Turkish Journal of Veterinary & Animal Sciences

Volume 44 | Number 1 Article 20

1-1-2020

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TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Short Communication

Turk J Vet Anim Sci
(2020) 44: 156-161

doi:10.3906/vet-1811-66

Effect of sesamin on protection of equine articular cartilage degradation in vitro

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Received: 25.11.2018 • **Accepted/Published Online:** 07.12.2019 • **Final Version:** 10.02.2020

Abstract: Black sesame seed is a potential herbal medicine for osteoarthritis treatment as it contains sesamin, which has antiinflammatory properties and has demonstrated chondroprotective effects in many species. Horses are particularly susceptible to osteoarthritis. Inflammatory mediators and matrix metalloproteinase are important factors causing cartilage degradation. This study aimed to investigate the protective potential of sesamin (0.5–2.0 μM) against interleukin (IL)-1β-induced equine cartilage explant degradation. The cartilage degradation was monitored by measuring the release of cartilage matrix molecules in culture media including sulfated-glycosaminoglycans and hyaluronan by calorimetric assay and ELISA assay, respectively. The remaining contents of collagen and uronic acid (UA) within the explant tissue were assessed by hydroxyproline assay and UA assay, respectively. Cartilage-degrading enzymes MMP-2 and MMP-3 were evaluated by gelatin zymography and ELISA assay, respectively. The results indicated that sesamin suppressed IL-1β-induced equine cartilage degradation by reducing the levels of sulfated-glycosaminoglycans and hyaluronan, and preserving the contents of UA and collagen within the explant tissues. The activity of MMP-2 and the quantity of MMP-3 were also suppressed by sesamin. The chondroprotective efficacy of sesamin was comparable to that of diacerein, an antiarthritic drug. Lactate dehydrogenase assay showed that sesamin at concentrations of up to 2 mM did not cause cytotoxicity to chondrocytes in cartilage explants. These results may lead to new options for osteoarthritis treatment in horses.

Key words: Sesamin, equine articular cartilage, cartilage degradation, chondroprotection

Joint diseases cause chronic health issues in athletic animals, including horses. In Europe, more than 44.8% of sport horses were retired and/or consequently euthanized due to osteoarthritis [1]. Injury to joints and surrounding tissues induces inflammatory processes. Proinflammatory mediators, especially interleukin (IL)-1β and proteolytic enzymes, are released into the synovial fluid, causing articular cartilage destruction [2]. Matrix metalloproteinases, including MMP-1 (collagenase 1), MMP-2 (gelatinase), MMP-3 (stromelysin 1), MMP-9 (gelatinase), and MMP-13 (collagenase 3), play major catalytic roles in the cartilage [3–9]. Osteoarthritis treatments usually combine medicine, surgery, and physical therapy. Alternative medicines such as acupuncture and herbal medicine are introduced in some cases in order to minimize the side effects of the main treatments and to increase the quality of life of the patient.

Black sesame is known as an herb that can be used as a health promotion supplement and as a medicine for clinical conditions such as dermatitis, migraine, and joint pain. Black sesame contains lignans such as sesamin and sesamolin [10]. Previous studies demonstrated the antiinflammatory effects of sesamin both in vivo [11–14] and in vitro [15]. It has been reported that sesamin extract reduced pathogenesis of arthritis by increasing articular cartilage thickness in inflammation-induced mice. Furthermore, the extract not only significantly reduced cartilage degradation but also enhanced both collagen and sulfated-glycosaminoglycan synthesis within the cartilage tissue [16].

It was therefore of interest to determine whether sesamin would have similar effects in horse cartilage. This study aimed to investigate the chondroprotective effect of sesamin on equine articular cartilage degradation induced by IL-1β in a cartilage explant model.

Equine articular cartilage samples were obtained within 6 h post mortem from an equine patient after consent was obtained from the owner. The animal had no clinical signs of joint disease. The articular cartilage was collected from the stifle joints of both hindlimbs. Full thickness cartilage biopsies were harvested aseptically and trimmed into

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cartilage explants of approximately 3 × 3 mm^2. The explants were then transported to the laboratory in Dulbecco's modified Eagle medium (DMEM; GIBCO) with 200 U/mL penicillin G sodium and 200 unit/mL streptomycin. The explants were rinsed at least three times with the same medium before being weighed and cultured in 12-well tissue culture plates containing 0.5 mL of serum-free DMEM with 200 U/mL penicillin G sodium and 200 U/mL streptomycin in each well. The cultures were maintained at 37 °C under 5% CO_2. The tissue culture media in the first 24 h were collected as day 0 samples. The media were replaced weekly, and the explants were cultured for 7 or 21 days with various treatments. Untreated control explants were cultured in media without any additional substances. The IL-1β group was treated with 10 ng/mL recombinant human IL-1β (Sigma-Aldrich). The sesamin groups received a co-treatment of 10 ng/mL recombinant human IL-1β in addition to 0.5, 1.0, or 2.0 μM sesamin (Sigma-Aldrich). Diacerein (TRB Chemidica), an antiarthritic drug, at a concentration of 20 μM was treated in parallel as the positive control. Collected tissues and culture media were kept at –20 °C until use.

Cartilage biomolecules that were released into the tissue culture media were evaluated. Sulfated-glycosaminoglycans were measured by a colorimetric dye binding assay [17]. Hyaluronan was measured by competitive inhibition-based ELISA [18]. IL-1β successfully induced significant release of sulfated-glycosaminoglycans and hyaluronan compared to the control. Sesamin at a concentration of 2 μM significantly suppressed the release of sulfated-glycosaminoglycans and hyaluronan induced by IL-1β, comparable to the diacerein group (Figure 1). In addition

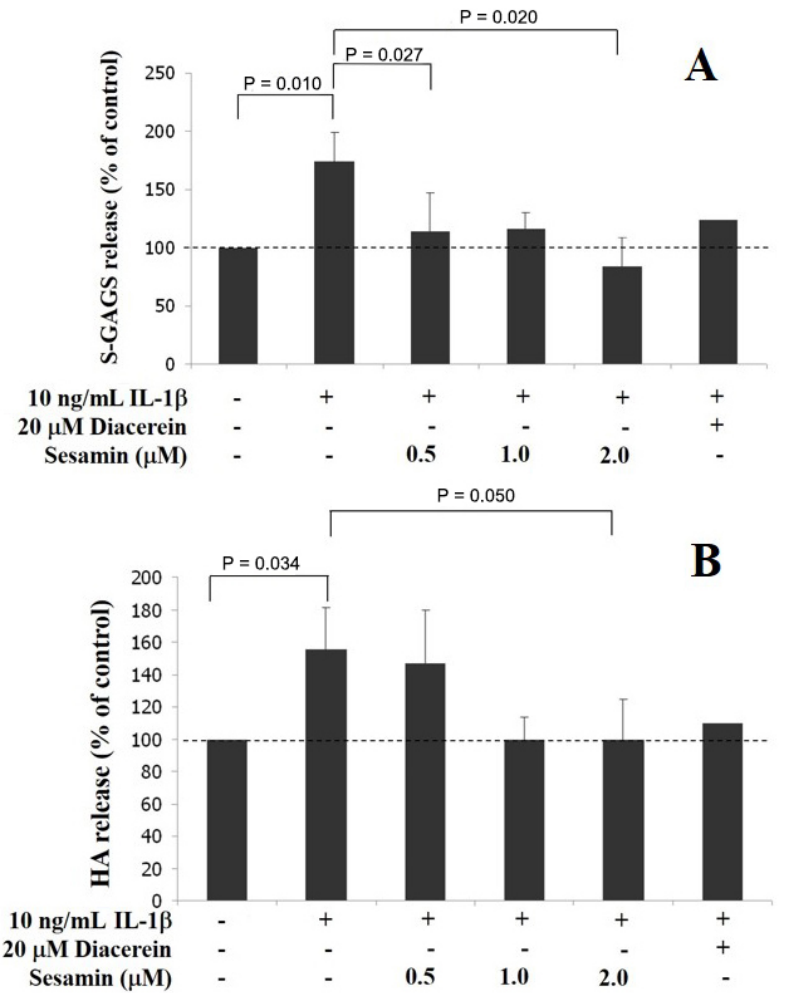

Figure 1. Sulfated-glycosaminoglycans (S-GAGS) (A) and hyaluronan (HA) (B) release percentages relative to the control groups. Comparison among the IL-1β group (10 ng/mL IL-1β), the diacerein group (20 μM diacerein), and the sesamin groups (0.5, 1.0, and 2.0 μM sesamin) on day 7 of the cartilage explant cultures. Data were analyzed using STATA version 9.2 with 95% confidence intervals ($P < 0.05$). The percentage concentration of sulfated-glycosaminoglycans and hyaluronan between the control and IL-1β group were compared by unpaired t-test, while the IL-1β group and the other treatment groups were compared by ANOVA.

to the detection of catabolic markers that were released into the tissue culture media, the remaining collagen and uronic acid (UA) contents within the explants were also analyzed on day 21 using a hydroxyproline assay [19] and a UA assay [18], respectively. The results showed an obvious but not statistically significant reduction of UA and collagen contents after IL-1β induction. Although there was no statistically significance, sesamin and diacerein showed the increasing trend of UA and collagen contents in the explants compared to the cytokine-treated group (Figures 2). These findings suggest that sesamin protects against IL-1β-induced release of cartilage matrix molecules similarly to diacerein, the standard antiarthritic drug.

Matrix metalloproteinase-2 (MMP-2) activity was measured by gelatin zymography [20], and the MMP-3 level was analyzed using a human MMP-3 test kit (Elabscience). The results from the gelatinolytic assay showed that tissue culture media of IL-1β-induced explants had higher MMP-2 activity. This activity increase was reduced by co-treatment with diacerein or 2 μM sesamin (Figure 3A). The MMP-3 level was increased after treatment with the proinflammatory cytokine IL-1β, but tended to be decreased with additional sesamin and diacerein (Figure 3B). However, these changes were not statistically significant. These results suggest that sesamin may attenuate the effects of IL-1β on activation of the production of MMPs, leading to the slowing of cartilage matrix degradation in the equine model comparably to diacerein, an IL-1β inhibitor [21–23], in our equine cartilage study.

Sesamin toxicity on cartilage explant cultures was tested by detecting lactate dehydrogenase (LDH), which indicates the death of cells [24], including chondrocytes. H_2O_2 at a concentration of 10 mM was used as the positive control. After culturing for 7 days, sesamin at concentrations of 0.5 to 2 μM did not alter LDH levels compared to the control group, while the explants that were treated with hydrogen peroxide showed highly significant increases in LDH. However, sesamin at 4 μM significantly increased LDH (Figure 4). Therefore, only 0.5 to 2 μM sesamin was applied in the experiments.

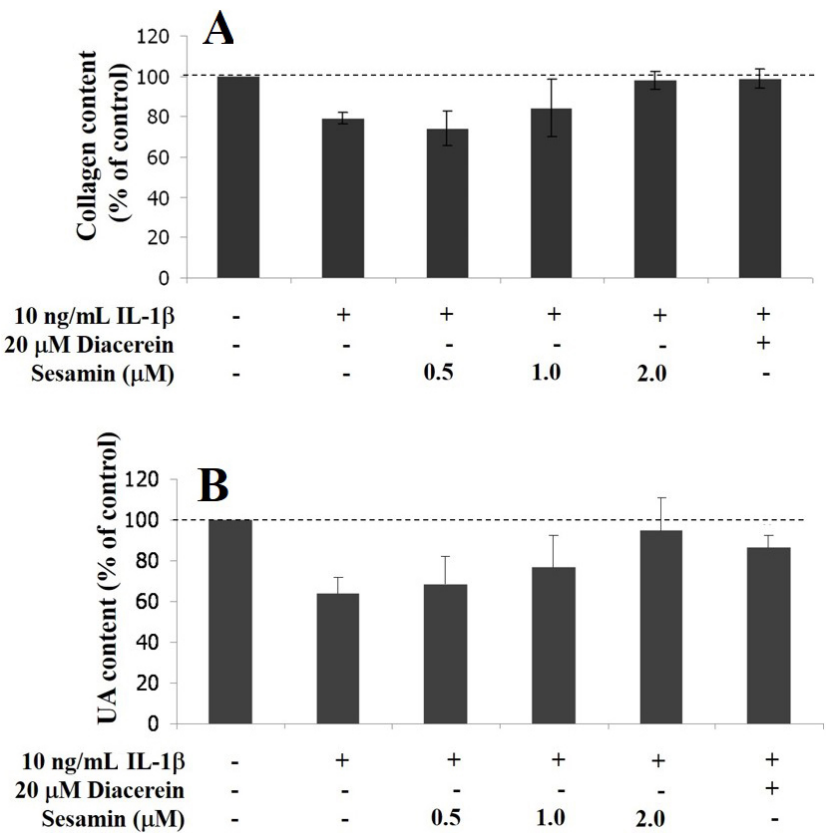

Figure 2. Percentage relative to the control group of UA (A) and collagen (B) content within the cartilage explants on day 21 of the cartilage explant cultures. Comparison among the IL-1β group (10 ng/mL IL-1β), the diacerein group (20 μM diacerein), and the sesamin groups (0.5, 1.0, and 2.0 μM sesamin). Data were analyzed using STATA version 9.2 with 95% confidence intervals ($P < 0.05$). The percentage concentrations of UA and collagen between the control and IL-1β group were compared by unpaired t-test, while the IL-1β group and the other treatment groups were compared by ANOVA.

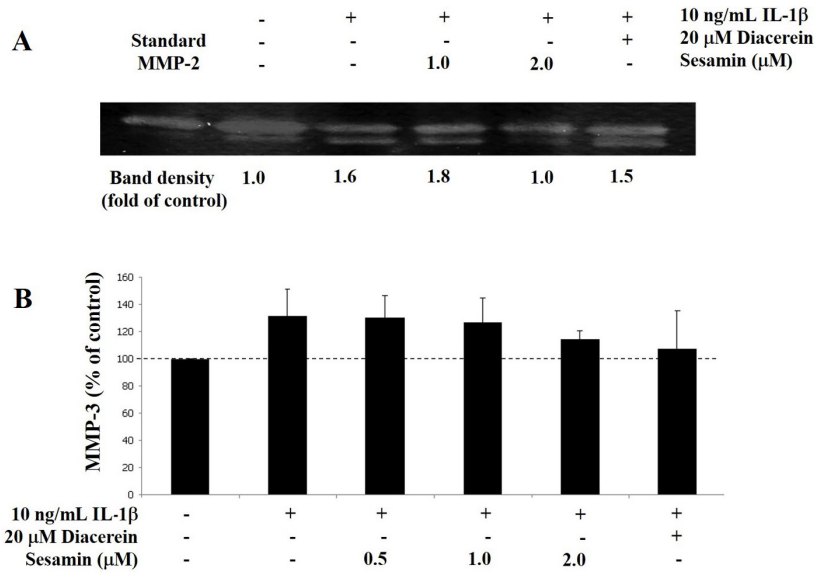

Figure 3. (A) Gelatin zymography of the activity of MMP-2 in the tissue culture media compared with the control group, the IL-1β group (10 ng/mL IL-1β), the diacerein group (20 μM diacerein), and the sesamin groups (0.5, 1.0, and 2.0 μM sesamin). Band densities were determined using TotalLab TL120 v2009 software and are represented as density fold compared to the control. (B) Percentage relative to the control of MMP-3 released into the tissue culture media. Comparison among the IL-1β group (10 ng/mL IL-1β), the diacerein group (20 μM diacerein), and the sesamin groups (0.5, 1.0, and 2.0 μM sesamin). The MMP-2 zymography result was not statistically tested. The percentage concentrations of MMP-3 between the control and IL-1β group were compared by unpaired t-test, while the IL-1β group and the other treatment groups were compared by ANOVA.

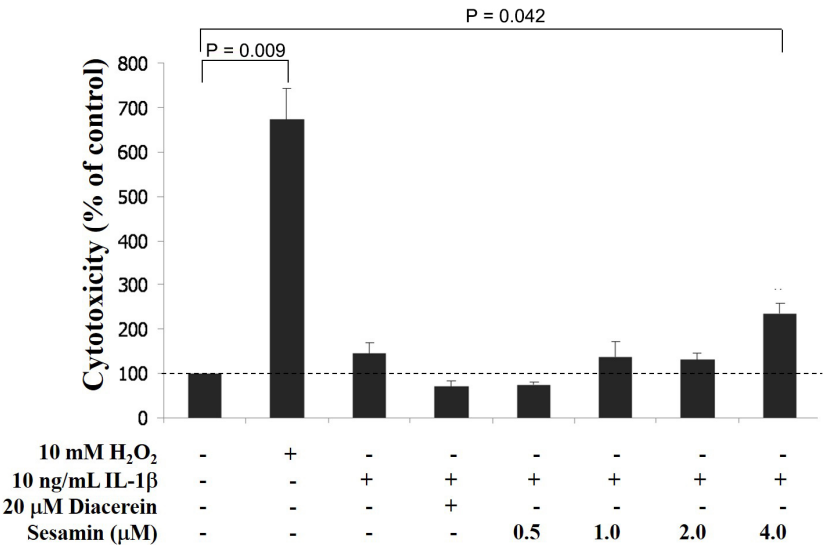

Figure 4. Lactate dehydrogenase (LDH) percentage relative to the control group. Comparison among treated group (10 mM H_2O_2), IL-1β group (10 ng/mL IL-1β), the diacerein group (20 μM diacerein), and the sesamin groups (0.5, 1.0, 2.0, and 4.0 μM sesamin). Data were analyzed using STATA version 9.2 with 95% confidence intervals ($P < 0.05$). The percentages of LDH between the control and IL-1β group were compared by unpaired t-test, while the IL-1β group and the other treatment groups were compared by ANOVA.

Taken all together, our results demonstrated the chondroprotective potential of sesamin in equine cartilage explant degradation induced by IL-1β, one of the key proinflammatory cytokines that involves pathogenesis of osteoarthritis. Accordingly, sesamin may be applied as an alternative treatment for osteoarthritis in human and animals, including horses.

Acknowledgments and/or disclaimers, if any
This work was supported by a Chiang Mai University research grant (2013). The authors would like to thank the Thailand Excellence Center for Tissue Engineering and Stem Cells, Chiang Mai University, for general support. We also thank the Large Animal Teaching Hospital of Chiang Mai University for the cartilage supply.

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