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ORIGINAL ARTICLE

Potential Therapeutic effect of Olive oil on Mice model of Osteoarthritis

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ABSTRACT

Osteoarthritis (OA) is a chronic painful, disabling condition affecting the synovial joints. It results from articular cartilage failure induced by a complex interplay of genetic, metabolic, biochemical, and biomedical factors with secondary components of inflammation. Olive oil (OO) was found to have anti-inflammatory properties. Therefore, we hypothesized that OO may change the pathogenetic events of experimentally-induced OA and may be used as an alternative therapy. To investigate the effect of OO on histological and biochemical changes in mice model of OA and its possible therapeutic use in OA. Thirty male mice were divided into three groupsof10 mice each. Control (C) group, in which animals were maintained without any treatment. Osteoarthritis (OA) group, in which animals had their knee joints immobilized in the extension position to induce OA. Osteoarthritis olive oil treated (OA+OO) group, in which animals had OA induced as in the OA group but, after the 4th week, they were given OO(0.2mL/animal/day, orally) for another 4 weeks. At the end of the 8weeksexperiment, animals were scarified under ether anesthesia and blood was collected and serum was investigated for interleukin (IL)1β, IL6, IL17, tumor necrosis factor (TNF)α. Also, histopathological examination of the knee was done. OO administration decreased the OA histopathological changes. This was possibly due to a significant decrease in serum levels of IL1β IL6, IL17 and TNFA in OA+OO group (85.15±2.01), (10.19±0.22), (137.36±1.68) and (153.03±2.13) in comparison to those in the OA group (103.1±1.97), (13.25±0.25), (170.46±1.18) and (186.08±1.47) respectively($p<0.001$ for each). OO delayed progression of OA through its anti-inflammatory anti-OA activity. Thus, it may be of value in therapy of OA.

Keywords: *Knee Osteoarthritis, Olive Oil,Histopathology,IL1β, IL6, IL17, TNFα, Proinflammatory Cytokines, Mice.*

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INTRODUCTION

Osteoarthritis (OA) is a common degenerative joint disease that causes arthralgia and elderly disability[1]. It is characterized by articular cartilage degeneration [2]. Like other severe forms of arthritis, OA causes inflammation, joints' damage and erosion of bone [3]. Bonnet and Walsh[4] stated that OA is a chronic painful and disabling disease that affects the synovial joints and caused by articular cartilage failure induced by genetic, metabolic, biochemical, and biomedical factors with secondary components of inflammation. Risk factors for knee OA include mechanical overload, obesity and trauma [5]. OA is progressed by inflammation that causes cartilage matrix breakdown, bone hypertrophy and thickening resulting in reduction of the OA joint shock absorbing capacity with formation of osteophytes in the joint [6]. Also, in OA, chondrocytes were found to exhibit numerous abnormal metabolic characters that include increased levels of proliferative, synthetic and degradative activity, thus, they are responsible for the development of the osteoarthritic process [7]. This is explained by Choy [8] who stated that in OA, cells of joint tissue as synovial fibroblasts, macrophages, and chondrocytes produce inflammatory

cytokines that cause joint damage.Interleukin1beta (IL1ß) is a cytokine that shares in the inflammatory response in OA as it upregulates cartilage-degrading factors[9]. Also, in OA, the synovial membrane releases other cytokines as tumor necrosis factor alpha (TNFα) and interleukin 6 (IL6) that induce new blood vessels formation, synovitis and pannus which causes bone destruction [8,9]. Interleukin17 (IL17) also shares in the OA pathogenesis. It is produced mainly by the stimulated CD4+ T cells and mast cells which infiltrated the joint and its synovial membrane via the blood vessels [10]. Chondrocytes are affected by IL17 causing expression of IL17R on their surface [11]. Chen et al.[10] found that serum level of IL17 in OA is elevated. The mechanisms by which IL17 shares in the pathogenesis of OA include, inhibition of the chondrocytes to synthesize proteoglycans, increasing production of enzymes of the matrix metalloproteinases (MMPs) group, affecting secretion of proinflammatory cytokines, that damage the joint cartilage, and increasing blood vessel network formation in the joint, causing hypertrophy of its synovial membrane [12-14]. Olive oil (OO) was found to have many beneficial effects in some diseases and this was due to its phytochemicals such as phenolic compounds, tocopherol and carotenoids, which have been shown to possess antimicrobial, antioxidant and anti-inflammatory properties[15]. Prevention and non-pharmacological interventions for OA including lifestyle, mainly diet, can improve the course and outcome these diseases[3]. The phenolic compounds derived from olives, virgin olive oil have received much attention because of their important anti-inflammatory, antiangiogenic, and anticancer properties. Oleuropein, exhibit a marked antioxidant activity in vitro, as it reduces endothelial adhesion molecule expression as VCAM-1 and inhibit the activation of transcription factors NF-kB and activator protein-1[16]. In the early study designed to investigate radiological and pathogenetic blood biomarkers (IL1β, IL6 and TNFα) in experimental OA upon simultaneous administration of OO in mice, it was found that OO had anti-inflammatory action through reducing the secretion of key regulatory proinflammatory cytokines (IL1β, IL6 and TNFα) that hindered the development of experimental knee osteoarthritis. Therefore, OO could have prophylactic activity against OA [17].This study was conducted to investigate the effect of OO on histological and biochemical changes in mice model of OA and its possible therapeutic use in OA.

MATERIALS AND METHODS

Thirty male albino mice, weighing 33- 37g, were obtained from Aljouf College of Medicine animal house, Sakaka, Saudi Arabia. They were provided water and fed *ad libitum*, with standard chow containing carbohydrates (40%), proteins containing all essential amino acids (30%) and lipids (30%). The animals were maintained under standard pathogen free laboratory conditions at 20–22°C, with a relative humidity of 40–60% and a photoperiod of 12h, light and dark. Experiments were performed according to national and institutional regulations for animal use. Mice were divided into three groups of 10 mice each. Control (C) group, in which animals were maintained without any treatment. Osteoarthritis (OA) group, in which animals had their knee joints immobilized in the extension position to induce OA [18]. Osteoarthritis olive oil treated (OA+OO) group, in which animals had OA induced as in the OA group but, after the 4th week, they were given OO(0.2mL/animal/day, orally) for another 4 weeks. At the end of the 8 weeks experiment, animals were scarified under ether anesthesia and blood was collected. Collected blood samples were allowed to clot at room temperature, then centrifuged at 3000 rpm for 10 min and serum was recovered and stored frozen at -80^{o}C till used for IL1β (minimum detection limit is 2.31 pg/mL), IL6 (minimum detection limit is 1.6 pg/mL, IL17 (minimum detection limit is 5 pg/mL) and TNFα (minimum detection limit is1.88 pg/mL, quantitative immunoassaying - using mouse-specific commercially available kits as recommended by the manufacturer (cat# SMLB00C, M6000B, M1700, MTA00B,Mouse Quantikine ELISA Kits, R&D Systems). The knee joints were isolated, formal in fixed and processedfor histological evaluations. The semi-quantitative histological grading criteria of Kraus' modified Mankin score and histopathology Osteoarthritis Research Society International (OARSI) system were used[19]. The knee joint from mice were excised, cleaned of soft tissue, placed into 10% formalin for 24–48 hours, and decalcified in EDTA. Paraffin-embedded histological sections were stained, using Hematoxylin and Eosin (H&E).

Statistical analysis

The data obtained for serum levels of IL1β,IL6, IL17 and TNFα was expressed as mean ± SEM and were compared among the experiment groups for significance using one-way ANOVA and Tukey HSD for Post-Hoc Multiple Comparisons using (IBM SPSS Statistics Version21 Software for Windows) for statistical significance at $P<0.05$.

RESULTS

Table-1 and figure-1 showed a significant increase in serum levels of IL1 β, IL6, IL17 and TNFα in OA group (103.1±1.97), (13.25±0.25), (170.46±1.18) and (186.08±1.47) in comparison to those in the C group (69.23±2.27), (6.85±0.13), (120.01±2.09) and (138.63±0.74) respectively. Also, there was a significant increase in serum levels of IL1β, IL6, IL17 and TNFα in OA+OO group (85.15±2.01), (10.19±0.22), (137.36±1.68) and (153.03±2.13) in comparison to those in the C group (69.23±2.27), (6.85±0.13), (120.01±2.09) and (138.63±0.74) respectively. On the other hand, there was a significant decrease in serum levels of IL1β, IL6, IL17 and TNFα in OA+OO group (85.15±2.01), (10.19±0.22), (137.36±1.68) and (153.03±2.13) in comparison to those in the OA group (103.1±1.97), (13.25±0.25), (170.46±1.18) and (186.08±1.47) respectively.

Figure-2 represented photomicrographs of paraffin-embedded H&E–stained mice knee joint sections. There were normal articular cartilage and normal bone ends in the C group (A). Also, there were surface irregularities (undulations), pannus and surface clefts to calcified zone score 5 in OA group (B). Moreover, there were flattened elongated chondrocytes of the upper zone, cell death in the articular cartilage superficial zone, and atrophy of articular chondrocytes with severe disorganization of bone in OA group (C). On the other hand, there were undulation and irregularities of bone surface, some bone areas appear destructed and the articular cartilage is normal in the OA+OO group (D).

Table-1: Effect of OO on serum levels of IL1β, IL6, IL17 and TNFα (in pg/ml)

	C	OA	OA+OO
IL1β	69.23±2.27	103.1±1.97[a]	85.15±2.01[a, b]
IL6	6.85±0.13	13.25±0.25[a]	10.19±0.22[a, b]
IL17	120.01±2.09	170.46±1.18[a]	137.36±1.68[a, b]
TNFα	138.63±0.74	186.08±1.47[a]	153.03±2.13[a, b]

Data was expressed as Mean±SEM. P<0.05 is considered statistically significant. [a]P<0.001 in comparison to (C) group. [b]P<0.001 in comparison to (OA) group. **C** is the control group, **OA** is the osteoarthritis group and **OA+OO** is the osteoarthritis olive oil treated group.

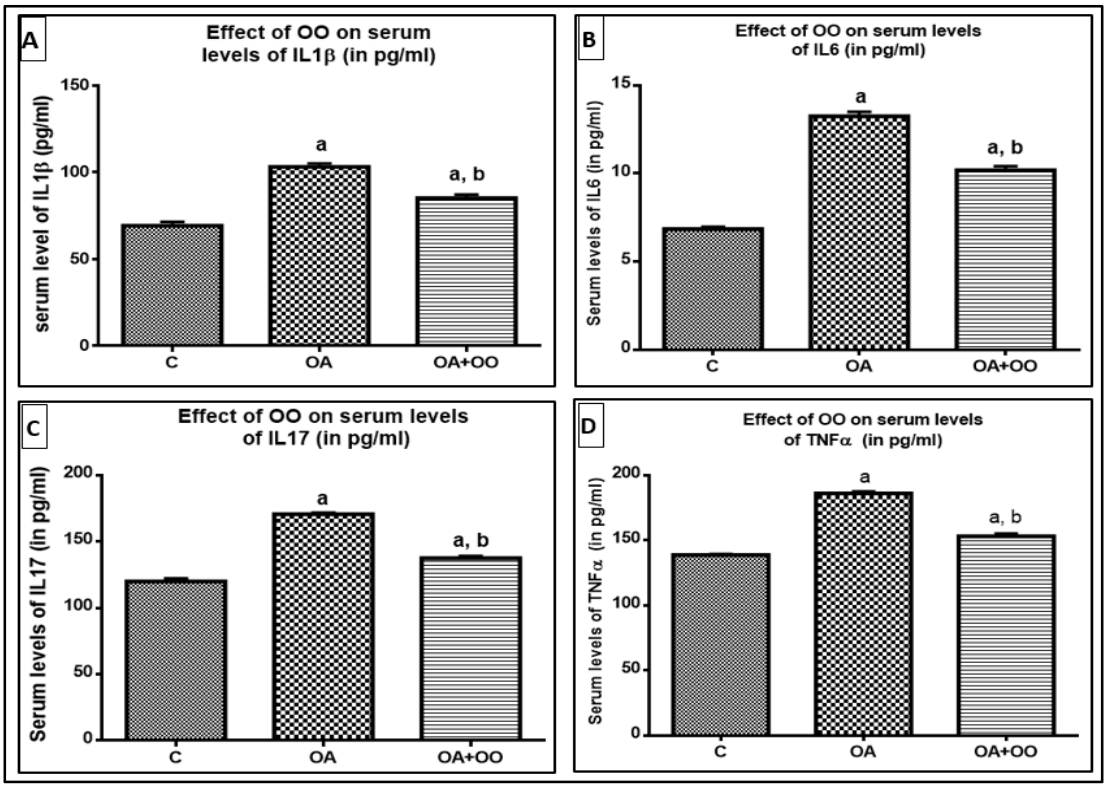

Figure-1: Effect of OO on serum levels (in pg/ml) of IL1β (A), IL6 (B), IL17 (C) and TNFA(D). Data was expressed as Mean±SEM. P<0.05 is considered statistically significant. [a]P<0.001 in comparison to (C) group. [b]P<0.001 in comparison to (OA) group. **C** is the control group, **OA** is the osteoarthritis group and **OA+OO** is the osteoarthritis olive oil treated group.

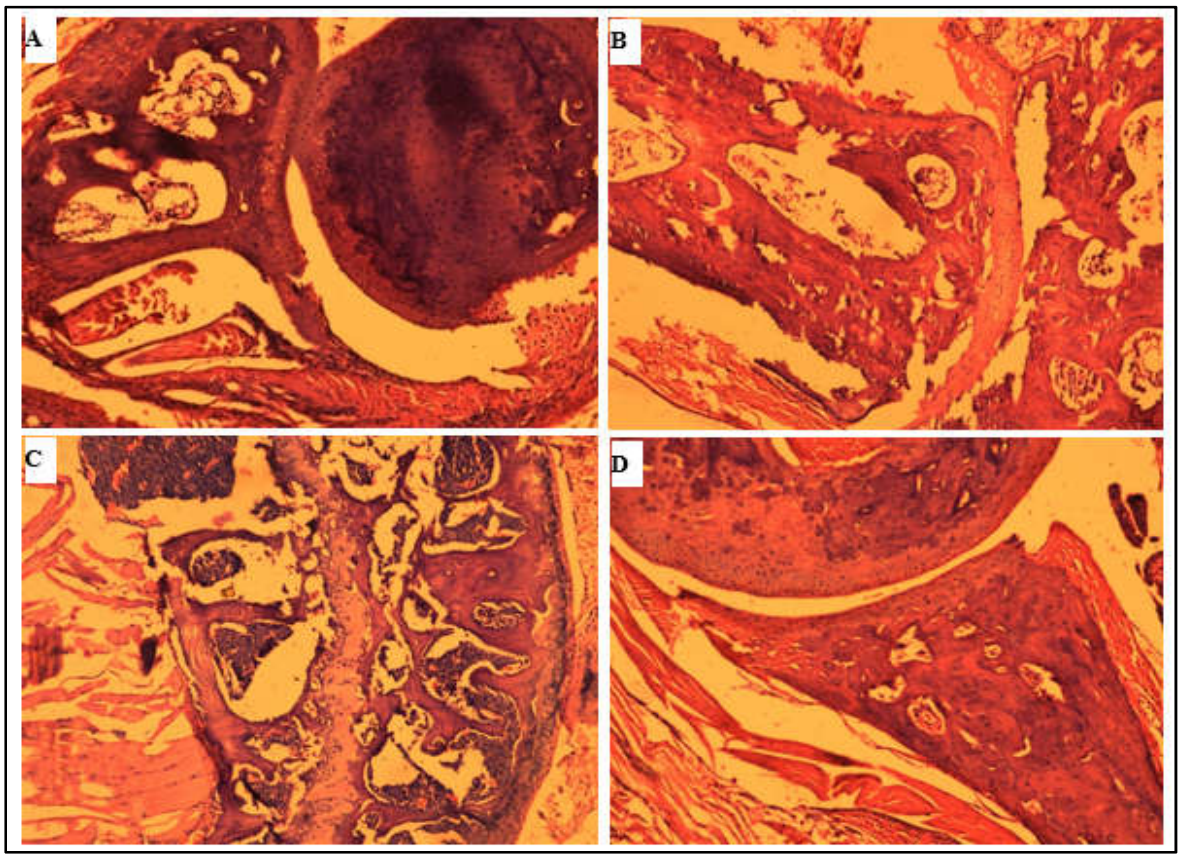

Figure-2: Photomicrographs of paraffin-embedded H&E–stained mice knee joint sections. (A) Mice knee joint section (H&E, 100X) from **C** group showing normal articular cartilage and normal bone ends. **(B)** Mice knee joint section (H&E, 100X) from**OA** group showing surface irregularities (undulations), pannus and surface clefts to calcified zone score 5. **(C)** Mice knee joint section (H&E, 100X) from **OA** group showing flattened elongated chondrocytes of the upper zone, cell death in the articular cartilage superficial zone, and atrophy of articular chondrocytes with severe disorganization of bone. **(D)** Mice knee joint section (H&E, 100X) from **OA+OO** group showing undulation and irregularities of bone surface, some bone areas appear destructed and the articular cartilage are normal. **C** is the control group, **OA** is the osteoarthritis group and **OA+OO** is the osteoarthritis olive oil treated group.

DISCUSSION

The results of this study showed a significant increase in serum levels of IL1β, IL6, IL17 and TNFA in OA group in comparison to those in the C group. These results confirmed the role of the proinflammatory cytokines in the pathogenesis of OA which emphasized the results of the early study on the prophylactic role of OO on OA [17]. This is supported by Fernandes, Martel-Pelletier, and Pelletier[20] who declared that increased IL1 and TNFα played an essential role in pathogenesis of chronic OA which was supported by McNulty et al.[21], who found that administration of either IL1Ra and/or TNF inhibitors decreased progression of OA. This is supported by Bigoni et al.[22]; Choy [8] and Magdalon et al.[23] who stated that in OA there is a joint damage began in the synovial membrane with release of tumor necrosis factor TNFA, IL6 and IL1β. Also, Choy [8] added, macrophages and lymphocyte activation increased IL17 production that enhanced synovitis and other cytokines and chemokines that increase the immune response. Furthermore, Chen et al. [10] found that serum level of IL17 in OA is elevated. The mechanisms by which IL17 shares in the pathogenesis of OA include, inhibition of the chondrocytes to synthesize proteoglycans, increasing production of enzymes of the MMPs group, affecting secretion of the pro-inflammatory cytokines, destroying the joint cartilage, and increasing blood vessel network formation in the joint causing hypertrophy of its synovial membrane [12-14]. Also, the results of this study showed a significant increase in serum levels of IL1β, IL6, IL17 and TNFA in OA+OO group in comparison to those in the C group but, there was a significant decrease in serum levels of IL1β, IL6, IL17 and TNFα in OA+OO group in comparison to those in the OA group. This was in agreement with Magdalon et al.[23] , Moreno [24], Singh, Devaraj, and Jialal [25], Singh and Jialal [26] and Scotece et al. [27] who found that OO through its content of the bioactive phenol secoiridoid oleocanthal reduced joint inflammation as it has been a strong

anti-inflammatory effect by decreasing IL6, IL1 and TNFA. This is also supported by Carluccio et al.[16]; Poudyal, Campbell, and Brown [28] and Impellizzeri et al.[29] who declared that the ameliorative effect of olive on arthritis was due to its content of oleuropein aglycone that modulated the inflammatory response as it reduced leukocyte infiltration in the affected joints. This is further supported by Cicerale et al.[15]; Martinez-Dominguez, de la Puerta, and Ruiz-Gutierrez[30]; Urpi-Sarda et al.[31] and Scoditti et al. [32] who stated that OO biophenols (OBP) inhibited the proinflammatory enzymes, cyclooxygenase (COX)-2, and inducible nitric oxide synthase (iNOS) with reduction of the proinflammatory cytokines. Moreover, Palmerini et al.[33] found that olive oil phenolic compounds increased Ca2+ release from intracellular stores that is important for different biological processes. In addition, Silva et al. [34] stated that phenolic compounds performed its anti-inflammatory actions through affecting both expression of pro-inflammatory genes, and activity of certain immune cells. Histopathological studies showed that there were surface irregularities (undulations), pannus and surface clefts to calcified zone score 5 in OA group. Moreover, there were flattened elongated chondrocytes of the upper zone, cell death in the articular cartilage superficial zone, and atrophy of articular chondrocytes with severe disorganization of bone in OA group. These results are supported by Choy [8] who found that mechanical stresses causes joint damage. This is further supported by Punzi et al., [35] who stated that OA caused structural changes in the joint cartilage causing loss of its function. Moreover, Pervaiz, et al., [36], Zhang, et al., [37] and Charalambous [38] found that there were severe articular cartilage histopathological changes in OA patients. Furthermore, Musumeci et al., [39] stated that OA showed osteophyte formation as well as loss of joint cartilage and subchondral bone. In addition Bolbos et al.[40] and Kawaguchi [41] found that OA development was indicated by the thickening of subchondral cortical bone and the decrease in the structure of underlying trabecular bone. On the other hand, in this study, there were less histopathological findings in the OA+OO group in the form of undulation and irregularities of bone surface, some bone areas appear destructed and the articular cartilage are normal. This result with the significant reduction of the proinflammatory cytokines in OA+OO group in comparison to OA group confirmed that OO delayed the progression of OA in OA+OO group in comparison to OA group. This is supported by Impellizzeri et al. [29] and Scoditti et al. [32] who suggested that the deteriorative effect of OO on chronic inflammatory joint diseases was due to the effect of its phenolic compounds on the inhibition of both COX-2 protein expression and metalloproteinase (MMP). This was also partly supported by Bitler et al.[42] who found daily living activities improvement in patients with OA after treatment with olive vegetation water for 8 weeks. This was further supported by Volker et al., [43] and Calder et al., [44] who found that diet type affected arthritis progression and they confirmed that oral oleic acid reduced the production of IL1β, IL6, and cytokine-induced neutrophil chemoattractants. In addition, Berbert et al., [45] and Bohlooli et al., [46] declared that olive oil supplementation improved intensity of joint pain in patients with OA. Also, van der Kraan and van den Berg [47] stated that OO may slow down progression of OA through inhibiting chondrocyte hypertrophy. These results declared that OO may be of value in treatment of OA which help in avoiding hazards of pharmacological treatments as hypertension induction or aggravation [48]. Further work should be done to confirm this result.

CONCLUSION

It is concluded that OO has an ameliorative effect on the progression of OA as it significantly decreased proinflammatory cytokines and histopathological changes in OA+OO group in comparison to OA group. Thus, it may be of a beneficial OA therapeutic value. Although, further work should be done to confirm these results.

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