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Effect of Gross Saponins of *Tribulus terrestris* on IL-1β-induced Chondrocyte Injury by Regulating miR-99a Expression

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ABSTRACT

The objective of this study was to investigate the effect of gross saponins of *Tribulus terrestris* (GSTT) on chondrocyte injury induced by IL-1β and its possible mechanism. Chondrocytes were isolated from rat knee joints and cultured. Chondrocytes were treated with IL-1β, and chondrocytes were treated with different concentrations of GSTT. ELISA was used to detect the levels of IL-6, TNF-α and IFN-γ. Flow cytometry was used to detect the apoptotic rate. The expressions of Bax, cleaved-caspase3 and Bcl-2 were detected by Western blot. qRT-PCR was used to detect the effect of GSTT on miR-99a expression level. The above methods were used to detect the effects of miR-99a overexpression on IL-1β-induced chondrocyte inflammatory factors and apoptosis rate. Anti-miR-NC and anti-miR-99a were transfected into chondrocytes, respectively, and treated with a culture solution containing GSTT and IL-1β for 24 h. Inflammatory factor levels and apoptotic rates were detected. After IL-1β treatment, the levels of inflammatory factors IL-6, TNF-α, and IFN-γ were significantly increased (P <0.05), and the apoptosis rate was significantly increased (P <0.05), the levels of Bax, cleaved-caspase3 protein were significantly increased (P <0.05), the protein level of Bcl-2 was significantly reduced (P <0.05), and the expression level of miR-99a was significantly reduced (P <0.05). After GSTT treatment, the levels of inflammatory factors IL-6, TNF-α, and IFN-γ were significantly reduced (P <0.05), the apoptosis rate was significantly reduced (P <0.05), and the protein levels of Bax and cleaved-caspase3 were significantly reduced (P <0.05), the protein level of Bcl-2 was significantly increased (P <0.05), the expression level of miR-99a was significantly increased (P <0.05), and there were statistically significant differences between different dose groups of GSTT (P <0.05) . After miR-99a overexpression, the levels of inflammatory factors IL-6, TNF-α, IFN-γ were significantly reduced (P <0.05), the apoptosis rate was significantly reduced (P <0.05), and protein levels of Bax and cleaved-caspase3 were significantly decreased (P <0.05), and the protein level of Bcl-2 was increased significantly (P <0.05). Inhibition of miR-99a expression could attenuate the effect of GSTT on IL-1β-induced chondrocyte injury. To conclude that GSTT could inhibit IL-1β-induced chondrocyte inflammation and apoptosis by up-regulating the expression of miR-99a.

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Key words
Gross saponins of *Tribulus terrestris*, miR-99a, Chondrocytes, Inflammatory factors, Apoptosis, IL-6, TNF-α, Bcl-2, Bax

INTRODUCTION

Osteoarthritis is a common chronic disease in clinic, and its main pathological features include significantly increased apoptosis rate of articular chondrocytes and significantly increased inflammatory response in cells (Gu *et al*., 2015; Deng *et al*., 2019). Therefore, inhibitubg chondrocyte apoptosis and inflammatory response may be an effective way to treat osteoarthritis. Gross saponins of *Tribulus terrestris* (GSTT) is the main active ingredient of Chinese medicinal herb, *Cenchrus echinatus*. Study has shown that GSTT can effectively treat cardiovascular disease, reduce endothelial cell injury and regulate endothelial cell function (Zhao *et al*., 2018; Cai *et al*., 2018). However, the mechanism of GSTT on chondrocyte injury has not been elucidated. Studies have shown that micro RNA-99a (microRNA-99a, miR-99a) is down-regulated in the cell model of Parkinson's disease, and overexpression of mir-99a can protect nerve cells from oxidative damage (Zhang and Liu, 2018). Related reports have suggested that miR-99a has a protective effect on endothelial cells damaged by lipopolysaccharides and can serve as a potential target for anti-inflammatory treatment of atherosclerosis (Bao *et al*., 2015). However, whether GSTT can affect chondrocyte injury by regulating the expression of miR-99a is unknown. Therefore, this study preliminarily explored the effect of GSTT on chondrocyte injury and its potential mechanism by focusing on the effect of interleukin-1β (IL-1β) on chondrocytes, observing the effects of different concentrations of GSTT on chondrocyte apoptosis and inflammatory response,

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analyzing its regulatory effect on miR-99a.

MATERIALS AND METHODS

Material and reagents

Twenty male SD rats of clean grade (Animal Qualification Certificate No.: SCXK (Shanghai): 2007-0008), with a body weight of 90-100g, were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. GSTT (Prododuct Code: 22153-44-2) was purchased from Chengdu Herbpurify Co., Ltd. DMEM, fetal bovine serum, type II collagenase were purchased from Gibco, USA. Trypsin and phosphate buffered saline (PBS) were purchased from Hyclone, USA. IL-1βwas purchased from Sigma, USA. Interleukin-6 (IL-6), Tumor Necrosis Factor-α (TNF-α), interferon γ (IFN-γ) enzyme linked immunosorbent assay (ELISA) were purchased from Wuhan BOSTER Biological Technology Co., Ltd. The apoptosis detection kit and Trizol reagent were purchased from Beijing Trans Gen Biotech Co., Ltd. Reverse transcription kit and real-time fluorescent quantitative PCR kit were purchased from TaKaRa, Japan. Lipofectamine 2000 was purchased from Invitrogen, USA. miR-99a oligonucleotide mimics (miR-99a mimics) and negative control mimic NC sequence (miR-NC), miR-99a specific oligonucleotide inhibitor (anti-miR-99a) and negative control (anti-miR-NC) were purchased from Guangzhou RiboBio Co., Ltd. RIPA lysate and bicinchoninicacid (BCA) protein quantitative assay kit was purchased from Shanghai Beyotime Biotechnology Co., Ltd. Rabbit anti-mouse Bcl-2 associated X protein (Bax), activated aspartic proteolytic enzyme 3 (cleaved caspase3) containing cysteine, and B-cell lymphoma-2 (Bcl-2) antibodies were purchased from CST, USA. Horseradish peroxidase (HRP -labeled goat anti-rabbit IgG secondary antibody was purchased from Santa Cruz, USA.

Isolation and culture of chondrocytes

The rats were sacrificed by necking method and soaked in 75% alcohol for 5 min. Then, the knee joints of the rats were cut, placed in a sterile plate and washed with PBS; the attached tissue around the joint was scraped and washed with PBS; cartilage was removed from the joint surface using the blade cutting and washed with PBS, cut into pieces (the volume of each piece was about 1 mm3), placed in glass bottles containing 0.2% type II collagenase (5 mL), digested for 2 h at 37°C water bath. Subsequently, the samples were filtered via a stainless steel mesh sieve (120 mesh), subjected to centrifugal treatment for 5 min at 4 °C by 1000 r/min, before discarding the supernatant and collecting the cell precipitate. Repeat the above steps 3 times, add DMEM medium to resuspend the cells, blow gently and mix well, inoculate in the culture bottle, and place in a 37 °C incubator. The culture medium was replaced every 2 days until monolayer cells appeared, and the third-generation chondrocytes were selected for subsequent experiments (Ye *et al*., 2015).

Drug treatment and experimental grouping

The logarithmic chondrocytes were digested by adding 0.25% trypsin, and then culture medium was added to stop digestion, resulting in the formation of the cell suspension. After that, the cell density was adjusted to 5×10^5 cells / mL, the cells were inoculated on 96-well plates (100 nm L/ well) and Il-1 containing 10 ng/mL was added for 24 h to induce chondrocyte apoptosis model (Lin *et al*., 2019), denoted as the IL-1β group. The cells were cultured in a medium containing IL-1βwith final concentrations of 10 mg/L, 30 mg/L, 100 mg/L GSTT and 10 ng/mL for 24 h (Zhang *et al*., 2010), and then denoted as IL-1β+GSTT-L group, IL-1β+GSTT-M group and IL-1β+GSTT-H group, respectively. According to the instructions of Lipofectamine2000 transfection reagent, miR-NC, miR-99a mimics were transfected to chondrocytes and cultured in medium containing 10 ng/mL IL-1β for 24h, denoted as IL-1β+miR-NC group and IL-1β+miR-99a group, respectively. To explore whether GSTT plays a role by regulating the expression of miR-99a in subsequent experiments, the subjects were divided into the IL-1β+GSTT+anti-miR-NC group (anti-miR-NC was transfected to chondrocytes and cultured with a medium containing 100 mg/L GSTT and 10 ng/mL IL-1β for 24 h) and the IL-1β+GSTT+anti-miR-99a group (anti-miR-99a was transfected to chondrocytes and cultured with a medium containing 100 mg/L GSTT and 10 ng/mL IL-1β for 24 h).

Determination of the concentrations of IL-6, TNF-α, IFN-γ by enzyme-linked immunosorbent assay (ELISA)

The logarithmic chondrocytes were digested with 0.25% trypsin and then the cell density was adjusted to 2×10^5 cells /mL. After that, the cells were inoculated on 6-well plates (100 nmL/ well), grouped according to "1.2.2" and cultured for 24 h after administration. Subsequently, the cells were digested by adding 0.25% trypsin and then centrifuged at 12000 r/min for 10 min at 4 °C. The levels of IL-6, TNF-α, IFN-γ were detected with reference to the Kit instructions, and the experiments were repeated 3 times for each group.

Detection of apoptosis rate by flow cytometry

The logarithmic chondrocytes of each group were collected, washed with precooling PBS, and centrifuged at 1000 r/min for 5 min at 4°C. Then, cell precipitators were collected and placed in a centrifuge tube, added with

Binding Buffer (500 °μL) suspended cells, 5μL of Annexin V-FITC and 5μL of PI, respectively for incubation at room temperature in dark for 20 min. After that, the apoptosis rate was measured by flow cytometry.

Detection of the expression level of miR-99a in the cells using quantitative real-time PCR (qRT-PCR)

The logarithmic chondrocytes of each group were collected, the total RNA in the cells was extracted by Trizol method, and the RNA concentration was determined by uv spectrophotometer. The total RNA was reversely transcribed into cDNA according to instructions for reverse transcription kit. The forward primer 5 '-AGCTCGCTTCTATGGGTCTG-3', reverse primer 5 '-ACGCTAGCTCTTGACATC-GA-3' of miR-99a as well as forward primer 5 '-ATTGGAACGATACAGAGAAGATT-3' and reverse primer 5 '-GGAACGCTTCAC GAATTTG-3' of U6 were synthesized by Sangon Biotech (Shanghai) Co., Ltd. CDNA was used as the template for qRT-PCR reaction, and the reaction system was configured according to the real-time fluorescence quantitative PCR kit. The reaction conditions were pre-denaturation at 95 °C for 2 min, denaturation at 95 °C for 30s, annealing at 58 °C for 30 s, extension at 72 °C for 30s, with a total of 40 cycles. Taking U6 as internal parameter, the $2^{-\Delta\Delta Ct}$ method was to calculate relative expression of miR-99a.

Detection of protein expressions of Bax, cleaved caspase3 and Bcl-2 by Western blot

The logarithmic chondrocytes of each group were collected, added with RIPA lysis cells for 30 min of reaction, and then subjected to centrifugal treatment at 12000 r/min at 4°C for 10 min before absorbing the supernatant (total protein). BCA method was used to detect the protein concentration, and the operation was conducted in strict accordance with the kit instructions. 5×SDS loading buffer was added to the protein sample before boiling for 10 min to denature the protein. 40 μg of the protein sample was taken for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After the reaction, the separated protein gel was transferred to the PVDF membrane, sealed at room temperature for 2h, then with addition of the primary antibody diluent (1:1000) and incubated at 4 °C for 24 h. Subsequently, the membrane was washed with TBST, then the secondary antibody diluent (1:2000) was added, ECL was added dropwise for exposure and development in the darkroom. Quantity one software was applied to detect the gray value of the band, and the relative protein expression amount = the gray value of the target protein band/the gray value of the internal reference band.

Statistical analysis

SPSS21.0 statistical software was used to analyze the data, and the measurement data were in normal distribution. T-test was used for comparison between two groups, and one-way ANOVA was used for comparison between multiple groups. All data were expressed in the form of $\bar{x}\pm s$, and the difference was considered statistically significant when $P < 0.05$.

RESULTS

Expression of IL-1β-induced chondrogenic inflammatory cytokines

Compared with the Control group, the levels of IL-6, TNF-α, IFN-γ in the IL-1βgroup were significantly increased ($P < 0.05$). Compared with the IL-1β group, levels of chondrocyte inflammatory cytokines IL-6, TNF-α, IFN-γ were significantly decreased in the IL-1β+GSTT-L group, IL-1β+GSTT-M group, and IL-1β+GSTT-H group ($P < 0.05$). The differences in IL-6, TNF-α, IFN-γ levels among the IL-1β+GSTT-L group, IL-1β+GSTT-M group, and IL-1β+GSTT-H group were statistically significant ($P < 0.05$), as shown in Table I.

Table I. Effect of gross saponins of *Tribulus terrestris* on expression of IL-1β-induced chondrogenic inflammatory cytokines ($\bar{x}\pm s$, n=9).

Group	IL-6(ng/L)	TNF-α(ng/L)	IFN-γ(ng/L)
Con	3.87±0.41	5.33±0.53	9.36±0.91
IL-1β	8.22±0.79[a]	31.26±3.14[a]	47.21±4.27[a]
IL-1β+GSTT-L	7.06±0.62[b]	22.36±2.27[b]	35.28±3.52[b]
IL-1β+GSTT-M	5.91±0.47[bc]	14.25±1.48[bc]	23.93±2.33[bc]
IL-1β+GSTT-H	4.74±0.48[bcd]	9.33±0.94[bcd]	16.47±1.65[bcd]
F	84.149	265.480	257.642
P	0.000	0.000	0.000

Note: Compared with Control group, [a]$P<0.05$; Compared with IL-1β group, [b]$P<0.05$; Compared with IL-1β+GSTT-L group, [c]$P<0.05$; Compared with IL-1β+GSTT-M group, [c]$P<0.05$.

IL-1β chondrocyte apoptosis

Compared with Control group,the apoptosis rate of chondrocytes in IL-1βgroup was significantly increased ($P<0.05$), the levels of Bax protein, cleaved-caspase3 protein increased significantly ($P<0.05$),the level of Bcl-2 protein decreased significantly ($P<0.05$); compared with IL-1β group,the chondrocyte apoptosis rate decreased significantly in IL-1β+GSTT-L group, IL-1β+GSTT-M group, and IL-1β+GSTT-H group ($P<0.05$), the levels of Bax protein, cleaved-caspase3 protein decreased significantly ($P<0.05$), the level of Bcl-2 protein increased significantly ($P<0.05$); compared with IL-1β+GSTT-L group, the

chondrocyte apoptosis rate decreased significantly in IL-1β+GSTT-M group and IL-1β+GSTT-H group (P<0.05), the levels of Bax protein and cleaved-caspase3 protein decreased significantly (P<0.05), the level of Bcl-2 protein increased significantly (P<0.05); compared with IL-1β+GSTT-M group, the chondrocyte apoptosis rate in IL-1β+GSTT-H group decreased significantly (P<0.05), the levels of Bax protein and cleaved-caspase3 protein decreased significantly (P<0.05), the level of Bcl-2 protein increased significantly (P<0.05), as shown in Figure 1 and Table II.

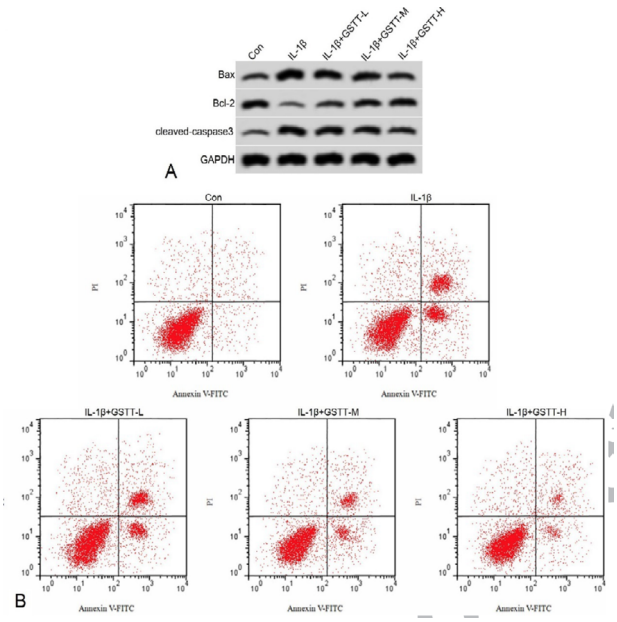

Fig. 1. Effect of gross saponins of *Tribulus terrestris* on IL-1β chondrocyte apoptosis. A, Expression of apoptosis-associated proteins; B, Apoptotic flow pattern.

miR-99a expression in IL-1β-induced chondrocytes

Compared with Control group, the expression level of miR-99a in chondrocytes was significantly decreased in the il-1β group (P<0.05); compared with IL-1β, the expression level of miR-99a in chondrocytes was significantly increased in IL-1β+GSTT-L group, IL-1β+GSTT-M group, IL-1β+GSTT-H group (P<0.05); compared with IL-1β+GSTT-L group, the expression level of miR-99a in chondrocytes was significantly increased in IL-1β+GSTT-M group, IL-1β+GSTT-H group (P<0.05); compared with IL-1β+GSTT-M group, the expression level of miR-99a in chondrocytes was significantly increased in IL-1β+GSTT-H group (P<0.05). Therefore, GSTT 100 mg/L was selected for subsequent studies, as shown in Table III.

Effect of miR-99a overexpression

Expression of IL-1β-induced chondrogenic inflammatory cytokines

Compared with IL-1β+miR-NC group, the levels of IL-6, TNF-α, IFN-γ were decreased significantly in IL-1β+miR-99a group (P<0.05), as shown in Table IV.

Il-1β-induced chondrocyte apoptosis

Compared with IL-1β+miR-NC group, the rate of chondrocyte apoptosis significantly decreased in IL-1β+miR-99a group (P<0.05), the levels of Bax protein and cleaved-caspase3 protein decreased significantly (P<0.05), the level of Bcl-2 protein increased significantly (P<0.05), as shwon in Figure 2 and Table V.

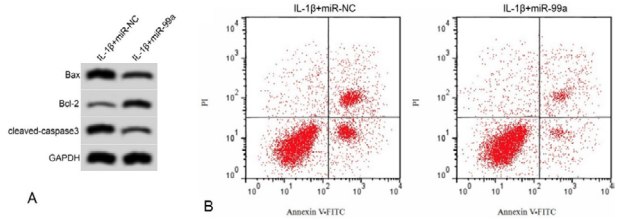

Fig. 2. Effect of miR-99a overexpression on il-1β-induced chondrocyte apoptosis. A, Expression of apoptosis-associated proteins; B, Apoptotic flow pattern.

Table II. Effect of gross saponins of *Tribulus terrestris* on IL-1β chondrocyte apoptosis ($\bar{x}$±s, n=9).

Group	Apoptosis rate (%)	Bax protein	Bcl-2 protein	Cleaved caspase 3 protein
Con	6.89±0.64	0.32±0.03	0.63±0.06	0.23±0.03
IL-1β	26.33±2.51[a]	0.79±0.07[a]	0.21±0.02[a]	0.68±0.06[a]
IL-1β+GSTT-L	20.36±1.84[b]	0.66±0.06[b]	0.32±0.03[b]	0.57±0.05[b]
IL-1β+GSTT-M	15.33±1.48[bc]	0.54±0.04[bc]	0.45±0.04[bc]	0.45±0.04[bc]
IL-1β+GSTT-H	9.41±0.91[bcd]	0.39±0.03[bcd]	0.57±0.05[bcd]	0.33±0.03[bcd]
F	216.204	139.727	150.400	154.042
P	0.000	0.000	0.000	0.000

Note: Compared with Control group, [a]*P*<0.05; compared with IL-1β group, [b]*P*<0.05; compared with IL-1β+GSTT-L group, [c]*P*<0.05; compared with IL-1β+GSTT-M group, [c]*P*<0.05.

Table III. Effect of gross saponins of *Tribulus terrestris* on miR-99a expression in IL-1β-induced chondrocytes ($\bar{x}$±s, n=9).

Group	MiR-99a
Con	1.00±0.06
IL-1β	0.37±0.04[a]
IL-1β+GSTT-L	0.49±0.05[b]
IL-1β+GSTT-M	0.63±0.06[bc]
IL-1β+GSTT-H	0.77±0.07[bcd]
F	167.556
P	0.000

Note: compared with Con group, [a]*P*<0.05; compared with IL-1β group, [b]*P*<0.05; compared with IL-1β+GSTT-L group, [c]*P*<0.05; compared with IL-1β+GSTT-M group, [c]*P*<0.05.

Table IV. Effect of miR-99 a overexpression on expression of IL-1β-induced chondrogenic inflammatory cytokines ($\bar{x}$±s, n=9).

Group	MiR-99a	IL-6(ng/L)	TNF-α(ng/L)	IFN-γ(ng/L)
IL-1β+miR-NC	1.00±0.06	8.41±0.79	33.12±3.14	47.13±4.57
IL-1β+miR-99a	2.89±0.27[a]	5.61±0.54[a]	12.32±1.33[a]	19.58±1.96[a]
t	20.500	8.778	18.299	16.621
P	0.000	0.000	0.000	0.000

Note: compared with IL-1β+miR-NC group, [a]*P*<0.05.

IL-1β-induced chondrocyte damage was reversed by inhibition of miR-99a

Compared with IL-1β+GSTT+anti-miR-NC group, the levels of IL-6, TNF-α, IFN-γincreased significantly in IL-1β+GSTT+anti-miR-99a group (P<0.05), and apoptosis rate increased significantly as well (P<0.05), the levels of Bax protein and cleaved-caspase3 protein increased significantly (P<0.05), the level of Bcl-2 protein decreased significantly (P<0.05), as shown in Figure 3 and Table VI.

DISCUSSION

Il-1β can promote chondrocyte apoptosis and participate in the development of osteoarthritis by increasing the level of matrix degrading enzyme and through mitochondrial pathway. Non-steroidal anti-inflammatory drugs are used for clinical treatment, but patients are prone to gastrointestinal bleeding and other complications. Previous studies have shown that some herbal medicines have anti-inflammatory, anti-oxidative stress and other effects, and can also inhibit chondrocyte apoptosis (Xia *et al*., 2019; Dai *et al*., 2015; Zhang *et al*., 2018). However, the mechanism by which Chinese herbal medicine works has not been clarified. Therefore, this study actively explores new Chinese herbal medicine and analyzes its molecular mechanism of chondrocyte damage, providing a new direction for the treatment of osteoarthritis.

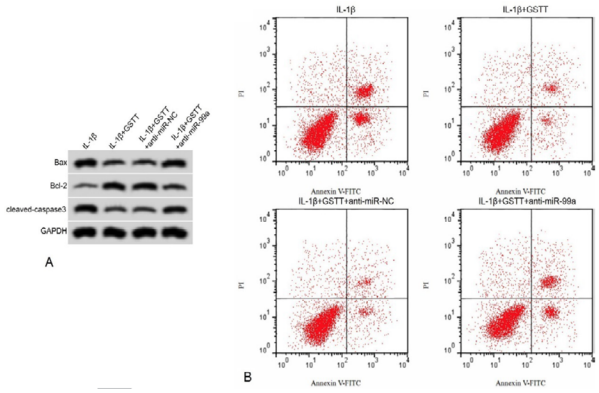

Fig. 3. The effect of gross saponins of *Tribulus terrestris* (100 mg/L) on IL-1β-induced chondrocyte damage was reversed by inhibition of miR-99a. A, Expression of apoptosis-associated proteins; B, Apoptotic flow pattern.

GSTT plays a role in protecting endothelial cells by inhibiting oxidative stress and inflammatory response of endothelial cells (Fan *et al*., 2019). Studies have shown that GSTT may inhibit inflammatory responses by inhibiting nuclear factor-κB (NF-κB) inflammatory signaling pathways, thereby reducing cerebral ischemia/reperfusion injury and ultimately playing a neuroprotective role (Zhai *et al*., 2015; Wang *et al*., 2019). In this study, after treating chondrocytes with il-1β, and the results showed that the levels of inflammatory cytokines IL-6, TNF-α, IFN-γ significantly increased, while the levels of IL-6, TNF-α, IFN-γ significantly decreased after treatment with different concentrations of GSTT, which is consistent with the results reported in relevant literatures (Dou *et al*., 2018). These results suggest that GSTT can inhibit the release of inflammatory cytokines and reduce il-1β-induced chondrocyte inflammatory damage in a dose-dependent manner. Chondrocyte apoptosis is closely related to the occurrence and development of osteoarthritis. Studies have shown that mitochondrial pathway is the main pathway of apoptosis. Bcl-2 belongs to the anti-apoptotic protein, of which the increased expression can inhibit cell apoptosis; while Bax belongs to the pro-apoptotic protein, of which the increased expression can promote the release of cytochrome C, activate cascade reaction of caspase, makes caspase-3 forms into cleaved-caspase3,

Table V. Effect of miR-99a overexpression on il-1β-induced chondrocyte apoptosis ($\bar{x}$±s, n=9).

Group	Apoptosis rate (%)	Bax protein	Bcl-2 protein	Cleaved-caspase3 protein
IL-1β+miR-NC	26.54±2.36	0.78±0.08	0.22±0.03	0.69±0.07
IL-1β+miR-99a	12.48±1.24[a]	0.42±0.04[a]	0.54±0.05[a]	0.38±0.03[a]
t	15.822	12.075	16.464	12.211
P	0.000	0.000	0.000	0.000

Note: compared with IL-1β+miR-NC group, [a]P<0.05.

Table VI. The effect of gross saponins of *Tribulus terrestris* (100 mg/L) on IL-1β-induced chondrocyte damage was reversed by inhibition of miR-99a ($\bar{x}$±s, n=9).

Group	miR-99a	IL-6 (ng/L)	TNF-α (ng/L)	IFN-γ (ng/L)	apoptosis rate (%)	Bax protein	Bcl-2 protein	cleaved-caspase 3 protein
IL-1β	1.00±0.06	8.19±0.81	32.11±3.15	49.65±4.28	27.11±2.71	0.78±0.07	0.22±0.03	0.67±0.06
IL-1β+GSTT	2.74±0.27[a]	4.62±0.41[a]	9.23±0.92[a]	15.39±1.52[a]	11.36±1.14[a]	0.37±0.03[a]	0.58±0.05[a]	0.32±0.03[a]
IL-1β+GSTT+anti-miR-NC	2.79±0.28	4.59±0.45	9.14±0.91	15.11±1.49	10.52±1.05	0.35±0.03	0.61±0.06	0.31±0.03
IL-1β+GSTT+anti-miR-99a	1.41±0.14[b]	7.06±0.71[b]	23.45±2.46[b]	38.46±3.84[b]	22.41±2.23[b]	0.69±0.06[b]	0.33±0.03[b]	0.58±0.05[b]
F	173.219	76.504	260.605	284.842	165.002	168.786	165.418	152.203
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: compared with IL-1β group, [a]P<0.05; compared with IL-1β+GSTT+anti-miR-NC group, [b]P<0.05.

thus inducing cell apoptosis (Shen *et al*., 2018). The results of this study showed that the apoptosis rate of chondrocytes was significantly increased after il-1IL-1β treatment, the expressions of Bax protein and cleaved-caspase3 protein were up-regulated, and the expression of Bcl-2 was down-regulated; however, the apoptosis rate of chondrocytes was significantly decreased after GSTT treatment, the expression levels of Bax protein and cleaved-caspase3 protein were down-regulated, and the expression of Bcl-2 was up-regulated. This suggests that GSTT may inhibit il-1β-induced chondrocyte apoptosis by regulating the expression of apoptosis-related proteins.

Overexpression of miR-99a can inhibit adipose tissue inflammation (Jaiswal *et al*., 2019). Studies have shown that miR-99a overexpression can inhibit endothelial inflammatory response by inhibiting the NF-κB signaling pathway (Bao *et al*., 2016). Related reports have suggested that miR-99a overexpression can reduce oxidative damage of nerve cells induced by lipopolysaccharide (Jing *et al*., 2017). The results of this study showed that the expression level of miR-99a in chondrocytes after il-1β treatment was significantly decreased, while that was significantly increased after GSTT treatment, suggesting that GSTT may play a role by up-regulating the expression of miR-99a. At the same time, the results of this study showed that mir-99a overexpression can significantly inhibit the IL-1β-induced chondrocytes inflammatory response and inhibit the IL-1β-induced chondrocytes apoptosis, suggesting that miR-99a overexpression can reduce IL-1β-induced chondrocytes damage. To explore whether GSTT can play a protective role on chondrocytes by regulating the expression of miR-99a, further analysis of this study showed that after inhibiting the expression of miR-99a, the inhibitory effect of GSTT on IL-1β-induced chondrocyte inflammation and apoptosis was significantly weakened. This suggests that GSTT may protect chondrocytes by up-regulating miR-99a and inhibiting il-1β-induced chondrocyte inflammation and apoptosis.

CONCLUSION

In conclusion, GSTT can inhibit il-1β-induced chondrocyte apoptosis and inhibit the secretion and release of inflammatory cytokines IL-6, TNF-α, IFN-γ. GSTT up-regulates the expression of miR-99a in chondrocytes by targeting Bax protein, cleaved-caspase3 protein and Bcl-2 protein, providing new ideas for the treatment of osteoarthritis. However, it is still necessary to verify whether GSTT has the same effect in animal models.

Statement of conflict of interests

The authors have declared no conflict of interest.

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