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Effect of Naringin on Monosodium Iodoacetate-Induced Osteoarthritis Pain in Rats

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF Qiang Xu
BE Zuo-fu Zhang
ABCEF Wei-xue Sun

Department of Joint Surgery, Yantai Yu Huang Ding Hospital, Yantai, Shandong, P.R. China

Corresponding Author: Qiang Xu, e-mail: xuqiang239@outlook.com
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Background: The aim of the current study was to evaluate the anti-osteoarthritic and anti-inflammatory effect of naringin in a monosodium iodoacetate (MIA)- induced osteoarthritis (OA) model in rats. The anti-osteoarthritic potential of naringin was evaluated against the MIA-induced OA rat model.

Material/Methods: Wistar rats were used for the study and were divided into the following groups: normal control (saline-treated); group II (MIA-treated): group III (MIA+Naringin), and group IV (MIA+Indomethacin). The potential effect of naringin was evaluated via its effect on the level of proinflammatory cytokines, measuring the weight-bearing distribution, and histopathological analysis.

Result: The anti-inflammatory effect of naringin was assessed *in vitro* in lipopolysaccharide-induced RAW 264.6 cells. The results suggest that naringin exerts an anti-inflammatory effect via reducing the production of the prostaglandin E_2 (PGE_2), nitric oxide (NO), interlukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) in LPS-induced RAW cells. Additionally, naringin also supported the recovery of hind-limb weight-bearing, reduced the generation or production of inflammatory mediator and proinflammatory cytokines, and protected the tissue from the damage in the OA model.

Conclusions: Naringin appears to be an effective therapeutic drug for the treatment of the OA and OA-related symptoms.

MeSH Keywords: Anti-Inflammatory Agents • Arthritis • Natural Springs

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Background

Osteoarthritis (OA) is a common musculoskeletal tissue disorder characterized by synovial inflammation, formation of osteophytes, degeneration of cartilage, and subchondral bone sclerosis [1–3]. OA causes joint damage, loss of cartilage function and structure, and dysregulation of anti-inflammatory and pro-inflammatory pathways [4, 5]. It mainly affects the subchondral bone and articular cartilage of synovial joints, resulting in the failure of joint movement, as well as causing joint pain during standing and walking [6]. Without treatment, OA can lead to devastating joint cartilage degradation [7,8]. Several methods can be effective in treating OA through management of joint pain, improving or maintaining joint mobility, enhancing joint strength, and reducing the disabling effects of OA. Although destruction of cartilage is the major effect of OA, collagen degradation is the fundamental cause of irreversible expansion of OA in connection with inflammation [9–12].

Several studies have reported that inhibiting the inflammatory reaction is effective in treating OA. It has been also found that herbal drugs may provide excellent protective effects in limiting the spread of OA in terms of cartilage destruction and promoting inflammation reaction in chondrocytes, via their effects on joint-linked tissues, resulting in the alleviation of joint pain [13].

Several researchers showed that citrus plants are good sources of flavonoids. Several flavonoids, such as naringin, hesperidin, nobiletin, naringenin, and narirutin, are isolated from citrus fruits [14]. These flavonoids were found to have powerful anti-inflammatory and antioxidant activities *in vitro* and *in vivo*. Due to the antioxidant nature of the compound, we evaluate the antioxidant, anti-osteoarthritic, and anti-inflammatory effects of naringin in rats with osteoarthritis (OA) induced by monosodium iodoacetate (MIA).

Material and Methods

Estimation of anti-inflammatory activity (*in vitro*)

The *in vitro* anti-inflammatory activity of naringin was determined in RAW 264.7 cells. The RAW 264.7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing FBS (5.5%) and antibiotics (1%). The RAW 264.7 cells were incubated in a humidified atmosphere of CO_2 (5%) at 37°C. to provoke the cells, the DMEM medium was changed with fresh DMEM, and lipopolysaccharide (1µg/ml) was added in the absence or presence of the SDS.

Estimation of prostaglandin (PGE_2), nitric oxide (NO), and proinflammatory cytokines

The cells were cultured with SDE and stimulated with LPS for 24 h. Griess reagent was used for to assess NO production. A previously reported protocol was used for the estimation of NO production [15,16]. The inflammatory mediator PGE_2 and proinflammatory mediators interlukin-6 (IL-6) and tumor necrosis factor (TNF-α) were measured using ELISA kits.

Evaluation of anti-osteoarthritic activity *in vivo*

Animals

For the current study, Sprague-Dawley (males, 6 weeks old) rats were used. The rats were procured from the Institutional Animal House and housed in standard environmental conditions with 12-h light/dark cycle at 20±5°C and 55±15% humidity. All rats received standard diet and water *ad libitum*. All experiments were performed in compliance with the National Institute of Health guidelines and were approved by the Departmental Animal Care and Use Committee. The study was approved by Yantai Yu Huang Ding Hospital.

Experimental study

The SD rats were randomly selected and divided into the following groups and each group contained 6 rats.
Group I: Normal control received saline;
Group II: OA control;
Group III: OA control treated with naringin (5 mg/kg);
Group IV: OA control treated with naringin (10 mg/kg);
Group V: OA control treated with indomethacin (2 mg/kg).

Monosodium iodoacetate (MIA) injection (3 mg/50 µL) was used to induce OA in the rats. The injection of MIA was administered in the articular space of anesthetized SD rats. All rats received oral administration of naringin and indomethacin 1 week before the administration of MIA injection for 4 weeks [17].

Estimation of hind-paw weight-bearing distribution

A capacitance tester was used for to assess changes in weight-bearing tolerance. After induction of OA, the SD rats demonstrated the reduced weight-bearing tolerance in hind paws. To assess hind-paw weight-bearing distribution, the rats were placed in a measuring chamber for 3 s for the determination of weight-bearing force. The weight distribution ratio of the rats was estimated using the following formula [18,19]:

$$\textit{Weight of the right hind limb} = \textit{Weight on right hind limb} + \textit{Weight on right hind limb} \times 100$$

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Estimation of proinflammatory cytokines

Blood samples were used determine levels of proinflammatory cytokines, and were further processed for collecting serum. The blood samples were centrifuged at 2000 rpm for 15 min; then the serum samples were collected and stored at –80°C until use. The inflammatory mediator PGE_2 and proinflammatory cytokines TNF-α, IL-1β, and IL-6 in the serum were determined using commercial ELISA kits [15,16].

Histopathological investigation

After completing the experimental study, rats in all groups were sacrificed by anesthesia. A knee joint sample was removed from all rats and processed by fixing in formalin (10%), embedding in paraffin, and serial slicing at 5 mm. The all tissue samples were stained with hematoxylin and eosin (HE). The prepared slides were examined under a light microscope.

Statistical analysis

Data are presented as a mean±standard deviation. All data analysis was performed using one-way analysis of variance (ANOVA), and Dunnett's test was performed to identify the significance using GraphPad Prism Software.

Results

Effect of naringin on inflammatory and proinflammatory mediators

In vitro, we have assessed the effect of the naringin on PGE_2 (Figure 1), NO (Figure 2), IL-6 (Figure 3), and TNF-α (Figure 4) in LPS-stimulated RAW 264.7 cells. All the cells were treated with naringin along with LPS or LPS alone for 24 h. Figures 1–4 confirm that naringin significantly reduced the production of PGE_2, NO, IL-6, and TNF-α. Also, naringin did not affect cell viability and showed no toxic effects on RAW 264.7 cells.

Effect of naringin on change in hind-paw weight-bearing distribution

Figure 5 demonstrates the effect of naringin on the change in hind-paw weight-bearing distribution. The distribution between the contralateral and sensitized hind limbs was used for the estimation of weight distribution and also used as an index of joint discomfort in the arthritic knee. Consequently, we assessed the effect of naringin on hind-paw weight-bearing using a capacitance tester for 21 days. The ratio of left and right limbs was used to evaluate the development of OA. The MIA control group rats showed quick reduction of weight-bearing distribution as compared to saline-treated normal group after administration of MIA injection, and the reduced level was maintained through the end of the experimental study. In contrast, naringin- and indomethacin-treated group rats showed decreased values at day 7 as compared with MIA group rats. In addition, naringin- and indomethacin-treated rats had balance between the left and right hind legs and returned to the normal control condition. These data show the significant restoration of hind- limb weight-bearing in the naringin-treated group.

Effect of naringin on inflammatory cytokine levels

Proinflammatory cytokines play a prominent role in the maintenance of tissue injury and chronic inflammation during the expression of OA. Therefore, we scrutinized the effect of naringin on inflammatory mediator such as PGE_2 (Figure 6) and proinflammatory cytokines, including IL-6 (Figure 7), IL-1β (Figure 8), and TNF-α (Figure 9) in MIA-induced OA rats. MIA control group rats showed enhanced levels of IL-6, IL-1β, TNF-α, and PGE_2; however, in the saline control group rats these levels were significantly reduced by naringin and indomethacin at an end of the experimental study. Our findings suggest that naringin protects cartilage in the MIA model via modifying these inflammatory mediators.

Effect of naringin on the histopathology

All groups of rats were assessed for histopathological changes. The rats were histopathologically examined for bone injury, synovial hyperplasia, and severity of inflammation. The saline control group rats showed normal joints. The MIA group rats exhibited histopathological changes such as inflammatory cells in the articular tissue, synovial space, cartilage erosion, and synovial hyperplasia. In contrast, treatment with naringin and indomethacin suppressed the synovial hyperplasia and tissue damage in joints. These histopathological changes confirm that naringin attenuates the severity of MIA-induced OA in rats.

Discussion

The available treatment for OA used in clinical practice mainly targets reduction of symptoms, protection of joint mobility, and limiting the failure of functional ability. Many researchers claimed that several herbal drugs and phytoconstituents used in the treatment of inflammatory arthritis improve OA symptoms [20,21]. Naringin shows various pharmacological effects; however, no evidence has been presented demonstrate the protective effect of naringin in OA treatment. Therefore, the present investigation was performed to assess the anti-osteoarthritic and anti-inflammatory effects of naringin in an MIA-induced OA model.

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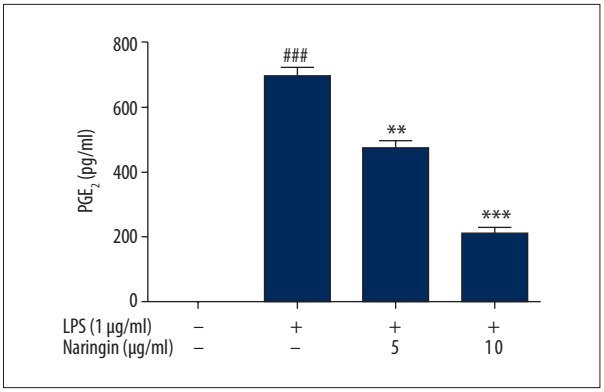

Figure 1. Effects of naringin on the production of PGE_2 in LPS-stimulated RAW 264.7 macrophages. Cells were treated with naringin (0, 5, and 10 μg/mL) plus LPS (1 μg/mL) or LPS alone for 24 h. Production of PGE_2 was determined by ELISA. Values are expressed as the means ±SD (n=3). ### p<0.001 versus untreated LPS and naringin; ** p<0.01, and *** p<0.001 versus LPS alone.

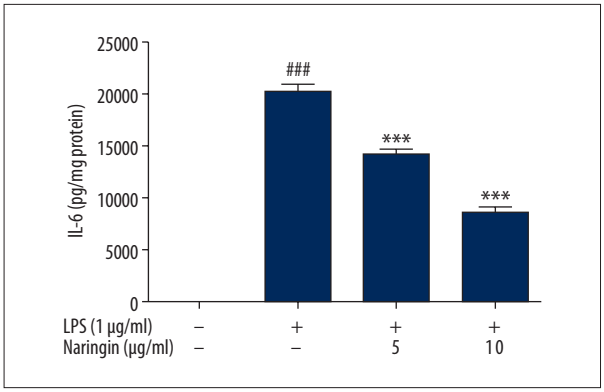

Figure 3. Effects of naringin on the production of IL-6 in LPS-stimulated RAW 264.7 macrophages. Cells were treated with naringin (0, 5 and 10 μg/mL) plus LPS (1 μg/mL) or LPS alone for 24 h. Production of IL-6 was determined by ELISA. Values are expressed as the means ±SD (n=3). ### p<0.001 versus untreated LPS and naringin; ** p<0.01, and *** p<0.001 versus LPS alone.

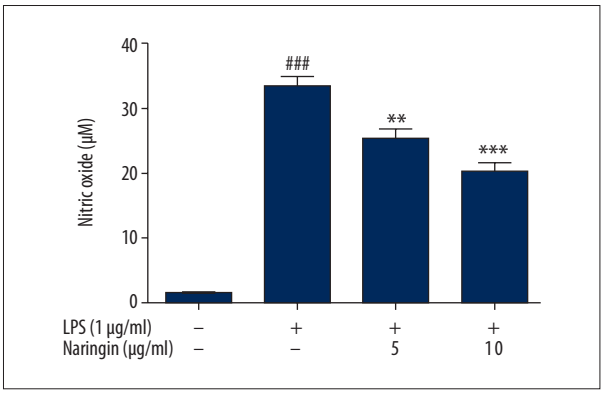

Figure 2. Effects of naringin on the production of nitric oxide in LPS-stimulated RAW264.7 macrophages. Cells were treated with naringin (0, 5, and 10 μg/mL) plus LPS (1 μg/mL) or LPS alone for 24 h. NO production was estimated using the Griess reagent. Values are expressed as the means ±SD (n=3). ### p<0.001 versus untreated LPS and naringin; ** p<0.01, and *** p<0.001 versus LPS alone.

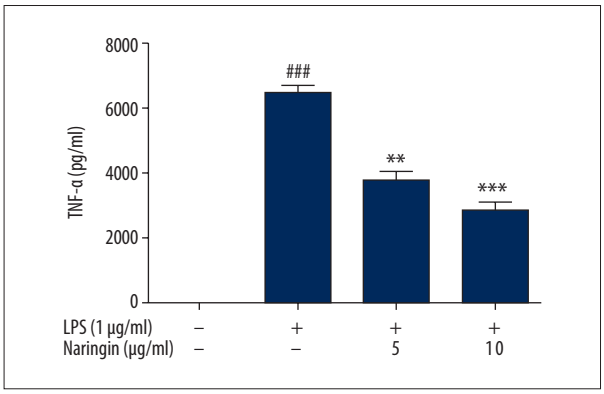

Figure 4. Effects of naringin on the production of TNF-α in LPS-stimulated RAW 264.7 macrophages. Cells were treated with naringin (0, 5, and 10 μg/mL) plus LPS (1 μg/mL) or LPS alone for 24 h. Production of TNF-α was determined by ELISA. Values are expressed as the means ±SD (n=3). ### p<0.001 versus untreated LPS and naringin; ** p<0.01, and *** p<0.001 versus LPS alone.

Several OA incidences confirmed that inflammatory mediators play a significant role in the progression and expansion of cartilage destruction [22]. The inflammatory mediator and proinflammatory cytokines showed a possible effect on the catabolic properties that contribute to the pathophysiological expansion of OA [23]. Our results confirm that naringin significantly suppresses the production of the PGE_2, NO, IL-6, and TNF-α in LPS-stimulated RAW 264.7 cells.

Various parameters are used to assess the anti-osteoarthritic effect of naringin in the MIA-induced model, including serum mediators, inflammatory cytokines, weight-bearing distribution, and histopathological characters. In the present study, we also evaluated the OA-associated pain-relieving effect of naringin in the MIA-induced OA model. OA-associated pain can be triggered by joint movement and typically results in reduced use and diminished joint mobility [24–27]. Our data confirmed that naringin significantly improves weight-bearing ability in MIA-induced OA rats, suggesting that naringin could be beneficial

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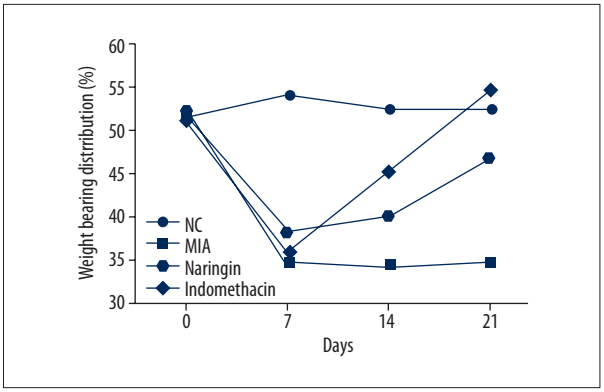

Figure 5. Effects of naringin on changes in the hind-paw weight-bearing distribution in MIA-induced OA in rats. The weight-bearing distribution ratio was estimated once a week for 21 days after the injection of MIA, using an incapacitance tester.

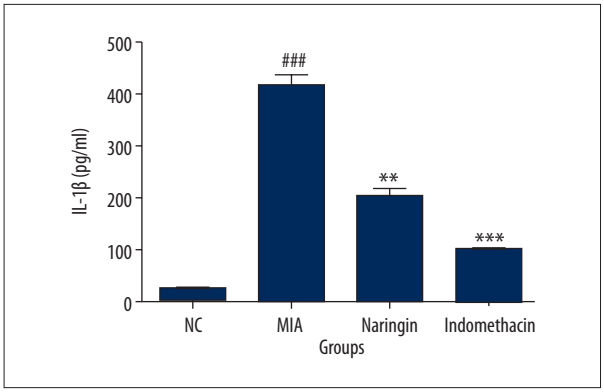

Figure 8. Effects of naringin on serum levels of IL-1β in MIA-induced OA in rats. IL-1β levels were measured by ELISA. ### $p<0.001$ versus saline; ** $p<0.01$, and *** $p<0.001$ versus MIA.

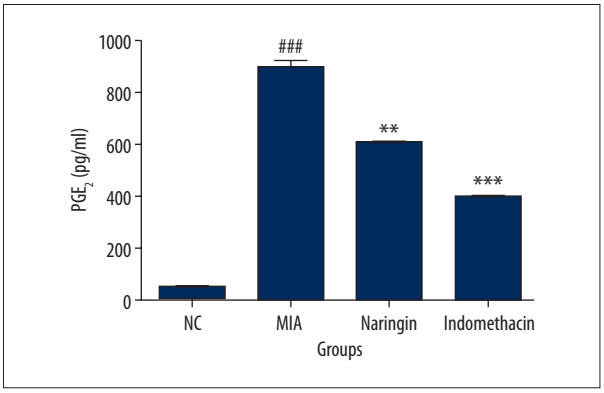

Figure 6. Effects of naringin on serum levels of PGE_2 in MIA-induced OA in rats. PGE_2 levels were measured by ELISA. ### $p<0.001$ versus saline; ** $p<0.01$, and ***$p<0.001$ versus MIA.

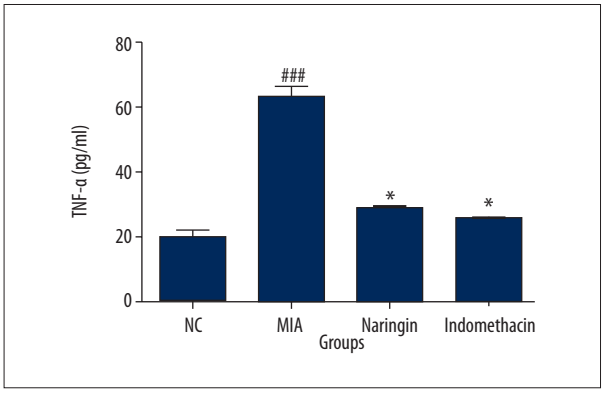

Figure 9. Effects of naringin on serum levels of TNF-α in MIA-induced OA in rats. TNF-α levels were measured by ELISA. ### $p<0.001$ versus saline; * $p<0.05$ versus MIA.

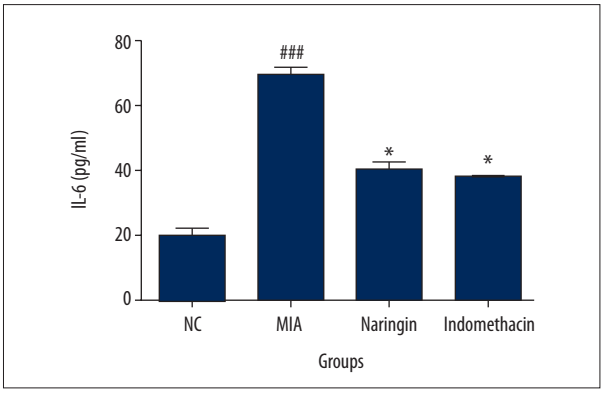

Figure 7. Effects of naringin on serum levels of IL-6 in MIA-induced OA in rats. IL-6 levels were measured by ELISA. ### $p<0.001$ versus saline; * $p<0.05$ versus MIA.

in the treatment of OA-related pain. Our result show that naringin significantly reduced inflammation in the MIA-induced OA model. Several studies confirmed that inflammation plays a significant role in the expansion of arthritis and confirm the chondroprotective effect. The present study revealed that the naringin exerts a chondroprotective effects in OA, induced by MIA in rats via inhibition of the inflammatory mediator and proinflammatory cytokines in serum.

Inflammation plays a role in the expansion of the OA and loss of the cartilage structure of joints. Proinflammatory cytokines and catabolic mediators such as PGE_2 and NO are generated through the inflamed synovium and modify the balance of the cartilage matrix repair and degradation [24,25]. These symptoms aggravate the joint degradation and clinical symptoms during OA. Therefore, targeting the inflammatory reaction can an effective approach to treating the progression and expansion of OA. In the present study, naringin significantly reduced the inflammatory reactions and prevented the progression of

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OA. Additionally, we also evaluated the histopathology of the joint, confirming the protective effect of naringin against damage to the synovial membrane and joints, as well as protecting against cartilage inflammation and synovial hyperplasia, as compared to MIA control group rats.

Conclusions

In conclusion, naringin demonstrated an anti-inflammatory effect via suppressing the production of PGE_2, NO, IL-6, and TNF-α in LPS-induced RAW cells. Naringin also attenuated stiffness and joint pain, reduced the production of inflammatory mediators and proinflammatory cytokines, and confined the subchondral bone and cartilage tissues in an MIA-induced OA model. Our results clearly show that naringin may be an effective therapeutic drug for the treatment of OA and OA-associated symptoms.

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