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Peimine inhibits IL-1β induced inflammatory response in mouse articular chondrocytes and ameliorates murine osteoarthritis

Key words:
Peiminine,
Nrf2/OH-1,
AKT/NF-κB,
osteoarthritis

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Introduction

Osteoarthritis (OA) is a common arthrosis characterized by degeneration and inflammation of articular cartilage. In recent decades, Peimine (Pm) is one of the active ingredients of fritillaria plants. According to reports, Pm has a potent anti-inflammatory effect in various diseases. However, the effects of anti-inflammatory by Pm in OA have not been previously reported. This research aims to evaluate the Pm anti-inflammatory effect on interleukin (IL)-1β-induced mice chondrocytes and chondroprotective effect in the OA model via surgical destabilization medial meniscus mouse.

Materials and methods

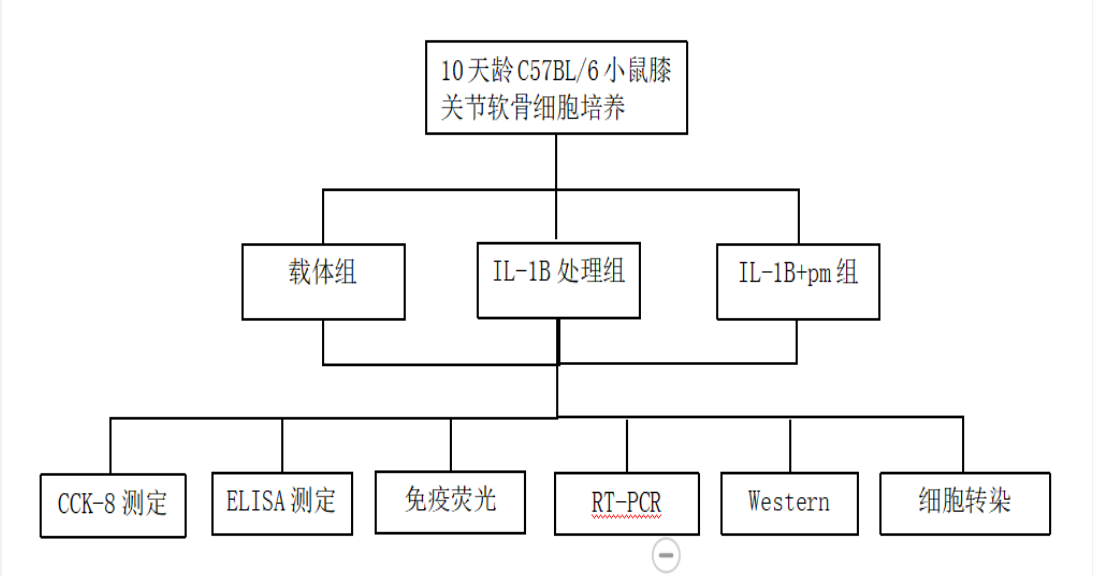
1、cell experiment

1.1 materials

cells come from the joint cartilage of 10-day C57BL/6 mouse

1.2 methods

As shown in the picture on the right



Materials and methods

2、animal experiment

2.1 materials

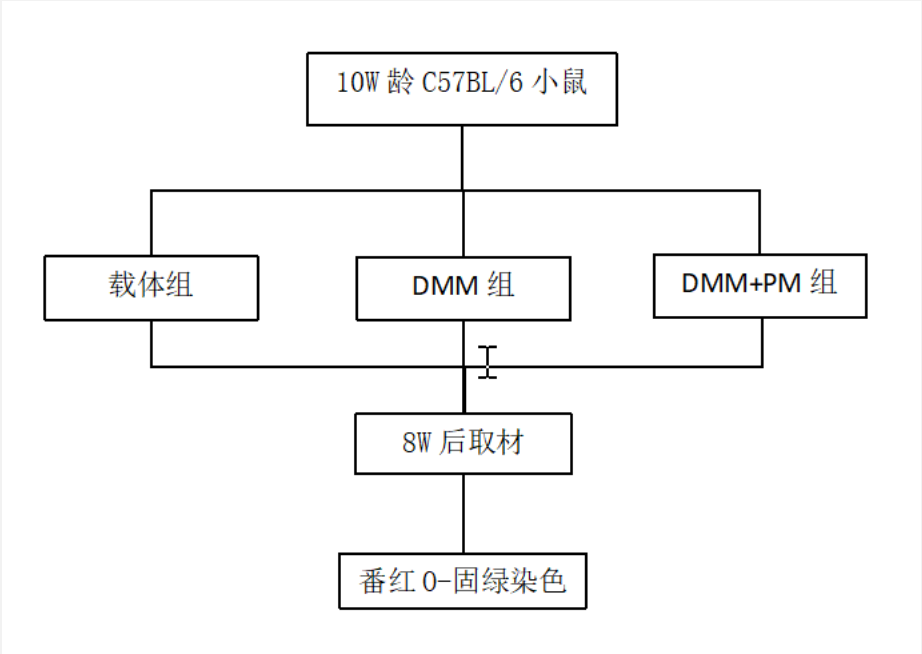
the right knee of C57BL/6 mouse

2.2 methods

DMM surgey in right knee

Analysis of histopathology

3、 Statistical analysis



Results

1、Effect of Pm on the chondrocyte viability

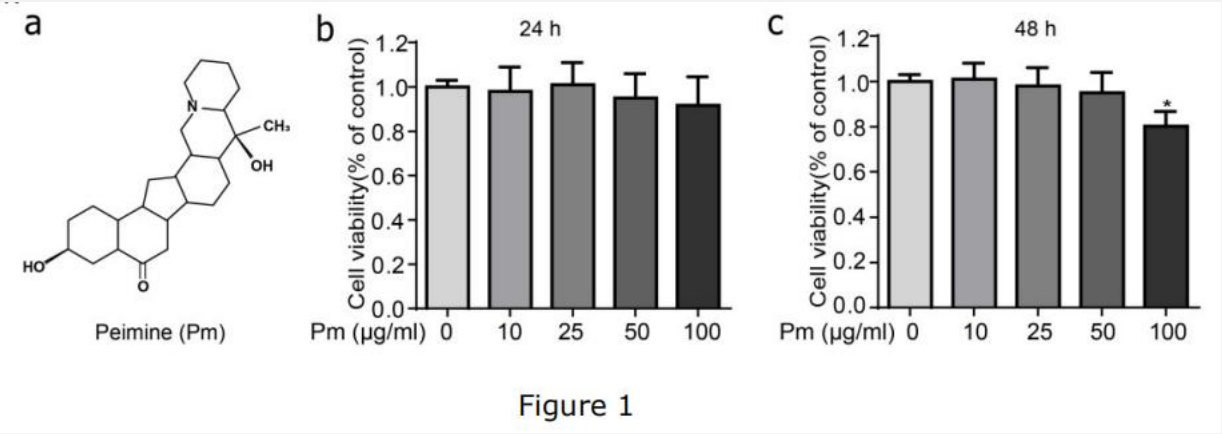
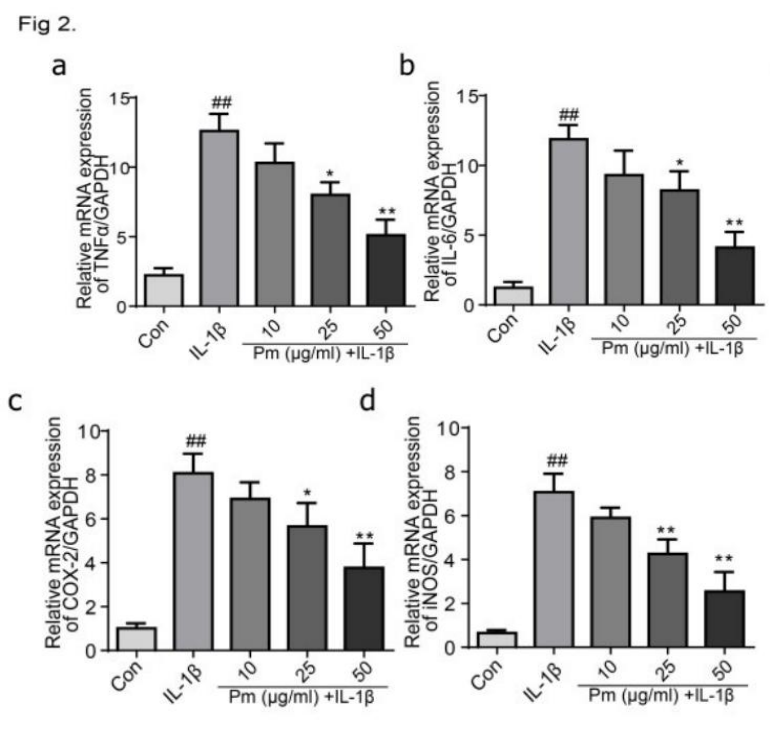


Figure 1

The chemical constitution of Pm is exhibited in Figure 1a.The CCK8 results suggest that Pm reduced cell viability significantly at 50 μg/ml after 48 h compared to untreated cells ($P < 0.05$). Pm had no cytotoxicity to mouse chondrocytes after 24 h or 48 h at concentration ≤50 μg/ml(Fig. 1b and c). Therefore, 10, 25, or 50 μg/ml Pm was used for succedent researches.

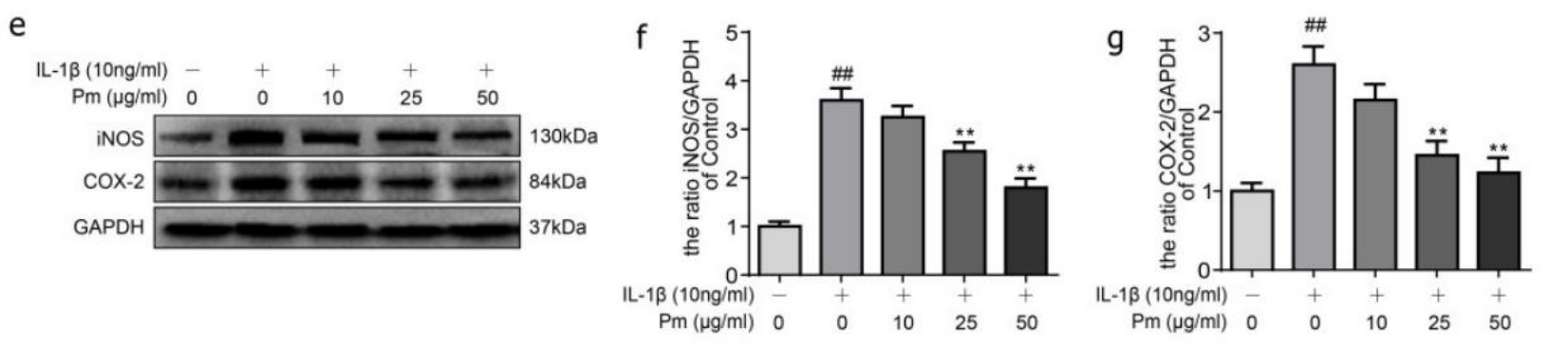
Results

2. The protective effects of Pm on IL-1β-induced COX-2, iNOS, NO, PGE2, IL-6 and TNF-α expression in mouse chondrocyte



The RT-PCR and western blot results shown that Pm inhibited the increase of IL-1β (10 ng/ml) induced mRNAs (Fig. 2a-d)

Results

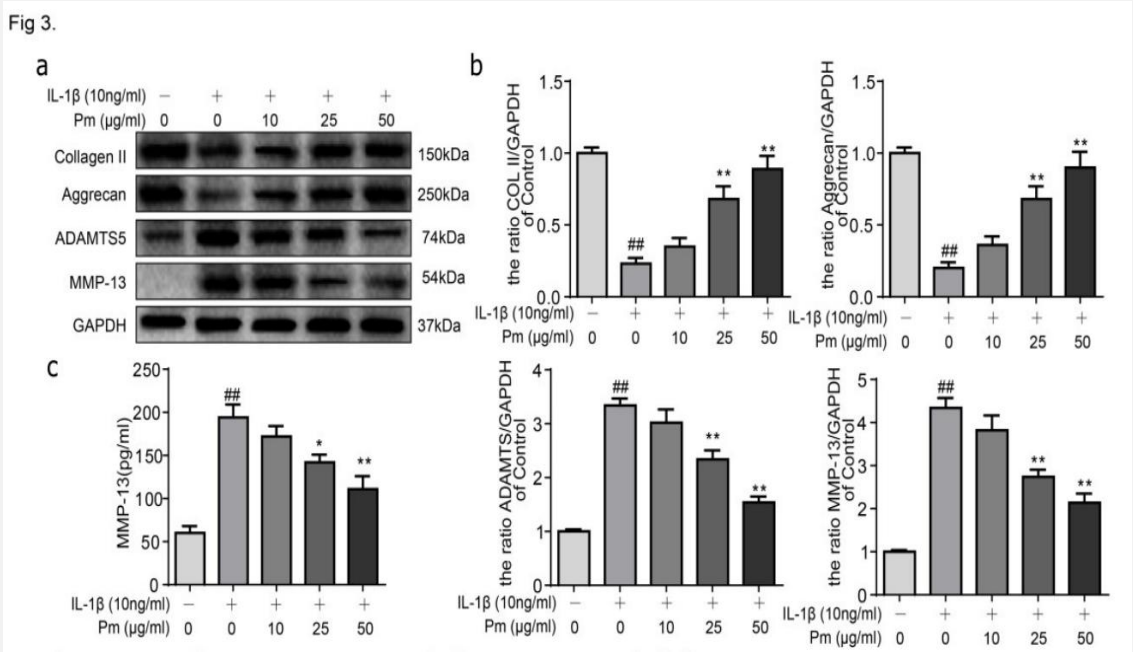


Meanwhile， Pm inhibited proteins (Fig. 2e-g) production of IL-6, TNF-α, iNOS and COX-2 in a dose-dependent pattern (25 and 50 μg/ml), but no significant difference was found in the 10 μg/ml Pm administration group and the IL-1β administration group at the levels of mRNA and protein

Results

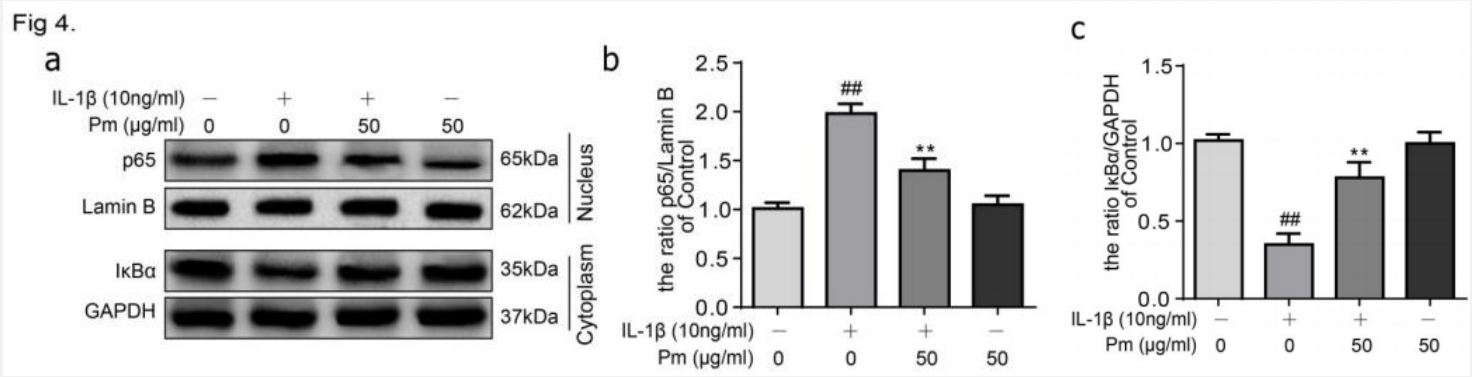
3、Pm prevents the loss of ECM synthesis in mouse chondrocytes induced by IL-1β.

As shown in Fig. 3a-c, Pm dramatically enhanced the expression of aggrecan and collagen-II and decreased the production of ADAMTS-5 and MMP-13 in a dose-dependent pattern, especially at 25 and 50 μg/ml ($P < 0.05$).



Results

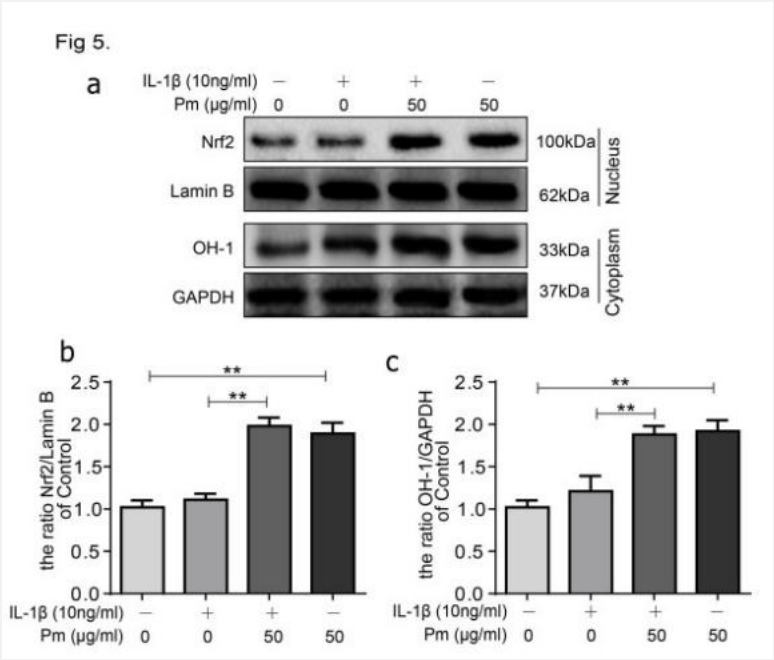
4、Pm inhibited IL-1β-induced NF-κB activation in mouse chondrocytes.



Pm pretreatment significantly inhibited IκBα expression in the cytoplasm descended and the p65 expression in the nucleus increased after IL-1β stimulation

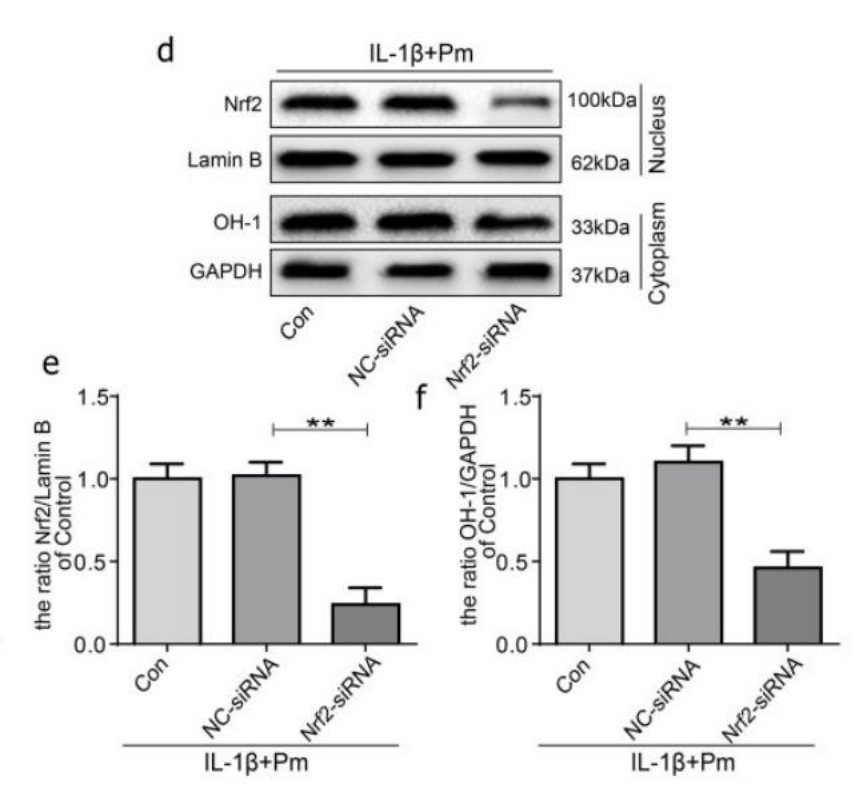
Results

5、Pm regulated the Nrf2/HO-1 signal pathway in chondrocytes



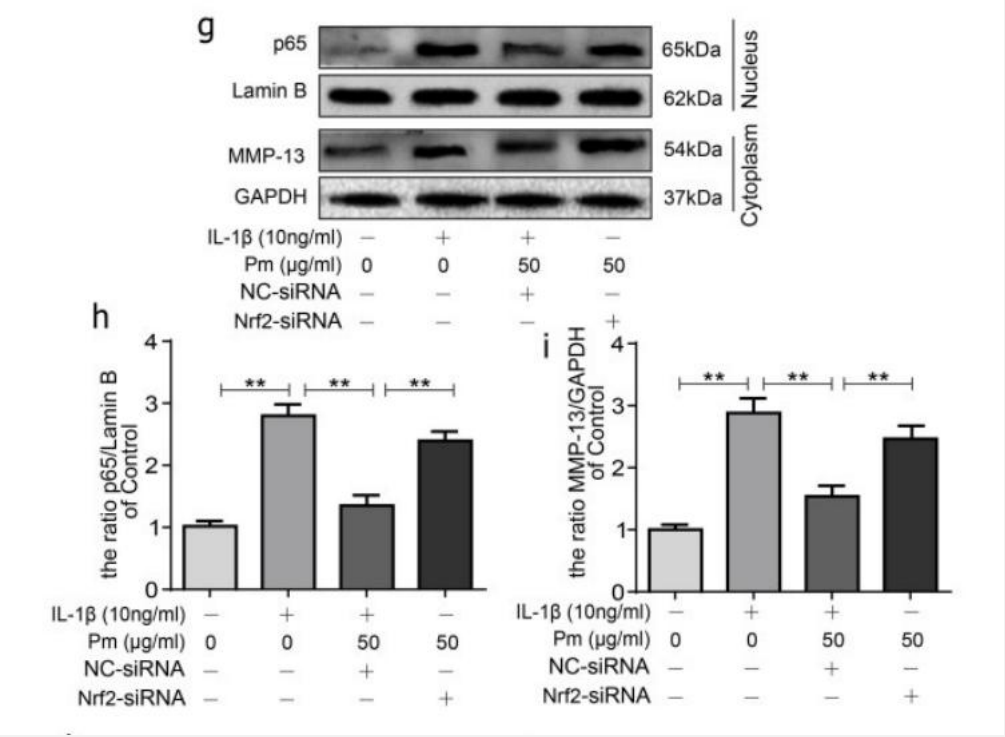
The results of Western blot showed that Pm pretreatment promoted nuclear translocation of Nrf2 and expression of HO-1 in the cytoplasm of chondrocytes treatment with or without IL-1β

Results



What's more, Nrf2 siRNA transfection significantly suppressed Pm-induced Nrf2/HO-1 signal activation in inflammatory conditions

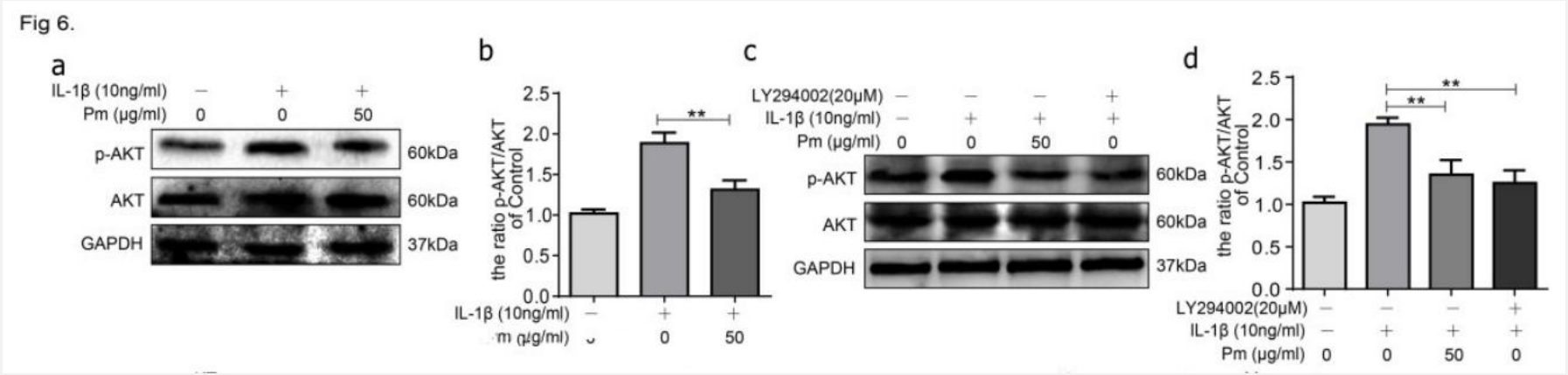
Results



After Nrf2 siRNA transfection, nuclear expression of p65 was up-regulated in chondrocytes cotreated with IL-1β and Pm, suggesting that the Nrf2/HO-1 signal pathway mediates Pm induced NF-κB signal inhibition

Results

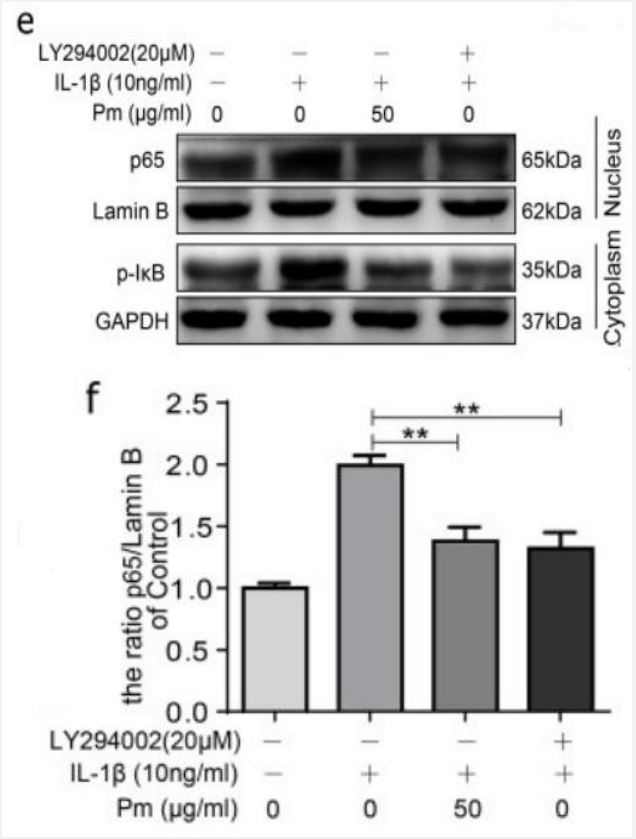
6、Effects of Pm on the AKT/ NF-κB signal pathway



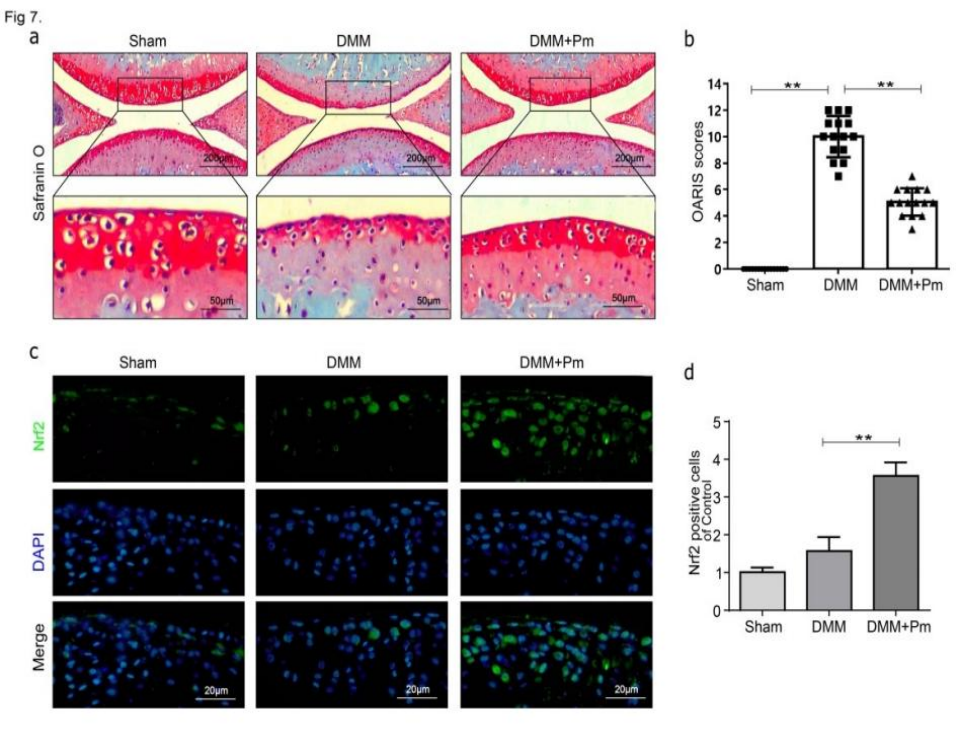
pretreatment of Pm inhibited IL-1β-induced the phosphorylation of AKT and IκB at 50 μg/ml and demonstrated by using AKT inhibitor (LY294002)

Results

Besides, the inhibition of AKT phosphorylation inhibited the IL-1β-induced p65 nuclear translocation in chondrocytes



Results



As shown by Safranin O staining, the articular cartilage surface was erosion and large loss of proteoglycan compared with the control sham group.

In the group of Sham and DMM, the fluorescence intensity of Nrf2 immunoreactivity of positive chondrocytes was less. However, Pm treatment increased the Nrf2 positive chondrocytes as compared to sham and DMM controls

Conclusion

Taken together, the present study has exhibited that Pm significantly decreased inflammatory reaction and catabolism induced by IL-1β via inhibiting AKT/NF-κB and stimulating Nrf2/HO-1 pathway in mouse OA chondrocytes. Meanwhile, in vivo show that Pm administration could reverse the apoptosis of chondrocytes and ECM degradation,indicating that Pm treatment could reduce OA progression, which is in agreement with the result in vitro. Altogether, although further study is needed, the data acquired in present study might support the potential use of Pm as a promising treatment for the OA therapy.

In conclusion,Pm suppressed the inflammation reaction induced by IL-1β in chondrocytes and ECM metabolism imbalance by activating Nrf2/HO-1 and blocking the Akt/NF-κB pathway.

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