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ORIGINAL ARTICLE

Protective Effects of Ginsenoside Rh1 on Intervertebral Disc Degeneration through Inhibition of Nuclear Factor Kappa-B Signaling Pathway

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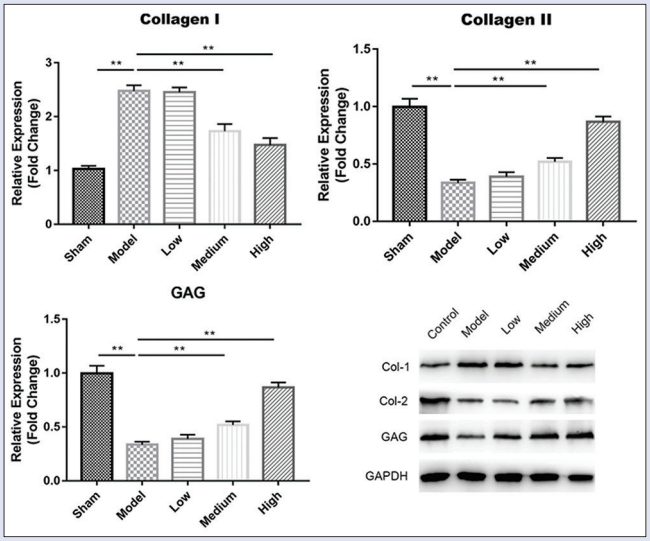
ABSTRACT

Background: In this study, we aim to explore the protective effect of ginsenoside Rh1 against intervertebral disc degeneration (IDD) and the related mechanism. **Materials and Methods:** IDD model in Sprague-Dawley rats was established and the animals were treated with different concentrations of ginsenoside Rh1 for 4 weeks, after this, the animals were sacrificed and the intervertebral disc of was collected for analysis using quantitative polymerase chain reaction. Western blot analysis was performed for quantifying the expression levels of glycosaminoglycans (GAGs) and Types I and Type II collagen. Moreover, serum samples were collected and the expression levels of some of the inflammatory cytokines such as interleukin (IL)-1 β and IL-6 were evaluated. Next, we collected the nucleus pulposus (NP) cells from the animals and were divided into five groups: control, IDD, treatment groups with different concentrations of ginsenoside Rh1 (10, 20, and 50 μg/mL). After treatment, the levels of IL-1 β and IL-6 in the cell culture supernatant were examined. Then, we performed western blot analysis to quantify the levels of B-cell lymphoma-2 (Bcl-2), B-cell lymphoma-extra-large (BCL-xL), and nuclear factor kappa-B (NF-κB) in different groups. **Results:** We observed that ginsenoside Rh1 significantly downregulated the expression of type I collagen and upregulated the expression of type II collagen and GAG under *in vivo* conditions. Moreover, the expression levels of IL-1 β and IL-6 in the serum samples of IDD rats and cell culture supernatant of NP cells isolated from the IDD rats were significantly increased. However, ginsenoside Rh1 significantly increased the levels of Bcl-2 and Bcl-xL and decreased the levels NF-κB both under *in vitro* and *in vivo* conditions. **Conclusion:** Ginsenoside Rh1 demonstrated protective effect against the IDD via regulation of IL-1 β/NF-κB signaling pathway.

Key words: Ginsenoside Rh1, interleukin-1β, intervertebral disc degeneration, nuclear factor kappa-B, nucleus pulposus cells

SUMMARY

- Ginsenoside Rh1 can play protective roles in the progress of intervertebral disc degeneration both under *in vitro* and *in vivo* conditions.
- Ginsenoside Rh1 can inhibit the nuclear factor kappa-B signaling pathway in intervertebral disc degeneration rats and nucleus pulposus cells.



Abbreviations used: IDD: Intervertebral disc degeneration; NP: Nucleus pulposus; qPCR: Quantitative polymerase chain reaction; IL-1 β: Interleukin-1 β; IL-6: Interleukin-6; Bcl-2: B-cell lymphoma-2; BCL-xL: B-cell lymphoma-extra large; NF-κB: Nuclear factor kappa-B; LBP: Lower back pain; SD: Sprague-Dawley; FBS: Fetal-bovine serum; ELISA: Enzyme-linked immunosorbent assay; SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PVDF: Polyvinylidene fluoride; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; HRP: Horseradish peroxidase; ANOVA: Analysis of variance.

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INTRODUCTION

Nowadays, lower back pain has become a common muscular problem worldwide. Intervertebral disc degeneration (IDD) results in low back pain (LPB).[1] In some cases, patients with IDD cannot work resulting in a poor quality of life.[1,2] With the increased life span of human beings and because of the high incidence rate, IDD has caused a huge burden

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to society, as well as to the healthcare system.[3] Unfortunately, the pathogenesis of IDD has not yet been fully elucidated and so far, there is no cure available to treat the disease. Therefore, it is important to search for new and effective medications for the treatment of IDD.

In recent years, great efforts have been made to explore the pathogenesis of IDD. Nucleus pulposus (NP), one of the three components of the intervertebral disc, has been shown to function as the primary component for maintaining the structure and the function of the intervertebral disc.[4,5] NP cells are the major types of resident cells that exist in the NP.[6] Previous studies have suggested that the abnormal apoptosis and dysfunction of the NP cells may closely be correlated with the incidence and progress of IDD.[7-11] Therefore, increasing the number and restoring the function of NP cells may be an effective method to alleviate the problems associated with IDD.

Ginsenoside Rh1 is one of the primary active components of traditional Chinese medicine ginseng.[12] In the clinical field, ginsenoside Rh1 has been proven to play protective roles in against various disorders of the cardiovascular system, immune system, pulmonary system, the liver, bones and other tissues and organs. In China, products related to ginseng were widely used by physicians for the treatment of wounds and fractures.[13] Previous studies have indicated that ginsenoside Rh1 shows therapeutic effect in bone formation by stimulating the signal transduction pathway, which in turn, activates or inhibits the expression of the downstream genes.[14] However, the effects of ginsenoside Rh1 on IDD has never been discussed in previous studies.

In this study, we will investigate the effect of ginsenoside Rh1 on IDD and the related mechanism both under *in vitro* and *in vivo* conditions, our results may provide novel evidence for the application of ginsenoside Rh1 as a new medication for the treatment of IDD.

MATERIALS AND METHODS

Animals

A total of 50 Sprague-Dawley (SD) rats (male, about 200 g, 8–9 weeks old) were used in this study. To induce IDD, we performed surgery to remove the left facet joint of the SD rats and then used a 21-gauge needle to insert into the endplates. After surgery, the skin and muscle of the rats were closed by suture. Then, the rats were randomly divided into five groups: control group and Sham group and ginsenoside Rh1 low, medium, and high groups (10, 20, or 40 mg/[kg/day], respectively). The control group or Sham group was treated with phosphate buffer saline (PBS). The study protocol was approved by the Ethical Committee of Yantai Yuhuangding Hospital.

Culture of the nucleus pulposus cell and treatment

NP cells were isolated from the control or IDD rats according to the methods described previously.[15] NP cells were maintained in F12-Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 100 mg/mL streptomycin, and 100 U/mL penicillin. In addition, at the cells were incubated at 37°C with 5% CO_2. NP cells were randomly divided into four groups: control group and treatment groups with different concentrations of ginsenoside Rh1 (0, 10, 20, and 50 μg/mL).

Enzyme-linked immunosorbent assay

The expression of interleukin-6 (IL-6) and interleukin-1 β (IL-1 β) in the serum of the rats and cell culture supernatants were evaluated using enzyme-linked immunosorbent assay (ELISA) kits (Beyotime, Haimen, China) based on the manufacturer's instructions.

Real-time quantitative reverse transcription-polymerase chain reaction

Total RNAs were isolated from the cells by the Trizol reagent based on the manufacturer's protocol. The expression of types I and II collagen, glycosaminoglycans (GAG), B-cell lymphoma-2 (Bcl-2), B-cell lymphoma-extra large (Bcl-xL), and nuclear factor kappa-B (NF-κB) were evaluated by SYBR Premix Ex Taq II (Takara, Dalian, China) following the reverse transcription quantitative-polymerase chain reaction (PCR) analysis (PrimeScript™ RT reagent Kit, Takara, Dalian, China) on ABI 7500 Real-Time PCR System (Applied Bioscience, MA, USA). glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalization.

Western blot

Protein samples from the different treatment groups was extracted from the cells, separated with 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and then transferred to polyvinylidene fluoride membranes (purchased from Millipore, Billerica, MA, USA). The membranes were then washed and blocked by nonfat milk and then treated by the primary antibodies (Abcam, Cambridge, MA, USA) overnight at 4°C. On day 2, the membranes were treated with horseradish peroxidase-conjugated secondary antibodies (Beyotime). Next, the membranes were treated with the BeyoECL Plus (Beyotime, Shanghai, China) kit and were photographed by ChemiDoc™ XRS + imaging system (Bio-Rad, California, USA). GAPDH was applied as the internal control.

Statistical analysis

Statistical analysis was conducted with SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). The data were expressed as the mean ± standard deviation. Comparisons among the multiple groups were analyzed by analysis of variance. $P < 0.05$ was considered statistically significant.

RESULTS

Protective effects of ginsenoside Rh1 on intervertebral disc degeneration *in vivo*

First, we created a rat IDD model and examined the effects of ginsenoside Rh1 on the expression of IDD markers types I and II collagen and GAG in this model. As shown in Figure 1, type I collagen was markedly increased, whereas type II collagen and GAG were dramatically decreased in lumbar NP tissue samples of the IDD rats. Moreover, the medium and high dose group showed an increase in both type II collagen and GAG, and showed a decrease in the level of type I collagen in NP tissues on both mRNA [Figure 1a-c] and protein [Figure 1d] levels. Furthermore, the expressions of IL-1 β and IL-6 in serum samples were examined by ELISA methods. As shown in Figure 2, IL-1 β and IL-6 were markedly increased in the serum samples of IDD rats, and the medium and high dose ginsenoside Rh1 can decrease the expression of IL-1 β and IL-6 in IDD rats in a dose-dependent manner.

Effect of ginsenoside Rh1 on nucleus pulposus cells *in vitro*

Next, NP cells were isolated from normal and IDD rats and the effects of ginsenoside Rh1 on these cells were examined. According to our results, compared with the normal NP cells, the secretion of IL-1 β and IL-6 was markedly increased in the cell culture supernatant of the NP cells

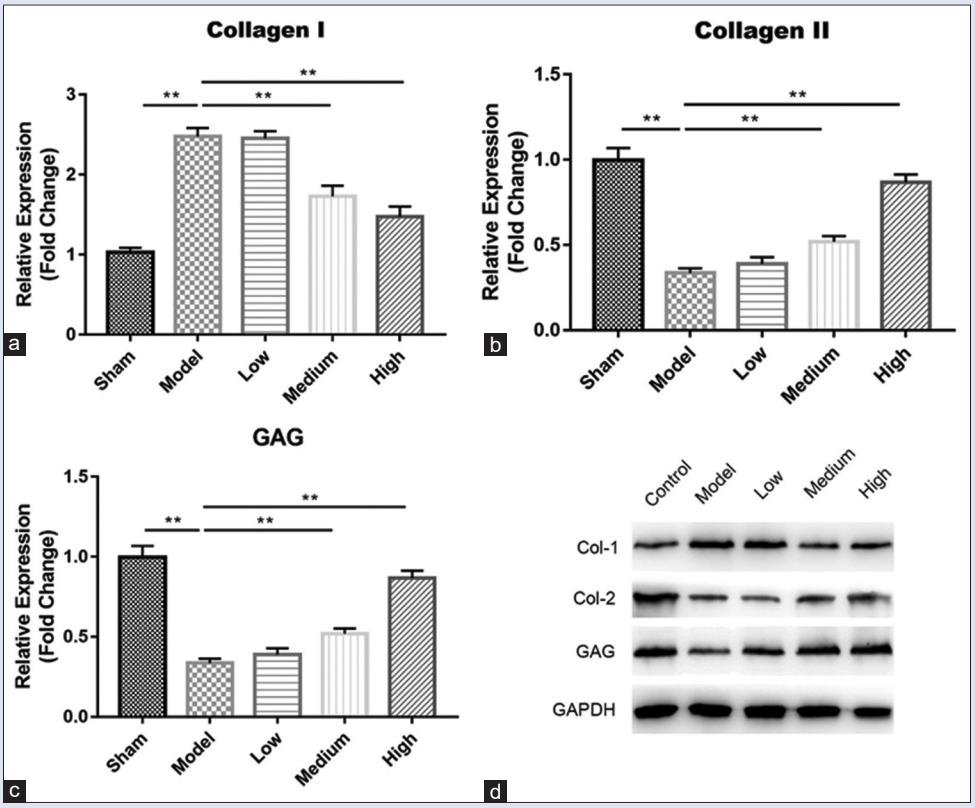

Figure 1: Effect of ginsenoside Rh1 on the mRNA (a-c) and protein (d) expressions of I collagen, type II collagen and glycosaminoglycans *in vivo*. $^{**}P < 0.01$

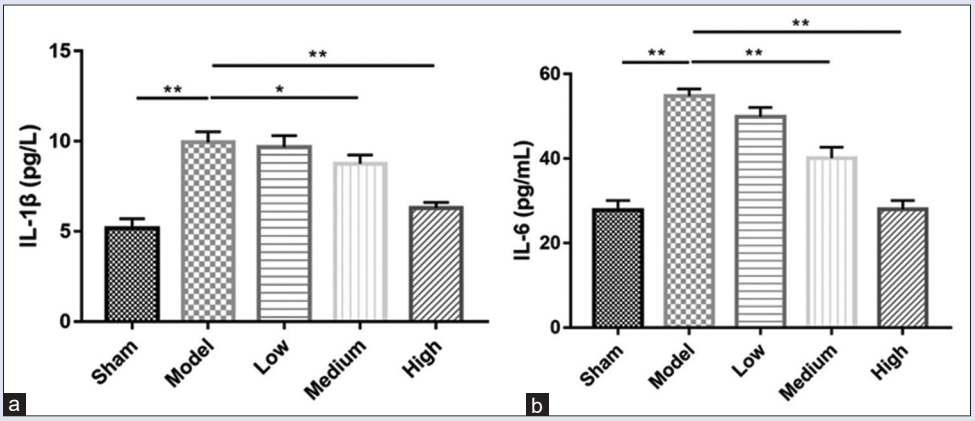

Figure 2: Effect of ginsenoside Rh1 on the expressions of interleukin-1 β (a) and interleukin-6 (b) *in vivo*. $^{*}P < 0.05$, $^{**}P < 0.01$

isolated from the IDD rats. Moreover, 20 and 50 μg/mL ginsenoside Rh1 treatment significantly decreased the secretion of IL-1 β and IL-6 in the cell culture supernatant of NP cells isolated from the IDD rats [Figure 3, $P < 0.05$].

Effects of ginsenoside Rh1 on the levels of nuclear factor kappa-B-related signaling molecules in intervertebral disc degeneration rats *in vivo* and nucleus pulposus cells *in vitro*

To further explore the underlying mechanism of ginsenoside Rh1-induced therapeutic effects against IDD, we investigated whether NF-κB signaling pathway was affected by ginsenoside Rh1. According to our results, the expression of NF-κB was markedly increased and the expression of Bcl-2 and Bcl-xL was decreased in NP tissue [Figure 4] and NP cells [Figure 5] isolated from the IDD rats. However, treatment with medium-and high-dose ginsenoside Rh1 induced dramatic increase in the expression levels of Bcl-2 and Bcl-xL and decreased the expression levels of NF-κB in NP tissue samples of IDD rats *in vivo* and NP cells *in vitro* [Figures 4 and 5, $P < 0.01$].

DISCUSSION

In this study, we aimed to determine the therapeutic effects of ginsenoside Rh1 in the treatment of IDD. According to our results, ginsenoside Rh1 can alter the expression of IDD markers both under *in vitro* and *in vivo* conditions, and we also proved that ginsenoside Rh1

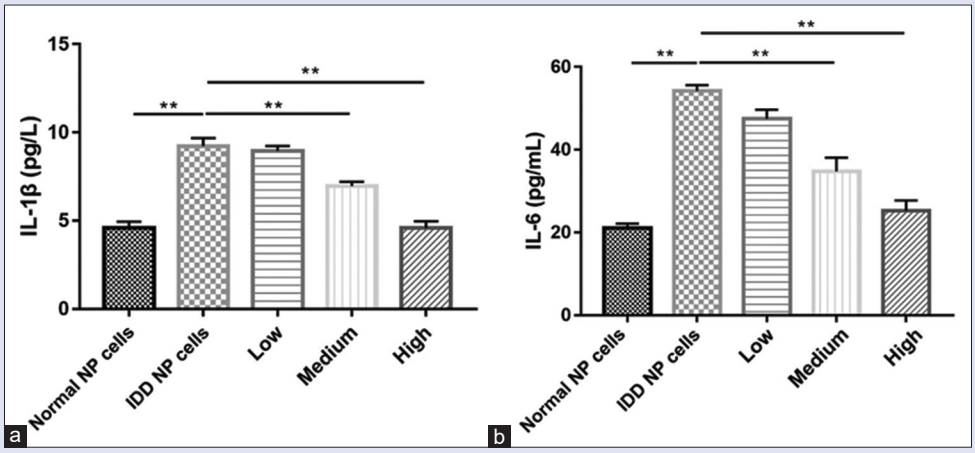

Figure 3: Effect of ginsenoside Rh1 on the expressions of interleukin-1 β (a) and interleukin-6 (b) *in vitro*. $^{**}P < 0.01$

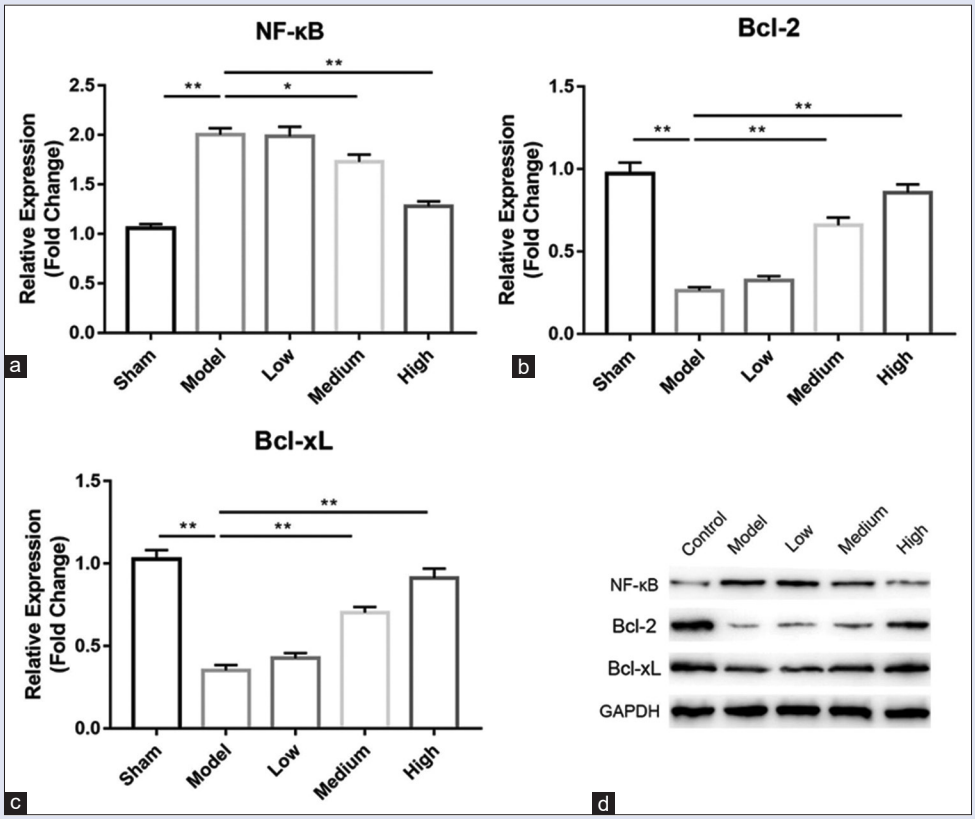

Figure 4: Effect of ginsenoside Rh1 on the mRNA (a-c) and protein (d) expressions of nuclear factor kappa-B related signaling molecules in intervertebral disc degeneration rats *in vivo*. $^{*}P < 0.05$, $^{**}P < 0.01$

can alleviate the inflammatory condition of IDD by regulating IL-1 β/NF-κB signaling. Our results suggest that ginsenoside Rh1 may serve as a potential alternative medication for the management of IDD.

In recent years, numerous reports on natural compounds in the treatment of bone diseases have been reported. Yuan *et al*. have reported that puerarin can prevent the bone loss of the ovariectomized mice models and also inhibit the formation of osteoclasts.[16] Caichompoo *et al*. have discussed the effect of extracts of *Schisandra chinensis* (Turcz.) on the proliferation of osteoblasts.[17] Hsieh *et al*. have discussed the roles of icariin in osteoblasts anabolism.[18] Tang *et al*. proved that Honokiol can alleviate IDD by suppressing TXNIP-NLRP3 inflammasome signal.[19] Zheng *et al*. have demonstrated that spermidine can lead to increased autophagy in NP and alleviate the symptoms of IDD.[20] Furthermore, the active components of ginsenoside (e.g., ginsenoside Rg1 and ginsenoside Rg3) have been shown to play protective effects in IDD.[21,22] However, investigation on the roles of ginsenoside Rh1 in IDD was limited, and it remains unclear whether ginsenoside Rh1 can exert the same effect as Rg1 or Rg3 in the treatment of IDD.

NP cells are a group of cells located in the intervertebral disc, and the results of previous studies have suggested that abnormal degradation of extracellular matrix (ECM) in NP cells can ultimately lead to IDD.[23] The ECM of NP cells consists of different types of collagen and proteoglycan,

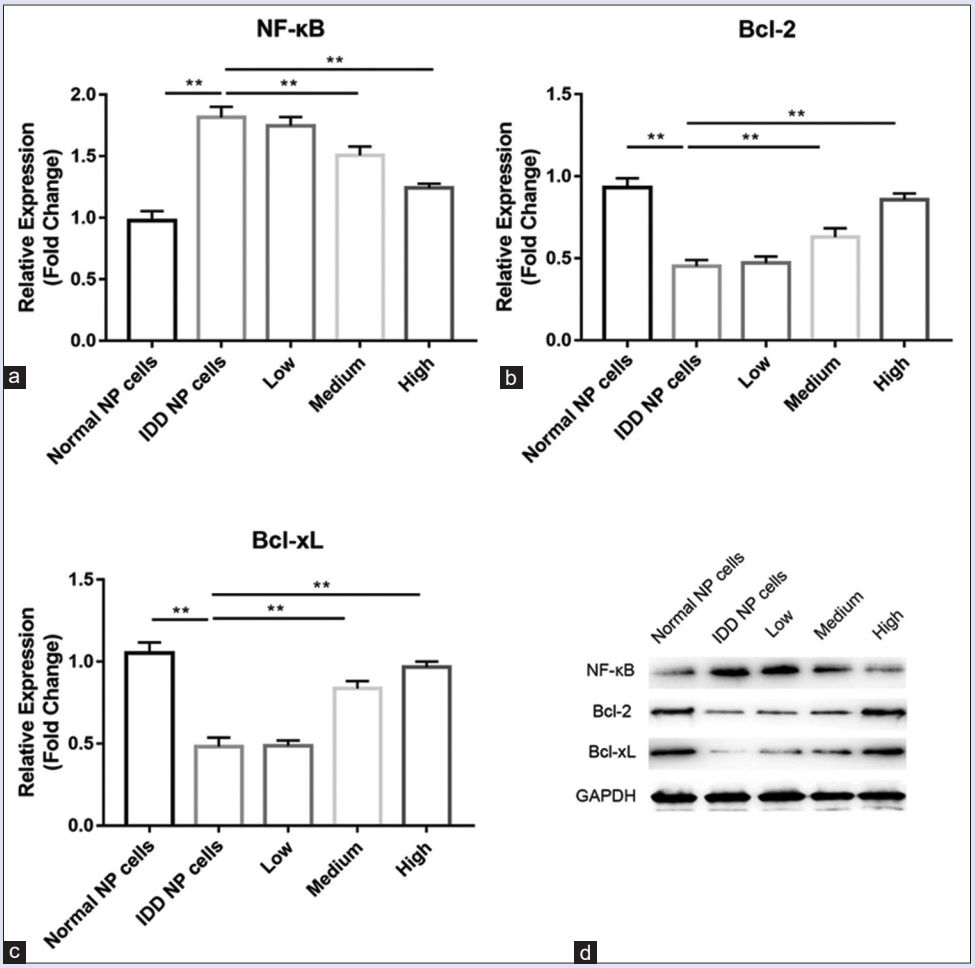

Figure 5: Effect of ginsenoside Rh1 on the mRNA (a-c) and protein (d) expressions of nuclear factor kappa-B related signaling molecules in nucleus pulposus cells *in vitro*. *$P < 0.05$, **$P < 0.01$

and patients with IDD exhibit dramatic changes in the expression level of these proteins.[23,24] In this study, we established rat IDD model and observed that the medium-and high-dose ginsenoside Rh1 significantly decreased the expression levels of type I collagen, and increased the expression levels of type II collagen and GAG in NP tissues of IDD rats, suggesting that ginsenoside Rh1 can alleviate IDD under *in vivo* conditions. Moreover, ginsenoside Rh1 can also change the expression levels of the above proteins in NP cells under *in vitro* conditions. In summary, these results suggest that ginsenoside Rh1 can alleviate IDD condition by suppressing the degradation of ECMs in NP cells.

The chronic inflammatory condition in the intervertebral disc is the other symptom of IDD, and in current clinical application, medications with anti-inflammatory functions have been applied for the treatment of IDD.[25] NF-κB has been known as an important regulator that exists in many types of cells and has been shown to be aberrantly activated in IDD, which may lead to chronic inflammatory condition and therefore contribute to the pathogenesis of the disease.[26-28] Previous studies have demonstrated the anti-inflammatory activity of ginsenoside Rh1;[13,29] therefore, in this study, we hypothesized that ginsenoside Rh1 may exert its protective effect in IDD by inhibiting the activation of NF-κB. To further investigate the underlying mechanism of ginsenoside Rh1-induced therapeutic effects, we examined the expression levels of NF-κB and the downstream molecules, as well as the related cytokines IL-1 β and IL-6. We found that the medium and high doses of ginsenoside Rh1 significantly decreased the levels of NF-κB and increased the level of Bcl-2 and Bcl-xL. These results suggest that ginsenoside Rh1 shows its therapeutic effects by suppressing the NF-κB signaling pathway.

This study has some limitations. First, we only performed cell-based assay and animal studies, and clinical studies should be performed in the future to confirm the effects of ginsenoside Rh1.

CONCLUSION

In conclusion, the results of this study demonstrate that ginsenoside Rh1 can alleviate IDD by regulating IL-1 β/NF-κB signaling pathway. Our results have provided novel evidence for the application of ginsenoside Rh1 as an effective medication for the treatment of IDD.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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