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Effect of *Toddalia asiatica* extract combined with miR-483 on proliferation, apoptosis and inflammatory factors expression of osteoarthritis chondrocyte

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Abstract: This study aims to investigate the effect of *Toddalia asiatica* extract combined with miR-483 on osteoarthritis chondrocyte proliferation and apoptosis and expression of inflammatory factors. Osteoarthritis chondrocytes were treated with *Toddalia asiatica* extract. Osteoarthritis chondrocytes were transfected with anti-miR-483 or miR-483, and *Toddalia asiatica* extract was applied. The levels of TNF-α, IL-6, IL-10 were determined by ELISA. Cell proliferation and cloning were evaluated by MTT and cloning experiment. Cell apoptosis was determined by flow cytometry. P21 and caspase-3 protein expression were analyzed by Western blot. The expression of miR-483 was detected by qRT-PCR. The application of *Toddalia asiatica* extract or inhibition of miR-483 significantly increased the cell survival rate, cloning count and IL-10 level of osteoarthritis chondrocytes, and significantly reduced the apoptosis rate, levels of P21, Caspase-3, TNF-α IL-6 and miR-483 expression level of osteoarthritis chondrocytes ($p<0.05$). Overexpression of miR-483 could reverse the promotion effect of *Toddalia asiatica* extract on osteoarthritis chondrocytes proliferation, clone formation and IL-10 level, as well as reserve the inhibition effect of *Toddalia asiatica* extract on osteoarthritis chondrocyte apoptosis and levels of P21, caspase-3, TNF-α, IL-6. The combination of *Toddalia asiatica* extract and miR-483 can promote the proliferation of osteoarthritis chondrocytes and inhibit apoptosis and expression of inflammatory factors.

Keywords: *Toddalia asiatica*, miR-483, chondrocytes, proliferation, apoptosis, inflammatory factors.

INTRODUCTION

Osteoarthritis is one of the most common degenerative diseases caused by joint instability in the elderly as well as one of the most common causes of disability (Heikal *et al*., 2019). Data show that osteoarthritis affects nearly 15% of the population, and the incidence of osteoarthritis worldwide is increasing by 100,000 people per year (Mandl, 2019). Many studies have shown that the reduction of articular chondrocytes and joint inflammation are the main causes of osteoarthritis, which can reduce the quality of life of patients (Cho *et al*., 2019). Therefore, effectively delaying the degeneration of chondrocytes is the key to prevent or treat osteoarthritis. As the root of *Toddalia asiatica* Lam. belonging to Rutaccac family. *Toddalia asiatica* has the effect of removing wind and pain, dispersing stasis and stopping bleeding, detumescence and detoxification, and can be used for the treatment of rheumatoid arthritis, fall-hit injury, wound bleeding, etc (Zhou *et al*., 2018; Zhang *et al*., 2017). It was found that the *Toddalia asiatica* extract had anti-inflammatory and analgesic effects, which can be used to down-regulate the expression of pro-inflammatory factors in mice, and had certain inhibitory effects on rheumatoid arthritis. MicroRNA (MicroRNA, miRNA/miR) is a small single-stranded non-coding RNA that binds to complementary target sequences of the 3' untranslated regions (3'-UTR) of mRNA so as to regulate stability and translation of mRNA (Hu *et al*., 2017). Previous studies have demonstrated that miRNA expression may play a key role in the development and progression of osteoarthritis (Xu, *et al*., 2019). According to relevant report, miR-483 was up-regulated in the cartilage of patients with osteoarthritis, and it was found that miR-483 may play an important regulatory role in osteoarthritis (Jing *et al*., 2016). However, the effects of *Toddalia asiatica* extract combined with miR-483 on proliferation and apoptosis of osteoarthritis chondrocyte and expression of inflammatory factors remain unclear. On this basis, this study investigates the effect of *Toddalia asiatica* extract combined with miR-483 on osteoarthritis chondrocyte proliferation and apoptosis and expression of inflammatory factors, providing promising drug targets for the clinical treatment of osteoarthritis.

MATERIALS AND METHODS

Reagents

Toddalia asiatica extract was purchased from Bozhou Sizhitang Pharmaceutical Co., Ltd.; DMEM/F12 medium was purchased from Gibco company, USA; anti-miR-NC, anti-miR-483, miR-NC, miR-483 were purchased from Guangzhou Ruibo Biotechnology Co., Ltd. Tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-10 (IL-10), Enzyme-Linked ImmunoSorbent Assay (ELISA） were purchased from eBioscience, USA. Annexin V-fluorescein isothiocyanate/ propidium iodide

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(Annexin □-FITC/PI) apoptosis detection kit and bicinchoninic acid (BCA) protein detection kit were purchased from Beyotime Biotechnology, Shanghai, China.

Preparation of Toddalia asiatica extract

After soaking *Toddalia asiatica* powders in 95% ethanol and reflux for 2-3h, extract for 3 times, combine with filtrate, reduce pressure and concentrate to make extract. After that, add 1% hydrochloric acid, and the resulting insoluble acidethyl acetate extract was adjusted with 5% sodium bicarbonate to neutral, followed by addition of ethyl acetate for extraction. Finally the extracts obtained were condensed and dried to obtain ethyl acetate extract of *Toddalia asiatica*.

Cell isolation and culture

Samples of osteoarthritis cartilage tissues from patients undergoing artificial knee arthroplasty as well as normal cartilage tissue samples were collected. According to method proposed by Du *et al*. (2016), chondrocytes in osteoarthritis were isolated. This study obtained the informed consent of the patient or their families, complied with the Helsinki Code of Ethics, and was approved by the Hospital Ethics Committee. Chondrocytes were cultured in DMEM/F12 medium containing 10% fetal bovine serum and 1% cyano-strepmycin DMEM/F12, and were grown at 37□ in 5% CO_2 in saturated and humid environment. When the chondrocytes grew to 80% confluence, trypsin was added for digestion and passage. The cells used in the experiment were second generation chondrocytes.

Cell transfection and grouping

Before transfection, chondrocytes from osteoarthritis were divided into control (normal cultured chondrocytes), *Toddalia asiatica* -L group (65 ug/mL *Toddalia asiatica* extract), *Toddalia asiatica*-M group (130 ug/mL ethyl acetate *Toddalia asiatica* extract), *Toddalia asiatica*-H group (260 ug/mL ethyl acetate *Toddalia asiatica* extract). The action time was 24 h for all groups.

During transfection, osteoarthritis chondrocytes were inoculated in 6-well plates. When the cells grew to 70% confluent, anti-miR-NC, anti-miR-483, miR-NC, miR-483 were transfected with Lipofectamine 2000 reagent. Among them, the cells transfected with miR-483 and miR-NC were treated with 260ug /mL *Toddalia asiatica* extract for 24 h. The transfected cells were classified as the anti-miR-NC group, anti-miR-483 group, *Toddalia asiatica* extract-H+miR-NC group, *Toddalia asiatica* extract-H+miR-483 group, respectively.

The levels of inflammatory cytokines TNF-α, IL-6 and IL-10 were detected by ELISA

The supernatant of human normal articular chondrocytes and osteoarthritis chondrocytes was collected. The levels of TNF-α, IL-6 and IL-10 were detected in accordance with the steps of TNF-α, IL-6 and IL-10 ELISA kit, respectively.

Methyl thiazolyl tetrazolium (MTT) was used to detect cell proliferation

Osteoarthritis chondrocytes were inoculated in 96-well plates, with a density of 1×104 cells per well. After 48 h of culture, 100 μL of MTT solution was added to each well and reacted at 37□ for 4 h. After that, 100 μL of dimethyl sulfoxide was added, and the absorbance (OD) value of chondrocytes was measured at 490 nm with a micrometer after 15 min of severe oscillation (Li *et al*., 2016). The cell survival rate was calculated as follows:

Cell survival rate (%) = OD value of the experimental group/OD value of the control group ×100%.

Cloning experiments detect cell cloning

Osteoarthritis chondrocytes were inoculated in a 60 mm culture dish and cultured in fresh medium for two weeks. When more than 50 cell colonies appeared in the petri dish, methanol was fixed for 20 min and jamsa staining was performed for 30 min to count the number of cell clones (Du *et al*., 2016).

Cell apoptosis was detected by flow cytometry

Annexin V-FITC binding solution was used to resuscitate osteoarthritis chondrocytes. The apoptosis of osteoarthritis chondrocytes were detected by flow cytometry after 20 min of light avoidance at room temperature.

Western blot was used to detect the protein expressions of P21 and Caspase-3 (cysteinyl aspartate specific proteinase 3)

Osteoarthritis chondrocytes were added with RIPA lysate to extract the total protein. After quantification with BCA protein detection kit, 12% sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) was performed. Subsequently, the solution was transferred to PVDF and sealed in 5% skim milk powder for 1 hour, incubated with P21 and Caspase-3 protein primary antibody (1:1000) overnight at 4□, and incubated with horseradish peroxidase-labeled secondary antibody (1:2000) at room temperature for 1 hour. Using ECL chemiluminescence kit and with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as internal reference, the gray values of P21 and Caspase-3 protein bands were analyzed (Qi *et al*., 2013).

Detection of expression of miR-483 by qRT-PCR

According to the manufacturer's protocol, the TRIzol reagent was used to extract total RNA from osteoarthritis chondrocytes. The reverse transcription kit was used to reverse transcription into cDNA, and then cDNA was used as a template to detect miR-483 level through real-time fluorescent quantitative PCR 7500 system using 2-ΔΔCt method. miR-483's primer sequence is 5,-CTGGGGCACAGATAACTCGAGCGGACTTTCCTGA

GAGGAGGGG-3'(positive), 5'-AGGGGCGGAATTTGCAGTGGTTTGGAAAATGTGTG-3' (negative). The primer sequence of internal reference GAPDH is 5'-GAAGGTGAAGGTCGGAGTC -3' (positive), 5'-GAAGATGGTGATGGGATTG-3' (negative) (Du *et al*., 2016).

STATISTICAL ANALYSIS

SPSS 22.0 software was used for statistical analysis of the data. The results were expressed as mean ± standard deviation (x±s). Independent-samples T test was used to compare data differences between two groups. One-way ANOVA was used to compare data differences between multiple groups. Snk-q test was used for multiple comparisons between groups. The difference was considered statistically significant when $p<0.05$.

RESULTS

Comparison of inflammatory factors between normal chondrocytes and osteoarthritis chondrocytes

According to ELISA data in table 1, compared with the normal human articular chondrocytes group, expression levels of inflammatory cytokines TNF-α and IL-6 in osteoarthritis chondrocytes were significantly increased, while the level of IL-10 was significantly decreased ($p<0.05$).

Effect of Toddalia asiatica extract on proliferation and apoptosis of osteoarthritis chondrocyte

Results of MTT, cloning experiment, flow cytometry and western blot analysis (table 2, fig. 1) show that compared with control group, the cell survival rate and clone formation number of chondrocytes in osteoarthritis were significantly increased, and the cell apoptosis rate, P21 and Caspase-3 protein expression levels were significantly decreased in *Toddalia asiatica* -L group, *Toddalia asiatica* -M group and *Toddalia asiatica* -H group, $p<0.05$.

Effect of Toddalia asiatica extract on inflammatory factors expression of osteoarthritis chondrocyte

According to the ELISA detection results in table 3, compared with control group, the expression levels of TNF-α and IL-6 were significant decreased, while the expression level of IL-10 was significant increased in *Toddalia asiatica* -L group, *Toddalia asiatica*-M group, *Toddalia asiatica*-H group, $p<0.05$.

Effect of Toddalia asiatica extract on expression of mir-483 in osteoarthritis chondrocyte

According to qRT-PCR detection data in table 4, compared with control group, the expression of miR-483 in osteoarthritis chondrocyte was significantly decreased in *Toddalia asiatica* -L group, *Toddalia asiatica*-M group, *Toddalia asiatica*-H group, $p<0.05$.

Effect of inhibiting mir-483 on proliferation, apoptosis and inflammatory factors expression of osteoarthritis chondrocyte

The results of qRT-PCR showed (table 5) that anti-miR-483 was transfected into osteoarthritis chondrocytes, and the expression level of miR-483 was much lower than that of the anti-miR-NC group ($p<0.05$), suggesting the successful construction of osteoarthritis chondrocytes with inhibited miR-483. The results of MTT, cloning experiment, flow cytometry, western blot and ELISA (tables 5-6 and fig. 2) showed that compared with the anti-miR-NC group, inhibition of miR-483 significantly increased the survival rate, number of clone formation and IL-10 level but significantly reduced the apoptosis rate, protein expression levels of P21, caspase-3, and expression levels of TNF-α and IL-6 ($p<0.05$).

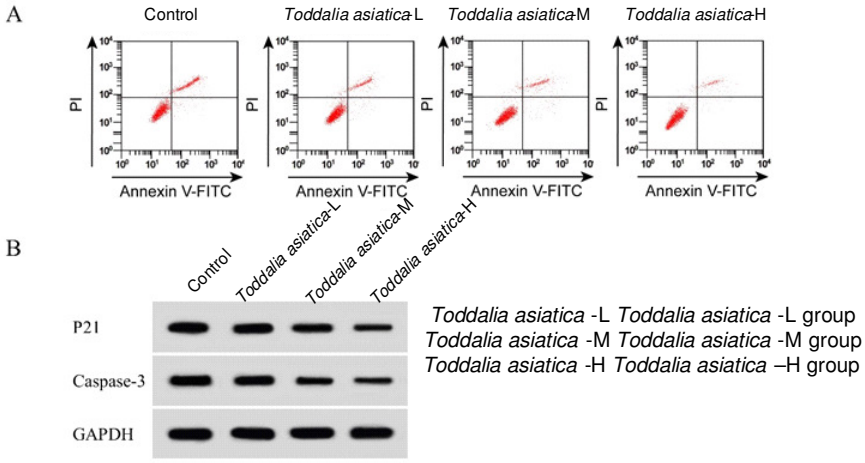

Fig. 1: Effect of *Toddalia asiatica* extract on proliferation and apoptosis of osteoarthritis chondrocyte (A: Effect of *Toddalia asiatica* extract on apoptosis of osteoarthritis chondrocyte; B: P21, Expression of P21 and Caspase-3)

Table 1: Comparison of inflammatory factors between normal chondrocytes and osteoarthritis chondrocytes ($\bar{x}$±s, n=9)

Group	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)
Normal human articular chondrocytes group	24.15±2.03	20.11±2.00	71.16±6.82
Osteoarthritis chondrocytes group	89.93±8.84*	64.32±6.15*	16.24±1.53*
t	21.757	20.509	23.572
p	<0.001	<0.001	<0.001

Note: Compared with the normal human articular chondrocytes group, *p<0.05.

Table 2: Effect of *Toddalia asiatica* extract on proliferation and apoptosis of osteoarthritis chondrocyte ($\bar{x}$±s, n=9)

Group	P21	Caspase-3	Cell survival rate (%)	Clone formation number	Cell apoptosis rate (%)
Control	0.75±0.07	0.63±0.06	51.36±5.23	73±7.11	27.13±2.45
Toddalia asiatica -L group	0.60±0.06*	0.48±0.05*	59.93±5.65*	92±8.64*	23.20±2.22*
Toddalia asiatica -M group	0.45±0.04*	0.32±0.03*	67.21±6.67*	131±12.48*	17.34±1.69*
Toddalia asiatica -H group	0.24±0.02*	0.18±0.02*	85.82±8.41*	169±15.82*	12.15±1.03*
F	162.514	184.986	44.388	123.308	104.877
p	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Compared with control group, *p<0.05. *Toddalia asiatica* -L group (65 ug/mL *Toddalia asiatica* extract), *Toddalia asiatica*-M group (130 ug/mL ethyl acetate

Table 3: Effect of *Toddalia asiatica* extract on inflammatory factors expression of osteoarthritis chondrocyte ($\bar{x}$±s, n=9)

Group	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)
Control	90.13±9.10	64.51±6.36	16.77±1.59
Toddalia asiatica -L group	75.14±7.31*	51.01±4.32*	21.15±2.10*
Toddalia asiatica -M group	51.26±5.14*	39.65±4.01*	37.89±3.66*
Toddalia asiatica -H group	33.69±3.57*	25.41±2.37*	56.94±5.45*
F	128.580	124.299	240.002
p	<0.001	<0.001	<0.001

Note: Compared with control group, *P<0.05.

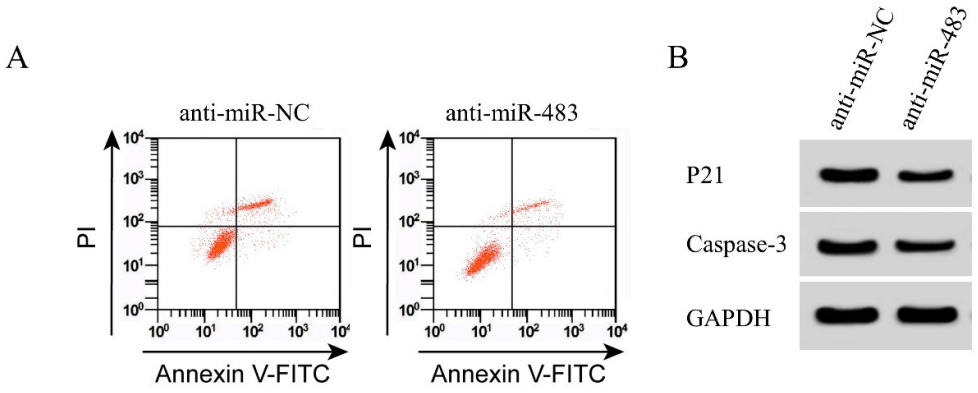

Fig. 2: Effect of inhibiting miR-483 on proliferation and apoptosis of osteoarthritis chondrocyte (A: Effect of inhibiting miR-483 on apoptosis of osteoarthritis chondrocyte; B: Protein expressions of P21 and Caspase-3)

Table 4: Effect of *Toddalia asiatica* extract on expression of miR-483 in osteoarthritis chondrocyte ($\bar{x}$ ±s, n=9)

Group	miR-483
Control	1.02±0.10
Toddalia asiatica -L group	0.81±0.08*
Toddalia asiatica -M group	0.64±0.06*
Toddalia asiatica -H group	0.40±0.04*
F	114.931
p	<0.001

Note: Compared with control group, *p<0.05.

The over-expression of miR-483 can reverse the influence of Toddalia asiatica extract on proliferation, apoptosis and inflammatory factors expression of osteoarthritis chondrocyte

The test results on cell proliferation, apoptosis and inflammation factors expression show that (tables 7-8, fig. 3), compared with *Toddalia asiatica* extract-H+miR-NC group, *Toddalia asiatica* extract--H+miR-483 group had significantly decreased cell survival rate, the number of clone forming, the IL-10 level, but significantly increased cell apoptosis rate, protein expression levels of P21 and Caspase 3, miR-483 expression amount, and levels of NF-α and IL-6 (p<0.05).

DISCUSSION

Osteoarthritis is a chronic painful arthritis, which can lead to decreased joint function and severe disability. As the population ages and due to risk factors such as obesity, the prevalence of osteoarthritis is also on the rise. The pathogenesis of arthritis is complex, which is thought to be the result of interactions among mechanical, genetic, metabolic and inflammatory mechanisms (Ghouri, conaghan, 2019). Currently, there is no satisfactory treatment. Traditional drugs such as NSAIDs and acetaminophen offer temporary relief, but the pathology is not delayed (Gulati 2017). Drugs used to treat osteoarthritis have a variety of side effects, including an increased risk of cardiovascular/gastrointestinal disease and adverse effects on cartilage (Rahman *et al*., 2018). The surgery does not reverse the destruction of articular cartilage, resulting in a loss of joint function (He *et al*., 2019). Currently, the use of herbal products as medicines and dietary supplements or as complementary and alternative medicines has become an important area of bone health research, especially in orthopedics, arthritis, rheumatism and musculoskeletal diseases (Choudhary *et al*., 2018). Herbal products have many therapeutic and dietary uses with low risk of side effects and toxicity. As a traditional Chinese medicine, *Toddalia asiatica* has a good application prospect.

Toddalia asiatica contains biological active ingredients such as coumarin and alkaloid (Cao *et al*., 2019), which has been widely used for the treatment of various diseases. Pharmacological studies have confirmed that *Toddalia asiatica* has a variety of biological activities, including anti-inflammatory, antibacterial, anti-tumor, antioxidant, analgesia, antiviral effects (He *et al*., 2018; Li *et al*., 2018). *Toddalia asiatica* extract has a certain therapeutic effect on the rheumatoid adjuvant arthritis, which can protect the synovial membrane damage of the joints of rats, inhibit the level of IL-6, and increase the level of IL-10 (Liu *et al*., 2018). However, the action mechanism of *Toddalia asiatica* extract on chondrocytes in osteoarthritis remains unknown. In this study, *Toddalia asiatica* extract significantly increased the cell survival rate, the number of cloning and the expression levels of anti-inflammatory factor IL-10 in osteoarthritis chondrocytes in a concentration-dependent manner, and significantly reduced the apoptosis rate, the protein expressions of P21 and caspase-3, as well as the levels of

Table 5: Effect of inhibiting miR-483 on proliferation and apoptosis of osteoarthritis chondrocyte ($\bar{x}$ ±s, n=9)

Group	miR-483	P21	Caspase-3	Survival rate (%)	Number of clone formation	Apoptosis rate (%)
Anti-miR-NC	1.01±0.10	0.74±0.07	0.62±0.06	51.35±5.21	74±7.16	27.24±2.47
Anti-miR-483	0.33±0.03*	0.30±0.03*	0.26±0.02*	79.96±8.02*	147±13.35*	14.04±1.39*
t	19.540	17.332	17.076	8.975	14.457	13.972
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Compared with anti-miR-NC group, *p<0.05.

Table 6: Effect of inhibiting miR-483 on inflammatory factors expression of osteoarthritis chondrocyte ($\bar{x}$ ±s, n=9)

Group	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)
Anti-miR-NC	90.11±9.12	63.98±6.31	16.51±1.52
Anti-miR-483	45.16±4.11*	33.18±3.27*	49.91±4.83*
t	13.481	13.001	19.789
p	<0.001	<0.001	<0.001

pro-inflammatory factors TNF-α and IL-6. It was suggested that the *Toddalia asiatica* extract could promote the proliferation of chondrocytes in osteoarthritis, inhibit apoptosis, and reduce inflammation, thus exerting the function of inhibiting osteoarthritis, which is consistent with former researcher's report (Li *et al*., 2018).

The main role of miRNA is to regulate target genes through translation inhibition and/or degradation of target mRNA (Liu *et al*., 2019). There is an increasing evidence that certain miRNAs can directly target different genes involved in the pathogenesis and development of osteoarthritis and play a critical role in regulating the activity and function of chondrocytes (Swingler *et al*., 2019). According to relevant report, miR-483 is involved in the occurrence and development of colorectal cancer, breast cancer, gastric cancer and other cancers (Niu *et al*., 2018; Yu *et al*., 2018; Cui *et al*., 2019). The expression level of miR-483-5p originating from miR-483 significantly increased in osteoarthritis chondrocytes, and the regulation of the expression of miR-483-5p may contribute to the maintenance of cartilage tissue (Qi *et al*., 2013). Over expression of miR-483-5p may increase the severity of experimental osteoarthritis in mice (Wang *et al*., 2017). However, the biological role of miR-483 in osteoarthritis remains unclear. In this experiment, the

Table 7: The over-expression of miR-483 can reverse the influence of *Toddalia asiatica* extract on proliferation, apoptosis and inflammatory factors expression of osteoarthritis chondrocyte ($\bar{x}$ ±s, n=9)

Group	P21	Caspase-3	Cell survival rate (%)	The number of clone forming	Cell apoptosis rate (%)
Toddalia asiatica extract -H+miR-NC	0.23±0.02	0.19±0.02	86.03±8.21	170±16.03	12.34±1.22
Toddalia asiatica extract -H+miR-483	0.66±0.06*	0.51±0.05*	55.85±5.46*	89±8.68*	24.11±2.36*
t	20.397	17.827	9.183	13.330	13.291
p	<0.001	<0.001	<0.001	<0.001	<0.001

Note: compared with *Toddalia asiatica* extract -H+miR-NC, *p<0.05.

Table 8: The over-expression of miR-483 can reverse the influence of *Toddalia asiatica* extract on inflammatory factors expression of osteoarthritis chondrocyte ($\bar{x}$ ±s, n=9)

Group	miR-483	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)
Toddalia asiatica extract -H+miR-NC	1.00±0.10	33.52±3.46	25.27±2.22	56.82±5.40
Toddalia asiatica extract -H+miR-483	2.39±0.22*	79.58±7.72*	56.14±5.51*	23.46±2.27*
t	17.256	16.334	15.590	17.085
p	<0.001	<0.001	<0.001	<0.001

Note: Compared with *Toddalia asiatica* extract -H+miR-NC, *p<0.05.

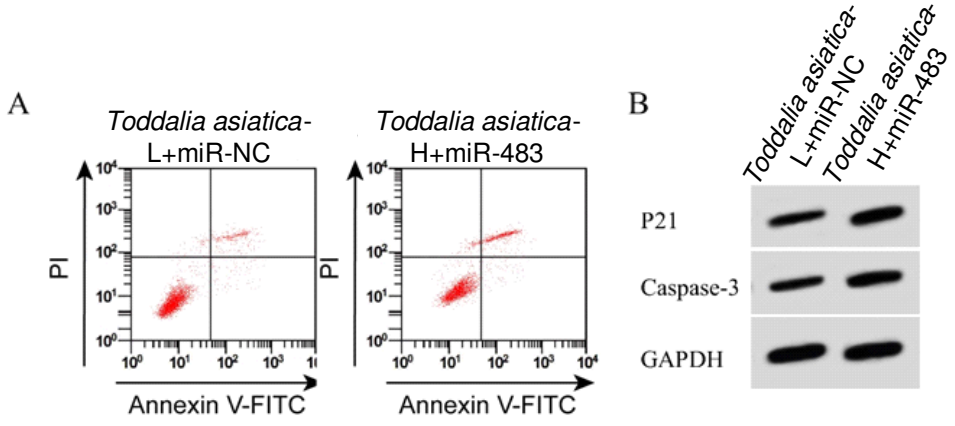

Fig. 3: The over-expression of miR-483 can reverse the influence of *Toddalia asiatica* extract on proliferation, apoptosis and inflammatory factors expression of osteoarthritis chondrocyte (A: The over-expression of miR-483 can reverse the influence of *Toddalia asiatica* extract on proliferation, apoptosis and inflammatory factors expression of osteoarthritis chondrocyte; B: protein expression of P21 and Caspase-3).

Toddalia asiatica extract reduced the expression level of miR-483 in a concentration-dependent manner, suggesting that the anti-osteoarthritis function of *Toddalia asiatica* extract might be achieved by down-regulating the expression level of miR-483. The results of functional experiments showed that the inhibiting miR-483 significantly increased the survival rate, the number of clone formation and Il-10 level of chondrocytes in osteoarthritis, but significantly reduced the apoptosis rate, protein expressions of P21 and caspase-3, and the expression levels of TNF-α and IL-6. This indicates that the down-regulation of miR-483 had the same effect as that of *Toddalia asiatica* extract, both of which could promote the proliferation of chondrocytes and inhibit the apoptosis of chondrocytes in osteoarthritis. In addition, over-expression of miR-483 could reverse the promoting effect of *Toddalia asiatica* extract on chondrocyte proliferation, clone formation and Il-10 level in osteoarthritis, as well as reverse its inhibitory effect on apoptosis, P21, caspase-3 protein expression, expression levels of TNF-α and IL-6. These results suggest that down-regulation of miR-483 expression in cells may be one of the important ways for the *Toddalia asiatica* extract to exert inhibition effect on osteoarthritis.

CONCLUSION

In conclusion, the *Toddalia asiatica* extract can promote the proliferation and clone formation of chondrocytes in osteoarthritis, inhibit cell apoptosis and reduce inflammation, showing certain anti-osteoarthritis activity, which is the same as the effect by inhibiting the expression of miR-483. In addition, the mechanism of action of the *Toddalia asiatica* extract is closely related to the regulation of miR-483 expression, providing a new therapeutic strategy for osteoarthritis.

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