5 sessions of walking and running on a treadmill for 5 to 10 min at the speed of 5 to 8 m/ min on a zero percent slope was considered. Then, the main exercise including running on a treadmill with no special slope for rodents with gradually increasing speed and with observance of the principle of overload from 25 min to 64 min was performed.

The exercise was performed in 5 sessions per week at the speed of 15 to 22 m/ min for 8 weeks (20). To warm up, the subjects at the beginning of each training session ran for 3 min at a speed of 7 m/ min, and to reach the target speed, the speed was then increased by 2 m/min every one minute. To cool the body at the end of each training session, the speed of the turntable decreased to the initial speed. Forty-eight hours after the last exercise session, the rats were killed with an intramuscular injection of ketamine (60 mg/kg) and xylazine (5 mg/kg). Then, the brain tissue samples were obtained and placed in a cold buffer (0.1 M phosphate-buffered saline, pH 7.4, containing the protease inhibitor cocktail, Roche). They were homogenized and centrifuged at 3500 rpm for 15 min, and supernatants were collected. Finally, indices of inflammation (TNF-α and IL-10) were recognized by specific enzyme-linked immunosorbent assay (ELISA) kits (ZellBio, Germany) (21, 22).

Statistical Analysis

The statistical comparison of the results was carried out using one-way ANOVA, followed by post-hoc Bonferroni's multiple comparison test. The results are shown as means ± S.E.M. A $P<0.05$ was used as the indicator of statistical significance.

Results

According to Figure 1, a one-way ANOVA results [$F(5, 42) = 27.62$, $P < 0.0001$] showed that there were significant differences between groups in terms of IL-10 levels. Bonferroni's multiple comparison post-hoc test revealed that there was a significant difference between the Ost ($P < 0.0001$), Sal ($P < 0.0001$), Mes ($P < 0.01$), and Tra ($P < 0.05$) groups in comparison with the Nav group. Interestingly, the results showed that there were significant differences between the Mes ($P < 0.001$), Tra ($P < 0.001$), and Tra

+ Mes ($P < 0.0001$) groups in comparison with the Ost group. Besides, the IL-10 levels in the Ost group did not indicate significant differences compared with the Sal group (ns). Furthermore, the data did not show a significant difference between the Mes and Tra groups (ns). According to Figure 2, a one-way ANOVA results showed that there were significant differences between groups [$F (5, 42) = 40.18$, $P < 0.0001$] in terms of TNF-α levels. Bonferroni's multiple comparison post-hoc test revealed that there was a significant difference between the Ost and Sal ($P < 0.0001$) groups in comparison with the Nav group.

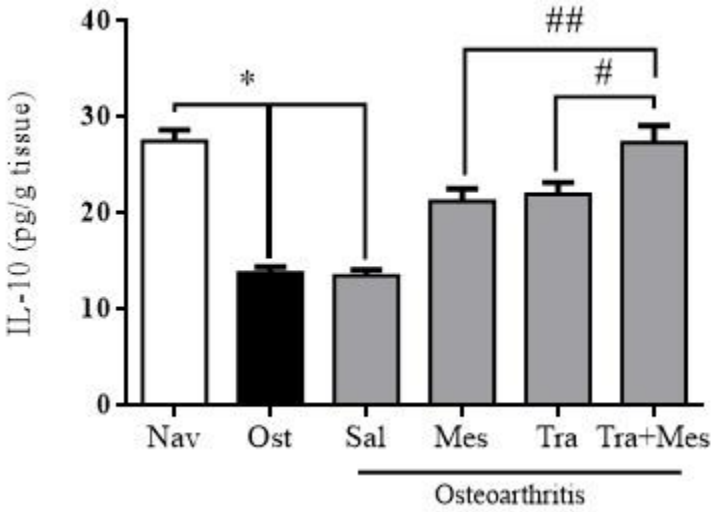

Figure 1. Effects of exercise and bone marrow mesenchymal stem cells on IL-10 levels in the brain of male osteoarthritis rats. Nav: nave or healthy control; Ost: osteoarthritis; Sal: slaine; Mes: mesenchymal cells; Tra: Training. *$P < 0.0001$ as compared with the Nav. #$P < 0.05$, ##$P < 0.01$ as compared with the Tra + Mes. Data are expressed as the mean ± S.E.M.

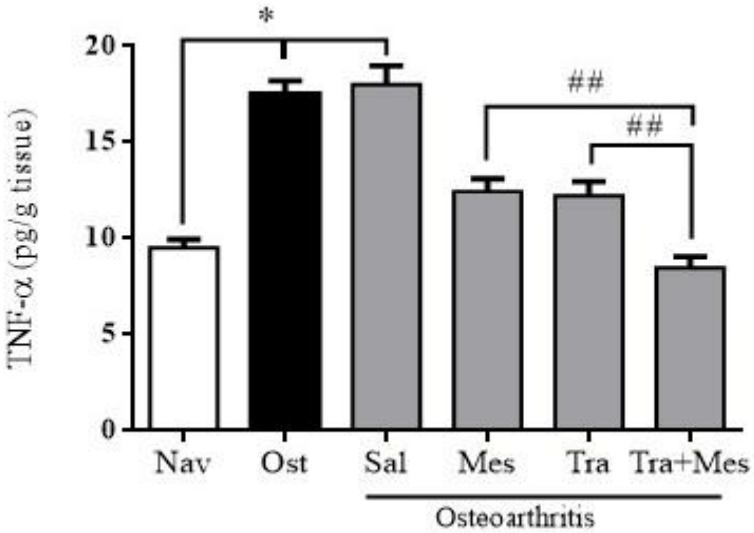

Figure 2. Effects of exercise and bone marrow mesenchymal stem cells on TNF-α levels in the brain of male osteoarthritis rats. Nav: nave or healthy control; Ost: osteoarthritis; Sal: slaine; Mes: mesenchymal cells; Tra: Training. *$P < 0.0001$ as compared with the Nav. ##$P < 0.01$ as compared with the Tra + Mes. Data are expressed as the mean ± S.E.M.

Interestingly, the results showed that there were significant differences between the Mes, Tra, and Tra + Mes groups ($P < 0.0001$) in comparison with the Ost group. Besides, the IL-10 levels in the Mes group did not indicate a significant difference compared with the Tra group (ns).

Discussion

The findings of this study indicated that eight weeks of aerobic exercise combined with treatment with bone marrow MSCs not only could increase the levels of IL-10 but also attenuated TNF-α levels in the brain of male rats with osteoarthritis.

It has been advised to use muscle-strengthening programs and functional exercises to relieve pain in patients with knee osteoarthritis. The long-term effects of exercise and weight loss as first-line treatments for osteoarthritis have been reported. Moreover, therapeutic exercises and physical activity are important for patients with knee osteoarthritis in any stage of the disease (23). In patients with medial knee osteoarthritis, an 8-week hip-adductor strengthening program as the home exercising program improved the function of the knee and reduced pain (24). In another study by Lee et al. (2004), an 8-week Tai Chi exercise program was found as a safe and effective nursing program to improve the risk factors for falls in older adults with osteoarthritis (25). The present results confirmed the previous investigations because we showed that an 8-week exercise can improve the release of inflammatory biomarkers in osteoarthritis rats.

Several authors have studied the cytokine response to physical exercise in recent years. For example, one study reported that ten weeks of moderate- to high-intensity resistance training reduced the systemic inflammatory milieu in sedentary elderly women (26). In another investigation, 16 weeks of resistance training in middle-aged healthy men did not affect IL-10 and TNF-α levels (27). This discrepancies may be explained by the difference in intervention duration and the ability to adapt to situations that may influence inflammatory response, subjects' gender, and age. Particularly, due to the effect of hormones, gender may result in different cytokine responses to exercise and age is associated with an increase in basal TNF-α levels (28). It has been reported that the anti-inflammatory effect of exercise training can be dependent on the intensity and duration of the exercise (29). In another study, it is well-established that in healthy rats, an 8-week moderate-intensity aerobic training down-regulated skeletal muscle production of cytokines involved in the onset, maintenance, and regulation of inflammation, and the response was heterogeneous according to fiber composition (30).

Stem cells are nonspecialized cells that can differentiate into some or all major specialized cell types of tissues or organs. Predominantly, adult stem cells, such as MSCs are responsible for daily tissue or cell maintenance, remodeling, and regeneration of multiple tissues (31). Exercise can play an important role in the function and fate of these adult stem cells by altering extracellular matrix composition, reducing inflammation, and promoting their migratory capacity demonstrated that MSC-derived extracellular vesicles ameliorate inflammation-induced preterm brain injury (32). OUR results confirmed their results because we have shown that exercise can decrease TNF-α levels in brain tissues.

Conclusion

Our findings indicated that the combination of eight weeks of aerobic exercise and treatment with bone marrow MSCs could increase the levels of IL-10 and attenuated TNF-α levels in the brain of male rats with osteoarthritis. Probably, the combined use of

eight weeks of aerobic exercise with bone marrow MSCs in subjects with osteoarthritis is more effective compared with the use of exercise and/or bone marrow MSCs therapy alone.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

This research was supported by the Islamic Azad University, Kermanshah, Iran, which was extracted from the Ph.D. thesis of Mohamad Fatahi. The authors would like to express their gratitude to the staff of the Laboratory of Animal Research Center, Islamic Azad University, Sari Branch, Iran, for helping us to carry out this project (N9.19.33.2018).

Financial Support

none

Author Contributions

Mohamad Fatahi performed the majority of the experiments and data collection. Naser Behpoor provided technical support. Parvin Farzanegi drafted the manuscript. And Sedighe Hosseinpourdelavar designed the experiments, conducted data analysis, and interpretation.

References

1. Jones IA, Togashi R, Wilson ML, Heckmann N, Vangsness CT. Intra-articular treatment options for knee osteoarthritis. Nat Rev Rheumatol. 2019;15(2):77-90. doi: 10.1038/s41584-018-0123-4.
2. Emery CA, Whittaker JL, Mahmoudian A, Lohmander LS, Roos EM, Bennell KL, et al. Establishing outcome measures in early knee osteoarthritis. Nat Rev Rheumatol. 2019;15(7):438-48. doi:10.1038/s41584-019-0237-3.
3. Han J, Neufer D, Pilegaard H. Exercise physiology: future opportunities and challenges. Pflugers Arch - Eur J Physiol 471, 381 (2019). doi: 10.1007/s00424-019-02263-6.
4. Khan MR, Smith RK, David F, Lam R, Hughes G, De Godoy R, et al. Evaluation of the Effects of Synovial Multipotent Cells on Deep Digital Flexor Tendon Repair in a Large Animal Model of Intra-Synovial Tendinopathy. J Orthop Res ®. 2020;38(1):128-38. doi: 10.1002/jor.24423.
5. Souza-Moreira L, Soares VC, Dias SdSG, Bozza PT. Adipose-derived Mesenchymal Stromal Cells Modulate Lipid Metabolism and Lipid Droplet Biogenesis via AKT/mTOR–PPARγ Signalling in Macrophages. Sci Rep. 2019;9(1): 1-11. doi: 10.1038/s41598-019-56835-8.
6. Bourzac C, Bensidhoum M, Pallu S, Portier H. Use of adult mesenchymal stromal cells in tissue repair: impact of physical exercise. Am J Physiol Cell Physiol. 2019;317(4):C642-C54. doi:10.1152/ajpcell.00530.2018.
7. Hu X, Garcia M, Weng L, Jung X, Murakami JL, Kumar B, et al. Identification of a common mesenchymal stromal progenitor for the adult haematopoietic niche. Nat Commun.2016;7(1):1-14. doi:10.1038/ncomms13095.
8. Fallahzadeh AR, Zarei M, Mohammadi S. Preliminary phytochemical screening, analgesic and anti-inflammatory effect of eryngium pyramidale Boiss. & Husson Essential Oil in Male Rat. Entomology and Applied Science Letters. 2016;3(5):140-7. doi:10.1248/bpb.b12-00905.

9. Asgari Nematian M, Yaghmaei P, Mohammadi S. Assessment of the antinociceptive, antiinflammatory and acute toxicity effects of Ducrosia anethifolia essential oil in mice. Sci J Kurdistan Univ Med Sci. 2017; 22:74-84. doi: 10.22102/22.3.74.
10. Galland S, Stamenkovic I. Mesenchymal stromal cells in cancer: a review of their immunomodulatory functions and dual effects on tumor progression. J Pathol. 2020;250(5):555-72. doi:10.1002/path.5357.
11. Hausmann JS. Targeting cytokines to treat autoinflammatory diseases. Clin Immunol. 2019; 206:23-32. doi: 10.1016/j.clim.2018.10.016.
12. El-Kader SMA, Al-Jiffri OH. Aerobic exercise modulates cytokine profile and sleep quality in elderly. A h s. 2019;19(2):2198-207. doi: 10.4314/ahs.v19i2.45.
13. Padoan A, Plebani M, Basso D. Inflammation and pancreatic cancer: focus on metabolism, cytokines, and immunity. Int J Mol Sci. 2019;20(3):676. doi:10.3390/ijms20030676.
14. Donath MY. Designer cytokine for the treatment of diabetes. Nat Metab. 2019:1-2. doi:10.1038/s42255-019-0130-z.
15. Newman RE, Yoo D, LeRoux MA, Danilkovitch-Miagkova A. Treatment of inflammatory diseases with mesenchymal stem cells. Inflamm Allergy Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy). 2009;8(2):110-23. doi:.2174/187152809788462635.
16. You T, Arsenis NC, Disanzo BL, LaMonte MJ. Effects of exercise training on chronic inflammation in obesity. Sports Med. 2013;43(4):243-56. doi:10.1007/s40279-013-0023-3.
17. Mohammadi S, Oryan S, Komaki A, Eidi A, Zarei M. Effects of hippocampal microinjection of irisin, an exercise-induced myokine, on spatial and passive avoidance learning and memory in male rats. Int J Pept Res Ther. 2020;26(1):357-67. doi:10.1007/s10989-019-09842-2.
18. Di K, Sui L, Yan X. Effects of tetramethylpyrazine combined with bone marrow mesenchymal stem cells transplantation on recovery of neural function in rats with spinal cord injury. Int J Clin Exp Med. 2018;11(12):13605-12. doi: 10.1136/annrheumdis-2014-205779.
19. Zhao Y, Liu B, Liu C-j. Establishment of a surgically-induced model in mice to investigate the protective role of progranulin in osteoarthritis. JoVE (J Visualized Exp). 2014 (84):e50924. doi: 10.3791/50924.
20. Hornberger Jr TA, Farrar RP. Physiological hypertrophy of the FHL muscle following 8 weeks of progressive resistance exercise in the rat. Can J Appl Physiol. 2004;29(1):16-31. doi: 10.1139/h04-002.
21. Mohammadi S, Oryan S, Komaki A, Eidi A, Zarei M. Effects of intra-dentate gyrus microinjection of myokine irisin on long-term potentiation in male rats. Arq Neuro-Psiquiatr. 2019;77(12):881-7. doi: 10.1590/0004-282x20190184.
22. Hollon TC, Pandian B, Adapa AR, Urias E, Save AV, Khalsa SSS, et al. Near real-time intraoperative brain tumor diagnosis using stimulated Raman histology and deep neural networks. Nat Med. 2020:1-7. doi: 10.1038/s41591-019-0715-9.
23. Messier SP, Loeser RF, Miller GD, Morgan TM, Rejeski WJ, Sevick MA, et al. Exercise and dietary weight loss in overweight and obese older adults with knee osteoarthritis: the Arthritis, Diet, and Activity Promotion Trial. Arthritis Rheum. 2004;50(5):1501-10. doi: 10.1002/art.20256
24. Sandal LF, Roos EM, Bøgesvang SJ, Thorlund JB. Pain trajectory and

exercise-induced pain flares during 8 weeks of neuromuscular exercise in individuals with knee and hip pain. Osteoarthr Cartil. 2016;24(4):589-92. doi: 10.1016/j.joca.2015.11.002.
25. Lee E-N, Yoo Y-W. Effects of an 8-week Tai Chi exercise program on the risk factors for falls in the elderly with osteoarthritis. J Health Sci. 2004;11(1):61-73. doi: 10.4103/1319-6308.112207.
26. Phillips MD, Flynn MG, McFarlin BK, Stewart LK, Timmerman KL. Resistance training at eight-repetition maximum reduces the inflammatory milieu in elderly women. Medicine & Science in Sci Sports. 2010;42(2):314-25. doi: 10.1249/MSS.0b013e3181b11ab7.
27. Bruunsgaard H, Pedersen BK. Effects of exercise on the immune system in the elderly population. Immunol Cell Biol. 2000;78(5):523-31. doi: 10.1111/j.1440-1711.2000.t01-14-.x.
28. Northoff H, Symons S, Zieker D, Schaible EV, Schäfer K, Thoma S, et al. Gender-and menstrual phase dependent regulation of inflammatory gene expression in response to aerobic exercise. Exerc Immunol Rev. 2008;14(86-103):134. doi:10.1111/j.1440-1711.2000.
29. Balducci S, Zanuso S, Nicolucci A, Fernando F, Cavallo S, Cardelli P, et al. Anti-inflammatory effect of exercise training in subjects with type 2 diabetes and the metabolic syndrome is dependent on exercise modalities and independent of weight loss. Nutrition, Drug Metab Dis. 2010;20(8):608-17. doi: 10.1016/j.numecd.2009.04.015.
30. Lira FS, Koyama CH, Yamashita AS, Rosa JC, Zanchi NE, Batista Jr ML, et al. Chronic exercise decreases cytokine production in healthy rat skeletal muscle. Cell Biochem Funct: Cellular biochemistry and its modulation by active agents or disease. 2009;27(7):458-61. doi: org/10.1002/cbf.1594.
31. Gehart H, Clevers H. Stem Cell-Derived Liver Cells: From Model System to Therapy. The Liver: Extra cell Matrix. 2020:1015-1021. doi: 10.1002/9781119436812.ch77.
32. Drommelschmidt K, Serdar M, Bendix I, Herz J, Bertling F, Prager S, et al. Mesenchymal stem cell-derived extracellular vesicles ameliorate inflammation-induced preterm brain injury. Brain, Behav Immun. 2017;60:220-32. doi: 10.1016/j.bbi.2016.11.011.