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ORIGINAL ARTICLE

# Intra-articular injection of an antioxidant formulation did not improve structural degeneration in a rat model of post-traumatic osteoarthritis

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**KEYWORDS**
antioxidant;
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quercetin;
vitamin C

**Summary** *Background/objective:* Oxidative stress plays an important role in osteoarthritis (OA), causing inflammation and matrix degradation in joints. Previous studies have shown that antioxidants such as quercetin and vitamin C are potential candidates for treating OA. We aimed to determine whether a formulation of quercetin and vitamin C, together with an iron chelator, could retard OA progression in a post-traumatic OA rat model.
*Methods:* Twelve rats received anterior cruciate ligament transection for OA induction. At 20 weeks postoperation, weekly intra-articular injection of 50 μL of either saline or a formulation of quercetin dehydrate, sodium-L-ascorbate, and deferoxamine mesylate was given consecutively for 4 weeks ($n = 5$). Gait analysis was performed at pretreatment, and at 1 week and 5 weeks post-treatment. Microcomputed tomography scanning and histological scoring were performed at 5 weeks post-treatment.
*Results:* Gait analysis showed that intra-articular injections of antioxidant formulation did not improve pain-associated Limb Idleness Index over time ($p = 0.449$, Friedman test). However, at 5 weeks post-treatment, the treatment group exhibited a significantly lower Limb Idleness Index than the control group ($p = 0.047$, Mann–Whitney $U$ test). At 5 weeks post-treatment, microcomputed tomography analysis revealed that there was no difference in any parameter between the treatment and control groups ($p > 0.05$, Student $t$ test). Severe OA histopathological changes were found in both groups. The Osteoarthritis Research Society International

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scores of the treatment and control groups were 20 (range, 20–26) and 20 (range, 9–26), respectively ($p = 0.382$, Mann–Whitney $U$ test).
*Conclusion:* Intra-articular injection of an antioxidant formulation containing quercetin, vitamin C, and deferoxamine did not retard OA progression in advanced-stage OA. Future studies should aim to determine whether giving antioxidants in early OA, with prolonged drug retention, would be effective in retarding OA progression.


## Introduction

Osteoarthritis (OA), a degenerative joint disease, affects the whole joint, causing pain, deformity, and loss of function. According to the World Health Organization, it is estimated that 9.6% of men and 18.0% of women aged over 60 years have symptomatic OA (http://www.who.int/chp/topics/rheumatic/en/). Currently, there is no promising treatment for OA. Increasing evidence showed that oxidative stress resulting from excessive production of reactive oxygen species (ROS) plays a role in the pathogenesis of OA [1]. Clinical studies demonstrated that oxidative stress was present in OA patients, where an abnormal antioxidant level was observed in the synovial fluid and serum of patients with primary and secondary OA [2,3]. Oxidative stress triggers inflammatory responses [4], induces extracellular matrix degradation, and inhibits matrix synthesis [5], and eventually leads to OA changes. ROS pathways are becoming one of the new treatment targets for OA [6]. To counteract the elevated oxidative stress, antioxidant therapy has been developed to reinforce the cellular antioxidant status. These include antioxidant supplements, dietary polyphenols, free radical scavengers, NADPH oxidase inhibitors, and iNOS inhibitors [7]. Combining multiple antioxidants that have different actions may be a better approach to tackle oxidative stress in OA. Of these, quercetin (Que) and vitamin C are two well-known antioxidants that possess potential beneficial effects on OA.

Que, a polyphenolic bioflavonoid found ubiquitously in food of plant origin, exhibits a wide range of effects on biological systems, particularly on anti-inflammation and antioxidation [8]. *In vivo* studies demonstrated that Que reduced the expression of inflammatory markers, restored antioxidant defence, subsided OA clinical signs, and delayed arthritis in rats with adjuvant arthritis [9,10]. The elevated inflammation in post-traumatic OA rats had also been reversed with Que treatment [11]. Furthermore, clinical studies on a dietary supplement consisting of Que had also shown favourable effects on OA-associated symptoms [12,13].

Another potent antioxidant, vitamin C (ascorbic acid), has a broad spectrum of antioxidative activities, owing to its ability to scavenge numerous ROS [14]. It also acts as a cofactor for numerous biochemical reactions and is essential for collagen biosynthesis [15]. A preclinical study on surgically induced OA in guinea pigs [16] and an epidemiological study on OA patients [17], both suggested that high dietary intake of vitamin C reduced the risk of cartilage loss and delayed disease progression. In addition, vitamin C level in joint fluids was significantly lower in patients with severe OA [3].

However, vitamin C can exhibit pro-oxidant activities, particularly in the presence of iron [18,19]. Iron level in the serum [20] and synovial fluid [21] of patients with OA was found to be elevated. It has been proposed that the high level of iron increases ROS, which leads to an upregulation of matrix metalloproteinases, thereby causing cartilage damage after joint trauma [22]. Iron chelators, such as deferoxamine (also known as desferrioxamine; DFO) and deferiprone, are commonly used chelating agents for removing excess iron from the body [23]. It can relieve oxidative stress in arthritic joint by inhibiting the iron-catalysed formation of destructive oxygen metabolites [24]. An animal study had also demonstrated that intra-articular (IA) injections of DFO reduce blood-induced knee joint damage [25].

Since Que, vitamin C, and DFO exhibit different mechanisms, we proposed to inject them as a formulation to treat OA knee in a post-traumatic rat model. In a previous pilot study on repeated IA injections of this formulation in OA rats, we observed a reduction in pain-associated limb idleness and less severe histopathological changes at 4 months post OA induction (Supplementary materials). These suggested that this antioxidant formulation may possess both symptom and disease-modifying potential. In this study, the same formulation was investigated to further prove its effectiveness in pain modulation and delaying degenerative changes in both the articular cartilage and the subchondral bone at a late stage OA in the knee.

## Materials and methods

### Anterior cruciate ligament transection for induction of post-traumatic OA

Animal experiments were approved by the Animal Experimentation Ethics Committee in The Chinese University of Hong Kong (Ref. no.: 11/008/DRG). Twelve female Sprague-Dawley rats at 12 weeks of age (average body weight 229.1 ± 13.2 g) underwent unilateral anterior cruciate ligament transection of the right knee for OA induction [26]. Under general anaesthesia, a medial parapatellar incision was made and the patella was dislocated to expose the

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