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Effect of Aerobic Exercise with and without Quercetin Supplementation on Rat's Knee Osteoarthritis

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Abstract

***Background*:** Osteoarthritis is the commonest form of arthritis and is considered to be one out of ten major causes of disability in the world. The objective of present study was to investigate the effect of treadmill exercise alone and in combination with quercetin supplementation on male rats' knee osteoarthritis.

***Materials and Methods*:** Thirty male Wistar rats (weight of 173±1 g, 8-weeks old) were randomly divided into 5 experimental groups (N=6): intact control, MIA only, training, quercetin, training plus quercetin. The Osteoarthritis model was induced by intra-articular injection of monosodium iodoacetate (MIA). Subjects then followed a moderate-intensity exercise program and quercetin supplementation for 28 days. Rats were killed after 28 days and histological assessment has been performed on their knee joints. One-way ANOVA ($p<0.05$) and post-hoc Tukey test was used for the statistical analysis.

***Results*:** Histological assessment including 1- Depth Ratio of Lesions ($p=0.001$), 2- Total Degeneration Width ($p=0.001$) and 3- Significant Degeneration Width ($p=0.001$) demonstrated a beneficial influence of moderate exercise combined with quercetin supplementation group, but this influence was not superior than the moderate exercise alone group.

***Conclusion*:** This study shows that a moderate exercise program and quercetin supplementation, either alone or in combination, exert a beneficial influence on rats' knee osteoarthritis. But it appears that moderate exercise alone has more effectiveness.

Introduction

Osteoarthritis (OA) or degenerative joint disease is the commonest form of arthritis and can result in joint pain, decrease in joint range of motion, loss of function and disability [1, 2]. Although its nature and symptoms are well-defined and its related risk factors have been studied precisely, there is no known cure for OA [3-5].

The treatment modalities for OA include non-pharmacological, pharmacological and ultimately surgery. The current treatments for osteoarthritis reduce pain and inflammation but have no significant effect on its progression. Recently, in addition to pharmaceuticals, the interest of using nutraceutical therapies is being highlighted. The antioxidant, anti-inflammatory and antiproliferative activities of naturally occurring polyphenols such as quercetin that are present in vegetables and fruits suggest that these compounds may be useful in the development of new treatments for OA [6-8]. According to recent guidelines of Osteoarthritis Research Society International (OARSI), optimal treatments of knee and hip OA should include both pharmacological and non-pharmacological modalities [9]. Some studies have been conducted on human and animal models that investigated the effects of pharmacological methods in prevention and treatment of OA [10]. Also several studies examined the effectiveness of non-pharmacological treatments, especially exercise, in management of OA symptoms [11-16]. However, reviewing past studies, there are few investigations on the combination of two modalities. For instance, 4 studies were found that have been performed on elderly patients and indicated contradictory results [14, 17].

It should be noted that these studies evaluated the effects of interventions using questionnaires or assessing some physical fitness measures. Hence the effects of treatment protocols on joint's cartilage are remained nearly unknown, because the researchers were unable to assess biochemical properties of the tissue In-vivo [18]. For this reason, there is a need to perform histopathological assessments that have to be done on animal models of OA resembling the condition in human models. According to our studies, intra-articular injection

of monosodium iodoacetate (MIA) in animal models (such as rats), results in pathological changes closely resembling those seen in human OA [19].

So the aim of present study was to examine the effects of moderate-intensity exercise in combination with quercetin supplementation on rats' knee osteoarthritis.

Materials and Methods

Thirty male Wistar rats (173±1 g, 8 weeks old) were obtained from Pasteur Institute (Amol, Northern Iran). The maintenance and care of the experimental rats were in accordance with the guidelines of the Helsinki convention. Subjects were kept in standard condition in individual plastic cages in a 12:12 light-dark cycle (light-on period, 6:00 AM-6:00 PM) in a controlled temperature of 22±2ºC and 50±5% humidity on sawdust bedding. They were fed a standard diet in pellet forms and had access to tap water ad libitum. Body weight was recorded at regular intervals. The animals were randomly divided into five groups (N=6): 1- Intact control, 2- MIA only (OA), 3- training, 4- quercetin, and 5- training plus quercetin.

The animals in training group were habituated on a motor-driven treadmill at a speed of 10 m/min for 10 min/day for 1 week to reduce their stress regarding the new environment [13]. OA was induced by intra-articular injection of monosodium iodoacetate (MIA); its injection into joints inhibits glyceraldehydes-3-phosphate dehydrogenase activity in chondrocytes, leading to disruption of glycolysis and eventual cell death. For this purpose, the animals were anesthetized with ketamine (90 mg/kg, i.p.) and xylazine (20 mg/kg, i.p.); MIA (Sigma-Aldrich, Germany) was injected with a U-100 insulin needle containing 1 mg of iodoacetate diluted in 50 µL saline solution into animals' right knee. In their left knee, 50 µL saline solution was injected [19].

Training program started 24 hours after OA induction. After the adaptation period, a program of moderate physical training once a day for 4 weeks with a speed of 18 m/min for 30 min/day was performed [12]. In order to assess therapeutic effects of quercetin (Acros Organics, Belgium) supplementation, it was administered by gavage with dose of 150 mg/subject (3 times per week and 65 mg/kg each time) for 4 weeks [21]. On day 28, animals were killed by cervical dislocation under anaesthesia. Whole knee joints were dissected, fixed in 10% formaldehyde solution in 50 ml vials and sent to pathology laboratory. The samples decalcified with 5% formic acid, dehydrated through a descending series of ethanol with the use of an automated tissue processing apparatus. After embedding in paraffin, serial sections with a thickness of 7 µm were prepared for histological examination. Frontal and sagittal sectioning have been prepared from tibiofemoral joints. The sections were stained with hematoxylin-eosin to observe cellularity.

The severity of OA lesions was graded on a scale adapted from OARSI histopathology instructions. Three histopathological measures used in this study according to OARSI recommendation included 1- depth ratio of lesions (DR), 2- total cartilage degeneration width (TDW) and 3- significant cartilage degeneration width (SDW). DR is a measurement of the depth of cartilage degeneration (e.g., including areas of chondrocyte and proteoglycan loss, which may have good retention of collagenous matrix and no fibrillation) that is taken at the midpoint in each of the three zones across the tibial surface.

TDW is the total width of the area of articular cartilage affected by any type of degenerative change (matrix fibrillation/loss, proteoglycan loss with or without chondrocyte death). And SDW is a measurement of the width of the tibial cartilage in which 50% or greater of the thickness (from surface to tidemark) is seriously compromised [20]. Histomorphological scores in micrometers were assigned to these three measurements for statistical analysis [19].

The data was analyzed using the SPSS-16. The One-way ANOVA (p<0.05) and post-hoc Tukey test was used for the statistical analysis.

Results

The results of histopathological assessments in experimental groups include 1) Depth Ratio of Lesions (p=0.001), 2) Total Degeneration Width (p=0.001) and 3) Significant Degeneration Width (p=0.001). In addition, histopathological assessment outcomes for each group will be stated separately.

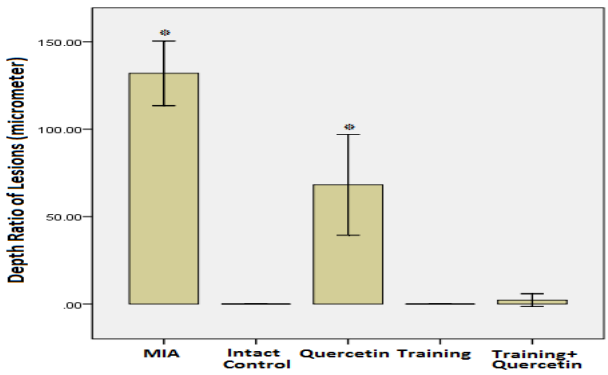

Figure 1. The mean±SD of histological scores related to Depth Ratio of Lesions measured in micrometers. Higher scores show greater severity of lesions. *Significant difference with training and training+quercetin

Depth Ratio of Lesions (DR): For this measure, there were no significant differences between training plus quercetin group and training group (p<0.05), whereas there was a significant difference between training plus quercetin and MIA only group (p=0.001) and also between training plus quercetin and quercetin group

(p=0.005). Also significant differences were observed between quercetin and MIA only group (p=0.007) and between quercetin and training group (p=0.003). Differences between training and MIA only group were significant (p=0.001). These findings are presented in figure 1.

Total Cartilage Degeneration Width (TDW): Regarding results related to TDW, there were no significant differences between training plus quercetin group and training group (p<0.05), but there was a significant difference between training plus quercetin and MIA only group and also between training plus quercetin and quercetin group (p=0.001). Also significant differences were observed between quercetin and MIA only group and between quercetin and training group (p=0.001). Differences between training and MIA only group were significant (p=0.001). These findings are presented in figure 2.

Significant Cartilage Degeneration Width (SDW): Also for this measure, there were no significant differences between training plus quercetin group and training group (p<0.05), but there was a significant difference between training plus quercetin and MIA only group and also between training plus quercetin and quercetin group (p=0.001). Significant differences were observed between quercetin and MIA only group and between quercetin and training group (p=0.001). Differences between training and MIA only group were significant (p=0.001). These findings are presented in figure 3.

Furthermore, photomicrographs of histomorphological changes of joint cartilage stained by Hematoxylin-Eosin for subjects in experimental groups are provided in figure 4. And figure 5 provides methods of assessing histopathological measures used in this study.

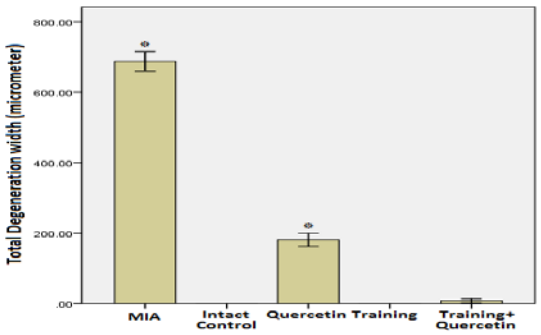

Figure 2. The mean±SD of histological scores related to Total Degeneration Width measured in micrometers. Higher scores show greater severity of lesions
* Significant difference with training and training+quercetin

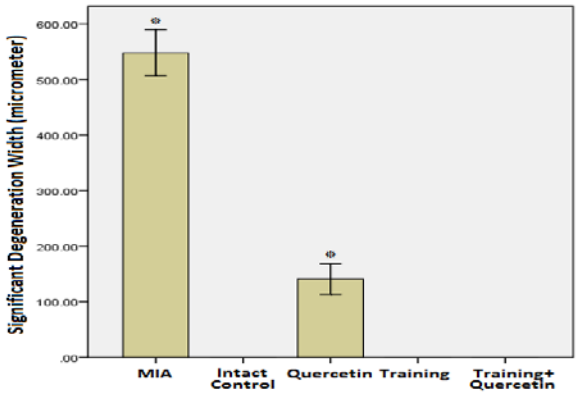

Figure 3. The mean±SD of histological scores related to Significant Degeneration Width measured in micrometers. Higher scores show greater severity of lesions
* Significant difference with training and training+quercetin.

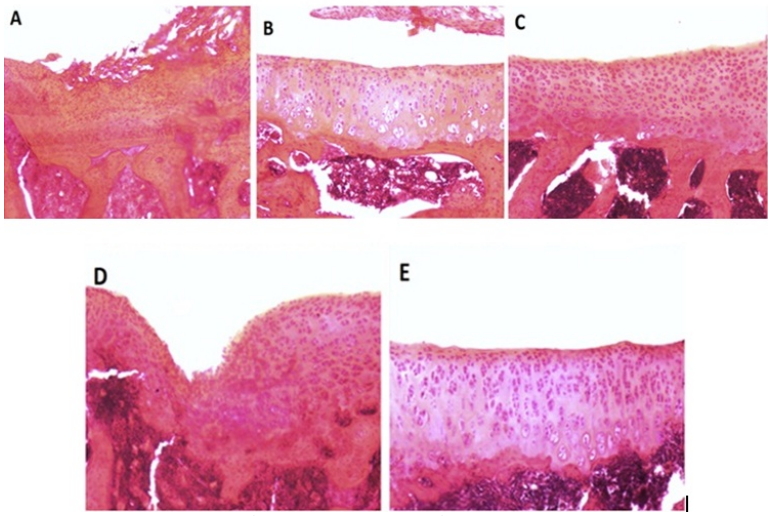

Figure 4. Photomicrographs of histomorphological changes of knee joint sections of subjects in each experimental groups. Cellularity and surface integrity was evaluated by Hematoxylin-Eosin staining (The original magnification is ×10). **A:** MIA only; OA lesions can be seen with tibial cartilage surface clefts and decrease in cellularity. Note the pink-red sites of lesions. **B:** Intact control; There are no observable changes in cartilage surface. **C:** Training; chondrocytes can be observed in many isogenic groups which is the indicator of cell division stimulation. **D:** Quercetin; there is a deep lesion in cartilage surface. **E:** Training+Quercetin; chondrocytes can be observed in many isogenic groups which is the indicator of cell division stimulation

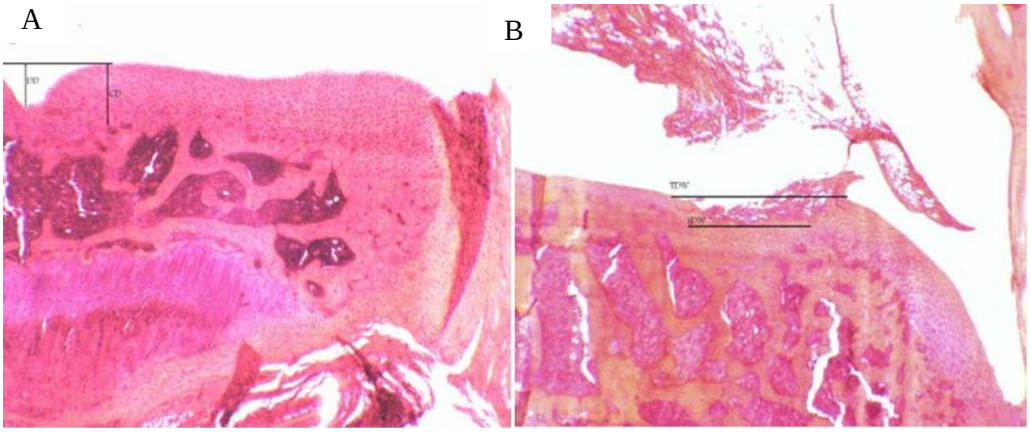

Figure 5. Method of histopathological assessment. **A:** Photomicrograph of quercetin group (The original magnification is ×3.2) showing method of assessing Depth Ratio of Lesions; DD=Degeneration Depth, CD=Cartilage Depth, and DD/CD=Degeneration Depth Ratio; score 1 stands for the most severe lesion. **B:** Photomicrograph of MIA only group (The original magnification is ×3.2) showing method of assessing Total Degeneration Width and Significant Degeneration Width; TDW= Total Degeneration Width and SDW= Significant Degeneration Width

Discussion

The present study aimed at examining the effects of moderate-intensity exercise in combination with quercetin supplementation on rats' knee osteoarthritis. This is one of the few investigations that assess the effects of exercise on rats' knee osteoarthritis. Findings of the present study showed that moderate exercise, either alone or in combination with quercetin supplementation could surprisingly treat symptoms of rats' knee osteoarthritis in 3 histopathological measures of depth ratio of lesions, total cartilage degeneration width and significant cartilage degeneration width. However, the effect of exercise in combination with quercetin supplementation was not greater than exercise alone. Furthermore, supplementation of quercetin had significant effects, but its effectiveness was fewer than the two other groups which followed an exercise program.

There are several studies that investigated the effects of exercise combined with nutraceuticals or pharmaceuticals on human OA [5, 10, 14, 17]. In fact, in order to precisely observe the changes made to cartilage tissue after interventions, histopathological assessment is needed. Obviously, these invasive methods are just applicable in animal models.

In one of the studies, Galois et al. examined the effect of different intensities of exercise (low, moderate, intense) on OA progression in rats. After histological assessment, researchers found that low and moderate-intensity exercise program had positive effects on cartilage lesion –which is in accordance with our findings– but intense exercise blocked these chondroprotective effects. They suggested that the positive effect of low and moderate exercise was probably because of decrease in levels of chondrocytes' death due to anti-apoptotic capacities of Heat Shock Protein 70 (HSP70) [12].

Another study conducted to observe efficacy of a moderate exercise program on rats' knee OA. Their results indicated that physical training contributes to the preservation of joint cartilage in rats with OA and to increase the defense mechanism against oxidative stress. Researchers suggested that this effect is presumably because of increase in activities of antioxidant enzymes such as Superoxide Dismutase (SOD) and Myeloperoxidase (MPO) [13].

Matsuda et al. investigated the effect of quercetin supplementation on rats' knee arthritis which is an inflammatory joint disease. They found that quercetin inhibited the production of TNF-α and nitric oxide (that have main role in inflammatory processes of arthritis) from activated macrophages. Additionally, quercetin decreased the phosphorylation and the activation of Jun N-terminal kinase/stress-activated protein kinase, leading to the suppression of AP-1 activation [21].

Osteoarthritis is a common joint disease and even though its symptoms are well-defined and its predisposing factors are well studied, there is no cure for it [17, 18, 21]. Unfortunately, OA is prevalent even among athletes due to many reasons such as injuries to ACL or menisci. It is reported that the risk of knee OA after ACL injury increases up to 100 % [22]. It should be noted that forty percent of all knee injuries in soccer belong to ACL [23]. There are several studies in the field of sports medicine that considered knee OA as the most important disease that can be occurred after injury to this joint [24-27]. This emphasizes the importance of addressing long-term consequences of sports injuries.

In fact, effective treatments that would reverse the disease have not been developed, even though a limited number of studies have suggested that some anti-arthritic

agents have the ability to block the pathologic process in humans and animals [6].

The mechanism of OA is multifactorial and in order to treat this disease it is essential to find a way that not only can protect the cartilage against degenerative damage by stimulating its intrinsic repair capacity of chondroprotection, but also neutralize the inflammatory/destructive potential of the mediators involved in oxidative-inflammatory stress as reactive oxygen species (ROS), nitric oxide (NO), proteolytic enzymes (such as metalloproteinases) and inflammatory cytokines (such as IL-1β) [7]. The results of our study suggest that aerobic exercise with moderate intensity combined with quercetin supplementation is effective in treatment of rats' knee OA, but this influence was not superior than the moderate exercise alone group. However, the present study had some limitations like incapability to control nocturnal activities of animals and effects of treadmill's electric shock on knee OA.

Regarding findings of this study, moderate exercise either alone or in combination with quercetin supplementation can exert positive effects on knee OA. Thus it is recommended that subjects with joint injury who are therefore prone to developing OA perform physical activity in moderate intensity combined with quercetin supplementation. Nevertheless, this claim needs more investigations and we suggest that further studies use older rats, utilize other models of OA, and also apply different exercise types such as swimming (with different intensities and duration) along with other nutraceuticals. In addition, analysis of inflammatory factors or serum enzymes may be beneficial.

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Conflict of Interest

Authors declared no conflict of interest.

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