Int J Clin Exp Med 2016;9(7):12880-12887
www.ijcem.com /ISSN:1940-5901/IJCEM0018489

Original Article

The protective effect of Hedyotis diffusa on collagen induced arthritis rats

Hongbo Shen[1], Yunjing Bai[2], Zejun Huo[1]

[1]*Department of Traditional Chinese Medicine, Peking University Third Hospital, Beijing, China;* [2]*Department of Rheumatism, PLA Army General Hospital, Beijing, China*

Received October 25, 2015; Accepted March 17, 2016; Epub July 15, 2016; Published July 30, 2016

Abstract: Objective: To investigate the protective effect of Hedyotis diffusa (HD) on collagen induced arthritis (CIA) rats. Methods: 40 male Wistar rats were randomly divided into four groups: normal group (blank control, n = 10), CIA model group, HD low dose group and HD high dose group. After CIA rats were prepared successfully, HD extract was administrated orally with the dose of 6 g/(kg. d) and 12 g/(kg. d) to low and high dose group at day 14; normal group was administrated with the same volume of saline. The arthritis index was evaluated weekly after treatment. Rats were sacrificed at day 42, their serum IL-1β, TNF-α levels and nitric oxide (NO) concentration in synovial tissues were measured and compared. The annexin I expression in each groups were measured. The active status of PI3K-AKT signal pathway was examined by western blot. Result: The arthritis index of HD low dose group was significantly lower than model group at day 35 and day 42 (P<0.05). The arthritis index of HD high dose group was significantly lower than model group at day 28, day 35 and day 42 (P<0.05). Histological analysis of joints showed that synovitis and osteoclastic bone resorption were attenuated by daily oral administration of HD. The expression of IL-1β and TNF-α in HD treatment groups were significantly lower than model group (P<0.05), as well as NO concentration in cartilage tissue. We observed significantly higher expression of annexin-I in HD treatment groups than model group (P<0.05). Conclusion: HD could reduce the expression of inflammatory cytokines IL-lβ and TNF-α and alleviate the inflammatory reaction and bone erosion in CIA rats.

Keywords: *Hedyotis diffusa* (HD), collagen induced arthritis (CIA), rat, annexin-I, IL-lβ, TNF-α

Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease with feature of erosive synovitis [1, 2], studies indicated that the pathogenesis of RA is related with genetic, infection, immune, endocrine factors and so on [3-5]. Currently, there is no satisfactory and effective cure for RA, and there are 4 major types of RA therapeutic drugs for clinical use: non-steroidal anti-inflammatory drugs (NSAIDS); drugs which improve the anti-rheumatic disease-modifying (DMARDs); corticosteroids and biological agents (such as TNF) [6, 7].

The occurrence of rheumatoid arthritis (RA) is related with variety of signal transduction pathways [8, 9]. Recent studies have found that phosphate ester phthalate inositol-3 kinase phosphoinositide 3 kinase (PI3K) and protein kinase B protein kinase B (PI3K-Akt) signal pathway [10, 11] is involved in a very fast signal transduction system, this pathway participated from the membrane to the nuclear signal transduction system. And its downstream pathways, Bad-caspase and NF-κB are important molecular pathways effecting RA [12, 13]. Hsieh et al found that PI3K/AKT signaling pathway was activated in early osteoarthritis process.

Traditional Chinese medicine has a long history of treating RA, folk prescription such as Yiqi Jiedu Huayu Decoction and BiZhongxiao decoction etc have effectively relieved the pain of RA patients, and Hedyotis diffusa (HD) was commonly used in these folk prescription. Modern experimental studies indicated that the single herb prescription of HD has related anti-inflammatory, antioxidant and immune regulation effect in treating RA. To further explore the therapeutic mechanism of HD in RA, collagen induced arthritis model in Wistar rat was adopt-

ed in this study, HD was administrated orally to collagen induced arthritis (CIA) rats to observe the anti-rheumatic effect of HD in PI3K/AKT pathway.

Materials and methods

Animals

The 40 male Wistar rats, 8 week old, with body weight of 200 ± 10 g, were purchased from the experimental animal research center of Chinese Academy of Medical Sciences. All the experiment protocol follows "Experimental Animal Management Regulations" of China.

After adaptive feeding for 2 weeks, the 40 rats were randomly divided into 4 groups according to related treatment: normal group received distilled water (N = 10); CIA model group received distilled water after induction; HD low dose group and HD high dose group were administrated orally with the HD dose of 6 g/(kg. d) and 12 g/(kg. d) after induction.

Collagen induced arthritis rats' model

The CIA rat model was established according to the instructions of Chondrex, Inc. (http://www.chondrex.com/protocol/Rat%20CIA.pdf). Briefly, 10 mg of bovine type II collagen (Chondrex, inc., Redmond, WA, USA) was dissolved in 5 ml of acetic acid solution (0.05 mol/L) with the concentration of 2 mg/ml. The prepared bovine type II collagen was diluted by complete Freund's adjuvant (Sigma-Aldrich, St. Louis, MO, USA) to the concentration of 1 mg/ml. A total of 0.2 mL bovine type II collagen was injected subcutaneously to rat tail root; The same concentration bovine type II collagen (1 mg/ml) was diluted by incomplete Freund's adjuvant (Sigma-Aldrich, St. Louis, MO, USA), and was injected again 7 days after first immunization. The CIA model was evaluated 2 weeks after first immunization, unsuccessful model animal was removed. The same dose of saline was injected in normal group.

Administration of medication

According to equivalent dose ratio of converted body surface area, Hedyotis diffusa tablet (approved serial number: Z36020632, Chinese herbal medicine company, Tianjin, China) administrated dose to a 70 kg adult was 30 g per day, the converted dose was 6 g/(kg. d) for rats. Finally, we adopted 6 g/(kg. d) and 12 g/(kg. d) as low and high HD dosage respectively.

14 days after first immunization, gastric administration was performed at 9:00 am in these rats with the medication or saline volume of 1 ml per day. The administration was continued to day 42.

Arthritis index observation

General observation including the mental state, hair, appetite activity, and the growth of body mass was observed and recorded every.

The arthritis index (AI) was evaluated 14 days after first immunization; AI was evaluated as 5 grades: 0, no arthritis phenomenon; 1, red swelling occurred in the little toe joints; 2, swelling occurred in joint of toe or foot plantar; 3, swelling occurred in lower part of ankle joint; 4, swelling occurred at ankle joint or over part of ankle joint. The AI index was the final sum score of all joints.

Histological analysis

After completing the experiment, mice were sacrificed and paws were fixed in 10% neutral buffered formalin and decalcified with 10% EDTA, pH 7.4. The paws were then embedded in paraffin and cut into 5-μm thick sections. The deparaffinized sections were stained with hematoxylin and eosin and observed under light microscope (ZEISS Co., Germany).

Serum IL-1β and TNFα levels

After administrated for 42 days, rats were fasting for 12 hours and were sacrificed by decollation. Blood was draw and the supernatant was removed by centrifugation The IL-1β and TNF-α level were detected by ELISA kit (Huamei Bio Engine Co., Ltd., Wuhan, China).

Expression of annexin I

Synovial tissue was taken from sacrificed rats, western blot method was used to detect the expression of annexin I. Briefly: the synovial membrane protein was extract by a total protein extraction kit (BsP003, Bio Engine Co., Ltd., Shanghai, China), the sample volume of 50 μg was loaded after measured the concentration, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed

at concentration of 10%, the transferred to Polyvinylidene Difluoride (PVDF) membrane. Incubated with Rabbit anti-annexin-1 polyclonal antibody (ZYMED Laboratories Inc. (Cambridge, U.K.)) at 4°C overnight (I:500), then incubated with AlexaFluor 647 goat anti-rabbit IgG (1:5000, Abcam plc, Cambridge, MA, USA) for 1 h at room temperature. An infrared imaging system for imaging (Odyssey® CLx, LI-COR Biosciences, Lincoln, NE, USA) was used for semi quantitative analysis. Annexin I expression was calculated as gray value in each group.

Pathological examination

After been sacrificed, bilateral knee joints of the rats were selected as the specimens, including the entire posterior jaw with the ankle joint. Specimens were fixed with 10% poly formaldehyde solution and prepared as paraffin sections, then stained by hematoxylin and eosin (HE) staining. The pathological changes were observed under light microscope.

Nitric oxide colorimetric assay

NO is an important biological molecule in the pathological and physiological state, NO can induce the apoptosis of chondrocytes, and is closely related to the pathological process of rheumatoid arthritis [14]. Cartilage tissue was washed repeated in Hanks solution, then was digested by type I collagenase (0.25%), cut and digested at 37°C for an hour.

NO was assessed indirectly by measuring the levels of oxidized forms (nitrites and nitrates) in samples using the Nitric Oxide Colorimetric Assay Kit (ab65328; Abcam, Cambridge, MA, USA). A standard curve was generated to measure levels between 1 and 100 μM of nitrite per well. Within 2 weeks of freezing, tissues were homogenized at 1:10 (w/v) using ice-cold homogenization buffer prepared in 20 mM Tris/HCl, containing 1 mM EDTA, 5 mM EGTA, 0.1 mM PMSF, 10 mM benzamidine and protease inhibitors (Complete® Protease Inhibitor Cocktail tablets, Roche Apply Science, Indianapolis, IN) (n = 6-8 rats/group). A 5-μl aliquot of homogenized tissue was used to determine total protein concentration using Bradford assay. A 200-μL aliquot of homogenized tissue was centrifuged at 10,000 g at 4°C for 2 min and the clarified supernatant recovered. The clarified sample was deproteinated to improve NO stability by adding ice-cold 5% metaphosphoric acid in a 1:1 ratio (v:v) (Sigma-Aldrich), mixing and spinning at 10,000 g for 5 min. Clarified deproteinated samples (supernatants) and standards were exposed to nitrate reductase and cofactors for 1 h at room temperature to transform nitrate to nitrite. Following application of the enhancer supplied by manufacturer, Griess reaction reagents were applied to convert nitrite to a purple azo chromophore compound and developed over 10 min providing a lower limit of detection of 1 μM at 540 nm using a linear model in a microplate reader.

Western blot analysis of PI3K, AKT and p-AKT expression

Cartilage tissue was homogenized fully, after lysis, protein was extracted by total protein extraction kit (BsP003, Bio Engine Co., Ltd., Shanghai, China); protein concentration was determined by BCA methods; denatured protein was separated in 10% SDS-PAGE, then protein was transferred to PVDF membrane, block with 5% skim milk for 1 hour. Anti-PI 3 Kinase p110 delta antibody (EPR386, abcam plc, Cambridge, MA, USA), Rabbit anti-AKT-polyclonal antibody and Rabbit anti p-AKT polyclonal antibody (Cell Signaling Technology, Inc., Beverly, MA, USA) were added and cultured at 4°C overnight, after been washed by TBS buffer 3 times, the horseradish peroxidase labeled secondary antibody was added and cultured at 37°C for 2 hours. After rinsed by TBS, the Chemiluminescence method was adopted; related strip was developed and fixed by x ray. The absorbance values of strips were measured by scanning. Expression of PI3K, AKT and p-AKT was calculated as relatively quantitative to β-actin (Zhongshan Gold Bridge Bio Co., Ltd., Beijing, China).

Statistical analysis

The normal distribution data was expressed as mean ± SD, data was analyzed by SPSS 17.0 software (IBM, Chicago, IL, USA). Multiple average values were compared by single factor analysis of variance. P<0.05 was considered as statistically significant.

Results

After immunization, 24 of 30 rats was assessed having CIA, the 24 rats were randomly divided

Table 1. Comparisons of the AI index (score) in each group

Group (N)	AI index (score)				
	Day 14	Day 21	Day 28	Day 35	Day 42
Normal (10)	5.53±1.09	5.71±0.99	5.84±0.95	5.48±1.05	5.66±1.10
Model (8)	7.59±1.22	10.42±1.16	10.09±1.12	9.52±1.17	7.33±1.23
HD low dose (8)	7.56±1.24	9.72±0.98	8.68±0.89	7.44±1.18*	6.15±1.21*
HD high dose (8)	7.27±1.09	9.13±1.24	7.78±1.15*	6.95±1.28*	5.78±1.04*

*, P<0.05, compared with model group at the same time point.

Table 2. Paw thickness (mm) comparison in HD high dose group with normal and model group

Group	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Normal	5.11±0.19	5.15±0.16	5.19±0.27	5.36±0.24	5.56±0.25	5.81±0.28	6.06±0.23
Model	5.26±0.37	5.61±0.94**	6.80±0.76**	7.56±0.52**	8.48±0.53**	8.71±0.67**	8.99±0.71**
HD high dose	5.24±0.40	5.63±0.94**	6.84±0.68**	7.64±0.54**	8.10±0.91**	7.90±0.81**,##	7.76±0.84**,##

**, P<0.01, compared with normal group at the same time point; ##, P<0.01, compared with model group at the same time point.

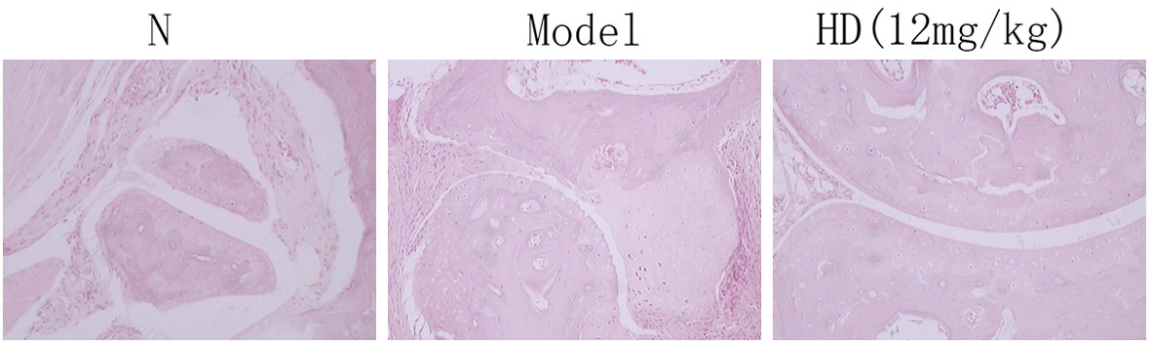

Figure 1. Oral administration of HD extract attenuated severity of arthritis in CIA rats. From left to right was HE staining of ankle joints from each group.

Table 3. Comparisons of serum IL-1β and TNF-α level (pg/ml) in each group

Group (N)	IL-1β	TNF-α
Normal (10)	19.41±0.92	21.81±1.55
Model (8)	60.30±2.46*	75.21±1.79*
HD low dose (8)	38.53±3.36*	42.97±1.64*
HD high dose (8)	29.74±1.03*,#	33.15±1.19*,#

*, *P*<0.05, compared with normal group; #, *P*<0.05, compared with model group.

into the model group, HD low dose group and HD high dose group, with 8 in each group.

AI index

As we can see from **Table 1**, the AI index (score) in HD low dose was significantly lower than those in model group at day 35 and day 42 (*P*<0.05); the AI index in HD high dose group were significantly lower than those in model group at day 28, day 35 and day 42 (*P*<0.05). In addition, the paw thickness in HD high dose group was decrease significantly when compared with model group in same time point (**Table 2**). Histopathologic examination of some of the arthritic joints of rats from the model group revealed extensive cartilage and bone erosions with massive infiltration of mononuclear cells and fibroblasts. In comparison with model group, histological analysis of joints showed that synovitis and osteoclastic bone resorption were attenuated by daily oral administration of HD (**Figure 1**).

Serum IL-1β and TNF-α levels

The serum IL-1β and TNF-α levels at day 42 was listed in **Table 3**, serum IL-1β and TNF-α levels in model group and HD treated groups were

Table 4. Comparisons of annexin I expression (gray value) in synovial tissue of each group

Group (N)	Gray value
Normal (10)	1.02±0.18
Model (8)	1.44±0.29*
HD low dose (8)	0.95±0.26#
HD high dose (8)	0.77±0.13#

*, *P*<0.05, compared with normal group; #, *P*<0.05, compared with model group.

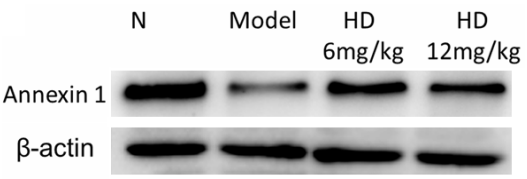

Figure 2. Western blot of Annexin I among four groups.

Table 5. NO level comparisons in cartilage tissue of each groups

Group (N)	NO (μmoll/L)
Normal (10)	14.68±2.19*
Model (8)	19.03±2.56*
HD low dose (8)	17.88±2.26
HD high dose (8)	15.19±2.13*

*, *P*<0.05, compared with model group.

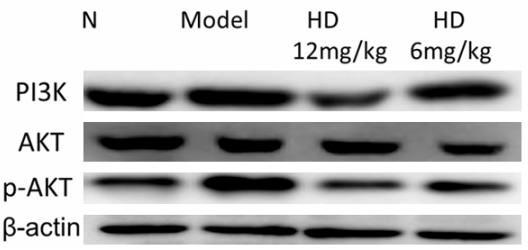

Figure 3. Western blot of PI3K, AKT and p-AKT among four groups.

both significantly higher than normal group (*P*<0.05); only in HD high dose group, the serum IL-1β and TNF-α levels were significantly lower than model group (*P*<0.05).

Annexin I expression

Annexin I expression in each group was evaluated as gray value (**Table 4** and **Figure 2**), the gray value in model group was higher than normal group with statistical difference, while HD treatment decrease annexin I expression, the gray value in HD low and high dose group were all lower than model group (*P*<0.05), but have no difference with normal group (*P*>0.05).

NO level detection

Measurement result showed the concentration of NO in the cartilage tissue of CIA rats was significantly higher than that in the normal group; we observed the concentration of NO was decreased in HD treated group, especially in high dose group (P<0.05, compared with model group, **Table 5**).

Pathological changes

14 days after the first immunization, there was no obvious abnormality in the synovial tissues of the rats, we observed inflammatory cell infiltration in model group with the prolongation time of immunization, large pannus formation could be observed at days 28, with discontinuous epithelial cell; at day 42, vascular proliferation could been seen, with the aggravation of arthritic inflammation, angiogenesis increased, blood vessel dilated, lymphocyte infiltration could be seen with a large number. In HD treated groups, a few angiogenesis and inflammatory cell infiltration could be seen, with the extension of treatment time, the neovascularization reduced gradually, inflammation reduced.

Western blot analysis

PI3K-AKT signaling pathway is considered to play a role in the pathogenesis of RA. Results showed the expression of PI3K, AKT and p-AKT in model group were significantly higher than normal group. While HD treatment reduced expression of PI3K, AKT and p-AKT significantly (**Figure 3**), our results suggest that at least in part HD act on RA through this pathway.

Discussion

Hedyotis diffusa is one of main decoction in the many traditional Chinese medicines; HD is commonly used in treating various types of inflammation. Literature research has demonstrated the pharmacological activity, chemical constituents and the quality control of HD [15, 16]. Studies have demonstrated HD has antitumor, anti-inflammatory, antibacterial activity, and HD could regulate immune response [17].

Compounds in HD include flavonoids, anthraquinone quinones, terpenoids, sterols, polysaccharides and phenolic acids etc. Trenth's research demonstrated type II collagen could produce antigen effect and cause tissue damage, the established collagen induced arthritis (CIA) model is very similar to human RA [18]. Currently, this animal model is commonly used in RA research.

Although the pathogenesis of RA remains unclear, researches have showed IL-1β and TNF-α play critical contributor in RA initiation and progression [19]; as two of important biomarker, levels of IL-1β and TNF-α were often been tested to monitor the therapeutic effect. Previous researches indicated that the mechanism of HD treating RA mainly through regulating immune, lowering inflammatory factors levels, and inhibiting angiogenesis. Zhang *et al* [20] found HD could inhibited the proliferation of synovial cells *in vitro* in RA patients. Wang *et al* [21] indicated that HD could inhibit the tumor necrosis factor (TNF α) and interleukin 1β (IL-1β), thus decrease the inflammation symptoms caused by collagen induction. Gu *et al* [22] demonstrated that HD could decrease redundant reactive oxygen species (ROS) in cells after oxidative damage, and reduce the oxidative damage in tumor bearing mice.

In our research, the diet and activity of CIA rats were increased after been treated by HD at day 28, their hair was shiny and the arthritis index of lowered when compared with that of the model group. Significantly decreased paw thickness and serum IL-1β, TNF-α revealed HD could reduce the swelling of ankle joint and inflammatory cytokines in CIA rats. The related mechanism might involve in down-regulate IL 1β and TNF-α level, inhibit the adhesion molecules, inhibit vascular endothelial growth factor and other cytokines, promote collagen synthesis, reduce the expression and activity of matrix metalloproteinases, and to alleviate joint inflammation, bone erosion and reduce the role of pannus formation [23-26]. Consistent with Wang' research and others [21]. Our study also found that the expression of membrane linked I protein in HD treated groups are significantly higher than that in model group, HD could upregulate the membrane annexin I expression. The potential reason might be that as a regulator of anti-inflammatory reaction, HD treatment increases the generation of annexin I. Through directly occupying the surface receptors of neutrophils, inhibiting the secretion of interleukin and plasma tumor necrosis factor, and annexin I inhibit adhesion, exudation and infiltration of inflammatory cell [27-29].

AKT is a serine/threonine protein kinase that plays a key role in the process of cell proliferation and apoptosis. Human AKT gene family includes AKT, AKT2 and AKT3. AKT involved in survival pathway and inhibit apoptosis of cells. AKT2 plays important role in insulin receptor signaling pathway, and mainly act in the regulation of glucose transport [30]. PI3K/AKT is an important pathway of inhibiting the apoptosis of cartilage [31]. PI3K, a membrane protein which can receive the incoming signal of the receptor of tyrosine kinase receptor on the membrane, and PI3K could directly or indirectly activate the downstream AKT [32]. AKT plays it's anti apoptotic effect through the following 3 ways: inhibiting the programmed cell death triggered by Bax~Bcl-2~Bim [33]; Isolation the soluble cytoplasm of Bad-14-3-3 [34]; and plays anti-apoptotic effect through XIAP factor [35].

Studies show when the growth factor binding the receptor in the cell membrane, which makes the phosphorylation of tyrosine residue, receptor tyrosine kinase tyrosine phosphorylation of R tyrosine kinase (RTK). At this time PI3K by P85 subunit SH2 (SRC homology 2) interaction and phosphorylation of tyrosine residues of 3 R P110 subunit active and allosteric regulation activated by PI3K catalyzed R activation of D phosphorylation and PIP3 PIP2 who produced all two intracellular second messenger [14, 15]. DPKB is one of the most important the downstream target kinase PI3K signal transduction pathway in the kinase is a protein product of retroviral AKT8 gene V-AKT encoding the so called AKT. AKT by PIP2 and PIP3 mediated transfer to the cell membrane and exposed T-Loop region Thr308 site DPDKI (3-phosphoinositide-dependent protein kinase > for lipid binding protein kinase, and PIP2 mediated by PIP3 guide and binding to the cell membrane, when the Ser473 site Thr308 site of PKb/AKT was at the end of the phosphorylation of PDKI and C regulatory region by PDK2 phosphorylation, PKb/AKT is activated. To sum up, the AKT in the PI3K/AKT pathway plays a leading role in the core, PI3K only as a medium of signal transmission.

In this paper, the activity of AKT in rat model of knee osteoarthritis was significantly lower than that in normal group, which indicate that PI3K/AKT signaling pathway was inhibited in rat RA model. PI3K/AKT in HD treated groups and normal group were higher than that in model group, this result showed HD could protect the cartilage cell function in RA rats, and could up-regulate the AKT signal transduction pathway.

In summary, HD can reduce the levels of inflammatory cytokines IL1β and TNF-α, and alleviate the inflammatory reaction in rats with collagen induced arthritis, and HD has protective effect on collagen induced arthritis in rats, the related mechanism might involve the up-regulate PI3K/AKT signal transduction pathway.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zejun Huo, Department of Traditional Chinese Medicine, Peking University Third Hospital, No. 49, Huayuan North Road, Haidian District, Beijing, China. Tel: +86 18601227122; E-mail: zejunhuo66@163.com

References

[1] Wallin K, Solomon A, Kareholt I, Tuomilehto J, Soininen H and Kivipelto M. Midlife rheumatoid arthritis increases the risk of cognitive impairment two decades later: a population-based study. J Alzheimers Dis 2012; 31: 669-676.

[2] Poh LW, He HG, Lee CS, Cheung PP and Chan WC. An integrative review of experiences of patients with rheumatoid arthritis. Int Nurs Rev 2015; 62: 231-247.

[3] Askling J, Fahrbach K, Nordstrom B, Ross S, Schmid CH and Symmons D. Cancer risk with tumor necrosis factor alpha (TNF) inhibitors: meta-analysis of randomized controlled trials of adalimumab, etanercept, and infliximab using patient level data. Pharmacoepidemiol Drug Saf 2011; 20: 119-130.

[4] Picerno V, Ferro F, Adinolfi A, Valentini E, Tani C and Alunno A. One year in review: the pathogenesis of rheumatoid arthritis. Clin Exp Rheumatol 2015; 33: 551-558.

[5] Dhaouadi T, Sfar I, Abelmoula L, Jendoubi-Ayed S, Aouadi H, Ben Abdellah T, Ayed K, Zouari R and Gorgi Y. Role of immune system, apoptosis and angiogenesis in pathogenesis of rheumatoid arthritis and joint destruction, a systematic review. Tunis Med 2007; 85: 991-998.

[6] Lin CC, Kuo CL, Lee MH, Hsu SC, Huang AC, Tang NY, Lin JP, Yang JS, Lu CC, Chiang JH, Chueh FS and Chung JG. Extract of Hedyotis diffusa Willd influences murine leukemia WEHI-3 cells in vivo as well as promoting T- and B-cell proliferation in leukemic mice. In Vivo 2011; 25: 633-640.

[7] Kim SJ, Kim YG, Kim DS, Jeon YD, Kim MC, Kim HL, Kim SY, Jang HJ, Lee BC, Hong SH and Um JY. Oldenlandia diffusa Ameliorates Dextran Sulphate Sodium-Induced Colitis Through Inhibition of NF-kappaB Activation. Am J Chin Med 2011; 39: 957-969.

[8] Sur Chowdhury C, Giaglis S, Walker UA, Buser A, Hahn S and Hasler P. Enhanced neutrophil extracellular trap generation in rheumatoid arthritis: analysis of underlying signal transduction pathways and potential diagnostic utility. Arthritis Res Ther 2014; 16: R122.

[9] Yang X, Li JS, Yang SQ, Zhang XX, Zhang TS, Zhou HY and Liu XG. [Influence of moxibustion on JAK-STAT signal transduction pathways of synovial cells in rheumatoid arthritis rabbits]. Zhen Ci Yan Jiu 2007; 32: 75-82.

[10] Wu MY, Yang RS, Lin TH, Tang CH, Chiu YC, Liou HC and Fu WM. Enhancement of PLGF production by 15-(S)-HETE via PI3K-Akt, NF-kappaB and COX-2 pathways in rheumatoid arthritis synovial fibroblast. Eur J Pharmacol 2013; 714: 388-396.

[11] Shibuya H, Yoshitomi H, Murata K, Kobayashi S, Furu M, Ishikawa M, Fujii T, Ito H and Matsuda S. TNFalpha, PDGF, and TGFbeta synergistically induce synovial lining hyperplasia via inducible PI3Kdelta. Mod Rheumatol 2015; 25: 72-78.

[12] Kwak HB, Sun HM, Ha H, Lee JH, Kim HN and Lee ZH. AG490, a Jak2-specific inhibitor, induces osteoclast survival by activating the Akt and ERK signaling pathways. Mol Cells 2008; 26: 436-442.

[13] Yang CF, Peng LX, Huang TJ, Yang GD, Chu QQ, Liang YY, Cao X, Xie P, Zheng LS, Huang HB, Cai MD, Huang JL, Liu RY, Zhu ZY, Qian CN and Huang BJ. Cancer stem-like cell characteristics induced by EB virus-encoded LMP1 contribute to radioresistance in nasopharyngeal carcinoma by suppressing the p53-mediated apoptosis pathway. Cancer Lett 2014; 344: 260-271.

[14] Lei J, Hui D, Huang W, Liao Y, Yang L, Liu L, Zhang Q, Qi G, Song W, Zhang Y, Xiang AP and Zhou Q. Heterogeneity of the biological properties and gene expression profiles of murine bone marrow stromal cells. Int J Biochem Cell Biol 2013; 45: 2431-2443.

[15] Wang X, Cheng W, Yao X and Guo X. Qualitative analysis of the chemical constituents in Hedyotis diffusa by HPLC-TOF-MS. Nat Prod Res 2012; 26: 167-172.

[16] Zhang P, Zhang B, Gu J, Hao L, Hu F and Han C. The Study of the Effect of Hedyotis diffusa on the Proliferation and the Apoptosis of the Cervical Tumor in Nude Mouse Model. Cell Biochem Biophys 2015; [Epub ahead of print].
[17] Zhang Y, Xie RF, Xiao QG, Li R, Shen XL and Zhu XG. Hedyotis diffusa Willd extract inhibits the growth of human glioblastoma cells by inducing mitochondrial apoptosis via AKT/ERK pathways. J Ethnopharmacol 2014; 158 Pt A: 404-411.
[18] Dynesius-Trentham RA, Rowland D, LaRoche E and Trentham DE. Rat leukocyte inhibitory factor (LIF): similarities to human LIF and generation by cells from rats with collagen-induced arthritis. Cell Immunol 1981; 63: 362-373.
[19] Wang D, Li Y, Liu Y and Shi G. The role of autoreactive T cell in the pathogenesis of rheumatoid arthritis and implications for T cell targeted vaccine therapy. Minerva Med 2015; 106: 157-167.
[20] Zhang L XG, Bai RX. Effect of Hedyotis diffusae on proliferation of rheumatoid arthritis synovial cell. Jiangxi Journal of Traditional Chinese Medicine 2008; 5: 64-66.
[21] Wang R TY. Experimental study of Hedyotis diffusa in treating rats with collagen induced arthritis. Modern Journal of Integrated Traditional Chinese and Western Medicine 2012; 21: 1277-1278.
[22] Gu SD CK. NF-kappa B, TNF-alpha, interleukin and rheumatoid arthritis. Chengde College Newspaper 2012; 3: 313-315.
[23] Lee A, Qiao Y, Grigoriev G, Chen J, Park-Min KH, Park SH, Ivashkiv LB and Kalliolias GD. Tumor necrosis factor alpha induces sustained signaling and a prolonged and unremitting inflammatory response in rheumatoid arthritis synovial fibroblasts. Arthritis Rheum 2013; 65: 928-938.
[24] Ulas T, Tursun I, Dal MS and Buyukhatipoglu H. Comment on: Oxidative stress in systemic lupus erythematosus and rheumatoid arthritis patients: relationship to disease manifestations and activity. Int J Rheum Dis 2013; 16: 98.
[25] Bao F, Wu P, Xiao N, Qiu F and Zeng QP. Nitric oxide-driven hypoxia initiates synovial angiogenesis, hyperplasia and inflammatory lesions in mice. PLoS One 2012; 7: e34494.
[26] Trenkmann M, Brock M, Gay RE, Michel BA, Gay S and Huber LC. Tumor necrosis factor alpha-induced microRNA-18a activates rheumatoid arthritis synovial fibroblasts through a feedback loop in NF-kappaB signaling. Arthritis Rheum 2013; 65: 916-927.
[27] Tu YH YX. Effect of ferulic acid in Chematis intricata Bunge on the treatment of rheumatoid arthritis. Journal of Yan'an University (Medical Science Edition) 2011; 4: 22-24.
[28] Pragasam SJ, Murunikara V, Sabina EP and Rasool M. Antiperoxidative potential of p-coumaric acid, a common dietary phenol, in adjuvant-induced arthritis in rats. Zhong Xi Yi Jie He Xue Bao 2012; 10: 932-938.
[29] Cai Y LQ, He GX, Yang B, Xie W, Xiong XG. Expression of annexin I protein in the synovial membrane of rats with collagen induced arthritis. Chinese Journal of Clinical Rehabilitation 2006; 5: 63-65.
[30] Martin KA, Merenick BL, Ding M, Fetalvero KM, Rzucidlo EM, Kozul CD, Brown DJ, Chiu HY, Shyu M, Drapeau BL, Wagner RJ and Powell RJ. Rapamycin promotes vascular smooth muscle cell differentiation through insulin receptor substrate-1/phosphatidylinositol 3-kinase/Akt2 feedback signaling. J Biol Chem 2007; 282: 36112-36120.
[31] Wang L, Gai P, Xu R, Zheng Y, Lv S, Li Y and Liu S. Shikonin protects chondrocytes from interleukin-1beta-induced apoptosis by regulating PI3K/Akt signaling pathway. Int J Clin Exp Pathol 2015; 8: 298-308.
[32] Zhao M, Zhou A, Xu L and Zhang X. The role of TLR4-mediated PTEN/PI3K/AKT/NF-kappaB signaling pathway in neuroinflammation in hippocampal neurons. Neuroscience 2014; 269: 93-101.
[33] Gauger KJ and Schneider SS. Tumour supressor secreted frizzled related protein 1 regulates p53-mediated apoptosis. Cell Biol Int 2014; 38: 124-130.
[34] Racz B, Gasz B, Gallyas F Jr, Kiss P, Tamas A, Szanto Z, Lubics A, Lengvari I, Toth G, Hegyi O, Roth E and Reglodi D. PKA-Bad-14-3-3 and Akt-Bad-14-3-3 signaling pathways are involved in the protective effects of PACAP against ischemia/reperfusion-induced cardiomyocyte apoptosis. Regul Pept 2008; 145: 105-115.
[35] Caron PL, Frechette-Frigon G, Shooner C, Leblanc V and Asselin E. Transforming growth factor beta isoforms regulation of Akt activity and XIAP levels in rat endometrium during estrous cycle, in a model of pseudopregnancy and in cultured decidual cells. Reprod Biol Endocrinol 2009; 7: 80.